UNIVERSITAT DE BARCELONA

Association between (poly)phenols and obesity: from the observational study to the clinical trial

Mercedes Gil-Lespinard

UNIVERSITAT DE BARCELONA

UNIVERSITY OF BARCELONA

FACULTY OF PHARMACY AND FOOD SCIENCES

Doctoral Thesis

Association between (poly)phenols and obesity: from the observational study to the clinical trial.

Mercedes Gil-Lespinard, 2024

UNIVERSITY OF BARCELONA

FACULTY OF PHARMACY AND FOOD SCIENCES

DOCTORAL PROGRAMME IN FOOD AND NUTRITION

Conducted at the Bellvitge Biomedical Research Institute (IDIBELL) within the Catalan Institute of Oncology (ICO)

Doctoral Thesis

Association between (poly)phenols and obesity: from the observational study to the clinical trial.

Thesis submitted by Mercedes Gil-Lespinard to obtain the degree of Doctor from the University of Barcelona

Mercedes Gil-Lespinard
PhD candidate

Dr. Raul Zamora-Ros
Director

Dr. Cristina Andrés-Lacueva
Academic Tutor

Mercedes Gil-Lespinard, 2024

Funding

The funding entity for this project was the Institute of Health Carlos III, Ministry of Science and Innovation, Spain. This support was made possible by the European Regional Development Fund under the slogan “A way to build Europe”, European Union.

Mercedes Gil-Lespinard: Doctoral grant Training Programme in Health Research (Programa de Formación en Investigación en Salud, PFIS) FI19/00185; and Mobility grant Mobility of Research Staff (Movilidad – Acción Estratégica en Salud, M-AES) MV22/00023.

Project: Health Research Fund (Fondo de Investigaciones Sanitarias, FIS) PI18/00191 with Dr Raul Zamora-Ros as Principal Investigator.

The EPIC study: financially supported by International Agency for Research on Cancer (IARC) and by the Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, which has additional infrastructure support provided by the NIHR Imperial Biomedical Research Centre (BRC). The national cohorts are supported by: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l’Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Centre (DKFZ), German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Federal Ministry of Education and Research (BMBF) (Germany); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy, Compagnia di SanPaolo and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS)—Instituto de Salud Carlos III (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra,

and the Catalan Institute of Oncology—ICO (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C8221/ A29017 to EPIC-Oxford), Medical Research Council (1000143 to EPICNorfolk; MR/M012190/1 to EPIC-Oxford) (United Kingdom). The funders of this study had no role in the decisions about the analysis or interpretation of the data, or preparation, review or approval of the manuscript.

Fenland study: The Fenland Study is core funded by the Medical Research Council (MRC), UK Research and Innovation.

The Bellvitge Biomedical Research Institute (IDIBELL) played a crucial role in making this project possible by facilitating the execution of the 4-year research contract and the project. The research was carried out in the Unit of Nutrition and Cancer within the Cancer Epidemiology Research Programme at the Catalan Institute of Oncology, Duran i Reynals Hospital, L'Hospitalet De Llobregat, Barcelona, with the support of the Departament de Salut of the Generalitat de Catalunya. This research was partially made possible thanks to the support of the staff from the Endocrinology and Nutrition Service from the Bellvitge University Hospital.

Part of this project was conducted at the MRC Epidemiology Unit, located in Addenbrooke's Hospital, University of Cambridge, UK, as part of a three-month internship linked to the PhD schedule. This collaboration provided valuable insights and perspectives from an international standpoint.

Acknowledgements

La finalización de mi tesis doctoral me llena de gratitud. Quiero destacar a las personas clave sin las cuales este logro no hubiera sido posible:

Mi más profundo agradecimiento a mi compañero de vida, Seán. Tu constante creencia en mí, infinita paciencia y apoyo incondicional fueron pilares fundamentales a lo largo de esta etapa. Junto con nuestras bellas Peluchina y Dutchie, que le dan calidez y alegría a nuestra casita.

A mi mamá, mi papá, y mis hermanas, les agradezco eternamente por confiar en mis habilidades y ser una fuente constante de amor y aliento.

A mi director, Raul. Tu orientación, sabiduría e *insights* fueron esenciales para mi formación como investigadora. Fue un privilegio aprender de vos y me considero muy afortunada.

Un agradecimiento de corazón a mis compañeras de trabajo y amigas, Lucía, Melissa, Carlota, Jazmín, Núria y Sara. Su amistad y colaboración enriquecieron enormemente mi investigación.

Extiendo mi aprecio a Paula J, Marta F, Marta C y Antonio A por su apoyo y valiosos aportes. Agradezco también a Enrique, Daniel y Paulina, y a todo el PREC, por crear un ambiente de investigación tan colaborativo. Un agradecimiento especial para Carlos González Svatetz quien fue mi profesor y me brindó la oportunidad de conocer a este grupo.

Quiero además agradecer a las personas que me ayudaron durante el ensayo clínico, Gema C, Núria V y Mònica M. Su experiencia y orientación fueron vitales para el éxito del proyecto.

My sincere gratitude to the people I had the privilege to work with during my stay in the MRC Epidemiology Unit in Cambridge: Nita, Fumiaki, Shayan, Divya, Anwesha, Solomon, and Meriel. Your knowledge, support and kindness were invaluable during this enriching experience.

A todas mis amistades y familia extendida, en Rosario, Cataluña, París, Cork, Ipswich y más allá. Su apoyo y comprensión fueron fundamentales.

Cada una de ustedes jugó un rol esencial en mi desarrollo académico y personal. Me considero muy afortunada de tener un círculo de apoyo tan increíble.

Table of Contents

Abstract

(Poly)phenols are bioactive compounds present in plant foods, that have been suggested to present antioxidant and anti-inflammatory properties for humans. These properties have been linked with prevention of different metabolic conditions, including obesity and related diseases. Adipose tissue metabolism and systemic oxidative stress have been proposed as potential mechanistic pathways by which these compounds could attenuate obesity-related parameters. However, the identification of specific (poly)phenols related with obesity is not yet clear. Increasing consumption of plant-based foods rich in (poly)phenols have proven effective in preventing or treating obesity. (Poly)phenols are not considered essential nutritional compounds, and daily intake recommendations have not yet been established. Consumption of (poly)phenols as healthy dietary components is consistent with the advice to eat five or more portions of fruits and vegetables per day, but it is currently difficult to recommend what type and dose of specific (poly)phenols should be consumed to derive maximum benefit. This epidemiological study aimed to evaluate the associations between dietary (poly)phenols and obesity parameters across various populations, and to identify specific (poly)phenols that may contribute to the improvement of obesity parameters.

In this doctoral thesis, the association between dietary intake of individual (poly)phenols and 5-year body weight (BW) change has been studied in 349,165 participants from a large European cohort, the European Prospective Investigation into Cancer and Nutrition (EPIC). In addition, the link between concentration of 36 (poly)phenols in plasma and 5-year BW change has been studied in a smaller sample of the EPIC population (n 761). Overall, these associations were assessed through general linear mixed models, adjusting for relevant confounders. An inverse association was observed between the majority of dietary (poly)phenols and 5-year BW change, highlighting the class flavonoids and those compounds found in whole grains, tea, fruits especially berries, and

cocoa. In addition, a tendency towards BW loss was observed for higher concentrations of plasma (poly)phenols, despite results not surpassing multiple comparison corrections and not reaching statistical significance.

Secondly, this thesis included the cross-sectional associations of dietary intakes of flavonoids and flavonoid subclasses with adiposity parameters, assessed in ~ 11,000 adult participants from the Fenland study, in the UK. Outcome variables included body fat percentage, visceral abdominal and subcutaneous abdominal fat thickness (VAT and SCAT, respectively), VAT:SCAT ratio, BMI, waist circumference (WC), waist-to-hip ratio (WHR), and a body shape index. Associations were assessed through robust linear regression analyses. Higher dietary intake of total flavonoids and most flavonoid subclasses was inversely associated with most outcomes, especially body fat percentage, VAT, BMI, WC and WHR.

Thirdly, a (poly)phenol-rich supplement was formulated considering different findings: results from the EPIC observational analyses, previous literature including cohort studies and randomised-controlled trials (RCTs), and compounds present in a Mediterranean dietary pattern. A 400mg (poly)phenol-rich supplement was formulated with seven different compounds from plant extracts. The aim was to assess this supplement on participants with severe obesity, in combination with the traditional weight-loss dietary approach. A double-blinded, placebo-controlled clinical trial was designed, that included 40 adult participants with severe obesity (body mass index (BMI) $\geq$ 40 kg/m^2). Participants were randomly assigned to the intervention group (IG, 3 supplement capsules (1,200 mg) + 1,200 kcal Mediterranean diet a day) or the control group (CG, 3 placebo capsules + 1,200 kcal Mediterranean diet a day). The duration of the trial was 12 weeks and participants were assessed in three opportunities: at baseline, after six weeks, and after 12 weeks. The primary outcomes were body weight and composition. Secondary outcomes included other anthropometric measurements, cardiometabolic and inflammatory biomarkers, metabolic pathways, and gut microbiota diversity. In the three visits, dietary data was

through a 24-hour dietary recall and a 3-day dietary record. Also, fasting blood and 24-hour urine samples was collected. Body weight and composition was measured through a bioimpedance scan, waist and hip circumferences using an ergonomic measuring tape, and blood pressure using a clinically validated blood pressure monitor. Faecal samples were collected at baseline and week 12. This trial was approved by the Bellvitge University Hospital Ethics Committee. Written and voluntary consent was obtained from each participant prior to the beginning of the study. The trial has been registered at www.ClinicalTrials.gov (NCT05428540).

Overall, and through different analyses and levels of scientific evidence, this doctoral thesis can conclude that higher dietary intakes of (poly)phenols through plant-based foods, particularly those classified as flavonoids, are associated with body weight maintenance and less adiposity and can be considered an interesting approach in the prevention or treatment of obesity and its associated cardiometabolic risk.

1. Introduction

1.1. Overweight and Obesity

1.1.1. Definition

Overweight and obesity are defined as an excessive fat accumulation with consistent body weight (BW) gain, commonly classified using body mass index (BMI) values, a measure that uses BW (kg) divided by the square of height (metres)(1). The World Health Organization (WHO) classifies obesity based on BMI in kg/m²: overweight ranges from 25 to 29.9, type I obesity from 30 to 34.9, type II obesity from 35 to 39.9, and type III or severe obesity is defined as 40 or above(2). Obesity mostly implies high levels of adiposity, and usually represents a risk factor for several diseases, such as cardiometabolic or musculoskeletal pathologies, as well as several types of cancer(3). There are several parameters that can be used to assess the metabolic risks associated with excess weight and adiposity related to this condition, that can be either correlated with or independent of BMI(4,5). Waist circumference (WC) is a measure of abdominal obesity expressed in centimetres, and recommended cut-offs for increased health risk are a WC >102 cm for men and >88 cm for women(6,7). Abdominal obesity has a much worse prognosis than gluteal-femoral obesity, and therefore one premise associated with adiposity is that its most important characteristic is its distribution(6). A measure that takes this distribution into account is the waist-to-hip ratio (WHR) that represents the circumference of the waist divided by that of the hips. WHR exceeding 0.90 in men and 0.85 in women imply a substantially increased risk of metabolic complications(6–8). In the context of disease prevention, understanding body fat (BF) percentage is another critical parameter to consider(6,9). A higher BF percentage, particularly when concentrated around vital organs, has been linked to an increased risk of metabolic disorders, cardiovascular diseases, type 2 diabetes, certain cancers, and overall mortality(6,9). The distribution of BF significantly impacts on disease risk. The Dual-Energy X-ray Absorptiometry (DEXA) scan stands out as the gold-standard method for analysing body composition, known for its accuracy and reliability(10,11). In addition, the analysis of various tissue compartments, including visceral and subcutaneous abdominal fat thickness (VAT and SCAT, respectively) may

play an important role in the measurement of obesity-related health risk, for example by the use of ultrasound or bioelectrical impedance analysis methods(12). Higher concentrations of visceral compared to subcutaneous adipose tissue have been associated with greater metabolic risks(13,14).

1.1.2. Epidemiology

According to the World Health Organisation (WHO), obesity is one of today's most blatantly clear – yet most ignored – public health problems: an escalating global epidemic that affects virtually all age and socioeconomic groups and threatens to overwhelm both developed and developing countries(1,15). As stated by the latest World Obesity Atlas Report (2023)(16), no country has reported a decline in obesity prevalence across their entire population, and none are on track to meet the WHO's target of 'no increase on 2010 levels by 2025'. In Europe, around 27% of adults presented obesity in 2020, and is predicted to affect ~10% more people by 2035. In Spain, about 16% of adult population presented obesity according to the Spanish National Health Survey and the European Health Survey carried out in 2017 and 2020, respectively(17). The estimates for global levels of overweight (BMI between 25.0 and 29.9) and obesity (BMI ≥ 30.0) suggest that over 4 billion people may be affected by 2035, compared with over 2.6 billion in 2020(16). Scientific projections indicate that the worldwide obesity prevalence is expected to rise from 14% to 24% by 2035.

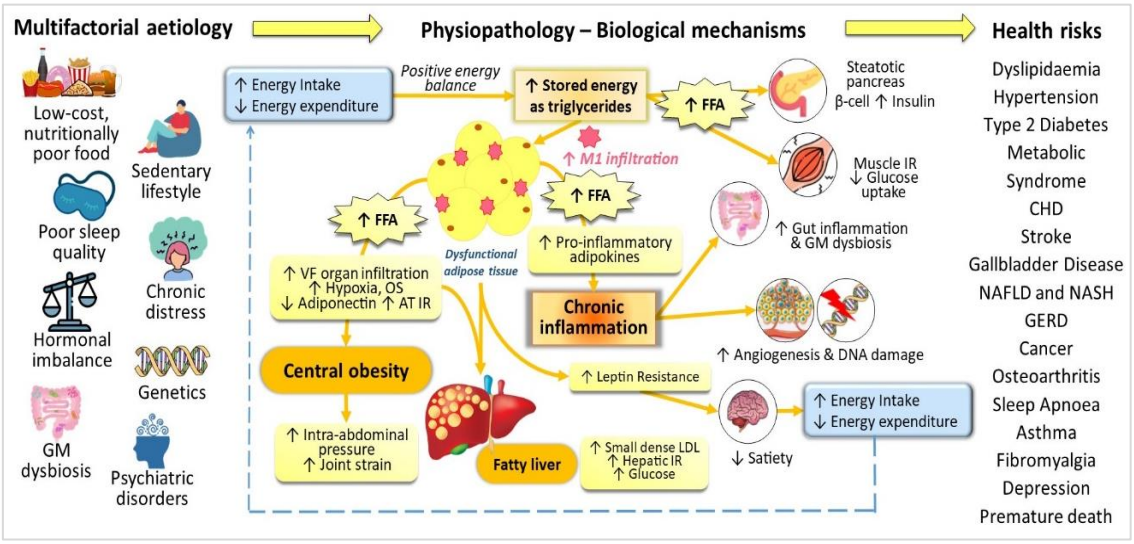
1.1.3. Clinical implications

Obesity is a human body's *normal* response to an *abnormal* environment, shaped by several factors that, over the past 40 years, have changed substantially and are rapidly transmissible(18,19). It has a multifactorial aetiology, including genetic, environmental, socioeconomic, and behavioural or psychological influences(20). Among these factors, the food system plays a crucial role. Increased supply of cheap, palatable and energy-dense, often ultra-processed foods, improved distribution systems to make food much more accessible, and more

persuasive food marketing(18,21) can be identified as one of the main drivers of this epidemic. Marketing environments that promote intake of hyper caloric, nutritionally poor foods rich in sugar, saturated fat and refined grains, often accompanied by sedentary behaviour (transport, work, and leisure activities) lead to an energy imbalance(21). In consequence, energy is stored in the form of triglycerides in the white adipose tissue (WAT), which expands by either hypertrophy (an increase in adipocyte size) or hyperplasia (an increase in adipocyte number due to the recruitment of new adipocytes) leading to dysfunctional WAT and BW gain. When the capacity of expansion is overwhelmed, fat accumulates in ectopic sites such as visceral depots, the liver, skeletal muscle, and pancreatic beta cells(22). An infiltration of new inflammatory cells, the major one initially being macrophages (M1 type, pro-inflammatory), accompanies the expansion of WAT(23). These changes create a pro-inflammatory state where adipocytes increase the secretion of cytokines such as tumour necrosis factor-alpha (TFN-α), interleukin 6 and 1-beta (IL-6 and IL-1β) or monocyte chemotactic protein-1 (MCP-1), while secretion of adiponectin, an insulin-sensitizing hormone, is decreased(24). The chronic and systemic inflammatory state characteristic of obesity, as well as the increase in visceral fat and BW, can lead to endothelial dysfunction, insulin resistance, and different pathways that promote angiogenesis and DNA damage. All these mechanisms increase the risk of serious non-communicable diseases, including type 2 diabetes mellitus, cardiovascular disease, hypertension and stroke(3). Obesity is also associated with certaintypes of cancer, sleep apnoea and osteoarthritis, as well as disorders of the digestive system such as gallbladder disease, non-alcoholic fatty liver disease, hepatic steatosis, or gastro-oesophageal reflux disease. Health consequences of obesity and obesity-related diseases range from increased risk of premature death to serious chronic conditions that reduce the overall quality of life(3,24). A study in the UK estimated, using mendelian randomisation(25), that a unit increase in BMI decreased quality-adjusted life years by 0.65% and increased the annual total healthcare costs by £42.23 per person(26). In Spain, observational data analyses showed that having a BMI ≥ 35 reduced health-related quality of life even in the absence of chronic diseases(27). **Figure 1**

shows a summary of the potential causes and physiopathology of obesity, as well as its associated health risks.

Figure 1. Aetiology, biological mechanisms, and health risks associated with obesity.



AT: adipose tissue; CHD: coronary heart disease; DNA: Deoxyribonucleic Acid; FFA: free fatty acids; GERD: gastro-oesophageal reflux disease; GM: gut microbiota; IR: insulin resistance; LDL: low-density lipoprotein; M1: type 1 macrophage; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; OS: oxidative stress; WAT: white adipose tissue. Images adapted from istockphoto.com. Content adapted from Cahit and den Hartigh, 2020 (24).

Obesity management encompasses diverse approaches. A meta-analysis of 72 studies involving 1,189,942 participants worldwide provided valuable insights into the prevalence of weight control efforts(28). The findings indicate that approximately 42% of adults in the general population and 44% among ethnic minority populations are actively attempting to lose weight, while about 23% reported trying to maintain their weight at some point. Mitigating obesity risk factors, including adherence to a nutritious dietary pattern, regular physical activity, stress management, and sufficient sleep, poses notable challenges, demanding substantial commitment and prolonged periods to observe discernible outcomes(29). Nonetheless, a multimodal lifestyle intervention constitutes the foremost preventive strategy against obesity(30). When addressing treatment interventions, other approaches are frequently pursued to complement lifestyle changes, such as pharmacological interventions. Prescribed medications can be used as an adjunct to diet and exercise, helping to suppress appetite or reduce fat absorption(31). Currently available and

commonly prescribed examples include orlistat(32), semaglutide (33) and liraglutide(34,35). Orlistat acts by reducing the absorption of dietary fat(32).

Semaglutide and liraglutide are both glucagon-like peptide-1 (GLP-1) receptor agonists that appear to act on the parts of the brain that regulate appetite. They work by attaching to GLP-1 receptors in brain cells, thereby increasing feelings of fullness and lowering feelings of hunger (33–35). A third option for obesity treatment, particularly for severe obesity, is bariatric surgery(36). Surgical procedures like gastric bypass or gastric sleeve can lead to significant weight loss and improvement in obesity-related health conditions(36). However, surgical interventions entail inherent risks and potential complications, necessitating thorough evaluation, comprehensive preoperative preparation, and diligent long-term post-operative care. Furthermore, it is crucial to recognize that not all people meet the criteria or express a preference for surgical interventions(36). To support these traditional approaches, a more recent strategy is the use of health behaviour change interventions (HBCIs)(37). It focuses on the understanding of the complexity behind people's decisions and engagement in behaviours that affect their health and well-being, including sustained weight management(37).

Obesity is a complex condition that not only impacts physical well-being but also tends to be accompanied by significant social and psychological challenges(38). Several studies have extensively documented the presence of detrimental stereotypes associated with body weight, specifically targeting people who present overweight or obesity(39). These stereotypes suggest that such people are characterized as lazy, lacking willpower, unsuccessful, unintelligent, lacking self-discipline, and noncompliant with weight loss treatments(39). The prevalence of this form of stigma remains largely unchallenged in our society and the public health implications associated with weight stigma have been largely disregarded(39). Instead, societal attitudes often assign blame to individuals with obesity for their body weight, with common perceptions suggesting that weight stigmatization is justified, or even necessary, due to the belief that people with obesity bear personal responsibility for their weight(38). The stigma of obesity has not been addressed as

a legitimate concern that requires the attention of those working to combat obesity and is not frequently discussed in the context of public health. Recognizing that individual behaviours are heavily influenced by the obesogenic environment is essential in shaping effective strategies to combat obesity(38).

The third Sustainable Development Goal for 2030 set by the United Nations Member States in 2015 is to ensure healthy lives and promote well-being for all at all ages(40). In 2019, the EAT-Lancet Commission presented a global planetary health diet that is healthy for both people and planet(41). It emphasizes a plant-forward diet where whole grains, fruits, vegetables, nuts, and legumes comprise a greater proportion of foods consumed. A healthy plant-based diet (PBD) includes, as mentioned, unprocessed plant foods and excludes or limits refined grains and sugars(42). Increasing consumption of healthy plant-based foods have proven effective in preventing or treating obesity(43,44). Higher adherence to the EAT-Lancet diet has been inversely associated with WC, and did not contribute with the development of obesity in a Danish population(45). An observational study has shown that a dietary pattern rich in healthy plant-derived foods is associated with reduced long-term obesity after a median follow-up of 10 years in Spanish participants(46). Likewise, cross-sectional results from the European Prospective Investigation into Cancer and nutrition (EPIC)-Oxford cohort showed that participants following dietary patters predominantly plant-based had lower BMI than meat-eaters(47). Clinical evidence has demonstrated that interventions with PBDs improve obesity-related parameters. An 18-week dietary intervention using a low-fat PBD in participants with overweight/obesity resulted in improved BW, lipid profile and glycaemic control(48). Likewise, five-arm RCT that compared effectiveness of different PBDs on BW showed that participants following a fully PBD (vegan) for 6 months had grated weight loss than more modest recommendations (i.e., vegetarian, pesco-vegetarian, semi-vegetarian and omnivorous)(49). In a 16-week RCT, a low-fat PBD intervention reduced BW and increased insulin sensitivity in participants with overweight or obesity(50).

Thus, evidence has proposed PBDs as an effective tool for prevention or treatment of obesity and obesity-related diseases.

1.2. (Poly)phenols

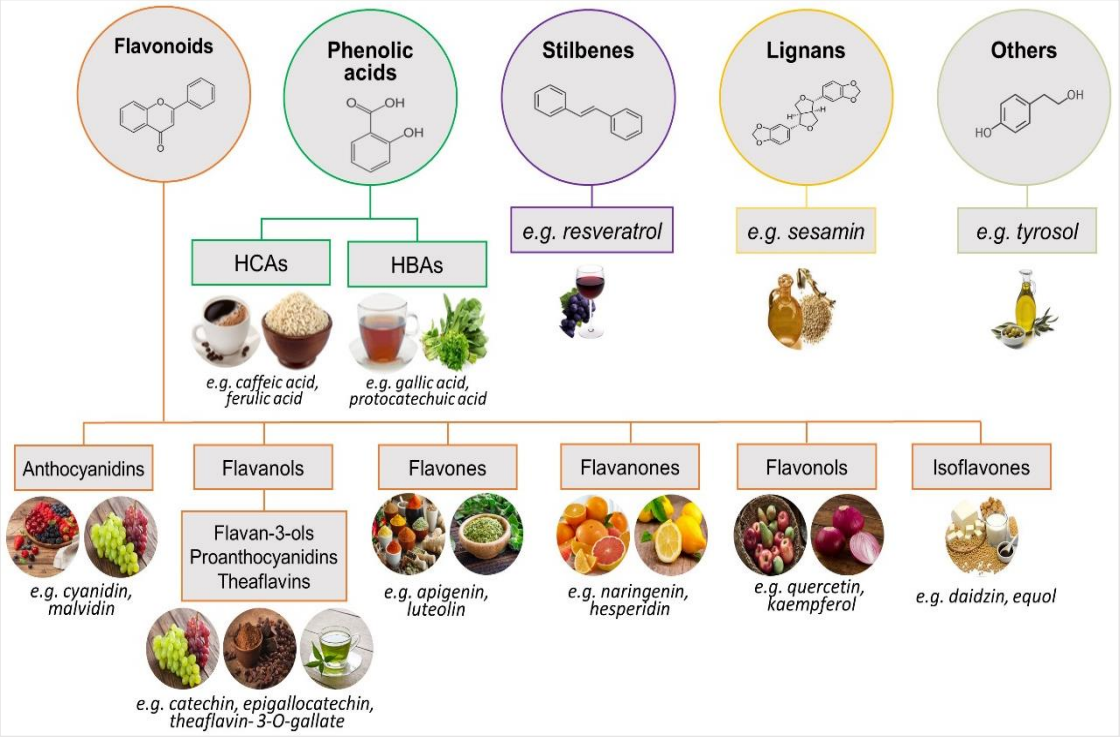
1.2.1. Definition and chemical structure

A particular class of compounds naturally occurring in plants, phenols and polyphenols, from now on mentioned as (poly)phenols, may be considered an important factor for the expression of PBD benefits on human health. (Poly)phenols constitute a large class of at least 10,000 organic compounds that have more than one aromatic ring structures with single or multiple hydroxyl (OH) groups bound to them(51,52). These natural products are highly diverse and, according to the quantity of phenol rings and the type of linkages that join multiple rings to each other, they can be classified in several classes. The predominant class found in human diet is phenolic acids, followed by flavonoids, stilbenes, lignans and others in minority(53–56). Flavonoids represent around 60% of all (poly)phenols from the human diet. They are commonly found in different fruits and vegetables, cocoa, soy, and teas. As for phenolic acids, they represent around 30% of (poly)phenols found in the diet, mainly present in foods like coffee, tea, wholegrain cereals, and seeds. Stilbenes represent a minor proportion, mainly present in grapes in the form of resveratrol, as well as lignans, found predominantly in seeds, cereals, and legumes. In addition, there are other (poly)phenols that represent a rather small proportion of compounds, among which, for example, tyrosol from olives or alkylresorcinols in cereals and cereal products stand out(57,58). **Figure 2** shows the main classes of (poly)phenols with their respective general chemical structure and main food sources according to the Phenol-Explorer database(59,60).

(Poly)phenols in plants are generally involved in defence against aggression by environmental factors, and constitute active substances and modulate the activity of a vast amount of enzymes and cell receptors(61). In the last decades, the presence of these compounds in the human diet through plant-based foods has led to an increased interest regarding their role in human health, as

they have shown to act as antioxidants and are thought to have anti-inflammatory properties(61,62). Growing scientific evidence is focusing on the establishment of the effects of (poly)phenol consumption on health and the identification of which of the hundreds of existing (poly)phenols are likely to provide the greatest protection in the context of preventive nutrition(62). (Poly)phenols are not considered essential nutrients, and daily intake recommendations have not yet been established(63). While thousands of molecules exist in nature, around 500 individual (poly)phenols have been identified in the human diet so far(59,62).

Figure 2. Chemical structure of major classes of (poly)phenols with individual examples, and main food sources.



HBAs: hydroxybenzoic acids; HCAs: hydroxycinnamic acids.

1.2.2. Bioavailability

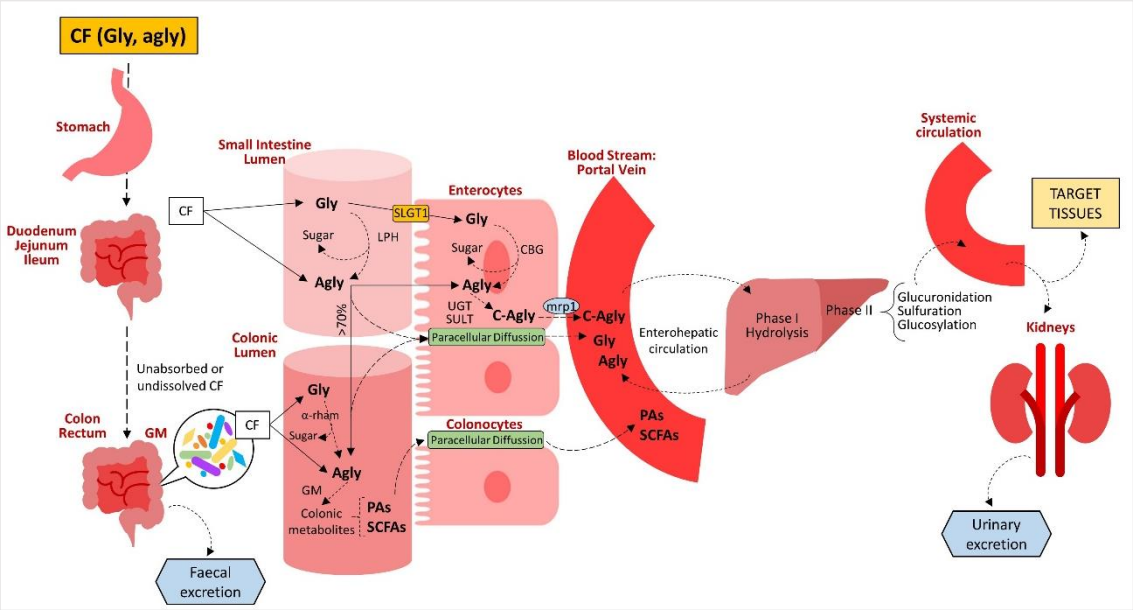
Once (poly)phenols enter the digestive system, they undergo several processes that can affect their bioavailability and health effects(64). Most of the (poly)phenols present in our diet exists as polymers or glycosides, with a sugar moiety (glycone) bound to an aglycone (the (poly)phenol). For these native forms to be absorbed, they need to be hydrolysed by the intestinal enzymes or by

the gut microbiota(64,65). Phenolic aglycones are partially absorbed by glucose transporters in the small intestine. Then, they are metabolized through sulfation, glucuronidation or methylation by phase II metabolic enzymes (sulfotransferases, uridine-5'-diphosphate glucuronosyltransferases, and catechol-O-methyltransferases) before glucose transport into the hepatic portal vein and circulation(66). Both the specific chemical structure of (poly)phenols and the type of sugar in the glycoside define their level and magnitude of intestinal absorption(64). Compounds with a high degree of polymerization that are not absorbed in at the small intestine reach the colon to undergo microbial catabolism. As a result, smaller compounds, such as phenolic acids, can be absorbed and, later, reach the liver to also be partially conjugated by phase II enzymes(67). Phase-II enzymes and microbial-derived metabolites either reach systemic circulation to be distributed to different organs and tissues or reach the kidneys to be excreted through urine. In addition, enterohepatic recirculation may result in some recycling back to the small intestine through bile excretion, with subsequent faecal excretion(66). **Figure 3** illustrates the processes of absorption, distribution, metabolism, and excretion (ADME) of (poly)phenols, using citrus flavanones as an example.

In humans, the bioavailability of (poly)phenols shows considerable intra-individual variability, meaning that the absorption and metabolism of these compounds can differ significantly from one occasion to another in the same person(65). Additionally, there is substantial inter-individual variability, with different people experiencing varying degrees of absorption and efficacy of (poly)phenols due to genetic, dietary, and environmental factors(65). These variations highlight the complexity of studying and understanding the health effects of polyphenols. Studies in humans have reported data on bioavailability of different (poly)phenol classes and subclasses, measuring metabolites mainly in plasma and urine(65). Regarding flavonoids, for example, after ingestion of 500 mg *Aronia melanocarpa* extract containing 9.02% anthocyanins, these compounds were detectable in overnight urine in healthy adults, former smokers (0.332 ± 0.136 mg mg−1 creatinine)(68). Likewise, in healthy adults, after ingestion of 250 ml of blackcurrant

juice containing 20% of anthocyanins, the urinary percentage of anthocyanins excreted after 120 min was 0.021 ± 0.003%(69).

Figure 3. ADME of (poly)phenols using citrus flavanones as example.



ADME: absorption, distribution, metabolism and excretion; Agly: aglycones; C-: conjugated; CBG: cytosolic beta-glucosidases; CF: citrus flavanones; Gly: glycosides; GM: gut microbiota; LPH: lactase-phlorin hydrolase; mrp1: multidrug resistance-associated protein 1; PAs: phenolic acids; SCFAs: short chain fatty acids; SLGT1: sodium/glucose cotransporter 1; SULT: sulphotransferases; UGT: uridin-diphospho--glucuronosyl-transferases.

Engler *et al* observed that after consumption of flavonoid-rich dark chocolate containing 213 mg procyanidins and 46 mg epicatechin, in healthy adults, plasma epicatechin concentrations were markedly increased at 2 weeks in the high-flavonoid group (204.4 ± 18.5 nmol/L, $p \leq 0.001$) versus control group(70). In a randomised controlled crossover trial, participants at risk of cardiovascular disease (CVD) were given 4.89 mg/kg body weight/day of enzymatic modified iso-quercetin(71). After 3 hours, quercetin metabolites concentration was significantly higher in plasma versus placebo group (quercetin aglycone 144.9 ± 12.3 nM vs 12.6 ± 12.3 nM; and isorhamnetin 245.5 ± 16.5 nM vs. 41.7 ± 16.5 nM) ($p < 0.001$). Flavanone-derived metabolites have also been detected in plasma after consumption of flavanone-rich food. Schär *et al* observed that after 5 hours of ~ 450 ml of orange juice consumption by men at CVD risk, 8 flavanones and 15 phenolic metabolites were significantly increased in plasma (including hesperidin, naringenin

and dihydroferulic glucuronides) – they collectively reached a concentration of 15.20 ± 2.15 μmol/L(72). As per phenolic acids, for example, in a crossover trial healthy adults received either 0.45 or 0.90 g of purified 5-chlorogenic acid (p5-CgA)(73). Authors observed that after 1 and 4 hours from the consumption of 0.90 g 5-CgA, total CgA metabolites reached 1.5 μM and 1.25 μM, respectively. After 0.45 g, metabolites reached 0.75 μM and 1 μM after 1 and 4 hours respectively. In both cases, the most representative CgA metabolite was 5-caffeoylquinic acid. In another study, healthy participants were given 400 ml of Arabica coffee containing 0.42 g of a combination of chlorogenic acids (containing, among others, 5-O-caffeoylquinic, caffeic and ferulic acid)(74). After 1 hour, plasma concentrations of ferulic and caffeic acid were 202.38 ± 12.87 nM and 49.76 ± 6.44 nM, respectively. Results on bioavailability of other classes such as stilbenes have also been reported. Wightman *et al* observed that after a daily consumption of 500 mg of pure trans-resveratrol for 28 days by adult participants, resveratrol 3-O-sulfate was the predominant metabolite in all volunteers, contributing 73–77% of total metabolites in plasma(75). Total resveratrol metabolites increased in plasma from 3 to 13 μM 110 minutes after administration.

1.2.3. Dietary intake and safety

Consumption of (poly)phenols as healthy dietary components is consistent with the advice to eat five or more portions of fruits and vegetables per day(76), but it is currently difficult to recommend what type and dose of specific (poly)phenols should be consumed to derive maximum benefit. Dietary intake of (poly)phenols has been studied in different large populations, with high variabilities in the amount consumed. In a large population sample from 10 Western European countries, mean total daily (poly)phenol intake varied between ~ 1,700 mg in Denmark and ~ 700 mg in Greece(53). In a Japanese population, (poly)phenol dietary intake was, on average, ~ 750 mg per day(77). Similarly, in large cohort from Mexico, consumption of (poly)phenols varied between 530 and 750 mg/d across this country(78).

This is a relatively new topic of research, and safety and health claims of only a few polyphenols have been officially established. The European Food Safety Authority (EFSA) has published different Scientific Opinion (SO) Reports regarding safety of several (poly)phenols and (poly)phenol-rich extracts for human consumption. In 2011, a SO on (poly)phenols in olives highlighted their safety for human consumption as well as their protective effects against oxidative stress. They also concluded that, to reach these beneficial effects, a daily dose of 5 mg of hydroxytyrosol and its derivatives in olive oil should be consumed(79). A SO on quercetin was also published, where they concluded that it is safe for general population and that it is associated with protection against oxidative damage(80). Resveratrol was also considered a safe compound for human consumption – the panel concluded that 150 mg/d of trans-resveratrol was safe for human adults(81). In 2018, a SO on the safety of green tea catechins(82) concluded that, when prepared in a traditional way or when reconstituted with an equivalent composition to traditional green tea infusions, green tea catechins are in general considered to be safe. In addition to EFSA, the European Medicines Agency (EMA) evaluated the safety of some of these compounds. (Poly)phenols might be not only consumed from food products or extracts, but also at pharmacological doses, for example, in dietary supplements. The EMA reported in 2017 that olive leaf extract is safe for consumption in adults as a herbal medicinal product, except during pregnancy or lactation(83). In addition, different botanical databases on herbal compounds assessed for human consumption have included (poly)phenol-rich extracts and individual compounds. One such example would be the EFSA Compendium, that includes different botanicals and botanical preparations intended for use in food, with its correspondent dosage and concerns for human consumption, to help with their safety assessment(84). Another example is Belfrit database, developed by the national authorities of Belgium, France and Italy, that aims to provide a list of plants that are permitted for use in food supplements based on the lists established by each of the three countries(85).

1.2.4. Measurement of (poly)phenol intake

The measurement of (poly)phenol consumption involves a spectrum of methodologies, ranging from subjective dietary assessments to more objective laboratory measurements(86,87). Subjective dietary assessment methods vary in their focus, with some measuring habitual diet, assessing long-term dietary patterns, and others capturing acute or specific day intake, offering a detailed view of intake on a particular day(86,87). Subjective assessments include diverse methods, such as food frequency questionnaires (FFQ) to measure habitual diet. In FFQs, participants report how often they consume specific foods and beverages over a defined period(88,89). Another approach is 24-hour dietary recalls, where participants recall all foods and beverages consumed in the past day, providing a snapshot of their diet(86). Additionally, researchers employ food diaries, where participants meticulously record their daily food and drink intake for a specific duration (e.g., 3 days), offering detailed dietary insights(86). Databases play a crucial role in estimating dietary intake of (poly)phenols from foods collected through dietary assessment methods and their (poly)phenol content. Examples of reliable sources include the Phenol-Explorer(60) and the United States Department of Agriculture (USDA)(90) databases, which provide clear information on the flavonoid composition in different food and beverages. The Phenol-Explorer database, one of the most important and widely used, contains data on 502 (poly)phenol glycosides, esters, and aglycones in 452 food items(60).

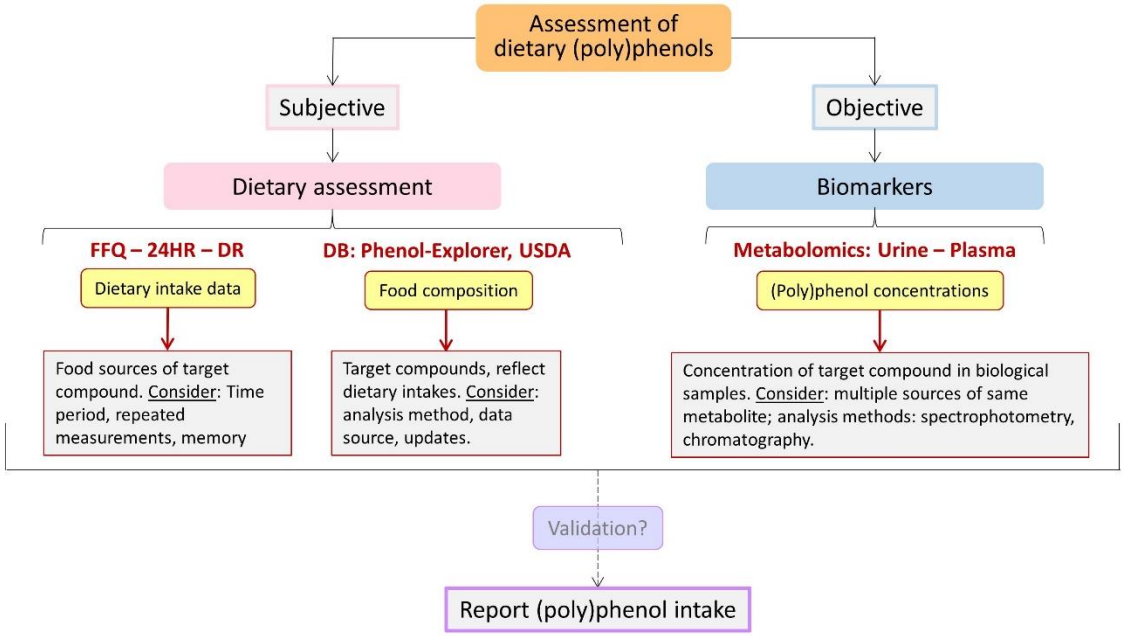
While self-reported dietary data is susceptible to bias and tends to underreporting(91,92), and databases often provide limited information on food (poly)phenol content and factors affecting the (poly)phenol content of foods such as species, processing or storage(87), these data can be compared and improved with the use of objective methods. Biomarkers in biological samples, including plasma and urine, serve as valuable resources to measure (poly)phenol metabolites, providing insights into absorption, systemic circulation, and bioavailability(93). Chemical analyses encompass a range of methodologies, starting with broad spectrophotometric approaches such as the Folin-Ciocalteu method, which measures total (poly)phenol content (94,95).

Progressing to more specific techniques, chromatography methods like high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) (96), and notably, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (97) are employed. LC-MS/MS, in particular, stands out for its exceptional analytical power, achieving excellent results with low quantification limits(97). Reaching the highest level of specificity, metabolomics offers a comprehensive analysis of the entire metabolite set in a biological sample(98). This advanced approach provides a holistic view of the metabolic impacts of (poly)phenol consumption, encapsulating the investigative progression from general to highly detailed analytical techniques(98). Using biomarkers to measure dietary intake of (poly)phenols comes with limitations. One major challenge is the complexity of (poly)phenol metabolism, which can vary widely among individuals due to genetic differences, gut microbiota composition, and other metabolic factors(93). This variability can lead to discrepancies between actual intake and what is measured by biomarkers. Additionally, (poly)phenols undergo extensive transformations in the body, and the biomarkers often measure only specific metabolites, not reflecting the complete intake or the diversity of consumed (poly)phenols(93). Furthermore, the short half-life of many (poly)phenols means that biomarkers may only provide a snapshot of recent intake, missing the broader context of dietary habits(93). These limitations suggest that while biomarkers are a valuable tool for assessing (poly)phenol consumption, they should be used in conjunction with other dietary assessment methods to obtain a more comprehensive understanding of (poly)phenol intake(93,99). Integrating subjective dietary assessments with biomarker analysis can offer a broader view of (poly)phenol consumption, triangulating data to enhance the reliability and accuracy of findings(99).

A very important aspect in nutrition epidemiological studies to ensure the accuracy and reproducibility of results is the validation of assessment methods(88,100,101). It involves assessing the performance of a method against a reference standard or established criteria. In (poly)phenol research, the validation of measurement methods is important to generate

trustworthy data, make meaningful comparisons across studies, and draw accurate conclusions about the impact of (poly)phenols in health(99,102). However, unlike other dietary components such as nitrogen, no gold standard biomarkers have been established for estimating (poly)phenol exposure(99). The Phenol-Explorer database used to estimate content of (poly)phenols in food items has undergone a validation process to ensure accuracy and reliability of its data(59,60,102). The validation included an extensive review of the scientific literature, standardization of the data as well as rigorous quality control measures to identify potential errors (e.g., cross-referencing data with multiple sources and applying statistical methods to identify outliers or discrepancies). In addition, it includes expert review, an update mechanism (the database is regularly revised and expanded), as well as user feedback and collaboration. This multifaceted approach ensures that the database is the best alternative to date as a resource of information(59,60,102). **Figure 4** provides a summary of the most common methods used to estimate dietary intake of (poly)phenols and highlights key considerations.

Figure 4. Most common methods used to estimate dietary intake of (poly)phenols.



DB: database; DR: dietary record; FFQ: food frequency questionnaire; 24HR: 24-hour dietary recall; LC/MS-MS: liquid chromatography tandem mass spectrometry; USDA: United States Department of Agriculture.

1.3. (Poly)phenols and Obesity

There is a growing research interest in identifying (poly)phenolic compounds that exhibit potent efficacy with minimal side effects in the management of obesity. Different mechanistic studies have suggested or demonstrated several biological pathways by which (poly)phenols may act as protective compounds against obesity and obesity-related conditions(103–105). Some of these include lowering oxidative stress, inflammation, fatty storage, lipid oxidation, and endothelial dysfunction, and improving microbiota composition. In addition, different levels of evidence have suggested or demonstrated the inverse association that higher intake of (poly)phenols may have with different obesity-related parameters, such as BMI, WC, or body weight(106). The latest evidence on observational and clinical studies is presented below.

1.3.1. Observational evidence

Analysing the consumption of (poly)phenols in large populations is the first step to suggest possible associations with improvement of obesity and obesity-related diseases. The Mediterranean healthy eating, aging and lifestyle (MEAL) study cohort examined the link between habitual intake of dietary flavonoids and obesity in ~ 2,000 adult participants from a Mediterranean area (southern Italy)(107). Participants with high intake of total flavonoids and flavonols resulted less likely to present obesity. Likewise, in around 13,000 participants from a French cohort, higher intakes of flavanones, flavones and lignans were inversely associated with 6-year changes in BMI and WC(108). Three prospective cohorts from the US including more than 124,000 participants observed that higher intake of foods rich in flavonols, flavan-3-ols, anthocyanins, and flavonoid polymers may contribute to BW maintenance after up to 24 years of follow-up(109). In a group of UK adults, mainly women (n~1,800), consumption of stilbenes and flavonoids was linked to lower odds of prevalent obesity, in part mediated by modulation of gut microbiome(110). These studies underscore the potential influence of consuming (poly)phenols in managing obesity. In the EPIC-PANACEA cohort, higher intakes of flavonoids and their subclasses were inversely associated with a modest body weight change, although the subclass

HCAs showed positive associations(111). A small number of studies have also objectively assessed intake of (poly)phenols through biological biomarkers. In the PREDIMED trial, for example, urinary (poly)phenol levels were inversely correlated with body weight and obesity, measured through BMI, WC and WHR, in an elderly population over 5 years(112). Another study assessed twenty-two (poly)phenol biomarkers in urine samples established and validated to effectively assess the habitual (poly)phenol intake in a population of Chinese adults. The levels of urinary (poly)phenol biomarkers, particularly those derived from gut microbial metabolites of (poly)phenols, showed an inverse association with overweight and obesity. The association was more pronounced in participants with inflammatory conditions, indicating the importance of maintaining high (poly)phenol biomarker levels or ensuring sufficient (poly)phenol intake in cases of obesity with chronic inflammation(113).

1.3.2. Clinical evidence

The association between the consumption of (poly)phenols and parameters associated with obesity has also been evaluated in numerous randomised controlled trials (RCTs). In such studies, the intake of (poly)phenols is frequently measured through the administration of supplements, capsules, or extracts derived from individual foods that contain specific classes, subclasses, or individual compounds. For example, capsules of berry extract containing anthocyanins(114); capsules of green tea extract rich in epigallocatechin-gallate and other catechins(115); tablets of pure trans-resveratrol(116); capsules of onion peel extract containing quercetin(117); or capsules of hydroxytyrosol from olives(118). A recent meta-analysis of human clinical trials included 40 RCTs published between 2010 and 2021 that measured the effectiveness of (poly)phenols in obesity management(106). Authors concluded that consumption of (poly)phenols decreased BW, BMI, and WC. In addition, they highlighted that the most effective class for decreasing obesity-related anthropometric measures was flavonoids. The synthesis of these findings underscores the potential role of (poly)phenols in obesity management. In contrast, a meta-analysis of RCTs examining the impact of (poly)phenol supplementation alongside calorie-restricted diets and/or

physical activity found that, while supplementation alone did not enhance weight or fat loss, isoflavone and soy products delivered additional anti-obesity effects in postmenopausal women(119). As we delve deeper in this thesis, subsequent sections will intend to provide a more comprehensive understanding of the complex interplay between (poly)phenols and obesity, through different levels of scientific evidence.

2. Hypothesis and Objectives

2.1. Hypothesis

(Poly)phenols are bioactive compounds prevalent in plant-based foods, renowned for their potential health benefits, especially regarding their antioxidant and anti-inflammatory properties. Experimental research has revealed that certain subclasses of (poly)phenols may also exhibit anti-obesogenic effects, even though human studies in this area remain scarce. Consequently, this project proposes the following hypothesis: a diet rich in (poly)phenols is anticipated to be associated with enhanced body weight management or greater body weight loss, less adiposity, and improved cardiometabolic indicators. This proposal is supported by a wide range of scientific evidence, encompassing both observational and interventional studies in humans.

2.2. Objectives

The main objective of this investigation was to evaluate the association between exposure to dietary (poly)phenols and anthropometric parameters associated with obesity in adults.

The specific objectives were:

1. To investigate the prospective association between individual (poly)phenols and body weight changes during follow-up in a sub-cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC).
2. To investigate the prospective associations between plasma concentrations of (poly)phenols as biomarkers of their dietary intake, and body weight changes during follow-up in a sub sample of the EPIC cohort.
3. To investigate the cross-sectional association between dietary intake of flavonoids and their subclasses, and adiposity-related parameters in the UK Fenland study.
4. To formulate a dietary supplement based on the data obtained from the observational analyses and the available literature, including a combination of potentially beneficial (poly)phenols, to improve obesity markers in adults with severe obesity.

5. To design and conduct a double-blinded, placebo-controlled randomised trial to assess the effects of such supplement in combination with a hypocaloric diet on severe obesity markers.

3. Material and Methods

3.1. Observational studies

3.1.1. EPIC study

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a massive cohort study that spanned 10 European countries, including Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, The Netherlands, and the United Kingdom, with 23 centres(121). It involved over half a million (521,000) participants and had a lengthy observational follow-up period of nearly 15 years, making it one of the largest and most extensive studies of its kind globally. EPIC aims to investigate the relationships between diet, nutritional status, lifestyle and environmental factors, and the incidence of cancer and other chronic diseases. Approval for this study was obtained from the ethical review boards of the International Agency for Research on Cancer (IARC) and from local institutions. The EPIC-Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating out of home And obesity (EPIC-PANACEA) study is a sub-cohort of the EPIC that included participants with data on BW at baseline and follow up, making it possible to assess BW changes. It excluded pregnant women, participants with extreme or implausible diet values (extreme ratio of reported energy intake/energy requirement), with unreliable anthropometric measures (height<1.3 m, BMI<16.0 kg/m2, WC<40 or >160 cm, WC<60 cm & BMI>25 kg/m2), and without lifestyle information at baseline, as well as participants from Greece, leaving a total of 349,165 participants included in the sub-cohort.

The EPIC cohort also allowed the conduction of nested case-control studies on different types of cancer. To assess plasma (poly)phenol concentrations, a convenient sample of cancer-free controls was selected from two EPIC nested case-control studies on colon and thyroid cancer (n=1,321)(122,123) with collection of plasma samples at baseline and available data on polyphenol concentrations. Participants with no plasma (poly)phenol measurements (n=430) and with no follow-up BW (n=73) were excluded. Also, data from Greece could not be included in these analyses (n=57). The final sample to assess the association between plasma (poly)phenols and BW change included 761 participants. Dietary intakes were estimated through validated FFQ

and the standardized EPIC Nutrient Database(124,125). To estimate (poly)phenol content, the Phenol-Explorer database was used(60). Plasma (poly)phenols were measured by a highly sensitive method based on differential isotope labelling with 12 and 13 C-dansyl chloride and an ultra-performance liquid chromatography–tandem mass spectrometry system (UPLC-MS/MS) with prior enzymatic hydrolysis(126). The explanatory variables assessed in the EPIC population included, firstly, dietary intake (mg/d) of 91 individual (poly)phenols. Secondly, plasma concentrations of 36 (poly)phenols (mmol/L). The outcome variable assessed in all EPIC analyses was BW change over 5 years. To calculate it, follow-up weight was subtracted from baseline weight. This result was then divided by years of follow up, and then multiplied by 5 (mean years of follow-up). Both individual dietary polyphenols (mg/d) and plasma biomarkers (mmol/L) underwent a log2 transformation to address right skewness. Baseline characteristics of population were stratified by quintiles (Q) of BW change (kg/5y) for dietary data (Q1 for loss, Q2 and Q3 for maintenance, Q4 for moderate gain, and Q5 for gain). For plasma biomarkers, population characteristics were reported by categories of BW change: loss, maintenance, and gain. For both studies, correlations were evaluated among compounds and different (poly)phenol-rich dietary sources. Missing values were omitted for continuous variables and classified as unknown for categorical variables. To assess the association of both dietary and plasma compounds with BW change, general linear mixed models were applied, adjusting for predefined confounders. The relationship between plasma (poly)phenol concentrations and BW change was further examined using multinomial logistic regression models, treating BW change as a categorical variable (i.e., loss, maintenance, gain). Post-hoc analyses for the study with dietary (poly)phenols included estimation of compounds according to energy intake (EI), as well as a sensitivity analysis excluding misreporters of EI (according to Goldberg(127) cut-off points). In addition, interactions were explored for sex, baseline age, BMI, menopausal status, smoking status at follow-up, and tertiles of fibre intake. Another analysis was performed excluding participants with chronic diseases at baseline (diabetes, cancer, stroke, or myocardial infarction). Finally, to clarify the role

of some (poly)phenols from coffee in BW change, further analyses were performed with coffee consumers and non-consumers, as well as adding certain food groups often consumed with coffee as confounders in the final model (i.e. sugar, milk, confectionery, and cake intake). For the study with plasma (poly)phenols, a sensitivity analysis was performed excluding participants with chronic diseases at baseline. In addition, interactions were explored with sex, baseline age, and smoking status at follow-up.

3.1.2. Fenland study

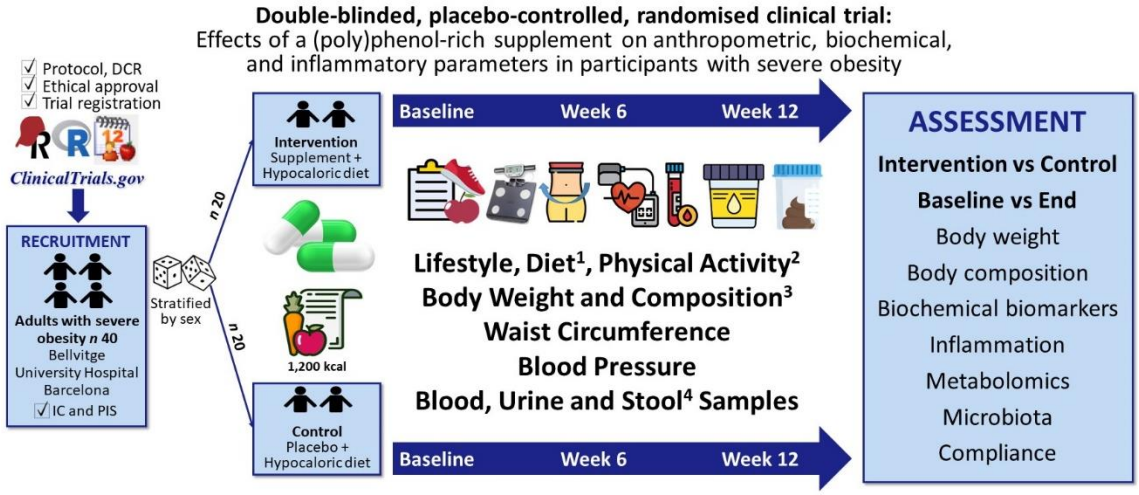
The Fenland Study, led by the MRC Epidemiology Unit from the University of Cambridge(128), is an on-going population-based cohort study designed to investigate the influence of lifestyle and genetic factors on the development of obesity, diabetes, and other metabolic disorders(129). Baseline measurements were conducted between 2005 and 2015 and included 12,435 participants, with a response rate of 27%. Eligible participants, born between 1950 and 1975, were invited from general practice lists in and around Cambridgeshire, in the East of England. Three locations were selected for recruitment and data collection: Cambridge, Ely, and Wisbech. Exclusion criteria included history of diabetes, psychotic or terminal illness, inability to walk unaided, and pregnancy or lactation. The Cambridge Local Ethics Committee approved the study, and all participants gave written informed consent. Participants underwent quantitative assessment of total and regional adiposity using ultrasound and DEXA scanning. In addition, key lifestyle determinants of metabolic disease were characterised objectively, such as free-living physical activity energy expenditure estimated by combined heart rate and movement sensing(129). The phase 1 of this study allowed evaluating cross-sectional relationships between different exposures and changes in metabolism. In addition, follow-up phases are being undertaken to be able to study these associations longitudinally(130). Dietary intakes were estimated using the FFQ EPIC Tool for Analysis (FETA)(131) that incorporates the standardized EPIC Nutrient Database(125). Specifically for flavonoid estimation, a food composition database for flavonoids that was based on US Department of Agriculture (USDA) databases was used(90). To assess cross-sectional

associations of flavonoid intake and adiposity parameters only participants classified as complete cases were included (n=11,568). The explanatory variable assessed in the Fenland study population was intake of total flavonoids, as well as flavonoid subclasses, including: flavanols (flavan-3-ol monomers, proanthocyanidins and theaflavins), anthocyanidins, flavonols, flavanones, flavones, and isoflavones. The outcome variables included different adiposity-associated parameters: the gold standard measure DEXA for body fat percentage, visceral and subcutaneous abdominal fat thickness (VAT and SCAT, respectively), VAT:SCAT ratio, ABSI, and conventional indicators like BMI, WC, and WHR. All data was collected at baseline, and therefore the associations assessed were cross-sectional. Continuous dietary intake of total flavonoids as well as the subclasses were log2-transformed to improve right skewness. Baseline characteristics of population were stratified by quintiles of total flavonoid intake. Total flavonoid intake was also assessed as a categorical variable (quintiles of intake). Correlations were evaluated among flavonoids and different (poly)phenol-rich dietary sources. Missing values were omitted and therefore a complete case analysis was performed. The cross-sectional association of flavonoids and adiposity was assessed through robust linear regression models adjusted for confounders defined based on biological plausibility. Post-hoc analyses included a further adjustment for BMI for all outcomes except BMI and ABSI. BMI was not included in the main model due to its high correlation coefficients with other adiposity parameters (e.g., WC). In addition, compounds were estimated according to energy intake (EI), and a sensitivity analysis was performed excluding misreporters of EI (top and bottom 1%). Three additional analyses were conducted, excluding participants with specific clinical conditions: HbA1c > 48 mmol/mol or taking oral antidiabetic medication; total cholesterol > 5.5 mmol/L or taking lipid-lowering medication; and average blood pressure ≥ 140 (systolic) or ≥ 90 (diastolic) mmHg or taking anti-hypertensive medication. Interactions were explored for sex, age, and smoking status.

3.2. Randomised-Controlled Trial

A comprehensive description of the methodology for the RCT titled "Effects of a (poly)phenol-rich supplement on anthropometric, biochemical, and inflammatory parameters in participants with morbid obesity" is presented below in the format of a scientific publication. Additionally, **Figure 5** visually illustrates a schematic representation of the trial design. The study employs a rigorous double-blinded, placebo-controlled, and randomised design, constituting a superiority trial. Its primary objective is to assess whether combining a conventional weight-loss treatment (hypocaloric diet) with a (poly)phenol-rich dietary supplement demonstrates superiority over the conventional treatment alone.

Figure 5. Schematic representation of the trial design.



DCR: data collection record; IC: informed consent; PIS: participant information sheet. [1]24-hour recall and 3-day dietary record. [2]Minnesota Leisure Time Physical Activity Questionnaire (Spanish shortened version)(132). [3]Bioelectrical impedance analysis. [4]Stool samples collected only at baseline and at week 12.

3.2.1. Manuscript 1: Effects of a (poly)phenol-rich supplement on anthropometric, biochemical, and inflammatory parameters in participants with morbid obesity: Study protocol for a randomised controlled trial.

Gil-Lespinard M, Capurro CB, Montserrat M, Virgili-Casas N, Zamora-Ros R. Effects of a (poly)phenol-rich supplement on anthropometric, biochemical, and inflammatory parameters in participants with morbid obesity: Study protocol for a randomised controlled trial. Contemporary Clinical Trials Communications [Internet]. 2022 Dec 1;30:101034. Available from: https://doi.org/10.1016/j.conctc.2022.101034

Impact Factor: 1.5

Category: Medicine, Research & Experimental. Q4 143/190

This work has been presented at:

- The 10th International Conference on Polyphenols and Health (ICPH 2022), held from April 20-23, 2022, at the Queen Elizabeth II Centre in London, United Kingdom, in the form of poster.
- The 7th Edition IDIBELL PhD Day, held on November 25, 2022, at the Bellvitge Biomedical Research Institute in L'Hospitalet de Llobregat, Spain, in the form of poster.
- The VI Congreso AND: Nutrición con el corazón: plant-based diets en el ejercicio profesional, held from November 24-25, 2023, at the School of Medicine, University of Barcelona in Barcelona, Spain, in the form of short oral communication.

Contemporary Clinical Trials Communications 30 (2022) 101034

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: www.elsevier.com/locate/conctc

Effects of a (poly)phenol-rich supplement on anthropometric, biochemical, and inflammatory parameters in participants with morbid obesity: Study protocol for a randomised controlled trial

Mercedes Gil-Lespinard [a], Carlos Bambarén Capurro [a], Mónica Montserrat [b], Núria Virgili-Casas [b], Raul Zamora-Ros [a,c,*]

[a] Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain
[b] Endocrinology and Nutrition Service, Bellvitge University Hospital-IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain
[c] Department of Nutrition, Food Sciences, and Gastronomy, Institute for Research on Nutrition and Food Safety (INSA), Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain

ARTICLE INFO

Keywords:
Morbid obesity
(poly)phenol supplement
Obesity
Cardiometabolic biomarkers
Clinical trial

ABSTRACT

Background: Morbid obesity (body mass index ≥40 kg/m^2) represents a severe health risk and implies the need of urgent therapeutic action. (Poly)phenols may play a relevant role in the management of this disease modulating physiological and molecular pathways involved in energy metabolism and adiposity. The purpose of this double-blinded, placebo-controlled, randomised trial is to determine if (poly)phenol supplementation, in combination with a dietary intervention, can improve anthropometric and cardiometabolic parameters in participants with morbid obesity.

Methods: Adults (n = 40) with morbid obesity, bariatric surgery candidates, will be recruited from the Bellvitge University Hospital, Spain, and randomly assigned (stratified by sex) to intervention (poly)phenol-rich supplement 1,200 mg/day + hypocaloric diet) or control group (placebo + hypocaloric diet) for 12 weeks. The primary outcome is body weight. Secondary outcomes are: other anthropometric markers and body composition measured through standardized methods and a bioimpedance analysis, cardiometabolic and inflammatory biomarkers, metabolic pathways, and gut microbiota diversity. Anthropometric parameters, dietary, physical activity and lifestyle questionnaires, blood pressure, and blood and urine samples will be collected at baseline, 6 weeks, and 12 weeks. Faecal samples will be collected at baseline and at 12 weeks. Informed consent of participants will be obtained before the start of the study.

Discussion:: The present study is expected to provide evidence on the effects of a combination of (poly)phenols on several well-established obesity and cardiometabolic markers, and to unravel possible underlying mechanisms by metabolomic analyses. Gut microbiota diversity will be considered as a potential future endpoint. The study will contribute to future strategies for prevention or treatment of obesity and related conditions.

1. Introduction

Obesity is a multifactorial disease defined as excess body fat accumulation, with a body mass index (BMI) higher or equal to 30 kg/m^2 [1]. It is considered to be one of the main risk factors for different chronic diseases such as type 2 diabetes, cardiovascular diseases and several types of cancer [2,3]. Worldwide, obesity has nearly tripled since 1975 [4]. The World Health Organisation reported in 2016 that 13% of world's adult population (aged 18 years and over) presented obesity [4]. In 2020, the European Health Survey estimated that about 16% of Spanish population presented obesity [5]. This condition is mainly a consequence of the high availability and consumption of low-nutritional quality and highly caloric foods, and the shift towards sedentary lifestyles [1]. Different health protection and promotion programmes have aimed to address this health problem. Unfortunately, the prevalence of obesity is reaching unprecedented levels, turning into one of the major

* Corresponding author. Department of Nutrition, Food Sciences and Gastronomy, Faculty of Pharmacy and Food Sciences, University of Barcelona, Avinguda Joan XXIII, 27-31, 08028, Barcelona, Spain.
E-mail addresses: rzamora@idibell.cat, rzamora@ub.edu (R. Zamora-Ros).

https://doi.org/10.1016/j.conctc.2022.101034
Received 3 July 2022; Received in revised form 19 October 2022; Accepted 7 November 2022
Available online 8 November 2022

global public health problems [1]. Morbid obesity is defined as having a BMI ≥40 kg/m^2 due to excess of body fat [6]. It is associated with several morbidities which are life-threatening, and indicates an urgent need of therapeutic action [6]. Body fat excess stimulates adipose tissue to release inflammatory mediators such as tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), and reduces the synthesis of adiponectin increasing oxidative stress and leading to a pro-inflammatory state [7]. Systemic inflammation leads to higher risk of tissue damage and dysfunction, consequently increasing metabolic disorders and chronic diseases [7].

Traditionally, strategies to prevent or treat obesity have been focused on the intake of total energy, macro and micronutrients, or pharmacological and surgical interventions that tend to be expensive and invasive. Anti-obesity medications often deliver insufficient efficacy and dubious safety, and they mostly act as a complement to behaviour modifications [8]. A randomised clinical trials (RCTs) studying the effects of short-term anti-obesity medications alone (i.e., appetite suppressants) showed 5% weight reduction after 12 weeks versus placebo [9]. A recent meta-analysis of RCTs with serotonin receptor agonists (i.e., *locarserin*) provided for ≥1 year showed a modest improvement on body weight, but with loss of maintenance of weight reduction after a certain time-point. In addition, these types of anti-obesity medications can often be linked to neuropathies as they interfere with serotonin pathways [10]. As per surgical interventions, different interventional studies in participants with morbid obesity concluded that clinical significant weight loss and improvements in risk factors achieved with surgery can also be achieved with lifestyle interventions [11,12]. Although non-surgical interventions may result in weight regain, clinical significant weight loss is possible when the conservative treatment is well planned and can be sustained in the long-term [13]. Recently, the use of functional foods and their bioactive compounds is considered a new approach in the management of obesity [14]. Several investigations have suggested that dietary components such as (poly)phenols may play a relevant role [14,15]. (Poly)phenols are bioactive phytochemicals that are widely present in plant-based foods [16]. Approximately 500 different individual (poly)phenols have been identified and, according to their chemical structure, they can be classified as flavonoids, phenolic acids, stilbenes, lignans, and others [16]. In Europe, daily intake of these compounds has been estimated to be around 1 g, with phenolic acids and flavonoids representing the most abundant classes [17]. (Poly)phenols are partially absorbed in the small bowel, metabolized by phase I and II enzymes and excreted in the bile or urine [18]. Those that remain in the lumen are metabolized by the gut microbiota when they reach the colon, and their secondary metabolites (simple phenolic compounds) can be absorbed at this level [18]. Many studies on cellular and animal models have shown the anti-obesity effects of different (poly)phenols, such as lowering total energy intake, reducing fat and glucose uptake into adipose tissue, and increasing caloric expenditure and glucose uptake into skeletal muscle [19]. Studies in humans indicated that (poly)phenols could reduce or maintain body weight [12–14]. Results from the Netherlands Cohort Study [20] suggested that flavonoid intake may contribute to body weight maintenance, particularly in female participants. Similarly, three cohort studies including US health professionals showed that higher intakes of flavonoid-rich foods contributed to body weight maintenance [21]. Results from the EPIC cohort (unpublished results) showed that several classes, subclasses and individual (poly) phenols, particularly flavonoids, were associated with prevention of body weight gain over 5 years. A systematic review of interventional studies observed a reduction in body weight of 1.5 kg over 12 weeks for several RCTs, adding (poly)phenol supplementation in participants with overweight or obesity compared to placebo [22]. A recent systematic review of RCTs evaluated the effect of (poly)phenol supplementation in combination with hypocaloric diets or physical activity interventions on body weight and obesity parameters [23]. It concluded that isoflavone supplementation together with weight-loss therapies, especially physical activity, may promote weight reduction, particularly in post-menopausal women. Associations between other phenolic compounds and anthropometric parameters were not observed, but the majority of studies found protective changes in different parameters associated with obesity, such as insulin sensitivity and inflammatory biomarkers [23]. In light of this evidence, the hypothesis of the current RCT is that the addition of a (poly)phenol-rich supplement to a traditional body weight-loss treatment (hypocaloric diet) will favour weight loss and improve health parameters associated with obesity (e.g., lipid profile, inflammatory biomarkers, blood pressure, insulin resistance).

2. Methodology

2.1. Ethics and consent

The present study has been approved by the Bellvitge University Hospital Ethics Committee. All protocol modifications will be reviewed and approved by the ethics board. Written and voluntary consent will be obtained from each participant prior to the beginning of the study. The trial has been registered at www.ClinicalTrials.gov (NCT05428540).

2.2. Design, participants, and setting

This study is a double-blinded, randomised, placebo-controlled trial that will be conducted at the Bellvitge University Hospital in the Barcelona area, Spain. Forty adult participants (≥18 y) with morbid obesity (BMI ≥40 kg/m^2) referred to the Unit of Endocrinology and Nutrition of the hospital for a weight-loss treatment (in most of the cases prior to bariatric surgery), will be randomly assigned to an intervention or control group (n = 20 per arm). They will receive either a (poly)phenol-rich supplement (1,200 mg/day) or placebo, respectively, for 12 weeks, in combination with a hypocaloric diet. An overview of the study is presented in Fig. 1. To detail the study protocol, the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [24] checklist was used (Table 1). Any protocol amendments will be discussed by the ethics committee before their application.

Before recruitment, a meeting with the medical staff from the Unit of Endocrinology and Nutrition will take place to widely discuss the aim, methodologies and technical aspects related to the development of this trial. After this meeting, the medical staff will start the assessment of potential participants and a detailed information sheet will be given to those plausible candidates meeting the inclusion criteria (see below). Candidates that demonstrate interest in the study will be given an informed consent reporting all the information on the intervention, and the protocol that they will be asked to undertake. Volunteers will be selected according to the inclusion and exclusion criteria (Table 2). Each participant enrolled will be assigned to an ID number and the encoding will be hidden to both the researchers involved in data collection and analysis, and the participants. All clinical and personal data of the participants will be collected and stored anonymously. The participants will be stratified by sex and randomly assigned to either supplement group (n = 20) or placebo group (n = 20).

2.3. Intervention

The trial duration will be 12 weeks. Each participant will attend three visits: baseline or visit 1 (V1, start of intervention), visit 2 after 6 weeks (V2), and visit 3 after 12 weeks (V3, end of intervention). Information regarding diet, physical activity, lifestyle factors, anthropometric measurements, blood pressure, and blood and urine samples will be collected in the three visits. In visits 1 and 3, faecal samples will be also collected. Recruitment and visits will take place in an outpatient clinic at the Bellvitge University Hospital. Trained staff will carry out questionnaires and anthropometric measurements, and fasting blood samples collection. Participants will bring 24 h urine and faecal samples collected the day before each visit (faecal samples only V1 and V3). Blood samples will be analysed in the clinical laboratory from the

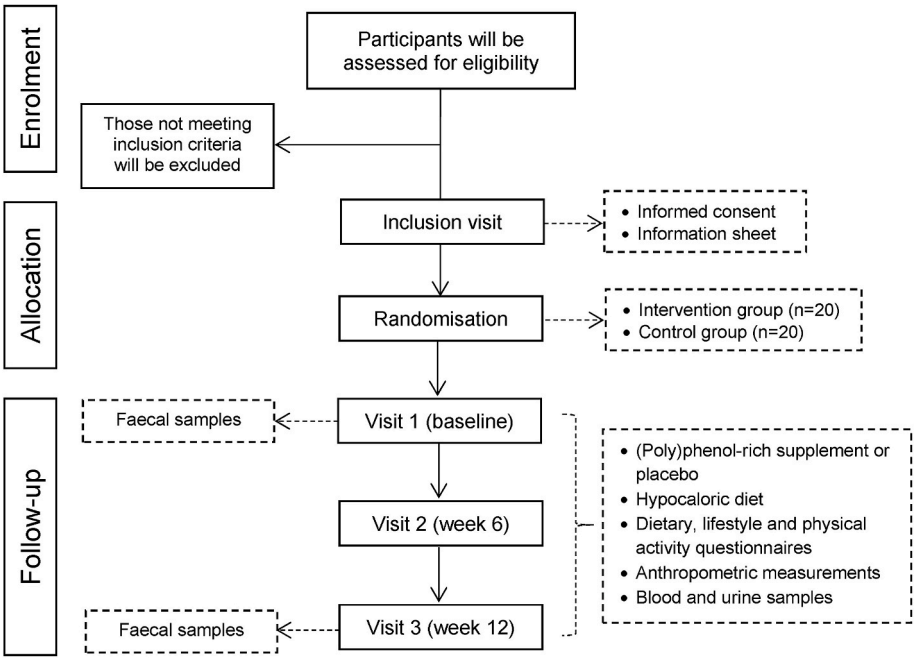


Fig. 1. Flow chart of the study design.

Table 1
Standard protocol items: recommendations for interventional studies (SPIRIT).

	Enrolment	Visit 1 (baseline)	Visit 2 (week 6)	Visit 3 (week 12)
Eligibility screening	X			
Informed consent	X			
Information sheet	X			
Capsules provision		X		
Hypocaloric diet explanation		X		
Dietary, physical activity, and lifestyle assessment				
Lifestyle and backgrounds		X		
Physical Activity		X	X	X
24-h Dietary Recall		X	X	X
3-day Dietary Record		X	X	X
Physical assessment				
Anthropometrics		X	X	X
Bioimpedance		X	X	X
Blood pressure		X	X	X
Biochemical assessment				
Blood lipids and sugar		X	X	X
Renal, hepatic, and thyroid function markers		X	X	X
Inflammatory biomarkers		X	X	X
Metabolomics		X	X	X
Microbiota composition		X		X

Table 2
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Age ≥18 years.	Record of type I diabetes mellitus.
Body mass index ≥40 kg/m2.	Endocrinopathy-related obesity.
Derived to the Unit of Endocrinology and Nutrition (Bellvitge University Hospital) for a weight-loss treatment, in many cases prior to bariatric surgery.	Severe infectious process that may affect the inflammatory state during the 4 weeks prior to inclusion.
	Acute metabolic complications.
	Cardiovascular event in the 6 months prior to the study.
	History of liver disease.
	Pregnant, breastfeeding or wishing to be pregnant in the 12 weeks following inclusion.
	Recent history of neoplasia (<5 years) except skin cancer or melanoma.
	Use of oral or intravenous glucocorticoids for more than 14 consecutive days in the 3 months prior to the study.
	Alcoholism, drug addiction, or major psychiatric disorder.

hospital. Urine and faecal samples will be stored in the ultra-freezers of the Unit of Nutrition and Cancer at IDIBELL. Urine samples will be analysed by the Nutritional Biomarkers and Metabolomics group from the University of Barcelona, Spain. Faecal samples will remain stored for future microbiota analyses. All data collected will be recorded on the Research Electronic Data Capture (REDCap) software platform [25].

The (poly)phenol-rich supplement selected for the present study is based on a combination of individual compounds commonly used in previous trials, such as green tea extract, or blueberry extract, that proved to be effective for improvement of obesity parameters [26–29]. In addition, the inclusion of compounds from typical (poly)phenol-rich foods characteristic of the Mediterranean dietary pattern, such as olive oil, fruit and vegetables (particularly citrus fruit and onions), red wine and grapes, and whole grain cereals [30] was also considered. An identification of the specific compounds was carried out, and the precise amounts were calculated to formulate the supplement taking into consideration safety and effective dosages. In addition, the ~1,000 mg a day mean intake of dietary (poly)phenols from the European population was considered to calculate the (poly)phenol supplementation [31]. Here, we aimed to double this amount by including 1,200 mg of extracts with ~850 mg of (poly)phenols. The Centre of Functional Food Research and Development (CIDAF by its Spanish initials, Granada, Spain) performed the quality assessment of the raw material, evaluated the stability and purity of compounds, and developed both the supplement and the placebo capsules.

2.3.1. *Composition*

The supplement comprises seven different extracts: 400 mg of green tea extract (10% catechins and 75% epigallocatechin-gallate (EGCG)); 200 mg of blueberry extract (50% anthocyanidins), 100 mg of olive leaf

extract (20% hydroxytyrosol and derivates); 100 mg of rice bran extract (98% ferulic acid); 200 mg of *citrus aurantium* extract (50% hesperidin); 100 mg of *polygonum cuspidatum* extract (100% resveratrol); and 100 mg of *sophora japonica* extract (70% quercetin dihydrate).

Participants in the intervention group will take a 400 mg capsule three times per day with meals (breakfast, lunch and dinner), a total of 1,200 mg of a combination of extracts (~830 mg (poly)phenols) a day. The placebo capsules consist of microcrystalline cellulose 101 (Avicel PH-101) and will be taken by the control group under the same conditions as the intervention group. Both the supplement and the placebo are optically identical opaque bicoloured gelatine capsules to ensure the double-blind design.

2.3.2. Bioavailability and safety of compounds

The bioavailability of (poly)phenols in humans can be affected by a series of factors, including food availability, food processing related factors, food matrix, interaction with other compounds, host-related factors and (poly)phenol-related factors [32]. As to this last point, mechanistic studies reported that different (poly)phenols may present synergistic interactions when consumed together, enhancing their bioavailability and activity [33,34]. Absorption, digestion, metabolism and excretion among (poly)phenols might differ due to their structural diversity. The 7 compounds included in this supplement present heterogenic characteristics. Some of them with low molecular weight (e.g., ferulic acid) are easily absorbed through the gut barrier. In contrast, large molecular weight (poly)phenols (e.g., anthocyanidins) have a much lower direct absorption, but they can be partly absorbed in the colon as small phenolic acids after microbiota metabolization [35]. Bioavailability varies among the different subclasses, ranking from resveratrol (~75–90%) > tyrosols (~75%) > phenolic acids (~25–40%) > flavonols (~20–40%) > catechins (~15–40%) > flavanones (~8%) > anthocyanidins (~2%) [34,36–38].

To formulate this supplement, we reviewed studies in humans regarding safety and safe doses of the extracts and compounds chosen, as well as official reports from the European Food Safety Authority (EFSA) for specific compounds. All of the compounds selected for this supplement are included in the European Botanical Databases: the BELFRIT list from Belgium, France and Italy [39]; the German Stoffliste [40]; and the EFSA Compendium [41]. There are EFSA Scientific Opinion reports that support the safety of green tea catechins, (poly)phenols from olives (hydroxytyrosol and derivates) and resveratrol for human consumption [42–44]. As per other compounds, i.e., anthocyanins from blueberries and citrus fruit flavonoids (including hesperidin), different interventional studies in humans have reported safe doses or no adverse effects [45] [–] [47]. Ferulic acid has shown low levels of toxicity in animal models [48] and recent literature has linked this compound to low toxicity in humans [49]. As per quercetin, the Food and Drugs Administration determined that its use in different dietary products can be classified as Generally Recognized As Safe [50].

2.3.3. Hypocaloric diet

Participants will take the capsules together with a hypocaloric diet, developed by dietitians/nutritionists from both the Bellvitge University Hospital and the Bellvitge Biomedical Research Institute (IDIBELL). It consists of a 1,200 kcal/d hypocaloric diet and includes a large variety of foods to ensure that all nutritional requirements are covered. Previous evidence supports that low-calorie diets start with a 4 to 12-week weight reduction phase and should be planned in order to set energy deficit using diet upon principles of balanced nutrition, proportion of nutrients and meal replacements to make it easier to return to habitual diets [51]. Here, participants will be given a printed guide with general dietary recommendations, food choices and portions to follow. Table 3 summarises the hypocaloric dietary guide.

Table 3
Hypocaloric dietary guide: food groups and portions and general recommendations.

Food group	Portions/ day	1 portion equivalent to (example)
Carbohydrates	2	· 2 slices of wholemeal bread · 4 tablespoons of brown rice/quinoa/ pasta (cooked)
Proteins	2	· 4 tablespoons of pulses (cooked) · 100 g tofu/chicken/fish · 1 egg
Fruits	2	· 1 piece standard size (apple, pear, banana) · 2 pieces medium/small size (plum, kiwi) · 1 glass of berries · 1–2 slices of watermelon
Vegetables	3	· ½ plate cooked vegetables · 1 bowl raw vegetables · 1 big size (aubergine, courgette) · 2 small size (onion, tomato, carrot)
Dairy/plant-based alternatives	2	· 1 cup of milk/plant beverage (no added sugar) · 1 small pot of yoghurt (no added sugar) · 1 small pot of white cheese · 2 slices of cheese
Nuts and seeds	1	· 1 handful of nuts (no fried, no added salt/sugar) · 3 whole walnuts · 1 tablespoon of seeds
Olive oil	2	· 1 tablespoon of extra virgin olive oil

General recommendations.
· More: whole (less processed) food, and combination of natural colours and textures; home-made foods; safe water as main drink choice.
· Less: ultra-processed foods, added sugar and saturated fat, red and processed meat, alcohol and sweetened beverages.

2.4. Data collection

2.4.1. General information

During the baseline visit, participants' personal and lifestyle data, and personal and family medical record will be collected through an initial questionnaire.

2.4.2. Anthropometric measurements

The primary outcome of this study is to assess the effect of the supplement on body weight. Secondary outcomes include other anthropometric parameters and body composition. In the three visits, body weight and body composition will be measured by trained staff using a highly accurate Multi Frequency Segmental Body Composition Analyser. Moreover, an ergonomic measuring tape will be used to collect data on waist and hip circumferences, and an integrated measuring rod will be used to measure height.

2.4.3. Dietary intake assessment

Data on food intake will be assessed using a 3-day dietary record that participants must have completed prior to each visit. In addition, a 24-h dietary recall will be carried out during each visit by a trained dietitian. To estimate nutritional intake, the Nutritional Calculation Programme (PCN) Pro 1.0 from the University of Barcelona [52] will be used. (Poly) phenol intake will be assessed using the Phenol-Explorer database [53], following the methodology used in the EPIC study [54].

2.4.4. Physical activity

During the three visits, a trained dietitian will collect information on participants' physical activity level through a shortened Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire [55], including information on both leisure-time and occupational physical activity.

2.4.5. Blood pressure

During the three visits, both systolic and diastolic pressure will be measured to each participant by a trained dietitian, obtained in a resting, seated position using an OMRON HBP-1300 semi-automatic blood pressure monitor.

2.4.6. Blood samples

Fasting blood samples will be collected in the three visits. Trained nurses will extract 20 ml from the antecubital vein of each participant following standardized procedures. Samples will be stored and analysed in the clinical laboratory of the Bellvitge University Hospital. They will be centrifuged for 15 min at 5000 g, stored in two aliquots, and frozen at −80 °C until analysis. Through these samples, we will measure metabolic and functional markers [i.e., blood count, lipid profile, thyroid (thyroxine, thyrotropin), liver (transaminases) and renal (creatinine) function, glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), glycosylated haemoglobin, uric acid, and vitamin D) and inflammatory biomarkers (i.e., TNF-α receptor I and II, IL-6, adiponectin, and ultra-sensitive CPR].

2.4.7. Urine samples

In the three visits, participants will bring a 24-h urine sample. Three aliquots (2 ml each) will be stored at −80 °C in the ultra-freezers of the Unit of Nutrition and Cancer, IDIBELL. One aliquot will be used to measure cortisol in the clinical laboratory of the Bellvitge University Hospital, only for visit 1. Another aliquot will be used to perform a metabolomic analysis by the Nutritional Biomarkers and Metabolomics group from the University of Barcelona. About 500 metabolites (e.g., (poly)phenols and derived microbiota metabolites, organic acids, energetic and protein metabolism compounds) will be quantified using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). This will allow us to monitor metabolite alterations derived from the (poly)phenol-rich supplement and associate these changes with improvements in clinical and biochemical outcome measurements.

2.4.8. Faeces samples

Participants will bring a faecal sample in the first and third visit. These samples will be analysed in future microbiota studies. They will be kept stored at −80 °C in the ultra-freezers of the Unit of Nutrition and Cancer at IDIBELL.

2.5. Sample size, randomisation and statistics

In the statistical power study, a minimum of 18 participants per arm will be needed to detect, with a study power of 0.85, a variation of 5–10% in anthropometric and laboratory measurements, in a population with 10% standard deviation, assuming an alpha error of 0.05. Assuming a possible drop-out rate of 15%, the required sample size is 40, with equal number of participants in each group (n = 20). Participants will be randomly divided by using a computer random number generator, with an allocation ratio of 1:1 for intervention and control group. They will be stratified based on sex (male and female). Randomisation and allocation will be performed by the principal investigator of the trial and blinded to the participants and researchers involved in both data collection and analysis. Compliance will be assessed primarily through number of capsules left in the third visit – with a 75% of the capsules taken and a remaining of ≤25%. Secondarily, it will be assessed through the analysis of urinary poly(phenol) concentrations. Statistical analyses will be performed with R Software [56].

2.5.1. Intention-to-treat and per-protocol population

The main analysis will be performed according to the intention-to-treat (ITT) principle. In the ITT analysis, all patients will be analysed according to their initially assigned study arm at baseline, regardless of the adherence to the study protocol. Participants who withdrew consent or participants with a protocol violation concerning eligibility will be excluded from the ITT analysis. Participants with missing baseline information or lost to follow-up (no outcome data available at any time point: visit 2 and 3) will be excluded from the ITT analysis. Differences in participant characteristics between those lost to follow-up and those included will be assessed. Participants without protocol violations and meeting the requirements of compliance will be included for a per-protocol (PP) analysis (Fig. 2).

2.5.2. Superiority trial

This trial is designed to evaluate if the addition of a (poly)phenol-rich supplement to a traditional weight-loss treatment (hypocaloric diet) is superior to the conventional treatment alone.

2.5.3. Missing data

Different methodologies will be assessed to deal with missing data. The definitive decision will be made depending on the type of missing data and the robustness of results. A priori, it is not expected that the number of missing exceeds 10%.

2.5.4. Baseline characteristics

Baseline information of included participants will be reported per randomisation group. The following characteristics will be presented: age, sex, lifestyle factors, anthropometric parameters, energy and food group intake, and biochemical and inflammatory biomarkers. Categorical variables will be expressed as number and percentage of participants for each category. Continuous variables will be presented as mean ± standard deviation in case of normal distribution, and as median and interquartile range in case of non-normal distribution. Normality will be

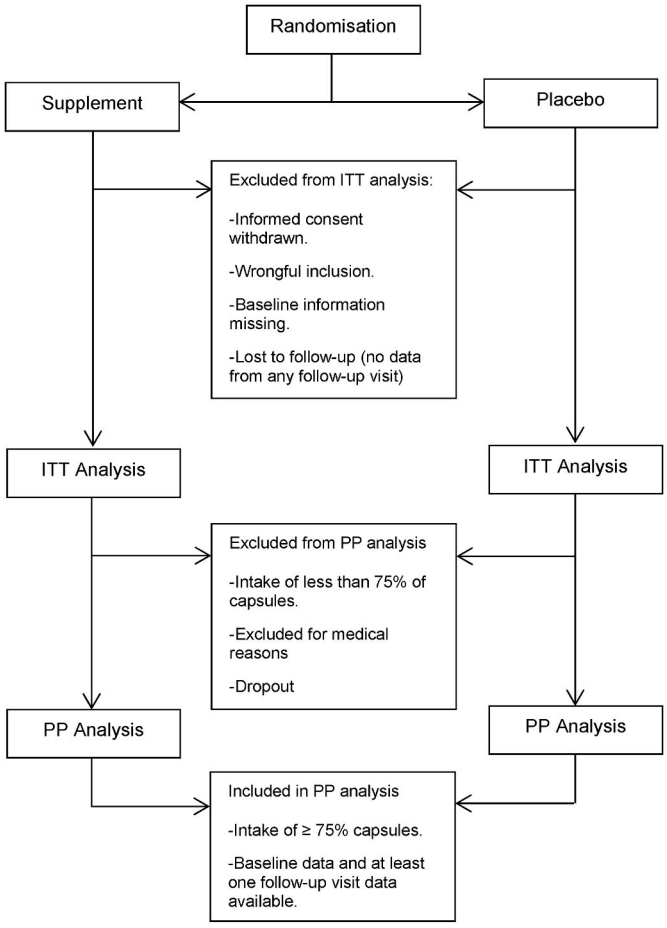


Fig. 2. Participant inclusion for intention-to-treat (ITT) and per-protocol (PP) analysis.

assessed by plotting distributions and by the Kolmogorov-Smirnov test. Differences between baseline characteristics of both groups will be assessed by the chi-square test or *t*-test for independent samples.

2.5.5. Outcome assessment

To analyse the differences in outcome variables between the two groups, the independent samples *t*-test will be used. Here, outcome variables will be expressed as differences between baseline and follow-up values (e.g., body weight change after 12 weeks). Besides, to assess pre- and post-intervention differences, the paired *t*-test will be used.

3. Discussion

This study will aim to evaluate the effects of a (poly)phenol-rich supplement, in combination with a hypocaloric diet, on anthropometric parameters, and cardiometabolic and inflammation biomarkers, in participants with morbid obesity. Previous evidence supports the association between the intake of different (poly)phenols, included in our supplement, and the improvement of several obesity parameters. For example, a combination of EGCG and resveratrol showed effectiveness downregulating pathways related to energy metabolism, oxidative stress, inflammation and immune system in participants with overweight and obesity [57]. Moreover, human intervention studies have shown individual anti-obesity effects of several (poly)phenol-rich foods including citrus fruits, green tea, berries, apples, and onions [14]. This is in line with what we propose to investigate in the current RCT. The main novelty of this intervention is precisely the combination of compounds that have shown to be effective alone (such as green tea catechins or blueberry anthocyanins) [17–20] and those that are included in a Mediterranean dietary pattern (such as citrus flavonoids, whole grain phenolic compounds, grapes and red wine resveratrol, and olive oil tyrosol) [30]. A recent review of RCTs on the effect of (poly)phenol-rich supplements combined with traditional weight-loss strategies (hypocaloric diet and/or physical activity interventions) on body weight loss and other obesity anthropometric parameters, concluded that (poly)phenol supplementation is not yet supported as a complementary approach for enhancing the effectiveness of traditional strategies [23]. Thus, through this study we aim to provide further evidence on this topic but studying participants with morbid obesity, a population with more need for treatment but for which evidence is still very little.

The sample size of this study has been calculated taking into account several assumptions, as mentioned above, to provide enough power to detect a mean difference in the primary outcome of 5–10% between the two groups. Sample size may be considered a limitation for some secondary outcomes and potential a posteriori subgroup analysis. Targeting bariatric surgery candidates with a BMI equal to or higher than 40 kg/m^2 may be a challenge when it comes to recruitment. Even so, this type of participant is often motivated and willing to participate, to have the opportunity to improve health, optimize quality of life, and lengthen lifespan [58,59]. Previous studies have reported that changes in body weight beginning at 2–5% can bring improvements in several associated risk factors [60,61]. Nonetheless, it should be bear in mind that weight loss *per se* should not be the only target, but general health improvement [61]. We will measure waist and hip circumference and therefore waist-to-hip ratio (WHR) as indicator of abdominal obesity and cardio-metabolic risk to provide a better assessment. In the EPIC study, a 5 cm larger waist circumference was associated with a 17% and 13% higher risk of death among men among women, respectively [62]. Likewise, a 0.1 unit higher WHR was associated with a 34% more risk of mortality among men and 24% among women [62]. Another characteristic that should be taken into consideration is that women are expected to outnumber men. In general, women tend to go through more frequent stigma experiences associated with body weight, often referred to negative beliefs/attitudes about the person, as well as perceived rejection, prejudice and discrimination (aroused from stereotypes and beliefs) [63]. In consequence, they are more likely to use weight loss programmes and strategies than men. Accordingly, despite not being a gender-based research, we will stratify randomisation by sex (female/-male) in order to create a balance between groups. As per risks associated with the intervention, ethical issues have been considered and according to the nature of the intervention, no side-effects are expected. Every potential participant will be provided with detailed information about it and informed consent will be obtained prior to inclusion. A major strength of this trial is its design: double-blinded, randomised, parallel, and placebo-controlled which enables to establish causation with an improved credibility and minimising biases. Through this trial, we may detect moderate/small effects that may be clinically relevant in participants with morbid obesity. The effects of (poly)phenols are usually stronger when measured in participants with higher needs; therefore, including participants with morbid obesity might allow us to see effects that may not be relevant after 12 weeks if measured in participants with small overweight or without excess of body fat, such as changes in cardiometabolic and inflammatory biomarkers. As length of the trial will be 12 weeks, the risk of loss to follow-up is very low. Moreover, as participants will be bariatric surgery candidates, we expect that the majority will accomplish adherence; the results of this trial will not affect participants' right to subsequently get the bariatric surgery according to medical opinion. This trial aims to be a pilot study that could open up new investigations. For example, it might be worth exploring their effectiveness in subjects with other degrees of overweight/obesity or in other outcomes, such as cardiovascular diseases or diabetes. Moreover, it could be interesting to test the efficacy of (poly)phenol-rich diets in future clinical nutrition research which could lead to enhancement of nutritional policies, as well as safer, more economic, and less aggressive approaches for morbid obesity treatment.

4. Conclusion

This study will evaluate the hypothesis that higher amounts of (poly)phenols could help reduce body fat, and therefore body weight, and lead to a cardiometabolic health improvement in participants with morbid obesity. Hence, this study will contribute to future strategies for prevention or treatment of obesity and related conditions.

Funding

The project is supported by the Institute of Health Carlos III, Spain [grant number PI18/00191] (co-funded by European Regional Development Fund, a way to build Europe). We also thank CERCA Program / Generalitat de Catalunya for the institutional support to IDIBELL. MG-L and RZ-R were supported by the PFIS [grant number FI19/00185] and Miguel Servet II [grant number CPII20/00009] programs, respectively, from the Institute of Health Carlos III (co-funded by European Social Fund, investing in your future).

Authors' contributions

RZ-R and NV-C designed the study. RZ-R acquired the funding. MG-L, CBC and RZ-R wrote the study protocol. MM and NV-C will recruit the study participants. MG-L will conduct the trial and the statistical analyses. MG-L and CBC wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures will be conducted in accordance with the ethical standards for human subject research set forth in the Declaration of Helsinki. Written informed consent will be obtained from all participants included in this trial. This trial was approved by the Clinical Investigation Ethics Committee of the Bellvitge (PR082/22) and is registered in the ClinicalTrials.gov database (NCT05428540). Personal information about potential and enrolled participants will be

maintained in a database in order to protect subjects confidentially. Investigators will communicate trial results to participants, healthcare professionals and other relevant groups via publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge all participants and members who will take part in this study.

Abbreviations

BMI	body mass index
CRP	c-reactive protein
EGCG	epigallocatechin-gallate
EPIC	European Prospective Investigation into Cancer and nutrition
HOMA-IR	homeostatic model assessment of insulin resistance
IDIBELL	Bellvitge Biomedical Research Institute
IL-6	interleukin-6
RCT	randomised controlled trial
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
TNF-α	tumour necrosis factor-alpha
UHPLC-MS/MS	ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry

References

[1] World Health Organization, Obesity, (n.d.). https://www.who.int/health-topics/obesity#tab=tab_2 (accessed September 15, 2021).
[2] S. Panico, L. Palmieri, C. Donfrancesco, D. Vanuzzo, P. Chiodini, G. Cesana, M. Ferrario, A. Mattiello, L. Pilotto, R. Sega, S. Giampaoli, J. Stamler, Preventive potential of body mass reduction to lower cardiovascular risk: the Italian Progetto CUORE study, Prev. Med. 47 (2008) 53–60, https://doi.org/10.1016/j.ypmed.2008.01.023.
[3] D.L. Roberts, C. Dive, A.G. Renehan, Biological mechanisms linking obesity and cancer risk: new perspectives, Annu. Rev. Med. 61 (2010) 301–316, https://doi.org/10.1146/annurev.med.080708.082713.
[4] Obesity, (n.d.). https://www.who.int/health-topics/obesity#tab=tab_2 (accessed September 15, 2021).
[5] Spanish Statistical Office, (n.d.). https://www.ine.es/en/index.htm (accessed March 11, 2022).
[6] Medical Subject Headings (MeSH), Obesity, Morbid, (n.d.). https://www.ncbi.nlm.nih.gov/mesh?Db=mesh&Cmd=DetailsSearch&Term=%22Obesity,+Morbid%22%5BMeSH+Terms%5D (accessed September 15, 2021).
[7] M.S. Ellulu, I. Patimah, H. Khaza'ai, A. Rahmat, Y. Abed, Obesity & inflammation: the linking mechanism & the complications, Arch. Med. Sci. 13 (2017) 851–863, https://doi.org/10.5114/aoms.2016.58928.
[8] T.D. Müller, M. Blüher, M.H. Tschöp, R.D. DiMarchi, Anti-obesity drug discovery: advances and challenges, Nat. Rev. Drug Discov. 21 (2022) 201–223, https://doi.org/10.1038/s41573-021-00337-8.
[9] J.G. Kang, C.Y. Park, J.H. Kang, Y.W. Park, S.W. Park, Randomized controlled trial to investigate the effects of a newly developed formulation of phentermine diffuse-controlled release for obesity, Diabetes Obes. Metabol. 12 (2010) 876–882, https://doi.org/10.1111/j.1463-1326.2010.01242.x.
[10] A.K. Singh, R. Singh, Efficacy and safety of lorcaserin in obesity: a systematic review and meta-analysis of randomized controlled trials, Expet Rev. Clin. Pharmacol. 13 (2020) 183–190, https://doi.org/10.1080/17512433.2020.1703109.
[11] C. Martins, M. Strømmen, O.A. Stavne, R. Nossum, R. Mårvik, B. Kulseng, Bariatric surgery versus lifestyle interventions for morbid obesity - changes in body weight, risk factors and comorbidities at 1 year, Obes. Surg. 21 (2011) 841–849, https://doi.org/10.1007/s11695-010-0131-1.
[12] B. Øvrebø, M. Strømmen, B. Kulseng, C. Martins, Bariatric surgery versus lifestyle interventions for severe obesity: 5-year changes in body weight, risk factors and comorbidities, Clin. Obes. 7 (2017) 183–190, https://doi.org/10.1111/cob.12190.
[13] J.W. Anderson, L. Grant, L. Gotthelf, L.T.P. Stifler, Weight loss and long-term follow-up of severely obese individuals treated with an intense behavioral program, Int. J. Obes. 31 (2007) 488–493, https://doi.org/10.1038/sj.ijo.0803423.
[14] M. Boccellino, S. D'Angelo, Anti-obesity effects of polyphenol intake: current status and future possibilities, Int. J. Mol. Sci. 21 (2020) 1–24, https://doi.org/10.3390/IJMS21165642.
[15] S. Wang, N. Moustaid-Moussa, L. Chen, H. Mo, A. Shastri, R. Su, P. Bapat, I. S. Kwun, C.L. Shen, Novel insights of dietary polyphenols and obesity, J. Nutr. Biochem. 25 (2014) 1–18, https://doi.org/10.1016/j.jnutbio.2013.09.001.
[16] R. Zamora-Ros, M. Touillaud, J.A. Rothwell, I. Romieu, A. Scalbert, Measuring exposure to the polyphenol metabolome in observational epidemiologic studies: current tools and applications and their limits, Am. J. Clin. Nutr. 100 (2014) 11–26, https://doi.org/10.3945/AJCN.113.077743.
[17] R. Zamora-Ros, V. Knaze, J.A. Rothwell, B. Hémon, A. Moskal, K. Overvad, A. Tjønneland, C. Kyrø, G. Fagherazzi, M.C. Boutron-Ruault, M. Touillaud, V. Katzke, T. Kühn, H. Boeing, J. Förster, A. Trichopoulou, E. Valanou, E. Peppa, D. Palli, C. Agnoli, F. Ricceri, R. Tumino, M.S. de Magistris, P.H.M. Peeters, H. Bas Bueno-De-Mesquita, D. Engeset, G. Skeie, A. Hjartåker, V. Menéndez, A. Agudo, E. Molina-Montes, J.M. Huerta, A. Barricarte, P. Amiano, E. Sonestedt, L. M. Nilsson, R. Landberg, T.J. Key, K.T. Khaw, N.J. Wareham, Y. Lu, N. Slimani, I. Romieu, E. Riboli, A. Scalbert, Dietary polyphenol intake in europe: the European prospective investigation into cancer and nutrition (EPIC) study, Eur. J. Nutr. 55 (2016) 1359–1375, https://doi.org/10.1007/s00394-015-0950-x.
[18] C. Manach, G. Williamson, C. Morand, A. Scalbert, C. Rémésy, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, Am. J. Clin. Nutr. 81 (2005), https://doi.org/10.1093/ajcn/81.1.230s.
[19] M. Meydani, S.T. Hasan, Dietary Polyphenols and Obesity, Nutrients. 2 (2010) 737, https://doi.org/10.3390/NU2070737.
[20] L.A.E. Hughes, I.C.W. Arts, T. Ambergen, H.A.M. Brants, P.C. Dagnelie, R. A. Goldbohm, P.A. Van Den Brandt, M.P. Weijenberg, Higher dietary flavone, flavonol, and catechin intakes are associated with less of an increase in BMI over time in women: a longitudinal analysis from The Netherlands Cohort Study, Am. J. Clin. Nutr. 88 (2008) 1341–1352, https://doi.org/10.3945/ajcn.2008.26058.
[21] M.L. Bertoia, E.B. Rimm, K.J. Mukamal, F.B. Hu, W.C. Willett, A. Cassidy, Dietary flavonoid intake and weight maintenance: three prospective cohorts of 124 086 US men and women followed for up to 24 years, BMJ (2016) 352, https://doi.org/10.1136/bmj.i17.
[22] G. Farhat, S. Drummond, E.A.S. Al-Dujaili, Polyphenols and their role in obesity management: a systematic review of randomized clinical trials, Phyther. Res. 31 (2017) 1005–1018, https://doi.org/10.1002/ptr.5830.
[23] F. Llaha, R. Zamora-Ros, The effects of polyphenol supplementation in addition to calorie restricted diets and/or physical activity on body composition parameters: a systematic review of randomized trials, Front. Nutr. (2020) 84, https://doi.org/10.3389/FNUT.2020.00084, 0.
[24] A.W. Chan, J.M. Tetzlaff, P.C. Gøtzsche, D.G. Altman, H. Mann, J.A. Berlin, K. Dickersin, A. Hróbjartsson, K.F. Schulz, W.R. Parulekar, K. Krleza-Jeric, A. Laupacis, D. Moher, SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials, BMJ 346 (2013), https://doi.org/10.1136/BMJ.E7586.
[25] P.A. Harris, R. Taylor, B.L. Minor, V. Elliott, M. Fernandez, L. O'Neal, L. McLeod, G. Delacqua, F. Delacqua, J. Kirby, S.N. Duda, The REDCap consortium: building an international community of software platform partners, J. Biomed. Inf. 95 (2019), 103208, https://doi.org/10.1016/J.JBI.2019.103208.
[26] M. Hibi, H. Takase, M. Iwasaki, N. Osaki, Y. Katsuragi, Efficacy of tea catechin-rich beverages to reduce abdominal adiposity and metabolic syndrome risks in obese and overweight subjects: a pooled analysis of 6 human trials, Nutr. Res. 55 (2018) 1–10, https://doi.org/10.1016/J.NUTRES.2018.03.012.
[27] C.H. Hsu, T.H. Tsai, Y.H. Kao, K.C. Hwang, T.Y. Tseng, P. Chou, Effect of green tea extract on obese women: a randomized, double-blind, placebo-controlled clinical trial, Clin. Nutr. 27 (2008) 363–370, https://doi.org/10.1016/J.CLNU.2008.03.007.
[28] D. Li, Y. Zhang, Y. Liu, R. Sun, M. Xia, Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients, J. Nutr. 145 (2015) 742–748, https://doi.org/10.3945/JN.114.205674.
[29] A.J. Stull, K.C. Cash, W.D. Johnson, C.M. Champagne, W.T. Cefalu, Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women, J. Nutr. 140 (2010) 1764–1768, https://doi.org/10.3945/JN.110.125336.
[30] S. Castro-Barquero, R.M. Lamuela-Raventós, M. Doménech, R. Estruch, Relationship between mediterranean dietary polyphenol intake and obesity, Nutrients 10 (2018), https://doi.org/10.3390/NU10101523.
[31] R. Zamora-Ros, V. Knaze, J.A. Rothwell, B. Hémon, A. Moskal, K. Overvad, A. Tjønneland, C. Kyrø, G. Fagherazzi, M.C. Boutron-Ruault, M. Touillaud, V. Katzke, T. Kühn, H. Boeing, J. Förster, A. Trichopoulou, E. Valanou, E. Peppa, D. Palli, C. Agnoli, F. Ricceri, R. Tumino, M.S. de Magistris, P.H.M. Peeters, H. Bas Bueno-De-Mesquita, D. Engeset, G. Skeie, A. Hjartåker, V. Menéndez, A. Agudo, E. Molina-Montes, J.M. Huerta, A. Barricarte, P. Amiano, E. Sonestedt, L. M. Nilsson, R. Landberg, T.J. Key, K.T. Khaw, N.J. Wareham, Y. Lu, N. Slimani, I. Romieu, E. Riboli, A. Scalbert, Dietary polyphenol intake in europe: the european prospective investigation into cancer and nutrition (EPIC) study, Eur. J. Nutr. 55 (2016) 1359–1375, https://doi.org/10.1007/s00394-015-0950-x.
[32] M. D'Archivio, C. Filesi, R. Varì, B. Scazzocchio, R. Masella, Bioavailability of the polyphenols: status and controversies, Int. J. Mol. Sci. 11 (2010) 1321–1342, https://doi.org/10.3390/ijms11041321.
[33] E. Brglez Mojzer, M. Knez Hrnčič, M. Škerget, Ž. Knez, U. Bren, Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects, Molecules 21 (2016), https://doi.org/10.3390/molecules21070901.
[34] C. Di Lorenzo, F. Colombo, S. Biella, C. Stockley, P. Restani, Polyphenols and human health: the role of bioavailability, Nutrients 13 (2021) 1–30, https://doi.org/10.3390/NU13010273.

[35] A. Scalbert, C. Morand, C. Manach, C. Rémésy, Absorption and metabolism of polyphenols in the gut and impact on health, Biomed. Pharmacother. 56 (2002) 276–282, https://doi.org/10.1016/S0753-3322(02)00205-6.
[36] C. Manach, G. Williamson, C. Morand, A. Scalbert, C. Rémésy, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, Am. J. Clin. Nutr. 81 (2005), https://doi.org/10.1093/ajcn/81.1.230s.
[37] M. Robles-Almazan, M. Pulido-Moran, J. Moreno-Fernandez, C. Ramirez-Tortosa, C. Rodriguez-Garcia, J.L. Quiles, Mc Ramirez-Tortosa, Hydroxytyrosol: bioavailability, toxicity, and clinical applications, Food Res. Int. 105 (2018) 654–667, https://doi.org/10.1016/j.foodres.2017.11.053.
[38] C.H. Cottart, V. Nivet-Antoine, C. Laguillier-Morizot, J.L. Beaudeux, Resveratrol bioavailability and toxicity in humans, Mol. Nutr. Food Res. 54 (2010) 7–16, https://doi.org/10.1002/mnfr.200900437.
[39] Search the Database - European Commission, (n.d.). https://ec.europa.eu/growth/tools-databases/tris/en/search/?trisaction=search.detail&year=2017&num=276 (accessed October 11, 2022).
[40] Federal Office of Consumer Protection and Food Safety - (BVL), List of Substances of the Competent Federal Government and Federal State Authorities, 2014, https://doi.org/10.1007/978-3-319-10732-5.
[41] Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements, EFSA J. 10 (2012), https://doi.org/10.2903/j.efsa.2012.2663.
[42] M. Younes, P. Aggett, F. Aguilar, R. Crebelli, B. Dusemund, M. Filipič, M.J. Frutos, P. Galtier, D. Gott, U. Gundert-Remy, C. Lambré, J.C. Leblanc, I.T. Lillegaard, P. Moldeus, A. Mortensen, A. Oskarsson, I. Stankovic, I. Waalkens-Berendsen, R. A. Woutersen, R.J. Andrade, C. Fortes, P. Mosesso, P. Restani, D. Arcella, F. Pizzo, C. Smeraldi, M. Wright, Scientific opinion on the safety of green tea catechins, EFSA J. 16 (2018), https://doi.org/10.2903/J.EFSA.2018.5239.
[43] N. and A. (NDA), EFSA Panel on Dietetic Products, Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), mainte, EFSA J. 9 (2011), https://doi.org/10.2903/j.efsa.2011.2033.
[44] Safety of synthetic trans-resveratrol as a novel food pursuant to Regulation (EC) No 258/97, EFSA J. 14 (2016), https://doi.org/10.2903/J.EFSA.2016.4368.
[45] M.F. Carvalho, A.B.A. Lucca, V.R. Ribeiro e Silva, L.R. de Macedo, M. Silva, Blueberry intervention improves metabolic syndrome risk factors: systematic review and meta-analysis, Nutr. Res. 91 (2021) 67–80, https://doi.org/10.1016/J.NUTRES.2021.04.006.
[46] M. Cheraghpour, H. Imani, S. Ommi, S.M. Alavian, E. Karimi-Shahrbabak, M. Hedayati, Z. Yari, A. Hekmatdoost, Hesperidin improves hepatic steatosis, hepatic enzymes, and metabolic and inflammatory parameters in patients with nonalcoholic fatty liver disease: a randomized, placebo-controlled, double-blind clinical trial, Phytother Res. 33 (2019) 2118–2125, https://doi.org/10.1002/PTR.6406.
[47] A. Garg, S. Garg, L.J.D. Zaneveld, A.K. Singla, Chemistry and pharmacology of the Citrus bioflavonoid hesperidin, Phytother Res. 15 (2001) 655–669, https://doi.org/10.1002/PTR.1074.
[48] C. Mancuso, R. Santangelo, Ferulic acid: pharmacological and toxicological aspects, Food Chem. Toxicol. 65 (2014) 185–195, https://doi.org/10.1016/J.FCT.2013.12.024.
[49] K. Zduńska, A. Dana, A. Kolodziejczak, H. Rotsztejn, Antioxidant properties of ferulic acid and its possible application, Skin Pharmacol. Physiol. 31 (2018) 332–336, https://doi.org/10.1159/000491755.
[50] Gras Notices, (n.d.). https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=341&sort=GRN_No&order=DESC&startrow=1&type=basic&search=341 (accessed December 15, 2021).
[51] A. Zubrzycki, K. Cierpka-Kmiec, Z. Kmiec, A. Wronska, The role of low-calorie diets and intermittent fasting in the treatment of obesity and type-2 diabetes, J. Physiol. Pharmacol. 69 (2018) 663–683, https://doi.org/10.26402/jpp.2018.5.02.
[52] Dipòsit Digital de la Universitat de Barcelona: Programa de Càlcul Nutricional Professional (PCN Pro), (n.d.). http://diposit.ub.edu/dspace/handle/2445/44329 (accessed April 26, 2022).
[53] V. Knaze, J.A. Rothwell, R. Zamora-Ros, A. Moskal, C. Kyrø, P. Jakszyn, G. Skeie, E. Weiderpass, M. Santucci de Magistris, C. Agnoli, S. Westenbrink, E. Sonestedt, A. Trichopoulou, E. Vasilopoulou, E. Peppa, E. Ardanaz, J.M. Huerta, H. Boeing, F. R. Mancini, A. Scalbert, N. Slimani, A new food-composition database for 437 polyphenols in 19,899 raw and prepared foods used to estimate polyphenol intakes in adults from 10 European countries, Am. J. Clin. Nutr. 108 (2018) 517–524, https://doi.org/10.1093/AJCN/NQY098.
[54] R. Zamora-Ros, V. Knaze, J.A. Rothwell, B. Hémon, A. Moskal, K. Overvad, A. Tjønneland, C. Kyrø, G. Fagherazzi, M.C. Boutron-Ruault, M. Touillaud, V. Katzke, T. Kühn, H. Boeing, J. Förster, A. Trichopoulou, E. Valanou, E. Peppa, D. Palli, C. Agnoli, F. Ricceri, R. Tumino, M.S. de Magistris, P.H.M. Peeters, H. Bas Bueno-De-Mesquita, D. Engeset, G. Skeie, A. Hjartåker, V. Menéndez, A. Agudo, E. Molina-Montes, J.M. Huerta, A. Barricarte, P. Amiano, E. Sonestedt, L. M. Nilsson, R. Landberg, T.J. Key, K.T. Khaw, N.J. Wareham, Y. Lu, N. Slimani, I. Romieu, E. Riboli, A. Scalbert, Dietary polyphenol intake in europe: the European prospective investigation into cancer and nutrition (EPIC) study, Eur. J. Nutr. 55 (2016) 1359–1375, https://doi.org/10.1007/s00394-015-0950-x.
[55] A. Ruiz, G. Pera, B. Díez, J. Miguel, X. Mundet, T. Alzamora, R. Elosua, P. Torán, A. Heras, R. Forés, M. Fusté, M. Fàbrega, A. Ruiz, G. Pera, J. Baena, X. Mundet, T. Alzamora, R. Elosua, P. Torán, A. Heras, Validación de una versión reducida en español del cuestionario de actividad física en el tiempo de Minnesota (VREM), Rev. Esp. Salud Publica 86 (2012) 495–508. https://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S1135-57272012000500004. (Accessed 26 April 2022).
[56] R. R Core Team, A Language and Environment for Statistical Computing, 2021. https://www.r-project.org/. (Accessed 31 January 2022).
[57] J. Most, I. Warnke, M.V. Boekschoten, J.W.E. Jocken, P. de Groot, A. Friedel, I. Bendik, G.H. Goossens, E.E. Blaak, The effects of polyphenol supplementation on adipose tissue morphology and gene expression in overweight and obese humans, Adipocyte 7 (2018) 190–196, https://doi.org/10.1080/21623945.2018.1469942.
[58] S.A. Jolles, E. Alagoz, N. Liu, C.I. Voils, G. Shea, L.M. Funk, Motivations of males with severe obesity, who pursue medical weight management or bariatric surgery, J. Laparoendosc. Adv. Surg. Tech. 29 (2019) 730–740, https://doi.org/10.1089/lap.2019.0219.
[59] E. Ahlich, C.L. Verzijl, A. Cunning, E. Wright, D. Rancourt, Patient motivations and goals for bariatric surgery: a mixed methods study, Surg. Obes. Relat. Dis. 17 (2021) 1591–1602, https://doi.org/10.1016/J.SOARD.2021.05.017.
[60] F. Magkos, G. Fraterrigo, J. Yoshino, C. Luecking, K. Kirbach, S.C. Kelly, L. De Las Fuentes, S. He, A.L. Okunade, B.W. Patterson, S. Klein, Effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in humans with obesity, Cell Metabol. 23 (2016) 591–601, https://doi.org/10.1016/j.cmet.2016.02.005.
[61] D.H. Ryan, S.R. Yockey, Weight loss and improvement in comorbidity: differences at 5%, 10%, 15%, and over, Curr. Obes. Rep. 6 (2017) 187–194, https://doi.org/10.1007/s13679-017-0262-y.
[62] T. Pischon, H. Boeing, K. Hoffmann, M. Bergmann, M. Schulze, K. Overvad, Y. van der Schouw, E. Spencer, K. Moons, A. Tjønneland, J. Halkjaer, M. Jensen, J. Stegger, F. Clavel-Chapelon, M. Boutron-Ruault, V. Chajes, J. Linseisen, R. Kaaks, A. Trichopoulou, D. Trichopoulos, C. Bamia, S. Sieri, D. Palli, R. Tumino, P. Vineis, S. Panico, P. Peeters, A. May, H. Bueno-de-Mesquita, F. van Duijnhoven, G. Hallmans, L. Weinehall, J. Manjer, B. Hedblad, E. Lund, A. Agudo, L. Arriola, A. Barricarte, C. Navarro, C. Martinez, J. Quirós, T. Key, S. Bingham, K. Khaw, P. Boffetta, M. Jenab, P. Ferrari, E. Riboli, General and abdominal adiposity and risk of death in europe, N. Engl. J. Med. 359 (2008) 2105–2120, https://doi.org/10.1056/nejmoa0801891.
[63] J. Spahlholz, N. Baer, H.H. König, S.G. Riedel-Heller, C. Luck-Sikorski, Obesity and discrimination - a systematic review and meta-analysis of observational studies, Obes. Rev. 17 (2016) 43–55, https://doi.org/10.1111/OBR.12343.

3.3. Statistical Analyses

The statistical analyses conducted for this research are reflected in each of the manuscripts included in the results section. Here, a summary of the methodology is presented.

In the EPIC study, to assess the associations of both dietary and plasma (poly)phenols with body weight change, multilevel mixed linear regression models with random effects on the intercept were performed. EPIC centres were taken as random effects to control for differences in follow-up procedures and questionnaire designs among centres. To evaluate associations between plasma (poly)phenols and body weight change, multinomial logistic regression models, using change in BW as a categorical variable (BW loss, maintenance, gain) were also performed.

In the Fenland study, to examine the cross-sectional associations between flavonoid intake and adiposity, robust multiple linear regression was used, minimizing the impact of outliers.

For both EPIC and Fenland studies, (poly)phenols were log2-transformed due to their right skewness, therefore results were presented as beta values for doubling the intake. The initial probability of false positive findings was set at 5%. However, given the number of tests conducted, a correction for multiple comparisons was applied using the false discovery rate (FDR) and all associations were considered significant if they surpassed this correction (FDR $q<0.05$). All statistical analyses were conducted using R (RStudio 2022.07.2)(120).

For the randomised clinical trial, a minimum of 34 participants was needed to detect, with a study power of 0.85, a variation of 5-10% in anthropometric and laboratory measurements, assuming an alpha error of 0.05. Assuming a possible drop-out rate of 15%, the required sample size was 40, with equal number of participants in each group (n = 20). Participants were randomly divided with an allocation ratio of 1:1 for intervention and control group. They were stratified based on sex (male and female). Randomisation and allocation were blinded to the participants and researchers involved in both data collection and analysis. Compliance was assessed primarily through number of capsules left in the third visit – with a 75% of the capsules taken and a

remaining of ≤25%. The main analysis will be performed according to the intention-to-treat (ITT) principle. Participants without protocol violations and meeting the requirements of compliance will be included for a per-protocol (PP) analysis.

4. Results

In this section, the manuscripts related to objectives 1, 2, and 3 will be presented. The first manuscript from the EPIC is in the form of scientific publication. The second one is currently accepted, in press. Additionally, the manuscript for the Fenland study is in the process of preparation for submission to a journal.

4.1. Manuscript 2: Dietary intake of 91 individual polyphenols and 5-year body weight change in the EPIC-PANACEA cohort.

Gil-Lespinard M, Castañeda J, Almanza-Aguilera E, Gómez JH, Tjønneland A, Kyrø C, et al. Dietary intake of 91 individual polyphenols and 5-year body weight change in the EPIC-PANACEA cohort. Antioxidants [Internet]. 2022 Dec 8;11(12):2425. Available from: https://doi.org/10.3390/antiox11122425

Impact Factor: 7.0

Category: Biochemistry & Molecular Biology. Q1 46/285

Category: Chemistry, Medicinal. Q1 6/60

This work has been presented as communications at:

- The 6th Edition IDIBELL PhD Day, held on October 22, 2021, at the Bellvitge Biomedical Research Institute in L'Hospitalet de Llobregat, Spain, in the form of poster.
- The Scientific Session of the Cancer Epidemiology Research Programme, held on February 14, 2022, at the Bellvitge Biomedical Research Institute in L'Hospitalet de Llobregat, Spain, in the form of oral communication.
- The 8th Edition IDIBELL PhD Day, held on November 10, 2023, at the Bellvitge Biomedical Research Institute in L'Hospitalet de Llobregat, Spain, in the form of oral communication as part of the talk ‘Impact of (Poly)phenols on Body Weight and Adiposity: Insights from Two European Cohorts’. Awarded 2nd Best Oral Communication.

antioxidants

MDPI

Article

Dietary Intake of 91 Individual Polyphenols and 5-Year Body Weight Change in the EPIC-PANACEA Cohort

Mercedes Gil-Lespinard [1], Jazmín Castañeda [1], Enrique Almanza-Aguilera [1], Jesús Humberto Gómez [2,3], Anne Tjønneland [4,5], Cecilie Kyrø [4], Kim Overvad [6], Verena Katzke [7], Matthias B. Schulze [8,9], Giovanna Masala [10], Claudia Agnoli [11], Maria Santucci de Magistris [12], Rosario Tumino [13], Carlotta Sacerdote [14], Guri Skeie [15], Cristina Lasheras [16], Esther Molina-Montes [3,17,18,19], José María Huerta [2,3], Aurelio Barricarte [3,20,21], Pilar Amiano [3,22,23], Emily Sonestedt [24], Marisa da Silva [25], Ingegerd Johansson [26], Johan Hultdin [27], Anne M. May [28], Nita G. Forouhi [29], Alicia K. Heath [30], Heinz Freisling [31], Elisabete Weiderpass [31], Augustin Scalbert [31] and Raul Zamora-Ros [1,*]

1 Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), 08908 Barcelona, Spain
2 Department of Epidemiology, Regional Health Council, IMIB-Arrixaca, 30008 Murcia, Spain
3 CIBER in Epidemiology and Public Health (CIBERESP), 28029 Madrid, Spain
4 Danish Cancer Society Research Center, DK-2100 Copenhagen, Denmark
5 Department of Public Health, Section of Environmental Health, Faculty of Health and Medical Sciences, University of Copenhagen, DK-1353 Copenhagen, Denmark
6 Department of Public Health, Aarhus University, DK-8000 Aarhus, Denmark
7 Department of Cancer Epidemiology, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany
8 Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, 14558 Nuthetal, Germany
9 Institute of Nutritional Science, University of Potsdam, 14469 Potsdam, Germany
10 Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network—ISPRO, 50139 Florence, Italy
11 Epidemiology and Prevention Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori Via Venezian, 20133 Milan, Italy
12 Department of Clinical and Experimental Medicine, Federico II University, 80131 Naples, Italy
13 Hyblean Association for Epidemiological Research (AIRE-ONLUS), 97100 Ragusa, Italy
14 Unit of Cancer Epidemiology, Città della Salute e della Scienza University-Hospital, 10124 Turin, Italy
15 Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, 9019 Tromsø, Norway
16 Department of Functional Biology, University of Oviedo, 33007 Oviedo, Spain
17 Department of Nutrition and Food Science, Campus of Cartuja, University of Granada, 18071 Granada, Spain
18 Instituto de Investigación Biosanitaria ibs.GRANADA, 18012 Granada, Spain
19 Institute of Nutrition and Food Technology (INYTA) 'José Mataix', Biomedical Research Centre, University of Granada, 18071 Granada, Spain
20 Navarra Public Health Institute, 31003 Pamplona, Spain
21 Navarra Institute for Health Research (IdiSNA), 31008 Pamplona, Spain
22 Ministry of Health of the Basque Government, Sub-Directorate for Public Health and Addictions of Gipuzkoa, 20013 San Sebastian, Spain
23 Public Health Division of Gipuzkoa, BioDonostia Research Institute, 20014 San Sebastian, Spain
24 Nutritional Epidemiology, Department of Clinical Sciences Malmö, Lund University, 22184 Malmö, Sweden
25 Register-Based Epidemiology, Department of Clinical Sciences Lund, Lund University, 22184 Lund, Sweden
26 Department of Odontology, Umeå University, 90187 Umeå, Sweden
27 Department of Medical Biosciences, Umeå University, 90187 Umeå, Sweden
28 Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, 3584 CX Utrecht, The Netherlands
29 MRC Epidemiology Unit, Institute of Metabolic Science, Cambridge Biomedical Campus, University of Cambridge School of Clinical Medicine, Cambridge CB2 0SL, UK
30 Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London W2 1PG, UK
31 International Agency for Research on Cancer (IARC-WHO), 69008 Lyon, France
* Correspondence: rzamora@idibell.cat

check for updates

Citation: Gil-Lespinard, M.; Castañeda, J.; Almanza-Aguilera, E.; Gómez, J.H.; Tjønneland, A.; Kyrø, C.; Overvad, K.; Katzke, V.; Schulze, M.B.; Masala, G.; et al. Dietary Intake of 91 Individual Polyphenols and 5-Year Body Weight Change in the EPIC-PANACEA Cohort. *Antioxidants* **2022**, *11*, 2425. https://doi.org/10.3390/antiox11122425

Academic Editor: Stanley Omaye

Received: 15 November 2022
Accepted: 6 December 2022
Published: 8 December 2022

Abstract: Polyphenols are bioactive compounds from plants with antioxidant properties that may have a protective role against body weight gain, with adipose tissue and systemic oxidative stress as potential targets. We aimed to investigate the dietary intake of individual polyphenols and their association with 5-year body weight change in a sub-cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC). This study included 349,165 adult participants from nine European countries. Polyphenol intake was estimated through country-specific validated dietary questionnaires and the Phenol-Explorer database. Body weight was obtained at recruitment and after a mean follow-up time of 5 years. Associations were estimated using multilevel mixed linear regression models. From 91 polyphenols included, the majority (n = 67) were inversely associated with 5-year body weight change after FDR-correction ($q < 0.05$). The greatest inverse associations were observed for quercetin 3-O-rhamnoside (change in weight for doubling in intake: −0.071 (95% CI: −0.085; −0.056) kg/5 years). Only 13 polyphenols showed positive associations with body weight gain, mainly from the subclass hydroxycinnamic acids (HCAs) with coffee as the main dietary source, such as 4-caffeoylquinic acid (0.029 (95% CI: 0.021; 0.038) kg/5 years). Individual polyphenols with fruit, tea, cocoa and whole grain cereals as the main dietary sources may contribute to body weight maintenance in adults. Individual HCAs may have different roles in body weight change depending on their dietary source.

Keywords: polyphenol; intake; body weight; obesity; cohort; EPIC

1. Introduction

Different studies have suggested that polyphenols, bioactive compounds from plants with antioxidant and anti-inflammatory properties, may play a protective role against obesity with adipose tissue and systemic oxidative stress as possible therapeutic targets [1,2]. Excessive production of reactive oxygen species (ROS) leads to oxidative stress which may generate lipid, protein, and DNA damage, inflammation, and alterations in energy homeostasis, as well as promoting adipocyte differentiation and adipogenesis associated with obesity development [2,3]. Approximately 500 individual polyphenols have been identified in the habitual human diet so far, in commonly and regularly consumed plant-based foods, such as fruits, coffee and tea, vegetables, cocoa, or whole grain cereals [4]. Depending on their chemical structure, they can be classified as flavonoids, phenolic acids, stilbenes, lignans, and others [5]. Bioavailability and bio-efficacy differs greatly from one polyphenol to another [6]. Thus, the most abundant polyphenols in our diet are not necessarily those leading to the highest concentrations in the target tissues or the most active ones [6].

Evidence on the link between polyphenols and body weight in humans is not clear, in part due to the heterogeneity between studies (design, populations, and supplements, among others) [7]. Previous observational studies have reported data on polyphenol intake and different anthropometric parameters (i.e., body mass index, waist circumference, and body weight change), mainly studying classes and subclasses. In general, higher intake of polyphenols have been associated with a lesser increase in those parameters. A longitudinal study from the Netherlands reported an inverse association between flavonoid intake and body mass index (BMI) in women [8]. Similarly, a Mediterranean cohort study showed that a higher dietary intake of flavonoids were significantly associated with lower body weight and obesity [9]. The SU.VI.MAX study also reported inverse associations between different flavonoid subclasses (flavanones, flavones), lignans and BMI [10]. They have also shown that those polyphenols, hydroxycinnamic acids (HCAs) and total polyphenols were inversely associated with waist circumference. In a previous investigation, we evaluated associations between the intake of total polyphenols, classes and subclasses, and body weight change in the EPIC-PANACEA cohort [11]. In this previous study, higher intake of flavonoids was inversely associated with body weight change. Conversely, HCAs were the

main contributors to total phenolic acids and total polyphenols, all of which were positively associated with body weight gain.

In general, individual polyphenols have been analysed in mechanistic studies that have shown how compounds even from the same class might act differently, or in interventional studies in humans mostly studying different doses of a single compound. For example, catechins, major tea and cocoa polyphenols could be potentially relevant for the prevention of body weight gain and could promote anti-obesity mechanisms, including control of adipocyte differentiation and lipid oxidation and modulation of human gut microbiota [2,12]. Likewise, epigallocatechin from green tea showed effectiveness on body fat and weight reduction in human trials [13]. A review on the relationship between dietary polyphenols from the Mediterranean diet and obesity highlighted the role of several individual compounds, including epigallocatechin gallate, hydroxytyrosol and resveratrol, in molecular mechanisms associated with obesity [7]. However, evidence on other different individual polyphenols and body weight and obesity in humans and from population-based studies is limited.

The current study aimed to delve deeper into the relationships between polyphenols and body weight control, focusing on polyphenols individually.

2. Materials and Methods

2.1. Population

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a multicentre cohort including ~521,000 men and women from 10 Western European countries with 23 centres, recruited between 1992 and 2000, mainly from the general population. Details of the recruitment and study design have been published previously [14]. The Physical Activity, Nutrition, Alcohol, Cessation of smoking, Eating out of home in relation to Anthropometry (PANACEA) project is a sub-cohort of the EPIC study, where body weight was collected at baseline and at one follow-up (between 2–11 years after the recruitment), making it possible to investigate body weight changes [15]. For the present study we excluded pregnant women, participants with extreme or implausible diet values, with unreliable anthropometric measures, and without lifestyle information at baseline (n = 23,713). We also excluded participants with missing body weight values at follow-up (n = 121,866) and with extreme/implausible values for annual weight change (≤ -5 or >5 kg/year) or body mass index (BMI) (<16 kg/m^2) at follow-up (n = 2066). Finally, data from Greece was not available for the current study (n = 24,638). The final analyses included 349,165 participants (Figure S1).

2.2. Dietary Data

At baseline, validated country/centre specific dietary questionnaires were used to collect dietary data regarding the previous year [16]. The standardized EPIC Nutrient Database [17] was used to estimate total energy and nutrient intake. Dietary intake of polyphenols was estimated using the Phenol-Explorer database [18], where more than 500 individual polyphenols have been included from over 400 different dietary sources, considering the effects of food processing and cooking. Further details were described previously [19].

2.3. Anthropometric Characteristics and Body Weight Change

Two weight measures were available for each participant: one at baseline and one at follow-up. At baseline, anthropometric characteristics were mostly measured by trained specialists using standardized methods [20]. The body weight and height of participants were measured at baseline in most centres, with participants wearing no shoes. The exceptions were the centres from France, Oxford (UK) and Norway, where baseline weight and height were self-reported. At follow-up body weight was mostly self-reported; it was measured only in participants from Doetinchen (the Netherlands) and Cambridge (UK) [20]. Assessment of self-reported weight was conducted through mailed questionnaires, with

several exceptions: Spain completed the questionnaire on the phone and Varese used a combination of postal surveys and telephone interviews [21]. The accuracy of self-reported body weight was improved with prediction equations derived from participants from EPIC-Oxford where body weight at baseline was both measured and self-reported [20]. To calculate body weight change, baseline weight was subtracted from follow-up weight, divided by follow-up years to obtain annual weight change and multiplied by 5 to obtain body weight change over 5 years.

2.4. Other Covariates

A broad spectrum of sociodemographic, lifestyle, and clinical characteristics were collected through standardized questionnaires [14] at baseline. Physical activity was collected through the validated EPIC-Physical Activity Questionnaire [22] and classified using the Cambridge index [23]. Smoking status was collected at both baseline and follow-up.

2.5. Statistical Analysis

Dietary data on 419 individual polyphenols were available for this study. For inclusion in the analysis, a cut-off point was made for polyphenols for which over 50% of the study participants were consumers and with a mean intake $\geq$ 1 mg/d. A total of 91 individual polyphenols met these criteria and were analysed as continuous variables. To improve right skewness, polyphenol data was log2-transformed, meaning that one unit increase corresponded to a doubling in the intake. To manage very small intakes, values below 0.0001 mg/d were transformed into zero. Then, half the minimum intake of the corresponding polyphenol was added to each zero value. Pearson's correlation coefficients were calculated between polyphenols and were considered strong when r $\geq$ 0.8. Correlated polyphenols were clustered in groups. From each group, those individual polyphenols presenting the strongest correlations and the highest median intake were selected (on behalf of the others) for the final analysis. Main food sources were selected from our previous EPIC study, where dietary data at baseline were linked to the Phenol-Explorer database [24]. Distributions of participants' characteristics were calculated according to quintiles of body weight change over 5 years, with quintiles 2 and 3 as reference categories (i.e., maintenance, range −2.23 to 1.77 kg/5 years). We performed multilevel mixed linear regression models with random effects on the intercept. EPIC centres were taken as random effects to control for differences in follow-up procedures and questionnaire designs among centres. ANOVA and Akaike's information criterion were used to verify their design. Model assumptions were checked visually by plotting residuals. Missing values were classified as unknown for categorical variables and omitted (n = 225) for continuous variables. Restricted cubic splines were used to evaluate linearity of the associations for continuous covariates. Baseline BMI and follow-up years showed a non-linear relationship with body weight change over 5 years. Thus, splines with 3 knots (percentile 10, 50 and 90) were included as covariates for these two variables. Knot positions were determined using the Harrell criteria [25]. We fitted several multivariable-adjusted models that controlled for potential confounders selected a priori, based on previous clinical and epidemiological evidence [26–30]. Model 1 was adjusted for sex, age, and BMI (at baseline). Model 2 was further adjusted for follow-up years, smoking status at follow-up, physical activity, education level, alcohol consumption, and menopausal status. Model 3 was additionally adjusted for energy intake and plausibility of energy intake reporting. The latter included three categories according to the ratio of reported energy intake to predicted basal metabolic rate (EI:BRM): under (EI:BMR < 1.14), plausible (1.14 to 2.1) and over reporters (>2.1), with the use of cut-off points proposed by Goldberg [31]. Finally, we fitted another fourth model adjusted for dietary factors as a proxy of diet quality: vitamin C and fibre intake. An additional analysis for model 4 was performed estimating polyphenols according to energy density. We divided polyphenols by energy intake and multiplied by 2000 (average daily caloric consumption for adults). Interactions were explored between individual polyphenols and different variables that have been shown to influence body weight changes [32–35]: sex (male vs. female), age

(< vs. ≥50 years), BMI categories (underweight, normal weight, overweight, obesity), menopausal status (pre-, post-, and peri-menopausal), smoking status at follow-up (never, former, current), and tertiles of fibre intake, in relation to body weight change. To explore interactions for both fibre and BMI, the categorical variable was added to model 4 (tertiles of fibre intake and BMI categories (underweight, normal weight, overweight, and obesity)). *p* values for interactions were calculated using the likelihood ratio test. Sensitivity analyses were performed excluding participants with chronic diseases at baseline (diabetes, cancer, stroke and/or myocardial infarction, *n* = 57,617), or with self-reported body weight at follow-up (*n* = 320,512). Further analyses were performed with coffee consumers and non-consumers to clarify the role of some polyphenols from coffee in body weight change. For these polyphenols in particular, in secondary analyses, model 4 was further adjusted for sugar, milk, confectionery, and cake intake. Coffee is often consumed together with these foods [36–38] which have been shown to increase calorie intake and have therefore been suggested to increase risk of body weight gain when chosen over healthier alternatives [37,39]. The false discovery rate (FDR) was computed to control for multiple comparisons. Differences were considered statistically significant at FDR $q < 0.05$. Statistical analyses were performed with R Statistical Software [40] (version 1.3.1093) using the LmerTest package [41].

3. Results

In total, 349,165 participants were included in this investigation, with female participants constituting 73.2% of the sample. Mean age (years) at recruitment was 51.7 (SD = 9.1); mean years of follow-up was 5.1 (2.3) and mean body weight change (kg) over 5 years was 2.6 (5.0). Table 1 shows the distribution of characteristics by quintiles of 5-year body weight change for the total population. The majority of polyphenols were consumed by more than 90% of the population (75 out of 91). The most consumed polyphenols were those from coffee (4-caffeoylquinic acid, median intake: 113 mg/d; 5-feruloylquinic acid: 22 mg/d; and 4-feruloylquinic acid: 16 mg/d), and ferulic acid (22 mg/d), mainly present in whole grain cereals (Tables 2 and S1). These were followed by hesperidin (16 mg/d) mainly present in citrus fruits and (+)-catechin (11 mg/d), mostly present in cocoa and tea. The highest level of non-consumers was observed for sanguiin H-6, a polyphenol widely present in raspberries (non-consumers = 48.6%), followed by some polyphenols present in black tea, such as theaflavin, or kaempferol and quercetin 3-O-glucosyl-rhamnosyl-glucoside (non-consumers = 40.4%) (Tables 2 and S1).

Table 1. Baseline characteristics of the study population by quintiles of body weight change after 5 years.

Characteristics	Total Population	Q1:	Q2 + Q3:	Q4:	Q5:
		Loss	Maintenance	Moderate Gain	High Gain
Participants (n)	349,165	69,834	139,665	69,842	69,824
Body weight change (kg/5 years) (range)	−24.99; 24.93	−24.99; −2.22	−2.23; 1.77	1.78; 4.16	4.17; 24.93
Body weight change (kg/5 years)	2.62 (5.03)	−6.01 (3.96)	−0.005 (1.09)	2.89 (0.68)	7.56 (3.45)
Age (years)	51.7 (9.1)	53.1 (9.0)	52.1 (9.0)	51.1 (9.1)	50.1 (9.3)
Weight (kg)	69.4 (13.4)	75.2 (14.3)	67.6 (12.7)	67.1 (12.4)	69.4 (13.1)
BMI (kg/m^2)	25.1 (4.1)	27.2 (4.5)	24.5 (3.8)	24.3 (3.7)	25.0 (4.0)
Follow-up time (years)	5.1 (2.3)	4.4 (1.9)	5.5 (2.4)	5.4 (2.4)	4.8 (2.1)
Alcohol intake (g/d), median (IQR)	11.8 (16.7)	13.6 (19.3)	11.9 (16.1)	11.2 (15.7)	10.7 (16.1)
Energy intake (kcal/d), median (IQR)	2076.2 (606.1)	2073.2 (615.4)	2086.1 (596.7)	2087.2 (602.3)	2048.6 (618.0)
Vitamin C intake (mg/d), median (IQR)	111.7 (73.6)	111.4 (75.5)	111.5 (72.8)	112.0 (72.0)	112.0 (75.9)

Table 1. *Cont.*

Characteristics	Total Population	Q1: Loss	Q2 + Q3: Maintenance	Q4: Moderate Gain	Q5: High Gain
Fibre intake (g/d), median (IQR)	22.0 (9.6)	22.0 (10.0)	22.2 (9.5)	21.9 (9.4)	21.7 (9.5)
Sex (female) (%)	73.2	67.2	73.6	75.2	76.5
Education level (%)					
None	4.2	7.9	3.2	2.8	3.6
Primary school	23.2	27.1	23.2	21.5	21.0
Technology school	22.2	22.9	22.2	21.5	22.2
Secondary school	21.7	15.7	21.6	24.7	25.1
Longer education	25.1	22.8	26.3	26.0	24.2
Unknown	3.6	3.6	3.5	3.5	3.9
Physical activity level (%)					
Inactive	18.6	22.6	17.5	17.2	18.4
Moderately inactive	34.2	33.9	34.4	34.8	33.5
Moderately active	27.4	24.1	28.0	29.6	28.5
Active	18.2	18.3	18.6	17.8	17.5
Unknown	1.6	1.1	1.5	1.6	2.1
Smoking at follow-up (%)					
Never	48.3	46.9	48.7	50.1	47.3
Former	30.2	28.6	29.0	30.0	34.2
Current	17.3	20.9	16.8	15.8	16.3
Unknown	4.2	3.6	5.5	4.1	2.2
Prevalent disease [1] (%)					
No	83.5	82.7	84.4	84.3	81.8
Yes	7.6	10.0	7.1	7.0	7.9
Unknown	8.9	8.3	8.5	8.7	10.3
Menopausal status [2] (%)					
Pre-menopausal	32.6	29.7	31.3	34.0	36.4
Post-menopausal	43.7	49.3	45.7	41.3	37.2
Peri-menopausal	20.6	17.2	19.9	21.8	23.6
Surgical post-menopausal	3.1	3.8	3.1	2.9	2.8

Data are expressed as mean (SD) and collected at recruitment if not stated otherwise. BMI: body mass index; IQR: interquartile range; Q: quintile. [1] Diabetes, cancer, stroke and/or myocardial infarction at baseline. [2] Only for female participants (n = 255,730).

Table 2. Change in body weight over 5 years according to baseline polyphenol intake in 349,165 participants from the EPIC-PANACEA cohort.

Individual Polyphenols (n = 62) [1]	Intake (mg/d), Median (p5; p95)	N-C (%)	Model 4 beta (95% CI) [2]	q-Value [3]	Main Food Sources [4]
(-)-Epicatechin 3-O-gallate	3.3 (0.0; 66.2)	0.2	−0.018 (−0.023; −0.013)	<0.001	Coffee, tea, SF
(+)-Catechin	10.9 (2.2; 35.7)	0.0	−0.060 (−0.076; −0.043)	<0.001	Apples, pears, chocolate
2,5-di-S-Glutathionyl caftaric acid	0.3 (0.0; 6.5)	23.4	−0.012 (−0.015; −0.009)	<0.001	Whole grain cereals, rice
3,4-DHPEA-EDA	0.4 (0.0; 4.1)	1.0	−0.022 (−0.031; −0.016)	<0.001	Tea, wine, FV
3-Feruloylquinic acid	8.0 (0.2; 22.7)	0.0	0.026 (0.018; 0.033)	<0.001	Coffee, SF, berries
3-p-Coumaroylquinic acid	1.0 (0.1; 11.4)	0.4	−0.030 (−0.040; −0.021)	<0.001	Whole grain cereals, rice
4-Caffeoylquinic acid	113.2 (2.9; 328.4)	0.0	0.029 (0.021; 0.038)	<0.001	Coffee, tea, legumes
4-Feruloylquinic acid	16.0 (0.1; 46.1)	0.1	0.017 (0.011; 0.023)	<0.001	Coffee, tea

Table 2. *Cont.*

Individual Polyphenols (n = 62) [1]	Intake (mg/d), Median (p5; p95)	N-C (%)	Model 4 beta (95% CI) [2]	q-Value [3]	Main Food Sources [4]
4-Hydroxybenzoic acid	0.4 (0.0; 4.0)	0.0	−0.033 (−0.044; −0.023)	<0.001	Wine, grapes
4-p-Coumaroylquinic acid	1.3 (0.0; 8.6)	2.6	−0.022 (−0.027; −0.015)	<0.001	Tea, apples, pears
5-Feruloylquinic acid	21.8 (0.2; 63.0)	0.1	0.019 (0.012; 0.025)	<0.001	Whole grain cereals, rice
5-Heneicosenylresorcinol	0.23 (0.0; 6.5)	19.9	−0.000 (−0.005; 0.004)	0.873	Wine, cocktails, sauces
5-Heneicosylresorcinol	9.5 (0.4; 28.8)	0.1	−0.039 (−0.050; −0.028)	<0.001	Apples, pears, FJ
5-Heptadecylresorcinol	2.1 (0.2; 17.1)	0.8	−0.038 (−0.048; −0.029)	<0.001	Soups, cakes, biscuits
5-Nonadecylresorcinol	8.5 (0.3; 30.4)	0.1	−0.037 (−0.048; −0.027)	<0.001	Coffee, cream desserts
5-O-Galloylquinic acid	1.8 (0.0; 104.2)	4.4	−0.012 (−0.016; −0.008)	<0.001	Whole grain cereals, olives, beer
5-Pentacosylresorcinol	1.0 (0.0; 5.8)	8.7	−0.011 (−0.017; −0.005)	0.002	Olives, legumes, bread
5-Tricosylresorcinol	2.4 (0.2; 8.8)	0.8	−0.034 (−0.044; −0.025)	<0.001	Coffee, beer, cream desserts
Apigenin 6,8-C-arabinoside-C-glucoside	1.8 (0.3; 7.6)	1.0	−0.023 (−0.032; −0.012)	<0.001	Wine, apples, pears
Apigenin 6,8-C-galactoside-C-arabinoside	2.5 (0.4; 9.6)	1.0	−0.023 (−0.033; −0.014)	<0.001	Vegetable oils, olives, sauces
Apigenin 6,8-di-C-glucoside	2.1 (0.1; 10.0)	2.1	−0.017 (−0.023; −0.011)	<0.001	FV, olives
Caffeic acid	1.9 (0.7; 6.5)	0.0	−0.058 (−0.084; −0.033)	<0.001	Coffee, cream desserts, beer
Caffeoyl tartaric acid	1.1 (0.0; 9.4)	0.7	−0.021 (−0.028; −0.015)	<0.001	LV, berries, FV
Cyanidin 3-O-glucoside	1.4 (0.1; 9.4)	0.2	−0.021 (−0.030; −0.009)	<0.001	Olives, berries
Cyanidin 3-O-rutinoside	4.0 (0.0; 48.9)	2.4	−0.007 (−0.013; −0.002)	0.009	Olives, sauces, mixed vegetables
Delphinidin 3-O-glucoside	0.7 (0.0; 5.4)	2.1	−0.007 (−0.015; −0.002)	0.024	Soya products, vegetarian dishes
Delphinidin 3-O-rutinoside	0.2 (0.0; 13.8)	17.6	−0.007 (−0.011; −0.003)	<0.001	Whole grain cereals, rice
Didymin	2.0 (0.1 9.2)	2.1	−0.017 (−0.023; −0.011)	<0.001	Wine, olives, beer
Dihydromyricetin 3-O-rhamnoside	0.5 (0.0; 10.4)	10.0	−0.013 (−0.017; −0.010)	<0.001	Wine, apples, pears
Ellagic acid	0.9 (0.0; 11.5)	1.9	−0.024 (−0.031; −0.018)	<0.001	Wine, cocktails, sauces
Ferulic acid	22.5 (0.2; 134.0)	0.0	−0.033 (−0.051; −0.014)	<0.001	Whole grain cereals, olives
Gallic acid	5.6 (0.2; 43.9)	0.1	−0.016 (−0.024; −0.009)	<0.001	Vegetable oils, sauces, bread
Hesperidin	16.5 (0.9; 9.2)	0.8	−0.017 (−0.023; −0.011)	<0.001	Citrus fruits, beer, olives
Kaempferol 3-O-glucoside	1.8 (0.1; 10.5)	0.3	−0.024 (−0.034; −0.015)	<0.001	Mixed vegetables, soups
Malvidin 3-O-(6-p-coumaroyl-glucoside)	0.7 (0.0; 13.5)	6.4	−0.008 (−0.012; −0.003)	<0.001	Berries, cakes, pastries
Naringin	0.5 (0.0; 9.0)	0.8	−0.020 (−0.026; −0.014)	<0.001	Legumes, soups, citrus fruits
Narirutin	2.8 (0.1; 14.2)	0.9	−0.017 (−0.024; −0.012)	<0.001	FJ, citrus fruits, soft drinks
O-Coumaric acid	0.7 (0.0; 5.2)	2.8	−0.004 (−0.009; 0.002)	0.178	Mixed vegetables, berries, LV
Oleuropein-aglycone	0.2 (0.0; 4.9)	1.0	−0.016 (−0.022; −0.009)	<0.001	Wine, sauces
P-Courmaric acid	2.2 (0.5; 6.1)	0.0	−0.003 (−0.022; 0.015)	0.733	Soups, berries, legumes
Pelargonidin 3-O-glucoside	2.2 (0.1; 13.5)	1.4	−0.012 (−0.019; −0.004)	0.003	FV, legumes
Phloridzin	1.0 (0.1; 3.8)	1.7	−0.024 (−0.032; −0.018)	<0.001	Berries, soft drinks, FJ
Phlorin	0.6 (0.0; 3.3)	1.6	−0.016 (−0.022; −0.009)	<0.001	LV, whole grain cereals, rice
Proanthocyanidin Polymers (>10 mers)	69.0 (18.2; 198.5)	0.1	−0.000 (−0.016; 0.014)	0.952	Spices, herbs, soups
Proanthocyanidins 04–06 oligomers	42.0 (11.0; 119.2)	0.1	−0.005 (−0.020; 0.011)	0.608	Sweets, bread, seeds
Proanthocyanidins 07–10 oligomers	30.0 (7.6; 82.8)	0.1	−0.011 (−0.026; 0.004)	0.178	Sweets, bread, seeds
Procyanidin dimer B3	3.4 (0.3; 23.2)	0.0	−0.056 (−0.068; −0.045)	<0.001	Berries, jams
Procyanidin dimer B4	3.9 (0.0; 23.9)	0.5	−0.030 (−0.036; −0.023)	<0.001	LV, vegetarian products
Procyanidin dimer B7	1.9 (0.2; 7.2)	0.4	−0.046 (−0.056; −0.035)	<0.001	Wine, spirits, cocktails
Prodelphinidin dimer B3	0.6 (0.0;14.9)	0.5	−0.019 (−0.025; −0.012)	<0.001	Sweets, bread, seeds
Protocatechuic acid	0.5 (0.1; 4.7)	0.0	0.006 (−0.010; 0.022)	0.434	FV, mixed vegetables, soups
Quercetin	1.2 (0.2; 4.9)	0.0	−0.041 (−0.056; −0.028)	<0.001	Beer, peanuts, mixed fruits
Quercetin 3,4-O-diglucoside	1.7 (0.3; 8.7)	0.2	−0.011 (−0.023; 0.002)	0.100	Spices, herbs, sauces
Quercetin 3-O-galactoside	1.5 (0.1; 7.9)	0.1	−0.049 (−0.058; −0.039)	<0.001	Berries, sweets, bread
Quercetin 3-O-glucoside	3.7 (0.3; 13.0)	0.0	−0.062 (−0.074; −0.051)	<0.001	FV, legumes
Quercetin 3-O-rhamnoside	1.1 (0.2; 3.9)	0.0	−0.071 (−0.085; −0.056)	<0.001	Beer, cocktails
Quercetin 3-O-rutinoside	0.4 (0.4; 16.6)	0.0	−0.050 (−0.064; −0.038)	<0.001	Soup, bread, flours

Table 2. *Cont.*

Individual Polyphenols (n = 62) [1]	Intake (mg/d), Median (p5; p95)	N-C (%)	Model 4 beta (95% CI) [2]	q-Value [3]	Main Food Sources [4]
Quercetin 4-O-glucoside	1.2 (0.2; 6.3)	0.2	−0.011 (−0.023; 0.001)	0.091	Berries, jams
Sanguiin H-6	0.1 (0.0; 4.2)	48.6	0.000 (−0.005; 0.007)	0.523	Sauces, condiments, soups
Sinapic acid	0.73 (0.11; 4.15)	0.1	0.021 (0.009; 0.033)	<0.001	Olives, FV, legumes
Stigmastanol ferulate	0.7 (0.0; 9.6)	16.1	0.004 (−0.000; 0.008)	0.074	Wine, spirits, cocktails
Tyrosol	1.1 (0.0; 8.5)	0.1	−0.038 (−0.046; −0.029)	<0.001	Mixed fruits

P5 and p95: percentile 5th and 95th; FJ: fruit juices; FV: fruiting vegetables; LV: leafy vegetables; N-C: non-consumers; SF: stone fruits. Overall mean 5-year weight gain corresponded to 2.6 (5.0) kg and negative beta-values indicate less weight gain (kg) over 5 years based on log2-transformed polyphenol intakes. [1] Selection criteria: consumers mean $\geq$ 1 mg/d; consumers $\geq$ 50%; Pearson's correlation coefficient < 0.8. [2] Multilevel linear mixed models with random effects on the intercept according to EPIC centre adjusted for age, sex, body mass index (3-knot restricted cubic spline), follow-up time in years (3-knot restricted cubic spline), alcohol intake at baseline (g/d), education level, physical activity level, smoking status at follow-up, menopausal status, total energy intake (kcal/d), plausibility of energy intake reporting, vitamin C intake (mg/d), and fibre intake (g/d). [3] false discovery rate q value considered statistically significant at $\leq$0.05. [4] Main food sources in descending order of polyphenol content according to our previous EPIC study.

Figure 1 shows q values (FDR) and beta coefficients for doubling in intake of the 91 polyphenols for model 4, with their respective main food source. Most polyphenols (n = 80) showed statistically significant associations with body weight change, of which 67 were inversely and 13 were positively associated with body weight change. Figure 2 shows polyphenol–polyphenol correlations for the 91 individual compounds, as well as their main food sources. After performing Pearson's correlations, 35 polyphenols with coefficients $\geq$ 0.8 were separated into six correlation groups. From each group, polyphenols with the highest median intakes were chosen. Thus, six individual polyphenols were selected on behalf of the 35, and therefore 29 were excluded (Table S1). A total of 62 polyphenols remained after excluding highly correlated polyphenols, of which 51 were significantly associated with body weight change and 11 showed null results after adjusting for potential confounders and correcting for multiple comparisons (Tables 2 and S2). From these, 46 polyphenols were inversely associated with 5-year body weight change for a doubling in intake. Their main dietary sources were fruits, tea, cocoa and whole grain cereals. The greatest inverse associations were observed for a doubling in intake of quercetin 3-O-rhamnoside: −0.071 kg/5 year (95% CI: −0.085; −0.056), quercetin 3-O-glucoside: −0.062 (−0.074; −0.051) and (+)-catechin: −0.060 (−0.077; −0.043) (Figure S2).

Only 5 out of 51 individual polyphenols showed positive associations with body weight gain, in general with coffee as their main food source, except for sinapic acid, for which the main food source was olives (Tables 2 and S2). Classified as a hydroxycinnamic acid (HCA), doubling in intake of 4-caffeoylquinic acid showed the greatest positive associations with body weight gain for model 4: 0.029 kg/5 year (95% CI: 0.021; 0.038). Results remained the same after further adjusting these five polyphenols for sugar, milk, confectionery, and cake intake. We performed extra analysis including only coffee non-consumers (n = 25,414), for which main dietary sources of these polyphenols were plums, berries and black tea. Median intakes of HCAs and 4-caffeoylquinic acid from other dietary sources than coffee, in coffee non-consumers, were 124 and 3 mg/d, respectively. Median intakes in coffee consumers were 520 mg/d for HCAs and 120 mg/d for 4-caffeoylquinic acid. Associations were not statistically significant for coffee non-consumers. In coffee consumers (n = 323,751), we observed positive associations with body weight gain for the five polyphenols (Table S3).

Our findings were robust and remained statistically significant for the majority of polyphenols after the sensitivity analyses, excluding participants with chronic diseases at baseline or with self-reported body weight at follow-up (Table S4). After estimating polyphenols according to energy density, results were consistent and very similar to those obtained in model 4 (Table S5). Only statistically significant interactions are reported in Tables S6–S11. According to sex, 29 polyphenols showed significant interactions: the

majority (n = 19) showed larger negative beta values for men versus women (Table S6). For age as a categorical variable (<50 vs. ≥50 years), significant interactions were observed for 27 polyphenols. The majority (n = 16) showed lower beta values for younger participants (Table S7). According to BMI categories, 35 polyphenols showed statistically significant interactions.

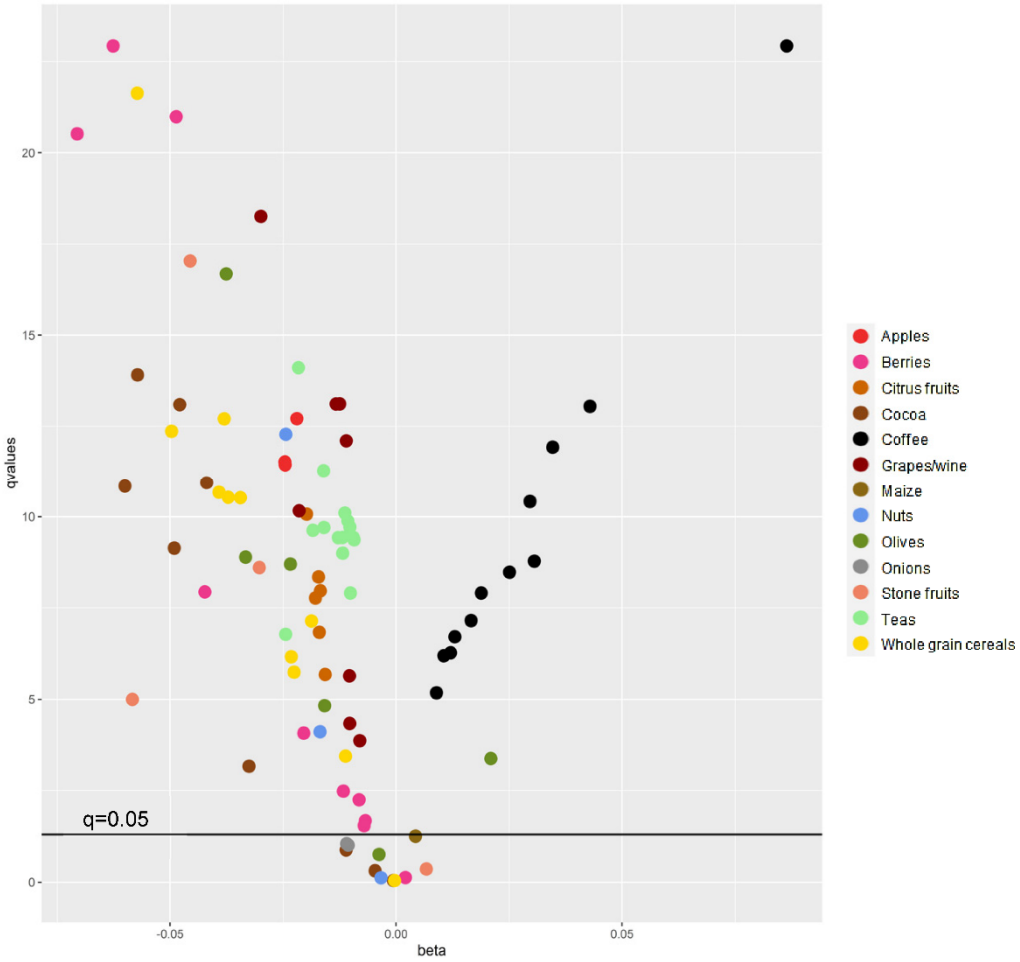


Figure 1. False discovery rate (FDR) q values and beta values (kg/5 year) of associations between 91 polyphenols and 5-year body weight change and their main food sources. Beta values correspond to a doubling in intake. q values (adjusted p value for FDR) are shown as -log10-transformed values to improve visualization. [1] Multilevel linear mixed model with random effects on the intercept according to EPIC centre adjusted for age, sex, body mass index (3-knot restricted cubic spline), follow-up time in years (3-knot restricted cubic spline), alcohol intake (g/d), education level, physical activity level, smoking status at follow-up, menopausal status, total energy intake (kcal/day), plausibility of dietary energy reporting, vitamin C intake (mg/d), and fibre intake (g/d) (Model 4).

The lowest beta values were observed for the categories normal and overweight, versus underweight or obesity (Table S8). Predominantly post-menopausal women had the biggest negative beta values when interactions were explored by menopausal status (Table S9). According to the category of smoking status at follow-up, the most noteworthy were those polyphenols classified as HCAs (4-caffeoylquinic acid, 3-feruloylquinic acid, 4-feruloylquinic acid, 5-feruloylquinic acid) which showed positive associations with body weight gain for non-smoking participants (Table S10). With regard to tertiles of fibre consumption, 30 polyphenols resulted in significant interactions. In general, higher intakes (tertiles 2 and 3) showed lower beta values than tertile 1 (Table S11). All interactions were also penalized for multiple testing using FDR.

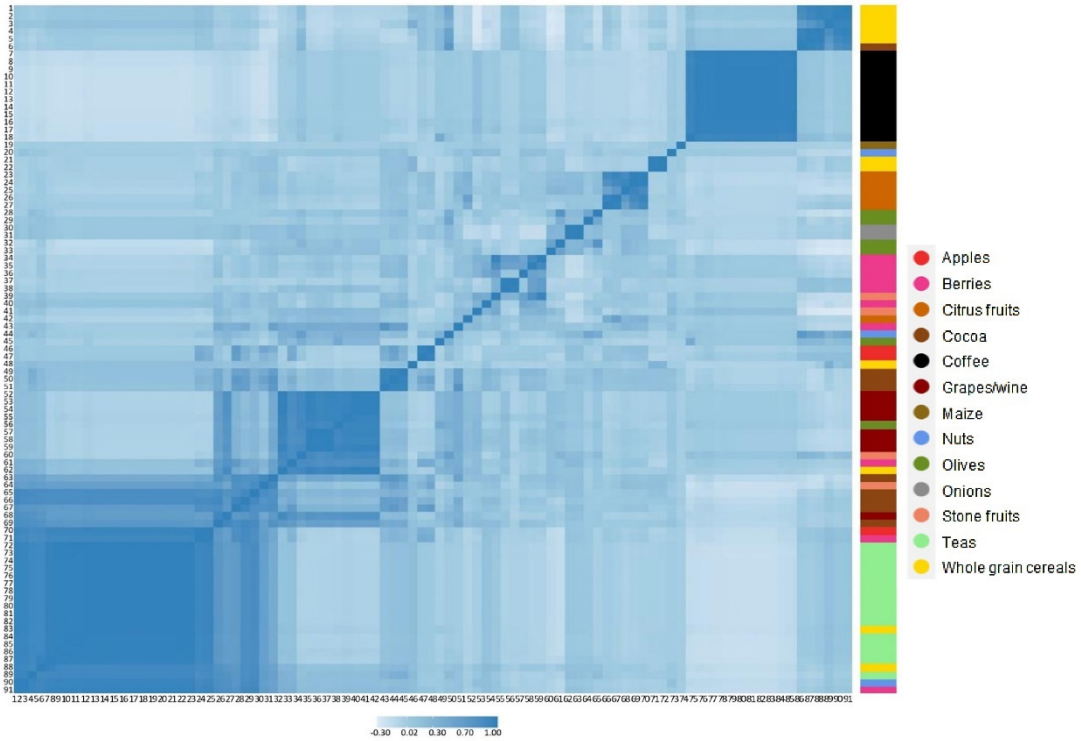


Figure 2. Pearson's correlation heat-map between the intakes of 91 polyphenols in the EPIC-PANACEA population. Numbers (1 to 91) represent individual polyphenols (Table S12). The right panel shows their main food sources.

4. Discussion

In this large prospective cohort, we found that of 51 individual polyphenols investigated, doubling in intake of 46 of these polyphenols was inversely associated with 5-year body weight change. Consumption of quercetin 3-O-rhamnoside, quercetin 3-O-glucoside, both included in the flavonol subclass, and (+)-catechin and procyanidin dimer B3, included in the flavanol subclass (all of them classified as flavonoids), showed the strongest inverse associations. Conversely, the intake of five polyphenols from the HCAs subclass (class phenolic acids) was positively associated with 5-year body weight gain. Investigating polyphenols individually enables the identification of how relatively similar compounds that belong to the same class, may behave differently in relation to body weight change. Unlike classes and subclasses, this approach can highlight some minor individual compounds that may not be the most abundant ones in the diet but may have a relevant association with body weight changes.

A pooled analysis of randomised-controlled trials (RCTs) showed that the current evidence on the effect of quercetin on body weight is inconclusive, and its effects on body weight have not yet been assessed as primary outcome for a large number of intervention studies [42]. As we observed that higher intakes of quercetin glycosides were inversely associated with body weight gain, it may be an interesting compound to study in future clinical trials on body weight. Our results also showed that (+)-catechin was correlated with (-)-epigallocatechin 3-O-gallate (EGCG), a flavanol widely present in tea. A meta-analysis of RCTs analysed the effects of green tea catechins supplementation on body weight [43] and found a positive effect on body weight loss and maintenance (average effect size = −1.31 kg; $p < 0.001$). A systematic review of RCTs showed that daily intakes of green tea, and therefore high doses of EGCG, presented beneficial results in 12-week weight loss interventions [13]. Likewise, an RCT showed that eight weeks of 44 mg daily of green tea catechins (~1 cup of tea) reduced body weight and increased energy expenditure and fat oxidation in participants with obesity [44]. A meta-analysis of RCTs concluded that supplementation with grape seed extract, mainly consisting of catechins,

epigallocatechins, and procyanidin dimers, demonstrated a significant improvement in obesity-related cardiometabolic biomarkers [45]. Grape seed extract is also rich in other polyphenols such as resveratrol. Even though we did not include it due to the intake cut-off point, several RCTs demonstrated that resveratrol intake reduces body weight, BMI, waist circumference and body fat [46]. It has been reported that dietary polyphenols have potential for acting on mitochondrial dysfunction and inflammation, as well as kidnapping free radicals, increasing the activity and expression of antioxidant enzymes and inhibiting ROS-producing ones [2]. In addition, polyphenols control adipocyte differentiation and lipid metabolism and oxidation through decrease in the activity of the pancreatic lipase and permeability of the intestine, and through their interaction with the gut microbiota [2]. Recent evidence suggests that gut microbiota-derived polyphenol metabolites may affect appetite control and body weight management [47], and can modulate the development of adipose tissue and the obesity-induced inflammatory genes [48]. However, as far as we know, several of the individual compounds included in this investigation are not yet studied neither by observational nor by interventional studies in humans assessing body weight changes, or evidence on their influence on body weight is still scarce.

Out of 51 individual polyphenols, five compounds classified as HCAs were positively associated with body weight gain: 4-caffeoylquinic acid, 3-feruloylquinic acid, 4-feruloylquinic acid, 5-feruloylquinic acid, and sinapic acid. Most of them have coffee as the main dietary source, except for sinapic acid (mainly from olives). For coffee non-consumers, there was little evidence that these polyphenols were associated with body weight change. As coffee is often part of the Western diet, a pattern rich in sugar and saturated fat [38] that has been associated with body weight gain and obesity [49], we performed an additional analysis adjusting for sugar, milk, confectionery, and cake intake. However, beta coefficients remained the same after this adjustment. In our previous study, we also analysed caffeinated and decaffeinated coffee as exposures, as caffeine is a dietary component that might be associated with body weight loss [50]. We observed higher values for decaffeinated (body weight gain for doubling in intake: 0.012 kg/5 year; 95% CI: 0.007, 0.016) versus caffeinated coffee (0.005; 95% CI: 0.002, 0.009) [11]. Beyond this, mechanistic evidence for these positive associations is lacking and further research is needed.

Despite having found significant interactions with different confounding variables, these results must be interpreted with caution. Differences between subgroups might be due to the large number of participants included in this study. Thus, a minimum change in beta values shows statistically significant values, even though, in general, categories of the same variable followed the same trend. Interactions have been discussed in more detail in our previous study regarding polyphenol classes and subclasses and body weight change in the EPIC-PANACEA cohort [11].

The prospective design of this investigation, its large sample of participants from different European countries, and its sufficient statistical power is a major strength. However, this study has some limitations. Assessing the relationship between diet and health outcomes is challenging in epidemiological studies, due to biases linked to dietary measurement errors [51]. To minimize them, we included validated dietary questionnaires and a standardized database to assess food and nutrient intakes [16,17], as well as the use of Phenol-Explorer database [18] to estimate polyphenol intakes. We had data on a large variety of variables related to diet and lifestyle, but most of them were only collected at baseline, and therefore changes during the follow-up were not accounted for. However, when possible, we took variables measured at follow-up, such as smoking status. As in every observational epidemiological study, residual confounding must be considered. Another limitation is that most of the centres used self-reported body weight at follow-up, a value which usually tends to be underestimated [52]. Average weight gain was higher in EPIC centres where follow-up weight was measured. Thus, Oxford-corrected body weight was used to improve the accuracy of these values. Moreover, after performing a sensitivity analysis excluding participants who self-reported body weight at follow-up, results followed the same trend as in the total population for the majority of polyphenols.

The large sample size of this study is both a strength and a limitation, as beta coefficients obtained should be interpreted with prudence: statistically significance is easy to obtain with such a large sample, even after FDR-correction. Still, a daily serving of polyphenol-rich foods can often provide a variety of individual polyphenols and higher quantities than the daily medians assessed in this investigation (e.g., 200 g of apple can provide ~3 mg of quercetin-3-O-rhamnoside [17], whereas its median intake in our population was ~1 mg/d).

5. Conclusions

Our results suggest that choosing polyphenol-rich foods, such as fruits, tea and whole grain cereals, may contribute to 5-year body weight maintenance (or less gain) in European populations. These results also suggest that individual HCAs may have different roles in body weight change depending on their dietary source. Our findings provide information about which individual polyphenols may be relevant for future mechanistic studies and interventional studies on body weight change.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11122425/s1, Figure S1: Flow chart of the study population; Figure S2: Flowchart of polyphenols selected and key compounds; Table S1: Correlated polyphenols according to Pearson Correlation Coefficient; Table S2: Change in 5-year body weight according to polyphenol intake in 349,165 participants from the EPIC-PANACEA cohort: comparison between models; Table S3: Associations between selected individual polyphenols and body weight change according to coffee consumption in the EPIC-PANACEA cohort; Table S4: Change in 5-year body weight according to polyphenol intake in participants without chronic diseases at baseline or with measured body weight at follow-up; Table S5: Change in 5-year body weight estimating polyphenols according to energy density in 349,165 participants; Table S6: Interactions by sex: change in 5-year body weight according to polyphenol intake in 349,165 participants; Table S7: Interactions by age: change in 5-year body weight according to polyphenol intake in 349,165 participants; Table S8: Interactions by BMI categories: change in 5-year body weight according to polyphenol intake in 349,165 participants; Table S9: Interactions by menopausal status: change in 5-year body weight according to polyphenol intake in 255,730 female participants; Table S10: Interactions by smoking status at follow-up: change in 5-year body weight according to polyphenol intake in 334,616 participants; Table S11: Interactions by tertiles of fibre consumption: change in 5-year body weight according to polyphenol intake in 349,165 participants; Table S12: Individual polyphenols represented in Figure 2.

Author Contributions: Conceptualization, R.Z.-R.; methodology, R.Z.-R., M.G.-L. and J.C.; validation, R.Z.-R.; formal analysis, M.G.-L.; investigation, resources and data curation, all EPIC co-authors; writing—original draft preparation, M.G.-L.; writing—review and editing, R.Z.-R., E.A.-A. and J.C.; visualization, M.G.-L.; supervision, project administration and funding acquisition, R.Z.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been funded by the Instituto De Salud Carlos III through the project PI18/00191 (Co-funded by European Regional Development Fund, ERDF, a way to build Europe). The PANACEA project was funded by the European Union, in the framework of the Public Health Programme (project number: 2005328). The coordination of EPIC is financially supported by International Agency for Research on Cancer (IARC) and also by the Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London which has additional infrastructure support provided by the NIHR Imperial Biomedical Research Centre (BRC). The national cohorts are supported by: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Federal Ministry of Education and Research (BMBF) (Germany); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy, Compagnia di San Paolo and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS)—Instituto de Salud Carlos III (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, and the Catalan Institute of Oncology—ICO (Spain); Swedish Cancer Society, Swedish Research Council and County Councils

of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C8221/A29017 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk; MR/M012190/1 to EPIC-Oxford). (United Kingdom). NGF acknowledges support from MRC Epidemiology Unit (MC_UU_0006/3) and from the NIHR Cambridge Biomedical Research Centre diet, nutrition and lifestyle theme (IS-BRC-1215-20014). She is an NIHR Senior Investigator. We thank the CERCA Program/Generalitat de Catalunya for the institutional support to IDIBELL. JC thanks the CONACYT predoctoral fellowship (ID 693636). MG-L, EA-A, and RZ-R were supported by the PFIS (FI19/00185), the "Sara Borrell" (CD20/00071), and the "Miguel Servet II" (CPII20/00009) programmes from the Instituto de Salud Carlos III (Co-funded by European Social Fund (ESF) investing in your future).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the International Agency for Research on Cancer (IARC) and local ethical committees pertaining to EPIC Centres (PR194/18).

Informed Consent Statement: Informed consent was obtained from all participants involved in the study.

Data Availability Statement: For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php (accessed on 1 December 2022).

Acknowledgments: We thank all EPIC participants and staff for their contribution to the study. We also thank Bertrand Hémon and Catalina Bonet for their valuable help with the EPIC database. We also thank the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; the University Paris-Saclay, University of Versailles Saint-Quentin-en-Yvelines (UVSQ), Institut national de la santé et de la recherche médicale (INSERM), Gustave Roussy, Centre de Recherche en Epidémiologie et Santé des Populations (CESP), Villejuif, France; and the Oxford University (UK) for their contribution and ongoing support to the EPIC Study.

Conflicts of Interest: The authors declare no conflict of interest. IARC DISCLAIMER: Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

References

1. Wang, S.; Moustaid-Moussa, N.; Chen, L.; Mo, H.; Shastri, A.; Su, R.; Bapat, P.; Kwun, I.; Shen, C.-L. Novel Insights of Dietary Polyphenols and Obesity. *J. Nutr. Biochem.* **2014**, *25*, 1–18. [CrossRef] [PubMed]
2. Pérez-Torres, I.; Castrejón-Téllez, V.; Soto, M.E.; Rubio-Ruiz, M.E.; Manzano-Pech, L.; Guarner-Lans, V. Oxidative Stress, Plant Natural Antioxidants, and Obesity. *Int. J. Mol. Sci.* **2021**, *22*, 1786. [CrossRef] [PubMed]
3. Zielinska-Blizniewska, H.; Sitarek, P.; Merecz-Sadowska, A.; Malinowska, K.; Zajdel, K.; Jablonska, M.; Sliwinski, T.; Zajdel, R. Plant Extracts and Reactive Oxygen Species as Two Counteracting Agents with Anti- and Pro-Obesity Properties. *Int. J. Mol. Sci.* **2019**, *20*, 4556. [CrossRef] [PubMed]
4. Zamora-Ros, R.; Touillaud, M.; Rothwell, J.A.; Romieu, I.; Scalbert, A. Measuring Exposure to the Polyphenol Metabolome in Observational Epidemiologic Studies: Current Tools and Applications and Their Limits. *Am. J. Clin. Nutr.* **2014**, *100*, 11–26. [CrossRef]
5. Guasch-Ferré, M.; Merino, J.; Sun, Q.; Fitó, M.; Salas-Salvadó, J. Dietary Polyphenols, Mediterranean Diet, Prediabetes, and Type 2 Diabetes: A Narrative Review of the Evidence. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 6723931. [CrossRef]
6. Di Lorenzo, C.; Colombo, F.; Biella, S.; Stockley, C.; Restani, P. Polyphenols and Human Health: The Role of Bioavailability. *Nutrients* **2021**, *13*, 273. [CrossRef]
7. Castro-Barquero, S.; Lamuela-Raventós, R.M.; Doménech, M.; Estruch, R. Relationship between Mediterranean Dietary Polyphenol Intake and Obesity. *Nutrients* **2018**, *10*, 1523. [CrossRef]
8. Hughes, L.A.; Arts, I.C.; Ambergen, T.; Brants, H.A.; Dagnelie, P.C.; Goldbohm, R.A.; van den Brandt, P.A.; Weijenberg, M.P. Higher Dietary Flavone, Flavonol, and Catechin Intakes Are Associated with Less of an Increase in BMI over Time in Women: A Longitudinal Analysis from the Netherlands Cohort Study. *Am. J. Clin. Nutr.* **2008**, *88*, 1341–1352. [CrossRef]
9. Marranzano, M.; Ray, S.; Godos, J.; Galvano, F. Association between Dietary Flavonoids Intake and Obesity in a Cohort of Adults Living in the Mediterranean Area. *Int. J. Food Sci. Nutr.* **2018**, *69*, 1020–1029. [CrossRef]
10. Adriouch, S.; Kesse-Guyot, E.; Feuillet, T.; Touvier, M.; Olié, V.; Andreeva, V.; Hercberg, S.; Galan, P.; Fezeu, L.K. Total and Specific Dietary Polyphenol Intakes and 6-Year Anthropometric Changes in a Middle-Aged General Population Cohort. *Int. J. Obes.* **2018**, *42*, 310–317. [CrossRef]

11. Castañeda, J.; Gil-Lespinard, M.; Almanza-Aguilera, E.; Llaha, F.; Gómez, J.-H.; Bondonno, N.; Tjønneland, A.; Overvad, K.; Katzke, V.; Schulze, M.B.; et al. Association between Classes and Subclasses of Polyphenol Intake and 5-Year Body Weight Changes in the EPIC-PANACEA Study. *Obesity*, 2023; *in press*.
12. Zhao, Y.; Zhang, X. Interactions of Tea Polyphenols with Intestinal Microbiota and Their Implication for Anti-Obesity. *J. Sci. Food Agric.* **2020**, *100*, 897–903. [CrossRef] [PubMed]
13. Vázquez Cisneros, L.C.; López-Uriarte, P.; López-Espinoza, A.; Navarro Meza, M.; Espinoza-Gallardo, A.C.; Guzmán Aburto, M.B. Effects of Green Tea and Its Epigallocatechin (EGCG) Content on Body Weight and Fat Mass in Humans: A Systematic Review. *Nutr. Hosp.* **2017**, *34*, 731–737. [CrossRef]
14. Riboli, E.; Hunt, K.; Slimani, N.; Ferrari, P.; Norat, T.; Fahey, M.; Charrondière, U.; Hémon, B.; Casagrande, C.; Vignat, J.; et al. European Prospective Investigation into Cancer and Nutrition (EPIC): Study Populations and Data Collection. *Public Health Nutr.* **2002**, *5*, 1113–1124. [CrossRef] [PubMed]
15. Freisling, H.; Noh, H.; Slimani, N.; Chajès, V.; May, A.M.; Peeters, P.H.; Weiderpass, E.; Cross, A.J.; Skeie, G.; Jenab, M.; et al. Nut Intake and 5-Year Changes in Body Weight and Obesity Risk in Adults: Results from the EPIC-PANACEA Study. *Eur. J. Nutr.* **2018**, *57*, 2399–2408. [CrossRef] [PubMed]
16. Margetts, B.M.; Pietinen, P. European Prospective Investigation into Cancer and Nutrition: Validity Studies on Dietary Assessment Methods. *Int. J. Epidemiol.* **1997**, *26*. [CrossRef]
17. Slimani, N.; Deharveng, G.; Unwin, I.; Southgate, D.A.T.; Vignat, J.; Skeie, G.; Salvini, S.; Parpinel, M.; Møller, A.; Ireland, J.; et al. The EPIC Nutrient Database Project (ENDB): A First Attempt to Standardize Nutrient Databases across the 10 European Countries Participating in the EPIC Study. *Eur. J. Clin. Nutr.* **2007**, *61*, 1037–1056. [CrossRef]
18. Neveu, V.; Perez-Jiménez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D.; et al. Phenol-Explorer: An Online Comprehensive Database on Polyphenol Contents in Foods. *Database* **2010**, *2010*, bap024. [CrossRef]
19. Knaze, V.; Rothwell, J.A.; Zamora-Ros, R.; Moskal, A.; Kyrø, C.; Jakszyn, P.; Skeie, G.; Weiderpass, E.; Santucci de Magistris, M.; Agnoli, C.; et al. A New Food-Composition Database for 437 Polyphenols in 19,899 Raw and Prepared Foods Used to Estimate Polyphenol Intakes in Adults from 10 European Countries. *Am. J. Clin. Nutr.* **2018**, *108*, 517–524. [CrossRef]
20. Spencer, E.A.; Appleby, P.N.; Davey, G.K.; Key, T.J. Validity of Self-Reported Height and Weight in 4808 EPIC–Oxford Participants. *Public Health Nutr.* **2002**, *5*, 561–565. [CrossRef]
21. Vergnaud, A.C.; Norat, T.; Mouw, T.; Romaguera, D.; May, A.M.; Bueno-de-Mesquita, H.B.; van der, A.D.; Agudo, A.; Wareham, N.; Khaw, K.T.; et al. Macronutrient Composition of the Diet and Prospective Weight Change in Participants of the EPIC-PANACEA Study. *PLoS ONE* **2013**, *8*, e57300. [CrossRef]
22. Peters, T.; Brage, S.; Westgate, K.; Franks, P.W.; Gradmark, A.; Diaz, M.J.T.; Huerta, J.M.; Bendinelli, B.; Vigl, M.; Boeing, H.; et al. Validity of a Short Questionnaire to Assess Physical Activity in 10 European Countries. *Eur. J. Epidemiol.* **2012**, *27*, 15–25. [CrossRef] [PubMed]
23. Wareham, N.J.; Jakes, R.W.; Rennie, K.L.; Schuit, J.; Mitchell, J.; Hennings, S.; Day, N.E. Validity and Repeatability of a Simple Index Derived from the Short Physical Activity Questionnaire Used in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *Public Health Nutr.* **2003**, *6*, 407–413. [CrossRef] [PubMed]
24. Zamora-Ros, R.; Knaze, V.; Rothwell, J.A.; Hémon, B.; Moskal, A.; Overvad, K.; Tjønneland, A.; Kyrø, C.; Fagherazzi, G.; Boutron-Ruault, M.C.; et al. Dietary Polyphenol Intake in Europe: The European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *Eur. J. Nutr.* **2016**, *55*, 1359–1375. [CrossRef] [PubMed]
25. Harrell, F.E. *Regression Modelling Strategies: With Applications to Linear Models, Logistic Regression and Survival Analysis*, 1st ed.; Springer: New York, NY, USA, 2001; pp. 13–102.
26. Mozaffarian, D.; Hao, T.; Rimm, E.B.; Willett, W.C.; Hu, F.B. Changes in Diet and Lifestyle and Long-Term Weight Gain in Women and Men. *N. Engl. J. Med.* **2011**, *364*, 2392–2404. [CrossRef] [PubMed]
27. Chiriboga, D.E.; Ma, Y.; Li, W.; Olendzki, B.C.; Pagoto, S.L.; Merriam, P.A.; Matthews, C.E.; Hebert, J.R.; Ockene, I.S. Gender Differences in Predictors of Body Weight and Body Weight Change in Healthy Adults. *Obesity* **2008**, *16*, 137–145. [CrossRef]
28. Ambikairajah, A.; Walsh, E.; Tabatabaei-Jafari, H.; Cherbuin, N. Fat Mass Changes during Menopause: A Metaanalysis. *Am. J. Obstet. Gynecol.* **2019**, *221*, 393–409.e50. [CrossRef]
29. Cohen, A.K.; Rai, M.; Rehkopf, D.H.; Abrams, B. Educational Attainment and Obesity: A Systematic Review. *Obes. Rev.* **2013**, *14*, 989–1005. [CrossRef]
30. Slavin, J.L. Dietary Fiber and Body Weight. *Nutrition* **2005**, *21*, 411–418. [CrossRef]
31. Goldberg, G.R.; Black, A.E.; Jebb, S.A.; Cole, T.J.; Murgatroyd, P.R.; Coward, W.A.; Prentice, A.M. Critical Evaluation of Energy Intake Data Using Fundamental Principles of Energy Physiology: Derivation of Cut-off Limits to Identify under-Recording. *Eur. J. Clin. Nutr.* **1991**, *45*, 569–581.
32. Vinke, P.C.; Navis, G.; Kromhout, D.; Corpeleijn, E. Age- and Sex-Specific Analyses of Diet Quality and 4-Year Weight Change in Nonobese Adults Show Stronger Associations in Young Adulthood. *J. Nutr.* **2020**, *150*, 560–567. [CrossRef]
33. Kabat, G.C.; Heo, M.; Allison, M.; Johnson, K.C.; Ho, G.Y.F.; Tindle, H.A.; Asao, K.; LaMonte, M.J.; Giovino, G.A.; Rohan, T.E. Smoking Habits and Body Weight Over the Adult Lifespan in Postmenopausal Women. *Am. J. Prev. Med.* **2017**, *52*, e77–e84. [CrossRef]
34. Dmitruk, A.; Czeczelewski, J.; Czeczelewska, E.; Golach, J.; Parnicka, U. Body Composition and Fatty Tissue Distribution in Women with Various Menstrual Status. *Rocz. Panstw. Zakl. Hig.* **2018**, *69*, 95–101.

35. Van Baak, M.A.; Mariman, E.C.M. Dietary Strategies for Weight Loss Maintenance. *Nutrients* **2019**, *11*, 1916. [CrossRef] [PubMed]
36. Drake, I.; Sonestedt, E.; Ericson, U.; Wallström, P.; Orho-Melander, M. A Western Dietary Pattern Is Prospectively Associated with Cardio-Metabolic Traits and Incidence of the Metabolic Syndrome. *Br. J. Nutr.* **2018**, *119*, 1168–1176. [CrossRef] [PubMed]
37. García-Montero, C.; Fraile-Martínez, O.; Gómez-Lahoz, A.M.; Pekarek, L.; Castellanos, A.J.; Noguerales-Fraguas, F.; Coca, S.; Guijarro, L.G.; García-Honduvilla, N.; Asúnsolo, A.; et al. Nutritional Components in Western Diet Versus Mediterranean Diet at the Gut Microbiota-Immune System Interplay. Implications for Health and Disease. *Nutrients* **2021**, *13*, 699. [CrossRef] [PubMed]
38. Van Dam, R.M.; Rimm, E.B.; Willett, W.C.; Stampfer, M.J.; Hu, F.B. Dietary Patterns and Risk for Type 2 Diabetes Mellitus in U.S. Men. *Ann. Intern. Med.* **2002**, *136*, 201–209. [CrossRef]
39. Poti, J.M.; Braga, B.; Qin, B. Ultra-Processed Food Intake and Obesity: What Really Matters for Health—Processing or Nutrient Content? *Curr. Obes. Rep.* **2017**, *6*, 420. [CrossRef]
40. R: A Language and Environment for Statistical Computing. Available online: http://www.r-project.org/index.html (accessed on 31 January 2022).
41. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. LmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **2017**, *82*, 1–26. [CrossRef]
42. Huang, H.; Liao, D.; Dong, Y.; Pu, R. Clinical Effectiveness of Quercetin Supplementation in the Management of Weight Loss: A Pooled Analysis of Randomized Controlled Trials. *Diabetes Metab. Syndr. Obes. Targets Ther.* **2019**, *12*, 553–563. [CrossRef]
43. Hursel, R.; Viechtbauer, W.; Westerterp-Plantenga, M.S. The Effects of Green Tea on Weight Loss and Weight Maintenance: A Meta-Analysis. *Int. J. Obes.* **2009**, *33*, 956–961. [CrossRef]
44. Auvichayapat, P.; Prapochanung, M.; Tunkamnerdthai, O.; Sripanidkulchai, B.O.; Auvichayapat, N.; Thinkhamrop, B.; Kunhasura, S.; Wongpratoom, S.; Sinawat, S.; Hongprapas, P. Effectiveness of Green Tea on Weight Reduction in Obese Thais: A Randomized, Controlled Trial. *Physiol. Behav.* **2008**, *93*, 486–491. [CrossRef] [PubMed]
45. Asbaghi, O.; Nazarian, B.; Reiner, Ž.; Amirani, E.; Kolahdooz, F.; Chamani, M.; Asemi, Z. The Effects of Grape Seed Extract on Glycemic Control, Serum Lipoproteins, Inflammation, and Body Weight: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Phyther. Res.* **2020**, *34*, 239–253. [CrossRef]
46. Tabrizi, R.; Tamtaji, O.R.; Lankarani, K.B.; Mirhosseini, N.; Akbari, M.; Dadgostar, E.; Peymani, P.; Asemi, Z. The Effects of Resveratrol Supplementation on Biomarkers of Inflammation and Oxidative Stress among Patients with Metabolic Syndrome and Related Disorders: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Food Funct.* **2018**, *9*, 6116–6128. [CrossRef] [PubMed]
47. Corrêa, T.A.F.; Rogero, M.M.; Hassimotto, N.M.A.; Lajolo, F.M. The Two-Way Polyphenols-Microbiota Interactions and Their Effects on Obesity and Related Metabolic Diseases. *Front. Nutr.* **2019**, *6*, 188. [CrossRef]
48. Corrêa, T.A.; Rogero, M.M. Polyphenols Regulating MicroRNAs and Inflammation Biomarkers in Obesity. *Nutrition* **2019**, *59*, 150–157. [CrossRef]
49. Rakhra, V.; Galappaththy, S.L.; Bulchandani, S.; Cabandugama, P.K. Obesity and the Western Diet: How We Got Here. *Mo. Med.* **2020**, *117*, 536–538. [PubMed]
50. Tabrizi, R.; Saneei, P.; Lankarani, K.B.; Akbari, M.; Kolahdooz, F.; Esmaillzadeh, A.; Nadi-Ravandi, S.; Mazoochi, M.; Asemi, Z. The Effects of Caffeine Intake on Weight Loss: A Systematic Review and Dos-Response Meta-Analysis of Randomized Controlled Trials. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 2688–2696. [CrossRef]
51. Kipnis, V.; Midthune, D.; Freedman, L.; Bingham, S.; Day, N.E.; Riboli, E.; Ferrari, P.; Carroll, R.J. Bias in Dietary-Report Instruments and Its Implications for Nutritional Epidemiology. *Public Health Nutr.* **2002**, *5*, 915–923. [CrossRef]
52. Gorber, S.C.; Tremblay, M.; Moher, D.; Gorber, B. A Comparison of Direct vs. Self-Report Measures for Assessing Height, Weight and Body Mass Index: A Systematic Review. *Obes. Rev.* **2007**, *8*, 307–326. [CrossRef]

4.2. Manuscript 3: Plasma concentration of 36 (poly)phenols and prospective body weight change in participants from the EPIC cohort.

Gil-Lespinard M, Almanza-Aguilera E, Castañeda J, et al. Plasma concentration of 36 (poly)phenols and prospective body weight change in participants from the EPIC cohort. Ann Nutr Metab. Published online January 25, 2024. doi:10.1159/000535803

Impact Factor: 3.9

Category: Endocrinology & Metabolism. Q2 64/145

Category: Nutrition and Dietetics. Q2 42/88

This work has been presented as communications at:

- The XL Reunión Anual de la Sociedad Española de Epidemiología (SEE), held from August 30 to September 2, 2022, at Donostia-San Sebastián, Spain, in the form of oral communication.
- The International Conference of Diet and Activity Methods 2023, held from 26-29 June 2023, at the University of Limerick, Ireland, in the form of poster.
- The 8th Edition IDIBELL PhD Day, held on November 10, 2023, at the Bellvitge Biomedical Research Institute in L'Hospitalet de Llobregat, Spain, in the form of oral communication as part of the talk ‘Impact of (Poly)phenols on Body Weight and Adiposity: Insights from Two European Cohorts’. Awarded 2nd Best Oral Communication.

Annals of Nutrition and Metabolism

Human Nutrition: Research Article

Ann Nutr Metab
DOI: 10.1159/000535803

Received: May 5, 2023
Accepted: November 25, 2023
Published online: January 25, 2024

Plasma Concentration of 36 (Poly)phenols and Prospective Body Weight Change in Participants from the EPIC Cohort

Mercedes Gil-Lespinard[a] Enrique Almanza-Aguilera[a] Jazmín Castañeda[a] Daniel Guiñón-Fort[a] Anne Kirstine Eriksen[b] Anne Tjønneland[b, c] Joseph A. Rothwell[d] Sanam Shah[d] Claire Cadeau[d] Verena Katzke[e] Theron Johnson[e] Matthias B. Schulze[f, g] Andreina Oliverio[h] Fabrizio Pasanisi[i] Rosario Tumino[j] Luca Manfredi[k] Giovana Masala[l] Guri Skeie[m] Marie Wasmuth Lundblad[m] Magritt Brustad[m, n] Cristina Lasheras[o] Marta Crous-Bou[a] Esther Molina-Montes[p, q, r, s] Sandra Colorado-Yohar[q, s, t, u] Marcela Guevara[q, v, w] Pilar Amiano[q, x, y] Ingegerd Johansson[z] Johan Hultdin[A] Nita G. Forouhi[B] Heinz Freisling[C] Mira Merdas[C] Charlotte Debras[C] Alicia K. Heath[D] Elom K. Aglago[D] Dagfinn Aune[D, E, F] Raul Zamora-Ros[a, G]

[a]Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain; [b]Danish Cancer Society Research Center, Copenhagen, Denmark; [c]Department of Public Health, University of Copenhagen, Copenhagen, Denmark; [d]Centre for Epidemiology and Population Health (CESP) U1018, French National Institute of Health and Medical Research (Inserm) "Exposome, Heredity, Cancer and Health" Team, University of Paris-Saclay, Versailles Saint-Quentin-en-Yvelines University (UVSQ), Villejuif, France; [e]Department of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [f]Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; [g]Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany; [h]Department of Epidemiology and Data Science, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy; [i]Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy; [j]Hyblean Association for Epidemiological Research (AIRE-ONLUS), Ragusa, Italy; [k]Centre for Biostatistics, Epidemiology, and Public Health (C-BEPH), Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy; [l]Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Florence, Italy; [m]Department of Community Medicine, UIT The Arctic University of Norway, Tromsø, Norway; [n]The Public Dental Health Service Competence Centre of Northern Norway, Tromsø, Norway; [o]Functional Biology Department, University of Oviedo, Oviedo, Spain; [p]Department of Nutrition and Food Science, Campus of Cartuja, University of Granada, Granada, Spain; [q]CIBER of Epidemiology and Public Health (CIBERESP), Madrid, Spain; [r]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [s]Biomedical Research Centre, Institute of Nutrition and Food Technology (INYTA) "José Mataix", University of Granada, Granada, Spain; [t]Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain; [u]Research Group on Demography and Health, National Faculty of Public Health, University of Antioquia, Medellin, Colombia; [v]Navarra Public Health Institute, Pamplona, Spain; [w]Navarra Institute for Health Research (IdiSNA), Pamplona, Spain; [x]Ministry of Health of the Basque Government, Sub-Directorate for Public Health and Addictions of Gipuzkoa, San Sebastian, Spain; [y]Public Health Division of Gipuzkoa, BioDonostia Research Institute, San Sebastian, Spain;

karger@karger.com
www.karger.com/anm

Karger
OPEN ACCESS

Correspondence to:
Raul Zamora-Ros, rzamora@ub.edu

[z]Department of Odontology, Umeå University, Umeå, Sweden; [A]Department of Medical Biosciences, Umeå University, Umeå, Sweden; [B]MRC Epidemiology Unit, Institute of Metabolic Science, Cambridge Biomedical Campus, University of Cambridge School of Clinical Medicine, Cambridge, UK; [C]Nutrition and Epidemiology Branch, International Agency for Research on Cancer (IARC-WHO), Lyon, France; [D]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK; [E]Department of Nutrition, Oslo New University College, Oslo, Norway; [F]Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital Ulleval, Oslo, Norway; [G]Department of Nutrition, Food Sciences and Gastronomy, Nutrition and Food Safety Research Institute (INSA), Food Innovation Network (XIA), Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain

Keywords
Plasma (poly)phenol · Body weight · Nutritional biomarker · Cohort · EPIC

Abstract

Introduction: Dietary intake of (poly)phenols has been linked to reduced adiposity and body weight (BW) in several epidemiological studies. However, epidemiological evidence on (poly)phenol biomarkers, particularly plasma concentrations, is scarce. We aimed to investigate the associations between plasma (poly)phenols and prospective BW change in participants from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. ***Methods:*** This study included 761 participants with data on BW at baseline and after 5 years of follow-up. Plasma concentrations of 36 (poly)phenols were measured at baseline using liquid chromatography-tandem mass spectrometry. Associations were assessed through general linear mixed models and multinomial logistic regression models, using change in BW as a continuous or as a categorical variable (BW loss, maintenance, gain), respectively. Plasma (poly)phenols were assessed as log2-transformed continuous variables. The false discovery rate (FDR) was used to control for multiple comparisons. ***Results:*** Doubling plasma (poly)phenol concentrations showed a borderline trend towards a positive association with BW loss. Plasma vanillic acid showed the strongest association (−0.53 kg/5 years; 95% confidence interval [CI]: −0.99, −0.07). Similar results were observed for plasma naringenin comparing BW loss versus BW maintenance (odds ratio: 1.1; 95% CI: 1.0, 1.2). These results did not remain significant after FDR correction. ***Conclusion:*** Higher concentrations of plasma (poly)phenols suggested a tendency towards 5-year BW maintenance or loss. While certain associations seemed promising, they did not withstand FDR correction, indicating the need for caution in interpreting these results. Further studies using (poly)phenol biomarkers are needed to confirm these suggestive protective trends.

Introduction

Phenols and polyphenols, referred to as (poly)phenols hereafter, are secondary metabolites from plants with bioactive properties [1]. More than 500 individual (poly)phenols have been identified from different plant foods in the human diet, with flavonoids and phenolic acids representing the major classes [1]. Fruits, vegetables, wholegrain cereals, olive oil, cocoa, tea, and coffee are some of the main dietary sources of (poly)phenols [2].

Several epidemiological studies estimating intakes through dietary questionnaires or urine biomarkers have reported associations between dietary (poly)phenols and body weight (BW) change [3–6]. A French cohort study observed an association between higher intake of different (poly)phenols and a smaller increase in body mass index (BMI) and waist circumference [3]. An Italian cohort study concluded that higher flavonoid intake was associated with a lower BW [4]. Despite not assessing specifically anthropometric outcomes, three recent cohort studies have reported data on dietary (poly)phenols and central obesity as marker of metabolic syndrome (MetS). In a Danish cohort, dietary intake of total (poly)phenols, flavonoids, and phenolic acid was associated with lower odds of MetS, defined as the presence of at least three markers including high WC, high plasma triglycerides or HDL-cholesterol, high systolic or diastolic blood pressure, and/or high HbA1c [7]. Likewise, higher flavonoid consumption in Chinese adults [8] and dietary intake of the subclass flavonol in a Polish cohort [9] were found to be potentially protective against MetS, probably mainly impacting central obesity.

We recently investigated the associations between dietary intake of (poly)phenols (i.e., total, classes, subclasses, and individually) and 5-year BW change in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort [5, 6]. In general, intakes of most of the individual (poly)phenols and total flavonoids were inversely associated with 5-year BW change, except for (poly)phenols from coffee (i.e., hydroxycinnamic acids).

Our motivation to investigate the hypothesis of an inverse association between plasma (poly)phenols and BW gain is multifaceted. Previous research has suggested that dietary (poly)phenols may possess anti-obesity properties [3–6, 8, 9], but reliance on self-reported dietary data can introduce bias [10]. To mitigate this and obtain a more objective measure of dietary intake, we turned to plasma biomarkers. These nutritional biomarkers offer a reliable quantification of (poly)phenol exposure, allowing for a more accurate assessment of their impact on BW changes. Notably, limited research has explored the relationship between plasma (poly)phenols and obesity markers, underscoring the need for a more comprehensive investigation. Utilizing data from approximately 800 participants with information on 36 well-characterized plasma (poly)phenols and BW change, our study aimed to elucidate the potential role of these (poly)phenol biomarkers in BW management.

Methods

Population and Data Collection

The EPIC study is a multicentre prospective cohort that includes over half a million participants from 10 Western European countries [11]. It primarily aimed to evaluate the association between dietary and lifestyle factors and the risk of cancer and cancer-related diseases. Details on recruitment and study design have been published previously [11]. For the present study, a convenient sample of cancer-free controls was selected from two EPIC-nested case-control studies (colon and thyroid cancer studies) (n = 1,321) [12, 13]. Participants with no plasma (poly)phenol measurement (n = 430) and with no follow-up BW were excluded (n = 73). Data from Greece were not available at the time of this study, and therefore, Greek participants were excluded (n = 57). The final sample included 761 participants (online suppl. Fig. S1; for all online suppl. material, see https://doi.org/10.1159/000535803).

The majority of lifestyle factors were collected at recruitment through standardized questionnaires, and medical history was self-reported at baseline [11]. For this study, we included data on baseline age, sex, physical activity, education level, menopausal status, and alcohol consumption. We also included data on changes in smoking status at follow-up. Dietary data were collected at baseline through validated country-specific dietary questionnaires [14]. To estimate nutrient and total energy intake, the standardized EPIC Nutrient Database was used [15]. Baseline BW and height were collected by trained staff using standardized methods, except for centres from in France, Oxford, and Norway, where they were self-reported [11]. At follow-up, BW was self-reported in all centres, with the exception of Doetinchem and Cambridge, where it was measured [16]. The accuracy of self-reported BW was improved with prediction equations derived from EPIC-Oxford participants, in which BW at baseline was both measured and self-reported [17]. To calculate the 5-year BW change, baseline BW was subtracted from follow-up BW for each participant. The result was then divided by the number of follow-up years to obtain the annual BW change and multiplied by 5 to finally obtain the BW change over 5 years.

Plasma (poly)phenol measurements were available to participants from 19 EPIC centres (from Denmark, Germany, Italy, Norway, Spain, Sweden, the Netherlands, and the UK). Blood samples were collected at baseline following standardized procedures [18]. Plasma concentrations of (poly)phenols (nmol/L) were measured by a highly sensitive method based on differential isotope labelling with 12 and 13 C-dansyl chloride and an ultra-performance liquid chromatography-tandem mass spectrometry system (UPLC-MS/MS) with prior enzymatic hydrolysis. This method allowed the quantification with high accuracy and reproducibility of a large selection of compounds representative of the main classes of dietary (poly)phenols in low volumes of plasma [18]. Limits of quantification (LOQ) for the individual (poly)phenols varied between 0.11 nmol/L for apigenin and 44.4 nmol/L for quercetin. Intra-batch coefficients of variability (CV) varied between 2.3 and 9.0%, and inter-batch CV was <20% for the majority of (poly)phenols, except for quercetin (23.4%) and enterodiol (20.3%). Details about the laboratory methodology have been published elsewhere [18].

Statistical Analysis

Baseline characteristics were calculated according to categories of BW change over 5 years. BW change was categorized into three groups: loss (<−1.5 kg/5 years), maintenance (from −1.5 to 1.5 kg/5 years), and gain (>1.5 kg/5 years). Data on 36 plasma (poly)phenols (nmol/L) spanning the majority of compounds found in the human diet were available for this study and were analysed as continuous variables. Right-skewed distributions were standardized using log2-transformations with zero values imputed with half the LOQ for each (poly)phenol. Therefore, results were expressed as doubling plasma (poly)phenol concentrations. Spearman rank coefficients were calculated to assess correlations between individual plasma (poly)phenol concentrations. Correlations were also evaluated between plasma (poly)phenol levels and the intake of the following 12 (poly)phenol-rich food groups: potatoes and other tubers, vegetables, legumes, cereals, fats and oils, condiments, fruits, nuts and seeds, olives, coffee, tea, and herbal teas.

Two general linear mixed models (GLMM) were fitted to evaluate the association between plasma (poly)phenols (nmol/L) and continuous BW change (kg/5 years). In addition, we fitted multinomial logistic regression models using BW change over 5 years as a categorical variable with three categories: BW maintenance (from −1.5 to 1.5 kg/5 years) as the reference category, BW loss (<−1.5 kg/5 years), and BW gain (>1.5 kg/5 years).

For model adjustment, we selected variables a priori [19–23]: age (continuous, years), sex, baseline BMI (continuous, kg/m^2), follow-up years (continuous), energy intake (continuous, kcal/day), fibre intake (continuous, g/day), vitamin C intake (continuous, mg/day),

physical activity using the Cambridge index [24] (categories: inactive, moderately inactive, moderately active, active), menopausal status (categories: pre, post and peri-menopausal), change in smoking status at follow-up (categories: stable never smoker, stable current smoker, starter, quitter, former at follow-up), laboratory batch, plausibility of energy intake reporting (categories according to the ratio of reported energy intake (EI) to predicted basal metabolic rate (BMR): under (EI:BMR <1.14), plausible (1.14–2.1) and over reporters (>2.1), with the use of cut-off points proposed by Goldberg et al. [25]), education level (categories: none, primary, technical, secondary, longer), alcohol consumption (continuous, g/day), EPIC centres, and type of cancer project (thyroid/colon). Some of these variables were excluded after stepwise regression screening (i.e., menopausal status, education level, and alcohol consumption). Participants with missing values in categorical adjustment variables were placed in a separate category, while continuous adjustment variables presented no missing values. Aikaike Information Criterion was used to compare and select the best-fitting models. Restricted cubic splines were used to evaluate the non-linearity of the associations for continuous covariates. BMI, follow-up years, and energy intake showed a non-linear relationship with BW change. Thus, splines with 3 knots (percentile 10, 50, and 90) were included as covariates for these three variables. Knot positions were determined using the Harrell criteria [26].

For GLMM, in Model 1, age, sex, and baseline BMI were used as covariates. EPIC centres and type of cancer project (thyroid or colon cancer control groups) were included as random effects to control for potential confounding due to differences in follow-up procedures, questionnaire design, and blood sample collection and analysis. Model 2 was additionally adjusted for years of follow-up, physical activity level, change in smoking status at follow-up, laboratory batch, energy intake, and plausibility of energy intake reporting. To account for certain dietary variables that may be related to both exposure and outcome, we fitted a third model, adding intake of fibre (g/day) and intake of vitamin C (mg/day) as further confounding variables. A sensitivity analysis was performed for each model to exclude participants with chronic diseases at baseline (diabetes, stroke, or myocardial infarction, $n = 92$, leaving $n = 669$ included). We further fitted interactions between (poly)phenol concentrations and sex, baseline age, and smoking status at follow-up [27, 28]. p values for interactions were calculated using the likelihood ratio test. The adjustment variables for multinomial logistic regression models were the same as the GLMMs, except for EPIC centres and type of cancer project that was included here as fixed effects.

For all models, p values were adjusted by computing the false discovery rate (FDR) to control for multiple comparisons and considered statistically significant at an FDR q value <0.05. All statistical analyses were performed with *R* Statistical Software version 4.2.2 [29].

Results

Descriptive Data Analysis

Table 1 shows characteristics of the 761 participants included in this study according to categories of BW change over 5 years. Overall, mean (SD) 5-year BW change was 0.6 (4.5) kg and ranged from − 18.2 to 21.8 kg. A total of 293 participants (38.6%) gained >1.5 kg of weight over 5 years, whereas 194 (25.5%) lost >1.5 kg/5 years. Baseline BW and BMI were higher in participants in the BW loss category. More than 50% of participants in the BW loss category had lower levels of education, whereas more than 50% of participants in the BW gain category presented higher educational levels. According to change in smoking status at follow-up, a high proportion of participants were classified as never smokers (48%), followed by former smokers (including never at baseline and former at follow-up, 23%). The majority of participants were females (77%), as the thyroid cancer control group included only female participants and represented 40% of the study population, while also 60% of the colon cancer group were females. Table 2 shows the median and 5th and 95th percentiles of plasma concentrations of (poly)phenols among participants. The highest plasma concentration values were observed for caffeic acid (median 367.0 nmol/L) and 4-hydroxyphenylacetic acid (287.0 nmol/L), whereas the lowest concentrations were observed for equol (0.4 nmol/L) and enterodiol (1.0 nmol/L). Figure 1 illustrates the statistically significant ($p \leq 0.05$) correlations between each of the plasma (poly)phenols and between plasma (poly)phenols and (poly)phenol-rich food groups. The strongest correlation was observed between 3,5-dihydroxybenzoic acid and 3,5-dihydroxy-phenylpropionic acid ($r = 0.90$). Correlation coefficients between plasma (poly)phenols and food groups were mostly low ($r < 0.40$), only highlighting correlations for tea (epicatechin $r = 0.46$; 3,5-dihydroxy-benzoic acid $r = 0.45$) and coffee (ferulic acid $r = 0.51$). The strongest inverse correlations were identified between protocatechuic acid and caffeic acid ($r = -0.52$), while isoharmentin displayed notable inverse correlations with three compounds: 3,4-dihydroxy-phenilacetic acid ($r = -0.52$), gallic acid ($r = -0.55$), and 3,4-dihydroxy-phenilpropionic acid ($r = -0.56$).

Plasma Concentration of (Poly)phenols and BW Change

We obtained nearly identical results for models 2 and 3. Consequently, we present the more extensively adjusted model, namely, model 3. Figure 2 shows results after modelling continuous 5-year BW change against log2-transformed plasma concentrations of (poly)phenols and adjusting for relevant confounders. Only plasma vanillic acid concentration appeared to be inversely associated with BW change in model 3 (*beta* per doubling concentration: −0.53 kg/5 years; 95% confidence interval [CI]: −0.99, −0.07), and a borderline association was

Table 1. Characteristics of 761 participants from the EPIC study according to categories of BW change over 5 years

Characteristic	BW loss (<−1.5 kg/5 years)	BW maintenance (−1.5 to 1.5 kg/5 years)	BW gain (>1.5 kg/5 years)	*p* value[a]
Participants, *n* (%)	194 (25.5)	274 (36.0)	293 (38.5)	0.001
Follow-up time, years	5.1 (2.7)	7.1 (3.2)	6.3 (2.9)	<0.001
BW change, kg/5 years	−4.7 (3.2)	0.1 (0.8)	4.5 (3.2)	<0.001
Age, years	54.8 (8.5)	53.5 (7.9)	52.9 (8.5)	0.059
Energy intake, kcal/day, median (IQR)	1,905 (745)	2,045 (855)	1,935 (790)	0.349
Alcohol consumption, g/day, median (IQR)	5.7 (14.0)	4.3 (15.1)	5.3 (15.0)	0.668
Fibre intake, g/day, median (IQR)	22.4 (9.1)	22.5 (9.9)	21.6 (9.5)	0.491
Vitamin C intake, mg/day, median (IQR)	123.8 (75.0)	118.8 (72.8)	110.3 (75.7)	0.026
BW, kg	73.6 (12.7)	67.1 (11.2)	68.7 (12.0)	<0.001
BMI, kg/m^2	28.2 (4.5)	25.4 (3.6)	25.3 (3.8)	<0.001
Sex, *n* (%)				0.358
Female	157 (80.9)	207 (75.5)	224 (76.5)	
Male	37 (19.1)	67 (24.5)	69 (23.5)	
Project, *n* (%)				0.436
Colon cancer control	117 (60.3)	155 (56.5)	181 (61.7)	
Thyroid cancer control	77 (39.7)	119 (43.5)	112 (38.3)	
Plausibility of energy intake reporting[b], *n* (%)				0.127
Under reporters	33 (17.0)	28 (10.2)	46 (15.7)	
Plausible reporters	148 (76.3)	216 (78.8)	219 (74.7)	
Over reporters	13 (6.7)	30 (11.0)	28 (9.6)	
Physical activity level, *n* (%)				0.001
Inactive	50 (25.8)	74 (27.0)	27 (19.5)	
Moderately inactive	76 (39.1)	103 (37.6)	123 (41.9)	
Moderately active	30 (15.5)	51 (18.6)	55 (18.8)	
Active	38 (19.6)	46 (16.8)	57 (19.5)	
Change in smoking status at follow-up, *n* (%)				<0.001
Stable never smoker	105 (54.0)	122 (44.0)	138 (47.0)	
Stable current smoker	33 (17.0)	30 (11.0)	34 (12.0)	
Starter	5 (2.5)	2 (0.8)	5 (1.5)	
Quitter	5 (2.5)	3 (1.2)	12 (4.0)	
Former at follow-up[c]	35 (18.0)	66 (24.0)	75 (25.5)	
Prevalent disease[d], *n* (%)				0.104
No	172 (88.6)	245 (89.4)	252 (86.0)	
Yes	12 (5.7)	14 (5.1)	11 (3.7)	
Education level, *n* (%)				0.004
None	37 (19.1)	25 (9.1)	23 (7.8)	
Primary school	73 (37.6)	103 (37.6)	111 (37.8)	
Technical school	21 (10.8)	35 (12.8)	39 (13.3)	
Secondary school	35 (18.0)	59 (21.5)	78 (26.6)	
Longer education	25 (12.9)	51 (18.6)	39 (13.3)	
Menopausal status[e], *n* (%)				0.001
Pre-menopausal	91 (46.9)	94 (34.3)	94 (32.1)	
Post-menopausal	97 (50.0)	177 (64.6)	186 (63.5)	
Peri-menopausal	6 (3.1)	3 (1.1)	13 (4.4)	

Data are expressed as mean (SD) and collected at recruitment if not stated otherwise. BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition; IQR, interquartile range (P25–P75). Percentages may not add up to 100 due to missing values. [a]*p* values by ANOVA, χ^2 or Kruskal-Wallis test among BW change categories for each variable. [b]Categories according to the ratio of reported energy intake (EI) to predicted basal metabolic rate (BRM): under (EI: BMR <1.14), plausible (1.14–2.1), and over reporters (>2.1), with the use of cut-off points proposed by Goldberg et al. [26]. [c]Includes never at baseline but former at follow-up. [d]Diabetes, stroke, or myocardial infarction at baseline. [e]Only for female participants (*n* = 588).

Table 2. Median and 5th and 95th percentiles of plasma concentration (nmol/L) of (poly)phenols among 761 participants from the EPIC study

(Poly)phenol	Median	P5th	P95th
(−)-Epicatechin	14.3	5.5	82.5
(−)-Epigallocatechin	11.1	11.1	48.3
(−)-Gallocatechin	11.1	11.1	15.6
(+)-Catechin	15.5	5.5	58.0
3,4-dihydroxy-phenylacetic acid	31.6	13.9	65.9
3,4-dihydroxy-phenylpropionic acid	146.5	12.5	321.8
3,5-dihydroxy-benzoic acid	23.2	6.6	158.8
3,5-dihydroxy-phenylpropionic acid	31.6	9.2	162.0
3-hydroxy-benzoic acid	19.7	6.8	62.4
3-hydroxy-phenylacetic acid	58.8	2.2	238.9
4-hydroxy-benzoic acid	272.0	160.1	518.8
4-hydroxy-phenylacetic acid	287.0	156.3	888.0
Apigenin	13.5	9.2	18.7
Caffeic acid	367.0	106.0	558.0
Daidzein	10.7	2.9	135.9
Enterodiol	1.0	0.2	8.9
Enterolactone	9.4	1.0	55.9
Equol	0.4	0.1	2.5
Ferulic acid	102.5	52.0	423.5
Gallic acid	28.0	12.2	82.6
Gallic acid ethyl ester	1.1	1.1	7.1
Genistein	5.4	1.3	59.7
Hesperetin	2.2	0.5	122.4
Homovanillic acid	80.5	51.0	162.5
Isorhamnetin	63.0	48.3	94.0
Kaempferol	87.0	59.1	126.9
m-Coumaric acid	8.10	0.5	76.4
Naringenin	3.70	1.1	77.9
Hydroxy-tyrosol	20.2	5.5	50.9
p-Coumaric acid	21.2	12.8	45.2
Phloretin	1.1	1.1	8.8
Protocatechuic acid	178.0	126.0	279.4
Quercetin	250.0	107.0	524.4
Resveratrol	2.6	1.1	13.6
Tyrosol	3.2	1.6	9.7
Vanillic acid	189.0	114.0	367.3

EPIC, European Prospective Investigation into Cancer and Nutrition.

observed for tyrosol (*beta*: −0.29 kg/5 years; 95% CI: −0.62, 0.00). It is worth mentioning that the majority of (poly)phenols displayed a suggestive trend towards inverse associations with BW gain. However, it is important to emphasize that, following the application of FDR adjustment, none of these associations reached statistical significance. Furthermore, similar trends were observed in the sensitivity analysis, which excluded participants with chronic diseases at recruitment (online suppl. Table S1). No statistically significant interactions were observed with respect to sex, age, or changes in smoking status at follow-up.

Table 3 shows the results from multinomial logistic regression analyses comparing BW loss versus BW maintenance (reference category). Most of the (poly) phenols showed a tendency towards BW loss. Plasma naringenin concentration was associated with BW loss in model 3 (odds ratio [OR]: 1.1; 95% CI: 1.0, 1.2). Additionally, plasma levels of ferulic acid (OR: 1.3; 95% CI: 1.0, 1.7), caffeic acid (OR: 1.7; 95% CI: 1.0, 3.0), and kaempferol (OR: 1.7; 95% CI: 1.0, 2.9) exhibited borderline associations. Table 4 presents results comparing BW gain with BW maintenance. We observed a pattern consistent with our previous analysis. Specifically, there were no statistically significant associations for BW gain, either before or after the application of the FDR correction, except for a suggestive inverse association for 3,5-dihydroxybenzoic acid (OR: 0.9; 95% CI: 0.8, 1.0).

Discussion

The current study suggested a tendency towards BW maintenance or reduction over 5 years at higher concentrations of plasma (poly)phenols. We would like to draw attention to the potential protective associations observed between plasma concentrations of vanillic acid and naringenin and BW loss. It is important to note that while the results related to vanillic acid are derived from GLMMs, those concerning naringenin are derived from multinomial models. It is noteworthy that both vanillic acid and naringenin have been proposed as metabolites of dietary flavonoids, as discussed below. Given their shared potential dietary sources, it is plausible that they may exhibit similar behaviours. However, we must approach these associations with caution due to the complexity of the relationships involved.

Even though both GLMMs and multinomial models included the same covariates and followed the same tendency, results were different. One possible explanation for these differences might be that plasma concentrations do not necessarily represent the same compounds present in foods. Natural vanillic acid is a phenolic compound widely present in vanilla beans and in different fruits and grains [30], but it can also be a derivative metabolite from different precursors. For example, as a result of the host metabolism, intake of caffeic acid from coffee has been shown to generate a high increase in urine levels of vanillic acid in vivo [31]. Likewise, one study using animal models observed that after red wine consumption, which is naturally rich in flavonoids and phenolic acids, vanillic acid was found in animal urine

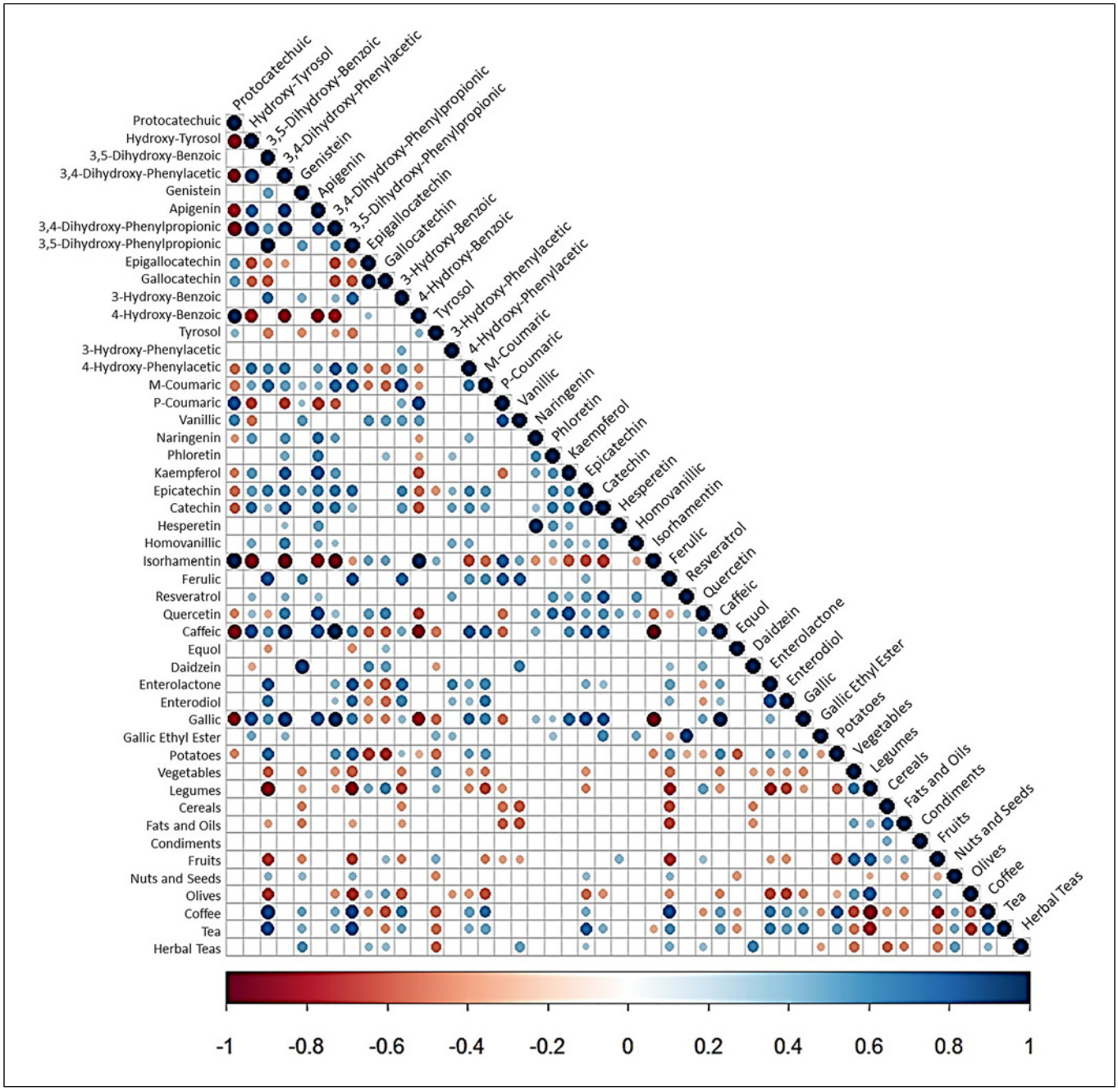


Fig. 1. Heat map showing Spearman rank correlations between plasma (poly)phenols and habitual intakes of selected food groups among participants from the EPIC study. Only significant correlations are shown ($p \leq 0.05$). Colour of the circles indicates the strength and direction of the correlations, and the size indicates the strength of the p value (the bigger, the stronger).

together with other microbial metabolites [32]. One study in vivo showed that after intake of an oral dose of cyanidin 3-glucoside (an anthocyanin widely found in fruits, particularly berries), vanillic acid was present in both human urine and serum [33].

Naringenin occurs naturally in an inactive form as naringin (the glycoside form) and is converted into its active form (aglycone) by bacteria belonging to the gut microbiome [34]. Naringin is a component of the everyday human diet, mainly present in citrus fruits. The

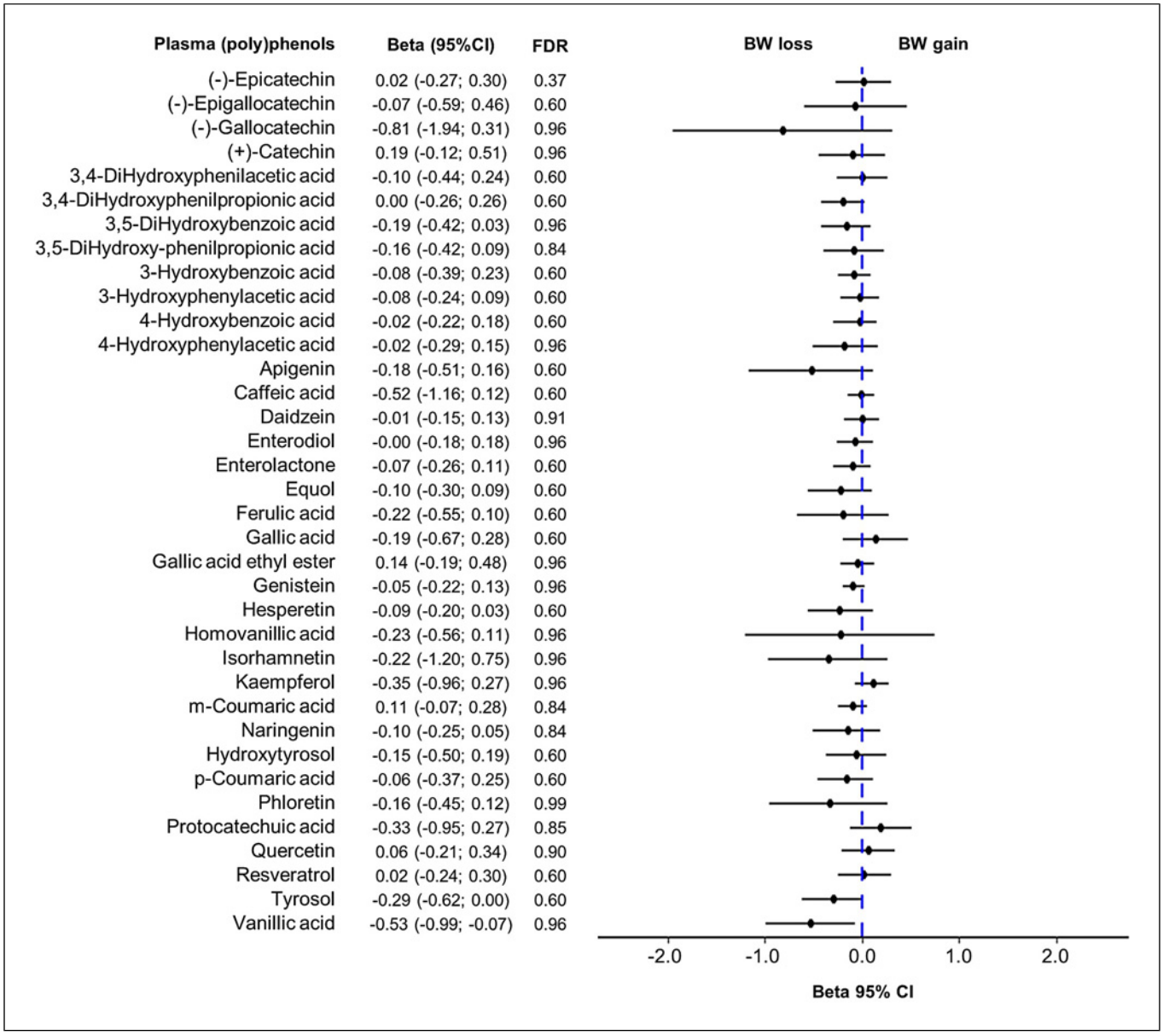


Fig. 2. *Beta* coefficients of BW changes associated with baseline log2-transformed plasma (poly)phenol concentrations for 761 participants from the EPIC study. General linear mixed models with the cancer project and EPIC centre as random effects. *Beta* values indicate 5-year BW change (kg) based on log2-transformed (poly)phenol plasma concentrations and, therefore, correspond to a doubling in plasma concentration of (poly)phenols. Model 3: Adjusted for age, sex, BMI (3-knot restricted cubic spline), follow-up time in years (3-knot restricted cubic spline), physical activity level, change in smoking status at follow-up, (poly)phenols laboratory batch, total energy intake (3-knot restricted cubic spline), plausibility of dietary energy reporting, intake of fibre (g/day), and intake of vitamin C (mg/day). BW, body weight; CI, confidence interval; FDR, false discovery rate.

gut microbiota metabolizes naringin and breaks it down to naringenin which is absorbed in the gut [35]. One study using animal models observed that the major components in plasma and urine after 18 h of gastric gavage of naringenin were glucuronides as well as the colonic metabolite 3-(4-hydroxyphenyl) propionic acid [36]. Thus, compounds present in plasma are not necessarily classified as those precursors present in diet – while naringenin is classified as a flavonoid in diet, one of its major human gut microbial metabolites is 3-(4-hydroxyphenyl) propionic acid, classified as phenolic acid [37].

Table 3. ORs for BW loss[a] (n = 194) versus BW maintenance (reference category[b], n = 274) for log2-transformed plasma (poly)phenol concentrations in participants from the EPIC study

Plasma (poly)phenols	Model 1[c]			Model 3[d]		
	OR (95% CI)	p value	FDR	OR (95% CI)	p value	FDR
(−)-Epicatechin	1.0 (0.8; 1.3)	0.80	0.92	1.0 (0.8; 1.3)	0.20	0.68
(−)-Epigallocatechin	1.0 (0.7; 1.5)	0.95	0.97	1.0 (0.7; 1.5)	0.97	0.99
(−)-Gallocatechin	1.3 (0.6; 2.8)	0.52	0.89	1.3 (0.6; 2.8)	0.54	0.82
(+)-Catechin	0.9 (0.7; 1.1)	0.21	0.74	0.9 (0.7; 1.1)	0.21	0.68
3,4-dihydroxy-phenylacetic acid	1.0 (0.8; 1.3)	0.88	0.96	1.0 (0.8; 1.3)	0.83	0.93
3,4-dihydroxy-phenylpropionic acid	1.0 (0.9; 1.2)	0.69	0.91	1.0 (0.9; 1.3)	0.64	0.82
3,5-dihydroxy-benzoic acid	1.0 (0.8; 1.2)	0.97	0.97	1.0 (0.9; 1.2)	0.90	0.95
3,5-dihydroxy-phenylpropionic acid	1.0 (0.9; 1.3)	0.71	0.91	1.1 (0.9; 1.3)	0.61	0.82
3-hydroxy-benzoic acid	1.1 (0.9; 1.3)	0.41	0.84	1.1 (0.-9; 1.3)	0.52	0.80
3-hydroxy-phenylacetic acid	1.0 (0.9; 1.2)	0.56	0.91	1.1 (0.9; 1.2)	0.36	0.76
4-hydroxy-benzoic acid	1.0 (0.9; 1.1)	0.81	0.92	1.0 (0.9; 1.1)	0.87	0.95
4-hydroxy-phenylacetic acid	1.0 (0.8; 1.2)	0.82	0.92	1.0 (0.8; 1.2)	0.80	0.93
Apigenin	1.1 (0.8; 1.6)	0.43	0.84	1.2 (0.8; 1.6)	0.39	0.77
Caffeic acid	1.8 (1.0; 3.1)	0.05	0.47	1.7 (1.0; 3.0)	0.05	0.51
Daidzein	0.9 (0.8; 1.0)	0.05	0.47	0.9 (0.8; 1.0)	0.07	0.51
Enterodiol	1.0 (0.9; 1.1)	0.59	0.91	1.0 (0.9; 1.1)	0.60	0.82
Enterolactone	0.9 (0.9; 1.1)	0.91	0.96	1.0 (0.9; 1.1)	0.99	0.99
Equol	1.0 (0.8; 1.1)	0.65	0.91	1.0 (0.8; 1.1)	0.68	0.84
Ferulic acid	1.3 (1.0; 1.7)	0.04	0.47	1.3 (1.0; 1.7)	0.15	0.51
Gallic acid	1.2 (0.8; 1.6)	0.39	0.84	1.2 (0.8; 1.7)	0.30	0.72
Gallic acid ethyl ester	0.9 (0.7; 1.2)	0.44	0.84	0.9 (0.7; 1.2)	0.51	0.82
Genistein	1.0 (0.9; 1.1)	0.75	0.92	1.0 (0.9; 1.2)	0.80	0.93
Hesperetin	1.0 (1.0; 1.1)	0.43	0.84	1.0 (1.0; 1.1)	0.43	0.78
Homovanillic acid	1.2 (0.8; 1.4)	0.66	0.91	1.1 (0.8; 1.4)	0.63	0.82
Isorhamnetin	0.7 (0.4; 1.4)	0.35	0.84	0.7 (0.3; 1.4)	0.30	0.72
Kaempferol	1.6 (0.9; 2.7)	0.09	0.59	1.7 (1.0; 2.9)	0.06	0.51
m-Coumaric acid	1.1 (1.0; 1.2)	0.28	0.84	1.1 (1.0; 1.2)	0.39	0.68
Naringenin	1.1 (1.0; 1.2)	0.05	0.47	1.1 (1.0; 1.2)	0.04	0.51
Hydroxy-tyrosol	1.1 (0.9; 1.4)	0.47	0.84	1.1 (0.9; 1.4)	0.43	0.78
p-Coumaric acid	1.2 (0.9; 1.5)	0.20	0.74	1.2 (0.9; 1.5)	0.21	0.68
Phloretin	1.1 (0.9; 1.3)	0.36	0.84	1.1 (0.9; 1.4)	0.23	0.68
Protocatechuic acid	1.3 (0.7; 2.6)	0.39	0.84	1.4 (0.7; 2.8)	0.35	0.76
Quercetin	0.8 (0.7; 1.0)	0.10	0.59	0.8 (0.7; 1.0)	0.12	0.66
Resveratrol	1.0 (0.9; 1.2)	0.67	0.91	1.0 (0.9; 1.3)	0.64	0.82
Tyrosol	1.2 (0.9; 1.4)	0.18	0.74	1.2 (0.9; 1.4)	0.20	0.68
Vanillic acid	1.3 (0.9; 1.8)	0.14	0.72	1.3 (0.9; 1.9)	0.15	0.66

CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; FDR, false discovery rate; OR, odds ratio. ORs correspond to a doubling in (poly)phenol concentration. [a]BW loss: weight change < −1.5 kg/5 years. [b]Reference category: BW maintenance from −1.5 to 1.5 kg/5 years. [c]Adjusted for age, sex, BMI (3-knot restricted cubic spline), cancer project, and EPIC centre. [d]Further adjusted for follow-up time in years (3-knot restricted cubic spline), physical activity level, change in smoking status at follow-up, (poly)phenols laboratory batch, total energy intake (3-knot restricted cubic spline), plausibility of dietary energy reporting, intake of fibre (g/day), and intake of vitamin C (mg/day).

As far as we know, there is a lack of evidence on plasma (poly)phenols and obesity-related parameters. Previous cohort studies have investigated similar relationships but with dietary or urinary (poly)phenols. The SU.VI.MAX study [3] concluded that higher dietary (poly)phenol intake, particularly flavonoids and their subclasses, may help reduce BW gain. The MEAL study observed that higher flavonoid intake was associated with a lower BW [4]. In line with this, the large randomized PREDIMED study concluded that higher (poly)phenol intakes, objectively measured through total urinary (poly)phenol excretion, were inversely correlated with BW and obesity [38]. Bertoia et al.

Table 4. ORs for BW gain[a] (n = 293) versus BW maintenance (reference category[b], n = 274) for log2-transformed (poly)phenol concentrations in participants from the EPIC study

Plasma (poly)phenols	Model 1[c]			Model 3[d]		
	OR (95% CI)	p value	FDR	OR (95% CI)	p value	FDR
(−)-Epicatechin	1.0 (0.8; 1.1)	0.61	0.98	1.0 (0.8; 1.1)	0.57	0.94
(−)-Epigallocatechin	0.9 (0.6; 1.2)	0.33	0.93	0.9 (0.6; 1.2)	0.33	0.93
(−)-Gallocatechin	0.9 (0.5; 1.8)	0.82	0.98	0.9 (0.5; 1.8)	0.74	1.00
(+)-Catechin	0.9 (0.8; 1.1)	0.56	0.97	0.9 (0.8; 1.1)	0.55	0.94
3,4-dihydroxy-phenylacetic acid	0.9 (0.8; 1.1)	0.51	0.96	0.9 (0.8; 1.1)	0.54	0.94
3,4-dihydroxy-phenylpropionic acid	1.0 (0.8; 1.1)	0.50	0.96	1.0 (0.8; 1.1)	0.52	0.94
3,5-dihydroxy-benzoic acid	0.9 (0.8; 1.0)	0.07	0.93	0.9 (0.8; 1.0)	0.07	0.93
3,5-dihydroxy-phenylpropionic acid	0.9 (0.8; 1.1)	0.29	0.93	0.9 (0.8; 1.1)	0.31	0.93
3-hydroxy-benzoic acid	1.0 (0.9; 1.2)	0.64	0.98	1.0 (0.9; 1.2)	0.64	0.96
3-hydroxy-phenylacetic acid	1.0 (0.9; 1.1)	0.42	0.96	1.0 (0.9; 1.1)	0.55	0.94
4-hydroxy-benzoic acid	0.9 (0.8; 1.1)	0.24	0.93	0.9 (0.8; 1.0)	0.24	0.93
4-hydroxy-phenylacetic acid	0.9 (0.8; 1.1)	0.23	0.93	0.9 (0.8; 1.1)	0.24	0.93
Apigenin	0.9 (0.8; 1.1)	0.46	0.96	0.9 (0.8; 1.1)	0.47	0.94
Caffeic acid	1.0 (0.7; 1.5)	0.80	0.98	1.1 (0.7; 1.5)	0.78	1.00
Daidzein	0.9 (0.9; 1.0)	0.12	0.93	0.9 (0.9; 1.0)	0.14	0.93
Enterodiol	1.0 (0.9; 1.1)	0.81	0.98	1.0 (0.9; 1.1)	0.84	1.00
Enterolactone	1.0 (0.8; 1.0)	0.11	0.93	0.9 (0.8; 1.0)	0.12	0.93
Equol	1.0 (0.9; 1.1)	0.86	0.98	1.0 (0.9; 1.1)	0.86	1.00
Ferulic acid	1.0 (0.8; 1.2)	0.90	0.98	1.0 (0.8; 1.2)	1.00	1.00
Gallic acid	1.0 (0.7; 1.3)	0.93	0.99	1.0 (0.7; 1.3)	0.97	1.00
Gallic acid ethyl ester	1.0 (0.8; 1.2)	0.98	0.99	1.0 (0.8; 1.2)	0.99	1.00
Genistein	1.0 (0.9; 1.1)	0.78	0.98	1.0 (0.9; 1.1)	0.77	1.00
Hesperetin	1.0 (0.9; 1.0)	0.33	0.93	1.0 (0.9; 1.0)	0.38	0.94
Homovanillic acid	0.9 (0.8; 1.1)	0.30	0.93	0.9 (0.7; 1.1)	0.31	0.93
Isorhamnetin	0.8 (0.5; 1.3)	0.37	0.94	0.8 (0.5; 1.3)	0.34	0.93
Kaempferol	1.1 (0.8; 1.6)	0.54	0.97	1.1 (0.8; 1.6)	0.53	0.94
m-Coumaric acid	1.0 (0.9; 1.2)	0.43	0.96	1.0 (0.9; 1.2)	0.41	0.94
Naringenin	1.0 (0.9; 1.1)	0.76	0.98	1.0 (0.9; 1.1)	0.87	1.00
Hydroxy-tyrosol	1.0 (0.8; 1.2)	0.90	0.98	1.0 (0.8; 1.2)	0.90	1.00
p-Coumaric acid	1.0 (0.8; 1.1)	0.67	0.98	1.0 (0.8; 1.1)	0.64	0.96
Phloretin	0.9 (0.7; 1.0)	0.11	0.93	0.9 (0.7; 1.0)	0.14	0.93
Protocatechuic acid	0.8 (0.6; 1.2)	0.28	0.93	0.8 (0.6; 1.1)	0.25	0.93
Quercetin	0.9 (0.8; 1.1)	0.24	0.93	0.9 (0.7; 1.1)	0.26	0.93
Resveratrol	1.0 (0.9; 1.2)	0.99	0.99	1.0 (0.9; 1.2)	1.00	1.00
Tyrosol	0.9 (0.7; 1.1)	0.34	0.93	1.0 (0.7; 1.1)	0.31	0.93
Vanillic acid	1.0 (0.8; 1.3)	0.84	0.98	1.0 (0.8; 1.3)	0.82	1.00

CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; FDR, false discovery rate; OR, odds ratio. ORs correspond to a doubling in (poly)phenol concentration. [a]BW gain: weight change >1.5 kg/5 years. [b]Reference category: BW maintenance from −1.5 to 1.5 kg/5 years. [c]Adjusted for age, sex, BMI (3-knot restricted cubic spline), cancer project, and EPIC centre. [d]Further adjusted for follow-up time in years (3-knot restricted cubic spline), physical activity level, change in smoking status at follow-up, (poly) phenols laboratory batch, total energy intake (3-knot restricted cubic spline), plausibility of dietary energy reporting, intake of fibre (g/day), and intake of vitamin C (mg/day).

[39] observed, in three prospective cohorts, that higher intake of foods rich in flavonoids may contribute to BW maintenance and prevention of obesity. A longitudinal study from the Netherlands found that a higher intake of flavonols and flavones was associated with lower increase in BMI over 14 years in the general female population [40].

In comparing our current study to our previous work utilizing dietary (poly)phenol data from the EPIC-PANACEA cohort [5, 6], we observed few distinct differences in the findings. Our previous dietary-based research found a strong, statistically significant inverse link between (poly)phenol intake and BW gain. However, in this study

using plasma (poly)phenol concentrations, associations were less pronounced and borderline statistically significant. Two key factors contribute to these disparities. Firstly, we had dietary data for a much larger subgroup (around 350,000) of the EPIC cohort, while plasma (poly)phenol analysis was limited to under 800 participants due to logistical constraints. This variation in sample size could affect our ability to detect associations. Secondly, using biomarkers like plasma (poly)phenols offers a more objective measure of diet but may not capture its full complexity due to individual differences in bioavailability and metabolism [41]. In contrast, dietary data provide a comprehensive view of dietary patterns [42]. In spite of these differences, both measurements (dietary data and nutritional biomarkers) provide evidence that (poly)phenols may have a protective impact in BW maintenance.

In addition, the discrepancies in explaining these associations can be attributed to differences in (poly) phenol bioavailability. In general, (poly)phenols show low bioavailability (<20%) which can be affected by several factors, including chemical structure (including sugar moiety), food matrix, interaction with other compounds, and individual conditions like intestinal activity, gut microbiota composition, sex, or age [41]. According to pharmacokinetic studies, (poly)phenols that are most absorbed in humans are isoflavones and phenolic acids, followed by catechins, flavanones, and quercetin glucosides. Low-molecular-weight (poly) phenols are easily absorbed through the gut barrier [43, 44]. When they reach the basal membrane of the enterocytes and when they enter the liver, they are conjugated to glucuronic acid, sulphate, and methyl groups to facilitate their transport and excretion and limit their potential toxicity [43, 44]. Conversely, large molecular weight (poly)phenols such as proanthocyanidins are poorly absorbed in the small intestine, and they reach the colon to be metabolized by the gut microbiota into lower molecular weight compounds, generally phenolic acids, which can be partially absorbed in the colon [45].

Increasing evidence supports the idea that the gut microbiome plays a key role in the relationship between (poly)phenols and metabolism, body fat, and obesity. It has been shown, for example, that diets high in (poly) phenol-rich whole plant-based foods improve gut microbiota profiles when compared to diets high in animal-based products [46]. Interactions between (poly)phenols and the gut microbiome have also been shown to decrease obesity-related conditions such as development of adipose tissue and obesity-induced inflammatory genes [47]. Plasma concentrations of different (poly)phenols may be a reflection of these metabolites resulting from dietary (poly)phenols-microbiome interactions [48].

The robust positive correlations observed between certain plasma (poly)phenols, such as the noteworthy correlation between 3,5-dihydroxy-benzoic acid and 3,5-dihydroxy-phenylpropionic acid, can be attributed to a complex interplay of dietary factors, metabolic processes, and individual variability. In a previous study assessing the pharmacokinetics of novel metabolites in urine, it was observed that these two compounds were new candidate biomarkers for whole-grain wheat and rye intake [49]. Therefore, both 3,5-dihydroxy-benzoic acid and 3,5-dihydroxy-phenylpropionic acid were proposed as potential biomarkers to increase the accuracy of whole-grain wheat and rye intake in epidemiology studies. These findings underscore the multifaceted nature of (poly)phenol metabolism and its dependence on dietary patterns, metabolic pathways, and individual characteristics, offering valuable insights for future research in (poly) phenol metabolism and its potential health implications. The strong correlations observed between certain compounds and tea and coffee may reflect some characteristics of dietary patterns in our population. Tea and coffee are frequently consumed by individuals as part of their daily routines [11]. Consistent and habitual consumption of these beverages may result in a continuous supply of (poly)phenols to the body, potentially leading to higher and steadier plasma levels over time [50].

As previously mentioned, one limitation of our investigation is the interpretation of human study data when working with these compounds. For example, as the occurrence of these metabolites in circulating blood is the result of digestive and hepatic activity, the complex interaction between (poly)phenols, individual gut microbiota, and host metabolism cannot be disregarded [1]. Another limitation is the relatively small sample size with available measurements of plasma (poly)phenols, particularly for subgroup analyses. The EPIC sampling characteristics do not allow the total generalizability of these findings to other populations. In addition, the fact that female participants constituted the vast majority of the study population means males were underrepresented. Concentrations of (poly)phenols were measured in single plasma samples at baseline; thus, intra-individual variations in circulating levels of these compounds were not available, which could also lead

to attenuation of the observed associations [51]. Because of the observational nature of the study design, although we were able to adjust our models for relevant lifestyle and dietary covariates, the possibility of residual confounding cannot be ruled out.

A major strength of this study is its prospective and multicentre design, including participants from different European countries with wide variation in diet. In addition, we had data on a relatively large number of (poly)phenols ($n = 36$), spanning all major classes found in the human diet. While plasma (poly)phenols in this study may indicate a plant-based diet, it is important to consider a broader context. While we incorporated fibre and vitamin C intake into our model, we recognize a lack of detailed knowledge regarding the potential biological mechanisms connecting plasma (poly)phenols to BW loss. Consequently, attributing our findings solely to a healthier diet may be overly simplistic. Nevertheless, further research is needed to explore how (poly)phenols and plant-based compounds influence BW regulation. In epidemiological studies, prediction of (poly)phenol intake mainly relies on food records and composition tables, often failing to assess total intake accurately [52]. Thus, assessing the relationship between diet-related compounds and health outcomes can be challenging. The measurement of plasma concentrations of (poly)phenols represents an objective measurement of a snapshot of internal exposure to these compounds that could come directly from different dietary sources or their precursors [51]. This has been a way of reducing the potential bias from dietary questionnaire-based data. While the evidence on the determination of plasma (poly)phenols is not extensive, a recent review described the common analytical technique utilizing LC-MS/MS [53]. However, the use of differential isotope labelling in our methodology is an extra advantage. This methodology reinforces the robustness, sensitivity, and specificity of our analytical approach and its applicability to quantifying low plasma (poly)phenol concentrations [54].

In conclusion, this prospective investigation suggested a tendency towards 5-year BW loss or maintenance at higher plasma (poly)phenol concentrations at baseline. Among specific (poly)phenols, vanillic acid and naringenin can be highlighted, which may act as biomarkers of flavonoid intake. While these specific associations seemed promising, they did not withstand FDR correction, indicating the need for caution in interpreting these results. These findings are preliminary, and further studies using (poly)phenol biomarkers are needed to better understand the potential observed trends. This study may contribute to the identification of specific (poly)phenols for future mechanistic studies or clinical trials on obesity-related pathways in humans.

Acknowledgements

We thank all EPIC participants and staff for their contribution to the study. We also thank Bertrand Hémon and Leila Luján Barroso for their valuable help with the EPIC database. We also thank Aarhus University, Aarhus, Denmark; the University Medical Center Utrecht, Utrecht, The Netherlands; the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; and Oxford University, UK, for their contribution and ongoing support to the EPIC Study. NGF acknowledges support from the MRC Epidemiology Unit (MC_UU_00006/3) and from the NIHR Cambridge Biomedical Research Centre nutrition, obesity, endocrinology, and metabolism (NOME) theme (NIHR203312). She is an NIHR Senior Investigator. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Statement of Ethics

The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and has obtained ethical approval from participating centres and the IARC Ethics Committee (reference number 20-02). Written informed consent was obtained from all study participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare. Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article, and they do not necessarily represent the decisions, policies, or views of the International Agency for Research on Cancer/World Health Organization.

Funding Sources

This study has been funded by the Institute of Health Carlos III through the grant PI18/00191, co-funded by the European Regional Development Fund, ERDF, as a way to build Europe. The PANACEA project was funded by the European Union in the framework of the Public Health Programme (project number: 2005328). The coordination of EPIC is financially supported by the International Agency for Research on Cancer (IARC) and also by the Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, which has additional infrastructure support provided by the NIHR Imperial Biomedical

Research Centre (BRC). The national cohorts are supported by: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Federal Ministry of Education and Research (BMBF) (Germany); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy, Compagnia di San Paolo and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS) – Institute of Health Carlos III (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, and the Catalan Institute of Oncology – ICO (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C8221/A29017 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk; MR/M012190/1 to EPIC-Oxford) (UK). We thank the CERCA Program/Generalitat de Catalunya for institutional support of IDIBELL. MG-L, EA-A, and RZ-R were supported by the PFIS (FI19/00185), "Sara Borrell" (CD20/00071), and "Miguel Servet II" (CPII20/00009) programmes, respectively, from the Institute of Health Carlos III (co-funded by the European Social Fund (ESF) investing in your future). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

R.Z.-R. designed research and wrote paper – reviewed and edited; M.G.-L. conducted research and wrote paper – prepared original draft; A.K.E., A.T., J.A.R., S.S., C.C., V.K., T.J., M.B.S., A.O., F.P., R.T., L.M., G.M., G.S., M.W.L., M.B., C.L., M.C.-B., E.M.-M., S.C.-Y., M.G., P.A., I.J., J.H., N.G.F., H.F., M.M., C.D., A.K.H., E.K.A., and D.A. provided essential material; M.G.-L. and D.G.-F. performed statistical analysis; and E.A.-A., J.C., and M.G.-L. had primary responsibility for final content. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

References

1 Zamora-Ros R, Knaze V, Rothwell JA, Hémon B, Moskal A, Overvad K, et al. Dietary polyphenol intake in europe: the European prospective investigation into cancer and nutrition (EPIC) study. Eur J Nutr. 2016;55(4):1359–75.
2 Koch W. Dietary polyphenols–important non-nutrients in the prevention of chronic noncommunicable diseases. A systematic review. Nutrients. 2019;11(5):1039.
3 Adriouch S, Kesse-Guyot E, Feuillet T, Touvier M, Olié V, Andreeva V, et al. Total and specific dietary polyphenol intakes and 6-year anthropometric changes in a middle-aged general population cohort. Int J Obes. 2018;42(3):310–7.
4 Marranzano M, Ray S, Godos J, Galvano F. Association between dietary flavonoids intake and obesity in a cohort of adults living in the Mediterranean area. Int J Food Sci Nutr. 2018;69(8):1020–9.
5 Castañeda J, Gil-Lespinard M, Almanza-Aguilera E, Llaha F, Gómez JH, Bondonno N, et al. Association between classes and subclasses of polyphenol intake and 5-year body weight changes in the EPIC-PANACEA study. Obes Silver Spring Md. 2023;31(4):1146–58.
6 Gil-Lespinard M, Castañeda J, Almanza-Aguilera E, Gómez JH, Tjønneland A, Kyrø C, et al. Dietary intake of 91 individual polyphenols and 5-year body weight change in the EPIC-PANACEA cohort. Antioxidants. 2022;11(12):2425.
7 Lanuza F, Zamora-Ros R, Bondonno NP, Meroño T, Rostgaard-Hansen AL, Riccardi G, et al. Dietary polyphenols, metabolic syndrome and cardiometabolic risk factors: an observational study based on the DCH-NG subcohort. Nutr Metab Cardiovasc Dis. 2023;33(6):1167–78.
8 Jin S, Liu J, Jia Y, Han T, Zhao X, Sun C, et al. The association of dietary flavonoids, magnesium and their interactions with the metabolic syndrome in Chinese adults: a prospective cohort study. Br J Nutr. 2021;126(6):892–902.
9 Popiolek-Kalisz J. The relationship between dietary flavonols intake and metabolic syndrome in polish adults. Nutrients. 2023;15(4):854.
10 Ravelli MN, Schoeller DA. Traditional self-reported dietary instruments are prone to inaccuracies and new approaches are needed. Front Nutr. 2020;7:90.
11 Riboli E, Hunt K, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002;5(6B):1113–24.
12 Zamora-Ros R, Lujan-Barroso L, Achaintre D, Franceschi S, Kyrø C, Overvad K, et al. Blood polyphenol concentrations and differentiated thyroid carcinoma in women from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Am J Clin Nutr. 2021;113(1):162–71.
13 Murphy N, Achaintre D, Zamora-Ros R, Jenab M, Boutron-Ruault MC, Carbonnel F, et al. A prospective evaluation of plasma polyphenol levels and colon cancer risk. Int J Cancer. 2018;143(7):1620–31.
14 Kroke A, Klipstein-Grobusch K, Voss S, Möseneder J, Thielecke F, Noack R, et al. Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. Am J Clin Nutr. 1999;70(4):439–47.
15 Slimani N, Deharveng G, Unwin I, Southgate DAT, Vignat J, Skeie G, et al. The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. Eur J Clin Nutr. 2007;61(9):1037–56.
16 Freisling H, Noh H, Slimani N, Chajès V, May AM, Peeters PH, et al. Nut intake and 5-year changes in body weight and obesity risk in adults: results from the EPIC-PANACEA study. Eur J Nutr. 2018;57(7):2399–408.
17 Spencer EA, Appleby PN, Davey GK, Key TJ. Validity of self-reported height and weight in 4808 EPIC–Oxford participants. Public Health Nutr. 2002;5(4):561–5.

18 Achaintre D, Gicquiau A, Li L, Rinaldi S, Scalbert A. Quantification of 38 dietary polyphenols in plasma by differential isotope labelling and liquid chromatography electrospray ionization tandem mass spectrometry. J Chromatogr A. 2018;1558:50–8.
19 Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med. 2011;364(25):2392–404.
20 Chiriboga DE, Ma Y, Li W, Olendzki BC, Pagoto SL, Merriam PA, et al. Gender differences in predictors of body weight and body weight change in healthy adults. Obesity. 2008;16(1):137–45.
21 Ambikairajah A, Walsh E, Tabatabaei-Jafari H, Cherbuin N. Fat mass changes during menopause: a metaanalysis. Am J Obstet Gynecol. 2019;221(5):393–409.e50.
22 Cohen AK, Rai M, Rehkopf DH, Abrams B. Educational attainment and obesity: a systematic review. Obes Rev. 2013;14(12):989–1005.
23 Slavin JL. Dietary fiber and body weight. Nutrition. 2005;21(3):411–8.
24 Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. 2003;6(4):407–13.
25 Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify underrecording. Eur J Clin Nutr. 1991;45(12):569–81.
26 Harrell FE. Regression Modeling Strategies: with applications to linear models, logistic regression and survival analysis. 1st ed. New York, NY: Springer; 2001; p. 1–9.
27 Rojo-Tirado MA, Benito PJ, Atienza D, Rincón E, Calderón FJ. Effects of age, sex, and treatment on weight-loss dynamics in overweight people. Appl Physiol Nutr Metab. 2013;38(9):967–76.
28 Audrain-Mcgovern J, Benowitz NL. Cigarette smoking, nicotine, and body weight. Clin Pharmacol Ther. 2011;90(1):164–8.
29 R Core Team. R: a language and environment for statistical computing; 2022. Available from: https://www.R-project.org/.
30 Ingole A, Kadam MP, Dalu AP, Kute SM, Mange PR, Theng VD, et al. A review of the pharmacological characteristics of vanillic acid. J Drug Deliv Ther. 2021;11(2-S):200–4.
31 Rechner AR, Spencer JPE, Kuhnle G, Hahn U, Rice-Evans CA. Novel biomarkers of the metabolism of caffeic acid derivatives in vivo. Free Radic Biol Med. 2001;30(11):1213–22.
32 Gonthier M-P, Cheynier V, Donovan JL, Manach C, Morand C, Mila I, et al. Microbial aromatic acid metabolites formed in the gut account for a major fraction of the polyphenols excreted in urine of rats fed red wine polyphenols. J Nutr. 2003;133(2):461–7.
33 De Ferrars RM, Czank C, Zhang Q, Botting NP, Kroon PA, Cassidy A, et al. The pharmacokinetics of anthocyanins and their metabolites in humans. Br J Pharmacol. 2014;171(13):3268–82.
34 Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr. 2014;5(4):404–17.
35 Choudhury R, Chowrimootoo G, Srai K, Debnam E, Rice-Evans CA. Interactions of the flavonoid naringenin in the gastrointestinal tract and the influence of glycosylation. Biochem Biophys Res Commun. 1999;265(2):410–5.
36 El Mohsen MA, Marks J, Kuhnle G, Rice-Evans C, Moore K, Gibson G, et al. The differential tissue distribution of the citrus flavanone naringenin following gastric instillation. Free Radic Res. 2004;38(12):1329–40.
37 Chen T, Wu H, He Y, Pan W, Yan Z, Liao Y, et al. Simultaneously quantitative analysis of naringin and its major human gut microbial metabolites naringenin and 3-(4'-hydroxyphenyl) propanoic acid via stable isotope deuterium-labeling coupled with RRLC-MS/MS method. Molecules. 2019;24(23):4287.
38 Guo X, Li X, Shi M, Li D, Medina-Remón A, Fitó M. Polyphenol levels are inversely correlated with body weight and obesity in an elderly population after 5 years of follow up (The randomised PREDIMED study). Nutrients. 2017;9(5).
39 Bertoia ML, Rimm EB, Mukamal KJ, Hu FB, Willett WC, Cassidy A. Dietary flavonoid intake and weight maintenance: three prospective cohorts of 124,086 US men and women followed for up to 24 years. BMJ. 2016;352:i17.
40 Hughes LA, Arts IC, Ambergen T, Brants HA, Dagnelie PC, Goldbohm RA, et al. Higher dietary flavone, flavonol, and catechin intakes are associated with less of an increase in BMI over time in women: a longitudinal analysis from The Netherlands Cohort Study. Am J Clin Nutr. 2008;88(5):1341–52.
41 D'Archivio M, Filesi C, Varì R, Scazzocchio B, Masella R. Bioavailability of the polyphenols: status and controversies. Int J Mol Sci. 2010;11(4):1321–42.
42 Shim J-S, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. Epidemiol Health. 2014;36:e2014009.
43 Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81(1 Suppl l):230S–42S.
44 Di Lorenzo C, Colombo F, Biella S, Stockley C, Restani P. Polyphenols and human health: the role of bioavailability. Nutrients. 2021;13(1):273–30.
45 Scalbert A, Morand C, Manach C, Rémésy C. Absorption and metabolism of polyphenols in the gut and impact on health. Biomed Pharmacother. 2002;56(6):276–82.
46 Sakkas H, Bozidis P, Touzios C, Kolios D, Athanasiou G, Athanasopoulou E, et al. Nutritional status and the influence of the vegan diet on the gut microbiota and human health. Med. 2020;56:88.
47 Corrêa TAF, Rogero MM, Hassimotto NMA, Lajolo FM. The two-way polyphenols-microbiota interactions and their effects on obesity and related metabolic diseases. Front Nutr. 2019;6:188.
48 Serra A, Macià A, Romero MP, Reguant J, Ortega N, Motilva MJ. Metabolic pathways of the colonic metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. Food Chem. 2012;130(2):383–93.
49 Zhu Y, Shurlknight KL, Chen X, Sang S. Identification and pharmacokinetics of novel alkylresorcinol metabolites in human urine, new candidate biomarkers for whole-grain wheat and rye intake. J Nutr. 2014;144(2):114–22.
50 Zhao Y, Zhang X. Interactions of tea polyphenols with intestinal microbiota and their implication for anti-obesity. J Sci Food Agric. 2020;100(3):897–903.
51 Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727–47.
52 Spencer JPE, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. Br J Nutr. 2008;99(1):12–22.
53 Białkowska A, Górnicka M, Zielinska-Pukos MA, Hallmann E, Hamulka J. Plasma carotenoids and polyphenols and their association with MetS: the need for nutritional interventions. Antioxidants. 2023;12(7):1336.
54 Achaintre D, Buleté A, Cren-Olivé C, Li L, Rinaldi S, Scalbert A. Differential isotope labeling of 38 dietary polyphenols and their quantification in urine by liquid chromatography electrospray ionization tandem mass spectrometry. Anal Chem. 2016;88(5):2637–44.

4.3. Manuscript 4: Associations between dietary intake of flavonoids and adiposity: cross-sectional findings from the Fenland Study, UK.

Gil-Lespinard M, Forouhi G. Nita, Imamura F, Zamora-Ros R. Associations between dietary intake of flavonoids and adiposity: cross-sectional findings from the Fenland Study, UK. Under preparation for journal.

This work has been presented as communications at:

- The Scientific Session of the Cancer Epidemiology Research Programme, held on October 23, 2023, at the Bellvitge Biomedical Research Institute in L'Hospitalet de Llobregat, Spain, in the form of oral communication.
- The 8th Edition IDIBELL PhD Day, held on November 10, 2023, at the Bellvitge Biomedical Research Institute in L'Hospitalet de Llobregat, Spain, in the form of oral communication as part of the talk ‘Impact of (Poly)phenols on Body Weight and Adiposity: Insights from Two European Cohorts’. Awarded 2nd Best Oral Communication.

Associations between dietary intake of flavonoids and adiposity: cross-sectional findings from the Fenland Study, UK.

Mercedes Gil-Lespinard[1,2], Nita G. Forouhi[2], Fumiaki Imamura[2], Raul Zamora-Ros[1,3*]

[1]Unit of Nutrition and Cancer, Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Spain.

[2]MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge, UK.

[3]Department of Nutrition, Food Sciences, and Gastronomy, Food Innovation Network (XIA), Institute for Research on Nutrition and Food Safety (INSA), Faculty of Pharmacy and Food Sciences University of Barcelona, Barcelona, Spain.

***Corresponding author:** Dr. Raul Zamora-Ros, Unit of Nutrition and Cancer, Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Spain; Department of Nutrition, Food Sciences, and Gastronomy, Faculty of Pharmacy and Food Sciences, University of Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona, Spain. E-mail: rzamora@ub.edu / rzamora@idibell.cat

Running title: Flavonoids and adiposity in the UK Fenland study.

Number of words: Abstract: 254. Main text: 3,425.

Number of references: 64

ABSTRACT

Prospective and experimental evidence supports beneficial effects of flavonoids on weight management and metabolic health, but their impact on specific adiposity parameters remains unclear. We aimed to investigate associations of total and subclasses of dietary flavonoids with adiposity. We evaluated cross-sectional data from 11,568 adults aged 30-65 years recruited to the Fenland Study between 2005 and 2015 in Cambridgeshire, UK. Habitual diets were evaluated using food frequency questionnaires. Flavonoid intakes were calculated using a British food composition database. The associations were examined using robust regression adjusted for relevant confounders and corrected for false discovery rate for multiple flavonoids and adiposity parameters. The median of flavonoid intake was 428 mg/d (interquartile range 282). Doubling in total flavonoid intakes was inversely associated with body fat (BF) (dual-energy X-ray absorptiometry) with $beta_{log2}$ -0.54% (95% CI -0.70; -0.40); visceral fat (VAT) -0.13 cm (-0.17; -0.08); subcutaneous fat (SCAT) -0.05 cm (-0.08; -0.02); VAT:SCAT ratio -0.02 (-0.04; -0.00); body mass index (BMI) -0.33 kg/m^2 (-0.44; -0.22); waist circumference (WC) -0.84 cm (-1.13; -0.55); and waist-to-hip ratio -0.004 (-0.006; -0.002). Most of flavonoid subclasses showed similar results, except isoflavones that were positively associated with BF [0.15 % (0.06; 0.23)], VAT [0.03 cm (0.01; 0.06)] and WC [0.19 cm (0.04; 0.35)]. Associations weakened when adjusted for BMI. Our study suggests that the influence of flavonoids on adiposity might be a potential pathway for the relationship between flavonoid-rich foods and metabolic risk. These findings should stimulate further investigation in prospective, interventional, and mechanistic studies to understand the link between flavonoids and adiposity.

Keywords: dietary flavonoids, adiposity, cross-sectional, Fenland study

INTRODUCTION

The role of diet as a prominent modifiable risk factor for obesity and adiposity-related conditions is widely recognized (1). Extensive evidence supports the relevant contribution of plant-based foods and their bioactive substances in preventing or treating these conditions (2,3). Flavonoids, polyphenolic compounds abundantly found in plant-based foods, such as fruits, vegetables, tea, and cocoa products, are of particular importance among these bioactive substances (4). Flavonoids are classified into six subclasses based on their chemical structure: flavanols, flavonols, flavanones, anthocyanidins, flavones, and isoflavones (4). These subclasses exhibit distinct profiles of bioavailability and bioactivity (5). There is a general agreement of observational studies suggesting an inverse association between total dietary flavonoid intake and body weight (BW) gain, body mass index (BMI), and waist circumference (WC) (6–10). However, further research is needed to clarify links, if any, between subclasses of flavonoids and specific adiposity parameters, such as body fat (BF) percentage and distribution. These associations have frequently been observed in specific flavonoid-rich foods, such as tea (rich in flavanols) or citrus fruits (rich in flavanones) (11,12). Several mechanistic studies have proposed potential metabolic pathways that explain causality of these associations (13,14). Flavonoids play a role in the increment of energy expenditure, the alteration of lipid metabolism, and some anti-inflammatory and antioxidant processes (13,14). Randomised clinical trials (RCTs) also have provided compelling evidence supporting that dietary flavonoids exert obesity lowering effects, particularly in relation to reducing BMI and WC (15). However, evidence for adiposity parameters such as visceral and subcutaneous abdominal fat thickness (VAT and SCAT, respectively) is still limited. We aimed to investigate the associations between dietary intake of flavonoids and different adiposity parameters, including total BF %, VAT and SCAT, VAT:SCAT ratio, body shape index (ABSI) and the traditional BMI, WC, and waist-to-hip ratio (WHR), in a large population-based study in the UK.

METHODS

Study design and population

The Fenland Study is an on-going population-based cohort study designed to investigate the influence of health behaviour or lifestyle and genetic factors on the development of obesity, diabetes, and other metabolic disorders (16). Between 2005 and 2015, 12,435 adult volunteers born between 1950 and 1975 (aged 30 to 65 years) were invited from general practice lists in and around Cambridgeshire, England, from three test sites: Cambridge, Ely, and Wisbech, with a response rate of 27%. Exclusion criteria included presence of diabetes, psychotic or terminal illness, inability to walk unaided, and pregnancy or lactation. In this current study, we excluded those with missing values of dietary intakes, outcomes or covariates (n=866), keeping 11,568 participants as analysis sample. The Cambridge Local Ethics Committee approved the study, and all participants gave written informed consent (16).

Dietary intakes

Participants self-reported habitual diet over the previous 12 months using a 130-item semi-quantitative food frequency questionnaire (FFQ), originally designed for the European Prospective Investigation into Cancer and Nutrition study in the UK (EPIC-Norfolk) (17). Validity of estimating foods and nutrients from the FFQ was assessed with 24-hour recalls and 7-day food records previously (17,18). Total energy, nutrient and flavonoid intakes were estimated using the in-house programme (19) that incorporated the standardised EPIC Nutrient Database (20). Specifically for this analysis, we used a food composition database for flavonoids (21), primarily based on US Department of Agriculture databases (22). The flavonoid databases were enriched by considering retention factors due to varied cooking methods, affecting flavonoid stability differently. Comprehensive analyses of entire recipes and estimation of missing values based on similar foods revealed subtle flavonoid variations, offering valuable insights into culinary practices' complexities and their influence on flavonoid intake (22). In this study, we expressed flavonoid intake as aglycones (mg/d) computed as total and by subclasses.

Anthropometric measures

Height and weight were measured with participants in lightweight clothing and barefoot. Weight was measured using a calibrated electronic scale (TANITA model BC-418 MA; Tanita, Tokyo, Japan). Height was assessed with a wall-mounted stadiometer (SECA 240; Seca, Birmingham, UK). BMI was calculated as weight (kg) divided by height squared (m^2). Using a non-stretchable fibreglass insertion tape (D loop tape; Chasmors Ltd, London, UK), WC was measured midway between the lower rib margin and the iliac crest and hip circumference was measured at the widest level over the greater trochanters. WHR was calculated as the ratio of the WC to the hip circumference. Total BF % was measured using dual energy x-ray absorptiometry (DEXA) (Lunar Prodigy Advanced fan beam scanner; GE Healthcare, Bedford, UK) as described previously (16). VAT and SCAT were measured using a LOGIQ Book XP ultrasound system (GE Healthcare, Bedford, United Kingdom) (16). VAT was defined as the depth (cm) from the peritoneum to the lumbar spine, and SCAT was defined as the depth (cm) from the skin to the linea alba. The VAT:SCAT ratio was calculated as an additional marker of health risk linked to abdominal obesity. We also calculated ABSI using the following formula: $ABSI = WC/(BMI^{2/3} \times Height^{1/2})$ with WC and height in metres and BMI in kg/m^2 (23).

Other variables

A standardised questionnaire was used to collect baseline data on self-reported age, sex, ethnicity, socioeconomic variables, and behavioural lifestyle factors, including alcohol and smoking history. Physical activity was assessed objectively using combined heart rate and uniaxial movement sensor for over 6 days (Actiheart, CamNTech, Cambridge, UK) with individual calibration for heart rate using a treadmill test (24). To estimate intensity time series, free-living data were pre-processed and modelled using a branched equation framework then summarised over time as daily physical activity energy expenditure (kJ/kg/d) (24).

Statistical Analysis

As exposures, we considered intake of total flavonoids as well as intake of its subclasses *i.e.* flavanols (flavan-3-ol monomers, proanthocyanidins and theaflavins), anthocyanidins, flavonols, flavanones, flavones, and isoflavones. Different adiposity-related parameters were assessed as outcomes, including total BF %, VAT, SCAT, VAT:SCAT ratio, BMI, WC, ABSI, and WHR. We reported characteristics of the population by quintiles of total flavonoid intake. We conducted Spearman correlations between dietary flavonoids (total and subclasses) and different food groups that would include plant-based items, comprising beans, cereal products, cocoa products, coffee, fats and oils, fruit, non-alcoholic beverages, nuts and seeds, potatoes, soups and sauces, soya products, teas, vegetables, and wine.

To examine the cross-sectional associations between flavonoid intake and adiposity, we used robust multiple linear regression (25). The exposure variables were log2-transformed due to their right skewness, therefore we presented results as beta values for doubling the intake of flavonoids. For total flavonoids, we also reported results by quintiles of intake testing linear trend by assigning the median of each quintile as a score. The initial probability of false positive findings was set at 5%. Given the number of tests conducted, we applied a correction for multiple comparisons using the Benjamini & Hochberg false discovery rate (FDR) (26,27). Associations were considered significant if they surpassed this correction (FDR $q<0.05$). Models were adjusted for potential confounders selected based on previous knowledge and biological plausibility (28–32). We fitted a first model adjusted for age and sex. We further adjusted for sociodemographic and behavioural factors including ethnicity (European origin/non-European origin), age at completion of full-time education (years), household income (£/year), site (Cambridge/Ely/Wisbech), smoking status (never/former/current), and physical activity energy expenditure (kJ/kg/d). Finally, we fitted a third model further adjusting for dietary factors including intake of: energy (kcal/d), fibre (g/d), vitamin C (mg/d), and different food groups (g/d): alcoholic beverages except wine, dairy products, red and processed meat, fats and creams, poultry and egg, sugar, sugar-sweetened beverages, and

artificially sweetened beverages. Food groups for adjustment were chosen because they generally lacked flavonoid content and may have a detrimental impact on adiposity (33). We visually assessed the linearity assumption by plotting residuals and fitting restricted cubic splines (knots at percentiles 10, 50, and 90) to the most adjusted model. To further manage confounding by energy intake (EI), apart from adjusting the standard multivariate model, we performed an exploratory analysis calculating flavonoids by energy density, standardising the EI in 2,000 kcal/d (flavonoids × 2,000/EI) (34). We also applied a regression residual technique for total EI adjustment (34). We performed further adjustments for BMI for all outcomes except BMI and ABSI. For outcomes like BF %, VAT, SCAT, WC and WHR, this further adjustment meant to isolate the influence of flavonoid intake on those specific aspects of body composition, independent of overall body size. Adjustment was not included for BMI and ABSI to avoid redundancy and collinearity as BMI is inherent in both measurements.

We conducted sensitivity analyses excluding 232 participants who were likely to misreport EI (participants in the bottom and top 1% of total EI). Such misreporting might be common and could introduce bias in estimating the relationship between dietary factors (e.g., flavonoid consumption) and adiposity-related parameters (35). Furthermore, we conducted three additional analyses, excluding participants with specific clinical conditions: HbA1c > 48 mmol/mol or taking oral antidiabetic medication (n=4,293); total cholesterol > 5.5 mmol/L or taking lipid-lowering medication (n=5,255); and average blood pressure ≥ 140 (systolic) or ≥ 90 (diastolic) mmHg based on three measurements of blood pressure, or taking anti-hypertensive medication (n=1,697). For a more comprehensive understanding of how the relationship between total flavonoid consumption and adiposity would vary across different subgroups (sex, age, and smoking status) (28,36), we conducted post hoc analyses to explore interactions. We incorporated the interaction term into our model using p-interactions as the assessment metric. We categorised participants according to sex (male/female), age (<48/≥48 years), and smoking status (never/former/current). We set the age cut-

off point based on the mean age of the study population (48 years). All analyses were conducted using R (RStudio version 1.2.5033) (37).

RESULTS

Descriptive characteristics

We included 11,568 adult participants, aged between 29 and 64 years. Median (IQR) of total flavonoid consumption was 428 (282) mg/d. In the highest quintile of total flavonoid intake (median (IQR) ~700 (135) mg/d) (**Table 1**), participants were more likely to be women (56%) than the lowest quintile (~170 (94) mg/d) (% women=50). Flavanols had the highest median intake [340 (252) mg/d], particularly the subclasses flavan-3-ols [163 (198) mg/d] and proanthocyanidins [139 (102) mg/d]. In contrast, isoflavones had the lowest median intake [1 (2) mg/d]. According to Spearman correlation analysis (**Figure 1**), most food groups showed positive correlations with different flavonoids. The strongest ones were observed for the food group 'teas' and theaflavins (coefficient 1.00), flavan-3-ols (0.97), flavonols (0.88), flavanols (0.85) and total flavonoids (0.80). The group 'fruit' also showed strong correlations with anthocyanidins (0.79) and proanthocyanidins (0.76). Isoflavones showed a moderate-strong correlation with cocoa products (0.65).

Flavonoid intake and adiposity

In the most adjusted model and after FDR correction, total flavonoid intake was inversely associated with the majority of the adiposity parameters assessed. Specifically, per doubling total flavonoid intake, total BF % was lower by $beta_{log2}$ -0.55 (95% CI: -0.70; -0.40); VAT -0.13 cm (-0.17; -0.08); and SCAT -0.05 cm (-0.08; -0.02) (**Figure 2** and **Table 2**). It also showed negative associations with BMI -0.33 kg/m² (-0.44; -0.22); WC -0.84 cm (-1.13; -0.55); and WHR -0.004 (-0.006; -0.002) (**Figure 2** and **Supplementary table 1**). The VAT:SCAT ratio showed $beta_{log2}$ -0.02 (95%CI: -0.04; -0.00)] with FDR q value = 0.05. When categorising total flavonoid intakes, similar associations were indicated (**Table 3**). Regarding subclasses, most of them displayed inverse

associations with various adiposity outcomes (**Figure 2** and **Table 2**) highlighting BF %, VAT, BMI, WC and WHR. The exception were the isoflavones, which showed positive associations with total BF %, VAT, and WC.

Other analyses

After calculating flavonoid intake according to energy density or taking residuals regressed on EI, results followed the same direction as the standard multivariate model (**Supplementary table 2**). BMI exhibited strong correlations with WC at 0.85 and total BF % at 0.78, along with moderate correlations with VAT at 0.60, WHR at 0.52 and SCAT at 0.54. Upon the additional adjustment for BMI, we observed some considerable changes (**Supplementary table 3**). Most associations were attenuated. We observed the most evident changes for WC, as associations of flavonoid variables with WC were substantially weakened or nullified after adjusting for BMI. Specifically, intakes of proanthocyanidins and anthocyanidins showed the strongest negative associations with BF %, VAT, VAT:SCAT, WC and WHR, independently of measured BMI.

After excluding probable misreporters of EI (n=11,336 remaining) (**Supplementary table 4**), following FDR correction, SCAT showed a notable change with non-significant results for all exposure variables. In addition, isoflavones showed stronger positive associations with total BF %, VAT, and WC. The exploratory analyses excluding participants with alteration of different metabolic parameters attenuated results (**Supplementary table 5**). In participants with levels of Hb1Ac < 48 mmol/mol or no oral antidiabetic use (n=7,275), total flavonoid intake, as well as most subclasses, showed log2-linear inverse associations with the majority of adiposity parameters, except ABSI, SCAT, and VAT:SCAT ratio, where associations were mostly null. Similar results were observed when only participants with total cholesterol levels <5.5 mmol/l or without use of lipid lowering medication were assessed (n=6,313). In participants with average blood pressure < 140 (systolic), < 90 (diastolic) mmHg or without use of antihypertensive medication (n=9,871), associations were very similar to those observed for total population. In interaction analyses

(**Supplementary tables 6 to 8**) when comparing sexes, total flavonoids intakes were inversely associated with VAT and VAT:SCAT ratio in women but not men (p interaction <0.05 for each), and inverse associations were stronger in older versus younger participants for VAT, VAT:SCAT, ABSI, and WHR (p interaction <0.05 for each). In addition, we observed inverse associations between total flavonoid intake and total BF %, SCAT, and BMI for never and former smokers, but not for current smokers (p interaction<0.05 for each).

DISCUSSION

Intakes of total flavonoids and most of flavonoid subclasses were associated with lower adiposity parameters, especially total BF %, VAT, BMI, WC, and WHR according to this cross-sectional analysis of the British population-based cohort. Exceptionally, positive associations were observed between isoflavone intakes and total BF %, VAT and WC. Whereas we could not establish causality, this study would suggest a negative association between total flavonoids and most of flavonoid subclasses and different adiposity parameters.

Previous observational studies have reported inverse associations between flavonoids and adiposity. In longitudinal findings from a sub-cohort of the EPIC study, evaluating approximately 350,000 European participants, higher intakes of total flavonoids and subclasses, as well as most individual compounds, were inversely associated with 5-year BW gain (7,8). Similarly, in three prospective studies in health professionals from the US, higher consumption of most flavonoid subclasses including flavonols, flavan-3-ols, anthocyanins and flavonoid polymers was inversely associated with BW change over 4-year time intervals (6). A cross-sectional study in Korea linked higher flavonoid intake to lesser abdominal obesity and overall obesity, determined by BF % in women, but not in men among whom a positive association was seen (38). Our analysis of flavonoid intake and adiposity parameters showed similar associations between men and women. Similar sex non-specific findings were observed in cross-sectional studies in Poland and the UK twin study (39,40). The latter UK study and ours are, to our knowledge, the only studies that

evaluated DEXA-derived adiposity measures. Evidence based on DEXA and other imaging instruments is required to advance our understanding of flavonoid-adiposity associations.

Different interventional studies have explored the link between flavonoids and adiposity parameters, interventions that often include a single flavonoid-rich food or supplementation with specific compounds. For example, an RCT including 30 Japanese healthy adults evaluated the effect of a daily dose of 50 or 100 mg theaflavin-rich supplement on BW, fat and muscle measured by bioelectrical impedance analysis (41). They observed a significant improvement of total BF and subcutaneous abdominal fat vs placebo after 10 weeks. A meta-analysis of RCTs assessed the effects of flavanol consumption in the form of supplement (e.g., capsules) or specific foods (e.g., tea) on different obesity-related outcomes (BMI, WC, BF %) (42). Flavanols decreased BMI and WC in participants with overweight or obesity (> 25 kg/m^2) and at doses ≥ 500 mg/day. This study reported a null effect of isoflavones that only significantly decreased BMI in subgroups of non-Asian populations and at doses ≥ 75 mg/day. These trials often tested pharmacological doses of flavonoids that are difficult to achieve through habitual dietary intake (43,44). Nonetheless, the combined data from clinical trials and observational studies support the benefits of consuming flavonoids for weight maintenance and favourable fat distribution. Further evaluation focusing on detailed adiposity measurements is warranted for certain flavonoids, such as isoflavones, with weak or unexpected evidence.

In our study, after further adjusting for BMI, results changed and some significant associations were lost, particularly with WC. These changes may reflect the limited power to detect an adiposity specific association independent of an overall body mass, because of their biological and statistical correlations (45). In this study, moderate to strong correlations were observed between BMI and WC (rho=0.85) and the other variables (rho=0.52 to 0.78), as reported in previous studies (45,46). Despite the correlations, proanthocyanidins and anthocyanidins intakes were associated with lower central and visceral adiposity measures, independent of measured BMI. Further mechanistic studies

would be required to explain these specific observations, also focusing on positively correlated bioactive compounds and food sources, such as flavanols or flavonols and teas, or anthocyanidins or proanthocyanidins and fruits.

Atypical results were observed for consumption of isoflavones, which showed positive associations with total BF % and WC. This would raise questions about the potential interplay between this subclass and other dietary factors, notably the correlation observed with cocoa products, and implied the need for accounting for the context of consumption (47). Previous studies primarily explored isoflavones in the context of soybean intakes in Asian populations (48,49). However, our results may have reflected a different setting, such as population characteristics and dietary choices.

Mechanistic studies have provided biologically plausible explanations for the observed inverse associations between flavonoids and adiposity (50), including reduction of food intake, by inducing satiety or reducing craving urges (51) and modulation of adipogenesis and adipocyte lifecycle (52). The latter effect would be likely observed in this study as we modelled an isocaloric condition (53). A significant portion of the consumed flavonoids remains unchanged and reaches the colon, where they are partially metabolised by gut microbiota (54,55). Some metabolites resulting from flavonoid metabolism in the gut include short-chain fatty acids and phenolic compounds that when reabsorbed may act as signalling molecules. They may influence the expression of genes related to lipid metabolism and adiposity, as well as the modulation of inflammation (55). Flavonoids may exhibit prebiotic properties, promoting growth and activity of beneficial gut bacteria, as well as enhancing the integrity of the gut barrier (56). This may prevent the leakage of harmful substances into the bloodstream which can trigger inflammatory responses associated with obesity (55). This flavonoid-microbiota crosstalk, emerging in the literature, underscores the potential role of flavonoids in the regulation of adiposity (13,55).

Strengths and limitations

Some strengths of this study are its large sample size (n=11,568) compared to other studies analysing similar associations (57–59), and the inclusion of several flavonoid subclasses, which allowed the investigation of potentially different associations with adiposity parameters for different flavonoids. The use of DEXA and body fat thickness measurements allowed for a more accurate assessment of BF distribution as markers of central adiposity related to metabolic risk (60). Adjustment for a wide range of potential confounders would be another strength of this study.

This study also has limitations. The cross-sectional design and residual confounding due to unmeasured or imprecisely measured factors limited inference for causal association. The source of confounding most difficult to control might be other constituents of flavonoid-rich foods (61). Despite performing several sensitivity analyses, errors due to dietary misreporting were possibly influential because of using self-reported methods of dietary assessment. In addition, it is important to acknowledge the potential underestimation of flavonoid intake as the flavonoid database did not encompass all potentially consumed flavonoid-rich foods, whereas we employed a comprehensive database with over 1,500 food items, including flavonoid sources in the UK (62,63). Validity, sensitivity and specificity of flavonoid intake estimates must be ascertained in future research. Finally, these results may not be generalizable to different populations (e.g., South Asia, South America or Africa) as the current study sample presented largely European origin, who might overall follow Westernised dietary patterns (64).

CONCLUSION

We observed an inverse association of total flavonoids and most of the flavonoid subclasses, with many adiposity parameters including total BF %, VAT, BMI, WC, and WHR. This suggests that abdominal adiposity might be a potential pathway for the inverse association between flavonoid-rich foods and adiposity-associated metabolic risk. These findings are important for hypothesis

generation and should stimulate further investigation in prospective studies, RCTs, and mechanistic studies of the link between flavonoids and adiposity.

Conflict of Interest

Authors report no conflict of interest.

Funding Disclosure

The Fenland study was funded by the Medical Research Council and the Wellcome Trust. The current work was supported by the Institute of Health Carlos III (co-funded by European Social Fund (ESF) investing in your future) through the grant MV22/00023, Movilidad Acción Estratégica en Salud 2022 Subprograma Estatal de Movilidad. MG-L and RZ-R were also supported by the Institute of Health Carlos III (co-funded by ESF investing in your future) through the PFIS (FI19/00185) and the Miguel Servet II (CPII20/00009) programmes, respectively. NGF and FI acknowledge support from MRC Epidemiology Unit (MC_UU_00006/3). NGF was also supported by the NIHR Cambridge Biomedical Research Centre (nutrition, obesity, endocrinology and metabolism (NOME) theme; NIHR203312). She is an NIHR Senior Investigator. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care. The funders had no role in the design, analysis or writing of this article.

Acknowledgements

We thank all Fenland Study participants for their willingness and time to take part, and to the General Practitioners and practice staff for their help with recruitment. We thank the Fenland PIs and all members of the following teams at the MRC Epidemiology Unit responsible for practical aspects of the study: Study Coordination, Field Epidemiology, Anthropometry Team, Physical Activity Technical Team, IT, Data Management, and Statistics. The authors also thank Emanuella De Lucia Rolfe for anthropometry, DEXA and ultrasound data.

Author contribution

The authors contributed to the present manuscript as follows: RZ-R and NGF designed the research; MG-L performed statistical analysis; MG-L wrote paper—prepared original draft and RZ-R, NGF and FI wrote paper—reviewed and edited the paper. MG-L had primary responsibility for final content. NGF is a Principal Investigator of the Fenland Study. All authors have read and agreed to the published version of the manuscript.

Ethics declarations

The participants in the Fenland study were recruited from general practice lists as the population-based sampling frame. The National Research Ethics Service (NRES), the body that approves the ethics of research involving NHS patients, considered and approved the study through its East of England Cambridge Central Committee. All participants provided written informed consent.

REFERENCES

1. WHO | Overweight and obesity [Internet]. 2014 [cited 2023 Jun 15]. Available from: https://www.who.int/gho/ncd/risk_factors/overweight/en/

2. Ahmad SR. Plant-based diet for obesity treatment. Frontiers in Nutrition [Internet]. 2022 [cited 2023 Feb 22];9. Available from: https://www.frontiersin.org/articles/10.3389/fnut.2022.952553

3. Eichelmann F, Schwingshackl L, Fedirko V, Aleksandrova K. Effect of plant-based diets on obesity-related inflammatory profiles: a systematic review and meta-analysis of intervention trials. Obes Rev. 2016 Nov;17(11):1067–79.

4. Iwashina T. The Structure and Distribution of the Flavonoids in Plants. Journal of Plant Research. 2000;113:287–99.

5. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. The American Journal of Clinical Nutrition. 2005 Jan 1;81(1):230S-242S.

6. Bertoia ML, Rimm EB, Mukamal KJ, Hu FB, Willett WC, Cassidy A. Dietary flavonoid intake and weight maintenance: Three prospective cohorts of 124 086 US men and women followed for up to 24 years. BMJ (Online) [Internet]. 2016 Jan 28 [cited 2021 Sep 7];352. Available from: https://pubmed.ncbi.nlm.nih.gov/26823518/

7. Gil-Lespinard M, Castañeda J, Almanza-Aguilera E, Gómez JH, Tjønneland A, Kyrø C, et al. Dietary Intake of 91 Individual Polyphenols and 5-Year Body Weight Change in the EPIC-PANACEA Cohort. Antioxidants. 2022 Dec;11(12):2425.

8. Castañeda J, Gil-Lespinard M, Almanza-Aguilera E, Llaha F, Gómez JH, Bondonno N, et al. Association between classes and subclasses of polyphenol intake and 5-year body weight changes in the EPIC-PANACEA study. Obesity (Silver Spring). 2023 Apr;31(4):1146–58.

9. Marranzano M, Ray S, Godos J, Galvano F. Association between dietary flavonoids intake and obesity in a cohort of adults living in the Mediterranean area. International Journal of Food Sciences and Nutrition. 2018 Nov 17;69(8):1020–9.

10. Adriouch S, Kesse-Guyot E, Feuillet T, Touvier M, Olié V, Andreeva V, et al. Total and specific dietary polyphenol intakes and 6-year anthropometric changes in a middle-aged general population cohort. International Journal of Obesity. 2018 Mar 1;42(3):310–7.

11. Wu CH, Lu FH, Chang CS, Chang TC, Wang RH, Chang CJ. Relationship among habitual tea consumption, percent body fat, and body fat distribution. Obes Res. 2003 Sep;11(9):1088–95.

12. Wang Y, Lloyd B, Yang M, Davis CG, Lee SG, Lee W, et al. Impact of orange juice consumption on macronutrient and energy intakes and body composition in the US population. Public Health Nutr. 2012 Dec;15(12):2220–7.

13. Mahboob A, Samuel SM, Mohamed A, Wani MY, Ghorbel S, Miled N, et al. Role of flavonoids in controlling obesity: molecular targets and mechanisms. Front Nutr. 2023 May 12;10:1177897.

14. Sandoval V, Sanz-Lamora H, Arias G, Marrero PF, Haro D, Relat J. Metabolic Impact of Flavonoids Consumption in Obesity: From Central to Peripheral. Nutrients. 2020 Aug 10;12(8):2393.

15. Akhlaghi M, Ghobadi S, Mohammad Hosseini M, Gholami Z, Mohammadian F. Flavanols are potential anti-obesity agents, a systematic review and meta-analysis of controlled clinical trials. Nutrition, Metabolism and Cardiovascular Diseases. 2018 Jul 1;28(7):675–90.

16. Rolfe EDL, Loos RJF, Druet C, Stolk RP, Ekelund U, Griffin SJ, et al. Association between birth weight and visceral fat in adults. Am J Clin Nutr. 2010 Aug;92(2):347–52.

17. Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R, et al. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. Public Health Nutr. 2001 Jun;4(3):847–58.

18. Lentjes MAH, McTaggart A, Mulligan AA, Powell NA, Parry-Smith D, Luben RN, et al. Dietary intake measurement using 7 d diet diaries in British men and women in the European Prospective Investigation into Cancer-Norfolk study: a focus on methodological issues. Br J Nutr. 2014 Feb 14;111(3):516–26.

19. Welch AA, Luben R, Khaw KT, Bingham SA. The CAFE computer program for nutritional analysis of the EPIC-Norfolk food frequency questionnaire and identification of extreme nutrient values. J Hum Nutr Diet. 2005 Apr;18(2):99–116.

20. Slimani N, Deharveng G, Unwin I, Southgate DAT, Vignat J, Skeie G, et al. The EPIC nutrient database project (ENDB): A first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. European Journal of Clinical Nutrition. 2007 Sep;61(9):1037–56.

21. Zamora-Ros R, Knaze V, Luján-Barroso L, Romieu I, Scalbert A, Slimani N, et al. Differences in dietary intakes, food sources and determinants of total flavonoids between Mediterranean and non-Mediterranean countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. British Journal of Nutrition. 2013 Apr;109(8):1498–507.

22. Bhagwat S, Haytowitz D. USDA Database for the Flavonoid Content of Selected Foods, Release 3.2. U.S. Department of Agriculture, Agricultural Research Service. US Department of Agriculture, Agricultural Research Service [Internet]. 2015 Sep; Available from: Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/flav

23. Krakauer NY, Krakauer JC. An Anthropometric Risk Index Based on Combining Height, Weight, Waist, and Hip Measurements. J Obes. 2016;2016:8094275.

24. Brage S, Ekelund U, Brage N, Hennings MA, Froberg K, Franks PW, et al. Hierarchy of individual calibration levels for heart rate and accelerometry to measure physical activity. J Appl Physiol (1985). 2007 Aug;103(2):682–92.

25. Møller SF, R. Bro J von F. Robust methods for multivariate data analysis. Journal of Chemometrics. 2006 May 5;19(10):549–63.

26. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological). 1995;57(1):289–300.

27. Verhoeven KJF, Simonsen KL, McIntyre LM. Implementing false discovery rate control: increasing your power. Oikos. 2005;108(3):643–7.

28. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in Diet and Lifestyle and Long-Term Weight Gain in Women and Men. New England Journal of Medicine. 2011 Jun 23;364(25):2392–404.

29. Cohen AK, Rai M, Rehkopf DH, Abrams B. Educational attainment and obesity: A systematic review. Obesity Reviews. 2013 Dec;14(12):989–1005.

30. Slavin JL. Dietary fiber and body weight. Nutrition. 2005;21(3):411–8.

31. Kirby JB, Liang L, Chen HJ, Wang Y. Race, Place, and Obesity: The Complex Relationships Among Community Racial/Ethnic Composition, Individual Race/Ethnicity, and Obesity in the United States. Am J Public Health. 2012 Aug;102(8):1572–8.

32. Kim TJ, Von Dem Knesebeck O. Income and obesity: what is the direction of the relationship? A systematic review and meta-analysis. BMJ Open. 2018 Jan;8(1):e019862.

33. Schlesinger S, Neuenschwander M, Schwedhelm C, Hoffmann G, Bechthold A, Boeing H, et al. Food Groups and Risk of Overweight, Obesity, and Weight Gain: A Systematic Review and Dose-Response Meta-Analysis of Prospective Studies. Adv Nutr. 2019 Mar;10(2):205–18.

34. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997 Apr;65(4 Suppl):1220S-1228S; discussion 1229S-1231S.

35. Mendez MA, Popkin BM, Buckland G, Schroder H, Amiano P, Barricarte A, et al. Alternative Methods of Accounting for Underreporting and Overreporting When Measuring Dietary Intake-Obesity Relations. American Journal of Epidemiology. 2011 Feb 15;173(4):448–58.

36. Graff-Iversen S, Hewitt S, Forsén L, Grøtvedt L, Ariansen I. Associations of tobacco smoking with body mass distribution; a population-based study of 65,875 men and women in midlife. BMC Public Health. 2019 Nov 1;19(1):1439.

37. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing.; 2022. Available from: https://www.R-project.org/

38. Kim SA, Kim J, Jun S, Wie GA, Shin S, Joung H. Association between dietary flavonoid intake and obesity among adults in Korea. Applied Physiology, Nutrition and Metabolism. 2020;45(2):203–12.

39. Popiolek-Kalisz J. The Relationship between Dietary Flavonols Intake and Metabolic Syndrome in Polish Adults. Nutrients. 2023 Feb 8;15(4):854.

40. Jennings A, MacGregor A, Spector T, Cassidy A. Higher dietary flavonoid intakes are associated with lower objectively measured body composition in women: evidence from discordant monozygotic twins. The American Journal of Clinical Nutrition. 2017 Mar 1;105(3):626–34.

41. Aizawa T, Yamamoto A, Ueno T. Effect of oral theaflavin administration on body weight, fat, and muscle in healthy subjects: a randomized pilot study. Bioscience, Biotechnology, and Biochemistry. 2017 Feb 1;81(2):311–5.

42. Akhlaghi M, Ghobadi S, Hosseini M, Gholami Z, Mohammadian F. Flavanols are potential anti-obesity agents, a systematic review and meta-analysis of controlled clinical trials. Nutrition, Metabolism and Cardiovascular Diseases. 2018 Apr 1;28.

43. Vogiatzoglou A, Mulligan AA, Lentjes MAH, Luben RN, Spencer JPE, Schroeter H, et al. Flavonoid intake in European adults (18 to 64 years). PLoS One. 2015;10(5):e0128132.

44. Knaze V, Zamora-Ros R, Luján-Barroso L, Romieu I, Scalbert A, Slimani N, et al. Intake estimation of total and individual flavan-3-ols, proanthocyanidins and theaflavins, their food sources and determinants in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br J Nutr. 2012 Sep 28;108(6):1095–108.

45. Pereira PF, Serrano HMS, Carvalho GQ, Ribeiro SMR, Peluzio M do CG, Franceschini S do CC, et al. Measurements of body fat distribution: assessment of collinearity with body mass, adiposity and height in female adolescents. Rev paul pediatr. 2015 Mar;33:63–71.

46. Gierach M, Gierach J, Ewertowska M, Arndt A, Junik R. Correlation between Body Mass Index and Waist Circumference in Patients with Metabolic Syndrome. ISRN Endocrinol. 2014 Mar 4;2014:514589.

47. Gherasim A, Arhire LI, Niță O, Popa AD, Graur M, Mihalache L. The relationship between lifestyle components and dietary patterns. Proc Nutr Soc. 2020 Apr;79(3):311–23.

48. Yamagata K, Yamori Y. Potential Effects of Soy Isoflavones on the Prevention of Metabolic Syndrome. Molecules. 2021 Sep 27;26(19):5863.

49. Akhlaghi M, Zare M, Nouripour F. Effect of Soy and Soy Isoflavones on Obesity-Related Anthropometric Measures: A Systematic Review and Meta-analysis of Randomized Controlled Clinical Trials. Adv Nutr. 2017 Sep;8(5):705–17.

50. Oliveira AK de S, de Oliveira E Silva AM, Pereira RO, Santos AS, Barbosa Junior EV, Bezerra MT, et al. Anti-obesity properties and mechanism of action of flavonoids: A review. Crit Rev Food Sci Nutr. 2022;62(28):7827–48.

51. Bhardwaj M, Yadav P, Vashishth D, Sharma K, Kumar A, Chahal J, et al. A Review on Obesity Management through Natural Compounds and a Green Nanomedicine-Based Approach. Molecules. 2021 May 28;26(11):3278.

52. Furuyashiki T, Nagayasu H, Aoki Y, Bessho H, Hashimoto T, Kanazawa K, et al. Tea catechin suppresses adipocyte differentiation accompanied by down-regulation of PPARgamma2 and C/EBPalpha in 3T3-L1 cells. Bioscience, biotechnology, and biochemistry. 2004 Nov;68(11):2353–9.

53. Tomova GD, Arnold KF, Gilthorpe MS, Tennant PWG. Adjustment for energy intake in nutritional research: a causal inference perspective. Am J Clin Nutr. 2021 Jul 27;115(1):189–98.

54. Williamson G, Kay CD, Crozier A. The Bioavailability, Transport, and Bioactivity of Dietary Flavonoids: A Review from a Historical Perspective. Comprehensive Reviews in Food Science and Food Safety. 2018;17(5):1054–112.

55. Xiong HH, Lin SY, Chen LL, Ouyang KH, Wang WJ. The Interaction between Flavonoids and Intestinal Microbes: A Review. Foods. 2023 Jan;12(2):320.

56. Del Bo' C, Bernardi S, Cherubini A, Porrini M, Gargari G, Hidalgo-Liberona N, et al. A polyphenol-rich dietary pattern improves intestinal permeability, evaluated as serum zonulin levels, in older subjects: The MaPLE randomised controlled trial. Clin Nutr. 2021 May;40(5):3006–18.

57. Makarem N, Mossavar-Rahmani Y, Sotres-Alvarez D, Hua S, Wong WW, Van Horn L, et al. The relation between polyphenols and body composition in US Hispanics/Latinos: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) Study of Latinos Nutrition And Physical Activity Assessment Study (SOLNAS). Current Developments in Nutrition [Internet]. 2017 Nov 1 [cited 2022 Apr 25];1(11). Available from: https://pubmed.ncbi.nlm.nih.gov/29955684/

58. Aali Y, Ebrahimi S, Shiraseb F, Mirzaei K. The association between dietary polyphenol intake and cardiometabolic factors in overweight and obese women: a cross-sectional study. BMC Endocr Disord. 2022 May 10;22:120.

59. Alshahrani SH, Al-Attar Z, Daabo HMA, Alshahrani NZ, Al-Shawi SG, Núñez EFD, et al. Dietary polyphenol intake, body composition and components of metabolic syndrome in a sample overweight and obese adults: a cross-sectional study. BMC Endocrine Disorders. 2023 Nov 27;23(1):261.

60. Jayedi A, Soltani S, Zargar MS, Khan TA, Shab-Bidar S. Central fatness and risk of all cause mortality: systematic review and dose-response meta-analysis of 72 prospective cohort studies. BMJ. 2020 Sep 23;370:m3324.

61. Arija V, Abellana R, Ribot B, Ramon J. Biases and adjustments in nutritional assessments from dietary questionnaires. Nutricion hospitalaria. 2015 Feb 27;31:113–8.

62. Flavonoids : USDA ARS [Internet]. [cited 2023 Sep 27]. Available from: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/methods-and-application-of-food-composition-laboratory/mafcl-site-pages/flavonoids/

63. Beking K, Vieira A. An assessment of dietary flavonoid intake in the UK and Ireland. International Journal of Food Sciences and Nutrition. 2011 Feb 1;62(1):17–9.

64. Imamura F, Micha R, Khatibzadeh S, Fahimi S, Shi P, Powles J, et al. Dietary quality among men and women in 187 countries in 1990 and 2010: a systematic assessment. Lancet Glob Health. 2015 Mar;3(3):e132-142.

TABLES AND FIGURES

Table 1. Characteristics of 11,568 participants from the Fenland study across quintiles of total flavonoid consumption.

Characteristics	Total flavonoid intakes by quintile values (Q)				
	Q1 (n=2,314)	Q2 (n=2,313)	Q3 (n=2,314)	Q4 (n=2,313)	Q5 (n=2,314)
Total flavonoid intake, mg/d (cut-off points)	≤ 253.8	>253.9 to 371.3	>371.3 to 481.3	>481.3 to 603.8	>603.8
Sex, % women	50	50	55	57	56
Site, %					
Cambridge	35	37	40	38	38
Ely	38	40	38	37	39
Wisbech	27	23	22	25	23
Ethnicity, % non-European origin	7	8	7	6	7
Household income, £/year					
<20,000	15	11	12	13	14
20,000 to 40,000	34	35	33	35	33
>40,000	51	54	55	52	53
Smoking status					
Never	51	55	58	57	56
Former	33	34	32	32	34
Current	16	11	10	11	10
Age, years	47.7 (12.3)	48.7 (12.6)	49.1 (12.1)	49.2 (11.8)	50.1 (11.0)
PAEE, kJ/kg/day	49.2 (29.8)	49.9 (27.6)	49.9 (28.8)	52.1 (28.3)	52.0 (29.4)
Age end of full-time education, years	17.0 (5.0)	18.0 (5.0)	18.0 (6.0)	18.0 (5.0)	18.0 (5.0)
Dietary intakes					
Energy, kcal/d	1,626 (662)	1,758 (686)	1,841 (702)	1,906 (726)	2,185 (943)
Fibre, g/d	4.7 (8.8)	5.6 (9.4)	5.7 (9.5)	5.5 (9.2)	5.6 (11.2)
Alcohol, g/d	13.4 (6.0)	15.4 (6.6)	17.0 (7.2)	17.6 (7.6)	21.6 (9.7)
Vitamin C, mg/d	90.6 (54.3)	106.0 (60.2)	117.7 (65.0)	119.2 (69.7)	155.8 (91.9)
BMI, kg/m^2	26.7 (6.3)	26.3 (5.8)	25.8 (5.7)	25.8 (5.4)	26.0 (5.6)
WC, cm	91.6 (18.6)	90.6 (19.0)	89.2 (18.7)	88.9 (17.8)	89.7 (18.2)
Flavonoid subclasses intakes					
Flavanols	122.4 (72.3)	246.9 (50.0)	342.2 (57.2)	445.9 (63.1)	572.4 (105.1)
Flavan-3-ols	29.3 (37.2)	145.0 (89.5)	164.9 (26.5)	265.5 (101.9)	239.8 (80.5)
Proanthocyanidins	75.7 (53.5)	112.2 (72.3)	145.3 (69.3)	158.4 (86.4)	232.0 (119.6)
Theaflavins	0.65 (4.0)	23.2 (19.2)	24.2 (20.5)	41.7 (18.5)	42.0 (13.9)
Anthocyanidins	10.9 (9.7)	15.0 (12.8)	18.1 (13.4)	18.5 (15.1)	27.5 (19.8)
Flavonols	14.8 (7.3)	24.8 (7.2)	32.6 (7.7)	41.5 (8.4)	50.9 (10.3)
Flavanones	12.0 (18.0)	19.7 (22.4)	22.4 (30.4)	22.5 (31.0)	38.7 (37.6)
Flavones	1.7 (2.1)	2.4 (2.6)	2.9 (2.8)	3.0 (2.7)	4.2 (3.0)
Isoflavones	1.3 (2.2)	1.4 (2.4)	1.5 (2.6)	1.5 (2.7)	1.6 (2.8)

Continuous variables expressed as median (IQR) if not stated otherwise. Abbreviations: BMI, body mass index; PAEE, physical activity energy expenditure; WC, waist circumference; WHR, waist-to-hip ratio.

Table 2. Associations of total dietary flavonoids and their subclasses with adiposity parameters in 11,568 participants from the Fenland study

Study variables	Body fat %		Visceral fat (VAT), cm		Subcutaneous fat (SCAT), cm		VAT/SCAT		ABSI‡	
	β (95% CI)*	Q†	β (95% CI)	Q	β (95% CI)	Q	β (95% CI)	Q	β (95% CI)×10^{-6}	Q
Mean (SD)	33.0 (10.4) %		4.8 (2.8) cm		2.7 (1.7) cm		1.8 (1.4)		0.08 (0.001)	
Total Flavonoids	-0.55 (-0.70, -0.40)	<0.001	-0.13 (-0.17, -0.08)	<0.001	-0.05 (-0.08, -0.02)	<0.001	-0.02 (-0.04, -0.00)	0.054	-9.1 (-19.2, 0.98)	0.101
Subclasses										
Flavanols	-0.45 (-0.58, -0.32)	<0.001	-0.10 (-0.14, -0.07)	<0.001	-0.04 (-0.06, -0.01)	<0.001	-0.02 (-0.04, -0.00)	0.058	-8.61 (-17.12, 0.04)	0.067
Flavan-3-ols	-0.22 (-0.31, -0.14)	<0.001	-0.05 (-0.08, -0.03)	<0.001	-0.03 (-0.04, -0.01)	<0.001	-0.01 (-0.02, 0.01)	0.367	-2.23 (-7.87, 3.32)	0.469
Proanthocyanidins	-0.64 (-0.81, -0.47)	<0.001	-0.16 (-0.20, -0.11)	<0.001	-0.02 (-0.06, 0.01)	0.163	-0.04 (-0.06, -0.02)	<0.001	-23.82 (-34.64, -13.07)	<0.001
Theaflavins	-0.03 (-0.05, -0.02)	<0.001	-0.01 (-0.01, -0.00)	<0.001	-0.00 (-0.01, -0.00)	0.011	-0.00 (-0.00, 0.00)	0.64	-0.44 (-1.41, 0.55)	0.426
Anthocyanidins	-0.24 (-0.37, -0.11)	<0.001	-0.06 (-0.10, -0.03)	<0.001	0.01 (-0.01, 0.04)	0.314	-0.03 (-0.05, -0.01)	<0.001	-18.63 (-26.50, -11.00)	<0.001
Flavonols	-0.32 (-0.49, -0.15)	<0.001	-0.08 (-0.13, -0.03)	<0.001	-0.04 (-0.08, -0.01)	0.011	-0.01 (-0.04, 0.01)	0.331	-0.03 (-11.32, 10.71)	0.996
Flavanones	-0.17 (-0.25, -0.09)	<0.001	-0.03 (-0.05, -0.00)	0.038	-0.00 (-0.02, 0.01)	0.754	0.00 (-0.01, 0.01)	0.727	-5.12 (-11.01, 0.09)	0.072
Flavones	-0.59 (-0.74, -0.44)	<0.001	-0.10 (-0.15, -0.06)	<0.001	-0.05 (-0.08, -0.03)	<0.001	-0.00 (-0.02, 0.02)	0.849	-4.15 (-13.63, 5.48)	0.433
Isoflavones	0.15 (0.06, 0.23)	<0.001	0.03 (0.01, 0.06)	<0.001	0.01 (-0.01, 0.03)	0.214	0.01 (-0.00, 0.02)	0.284	4.18 (-1.67, 9.90)	0.195

*Results for body mass index, waist circumference, and waist-to-hip ratio are presented in Figure 2 and Supplementary Table 1. Total flavonoids and subclasses were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. †Q value considered statistically significant at <0.05 after false-discovery correction for two-sided alpha=0.05 applied to 11 flavonoid variables and 8 adiposity parameters. ‡Mean and standard deviation for ABSI are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where *m* represents waist circumference in metres, $(kg/m^2)^{-2/3}$ is body mass index raised to -2/3, and $m^{-½}$ is height in metres raised to -1/2 (23). Abbreviations: ABSI, a body shape index; CI, confidence interval; SD, standard deviation.

Table 3. Associations of quintiles of total dietary flavonoids with adiposity parameters in 11,568 participants from the Fenland study

Study variables	β (95% confidence interval) per doubling total flavonoid intake across quintile (Q) groups					p-trend*
	Q1 *n* 2,314	Q2 *n* 2,313	Q3 *n* 2,314	Q4 *n* 2,313	Q5 *n* 2,314	
Total flavonoids median (IQR), mg/d	173 (94)	315 (57)	428 (54)	538 (58)	698 (135)	
Adiposity parameters						
Body mass index, kg/m2	Ref	-0.38 (-0.62; -0.13)	-0.55 (-0.80; -0.30)	-0.65 (-0.90; -0.41)	-0.50 (-0.76; -0.24)	<0.001
Waist circumference, cm	Ref	-0.80 (-1.46; -0.15)	-1.35 (-2.01; -0.70)	-1.61 (-2.26; -0.95)	-1.33 (-2.04; -0.61)	<0.001
Waist-to-hip ratio	Ref	-0.002 (-0.006; 0.002)	-0.004 (-0.008; -0.001)	-0.005 (-0.009; -0.002)	0.006 (-0.011; -0.002)	<0.001
Total body fat, %	Ref	-0.56 (-0.91; -0.21)	-0.86 (-1.21; -0.50)	-0.93 (-1.29; -0.58)	-1.12 (-1.51; -0.73)	0.002
VAF thickness, cm	Ref	-0.09 (-0.19; 0.01)	-0.15 (-0.25; -0.05)	-0.19 (-0.29; -0.09)	-0.26 (-0.37; -0.15)	<0.001
SCAF thickness, cm	Ref	-0.05 (-0.12; 0.01)	-0.10 (-0.16; -0.03)	-0.08 (-0.14; -0.01)	-0.09 (-0.16; -0.02)	0.013
VAT:SCAT ratio	Ref	-0.02 (-0.07; 0.02)	-0.01 (-0.05; 0.04)	-0.03 (-0.08; 0.02)	-0.06 (-0.01; 0.00)	0.054
ABSI‡ ($\times 10^{-6}$)	Ref	-3.23 (-25.82; 20.47)	-6.87 (-31.07; 17.25)	-5.16 (-29.05; 18.72)	-20.07 (-46.75; 5.33)	0.144

Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. *Linear trend test was performed by assigning the median of each quintile as a score. ‡ABSI coefficients are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where m represents waist circumference in metres, (kg/m2)-2/3 is body mass index raised to -2/3, and m-½ is height in metres raised to -1/2; multiplied by 10^{-6} to help improve readability and consistency of the table (23).

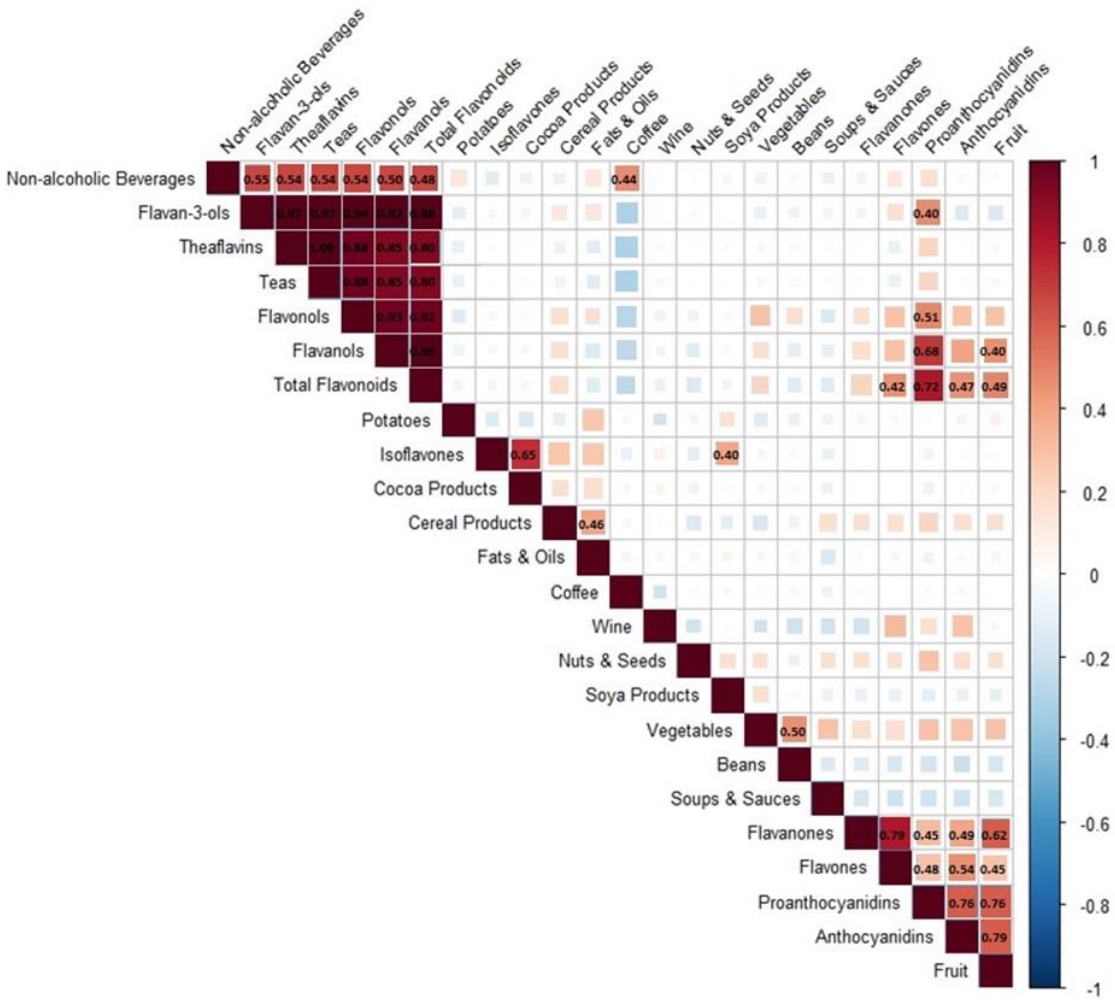


Figure 1. Spearman correlation coefficients among total flavonoids and subclasses, and habitual intakes of selected food groups among 11,568 participants from the Fenland study, UK.
The dietary items are arranged by hierarchical clustering order. Filling of cells indicates the strength of the p-value (the bigger, the stronger), whereas colour indicates the strength and direction of the correlations (red, positive; blue, negative).

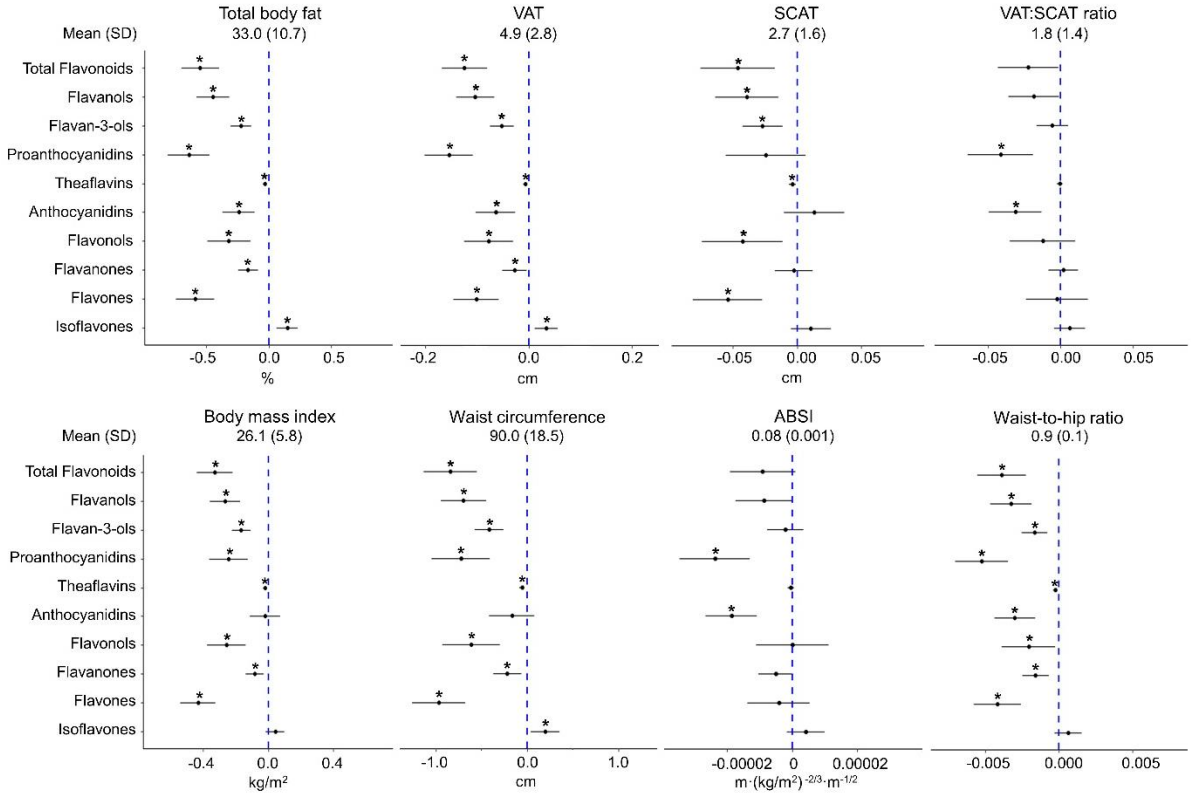


Figure 2. Associations of dietary flavonoids and subclasses with adiposity parameters in 11,568 participants from the Fenland study, UK.

Total flavonoids and subclasses were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Forest plots represent robust linear regression coefficients with their 95% CI adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages.

5. Discussion

This doctoral thesis focused on understanding how (poly)phenols, estimated both as dietary intakes and plasma concentrations, relate to changes in body weight in a European multi-country population. The study also investigated the connection between the intake of flavonoid classes and adiposity in a British population. Insights gained from the EPIC study are noteworthy and go along with previous investigations in the topic. These, together with the evidence from human intervention studies and an examination of the (poly)phenol profile of the Mediterranean diet, have guided the development of a (poly)phenol-rich dietary supplement. This supplement holds promise for potential anti-obesity effects, supported by previous scientific evidence. The results derived from the Fenland study provide new evidence to better understand the role of flavonoids, the most consumed class of (poly)phenols, in adiposity and therefore in cardiometabolic risk. They are valuable for hypothesis generation and should set the stage for future longitudinal and interventional studies.

Each of the studies presented in this doctoral thesis included a discussion section. Nevertheless, it is essential to further discuss several general considerations related to the different areas covered. In addition, it is important to indicate the potential strengths and limitations derived from this work.

5.1. Global discussion

The present study represents a synthesis of diverse scientific evidence, encompassing cross-sectional findings, prospective observations, and a design of a human intervention, with the protocol of a randomised-control trial. The observational component, carried out in various populations in Europe, revealed the following:

1) In the EPIC cohort, when analysing dietary intakes, a beneficial inverse association was observed between higher intake of the majority of individual dietary (poly)phenols, particularly

those classified as flavonoids, and BW change. Additionally, a positive association was observed between HCAs and BW gain.

2) In the EPIC cohort, and working with plasma biomarkers, a suggestive tendency towards body weight loss was noted at higher concentrations of plasma (poly)phenols.

3) In the Fenland study, a strong inverse cross-sectional association was found between higher intake of flavonoids and various adiposity parameters in a British population.

The prospective inverse associations observed in the EPIC population, combined with previous scientific evidence, facilitated the formulation of a dietary supplement incorporating seven (poly)phenolic compounds. These compounds show promise as an effective complementary treatment to diet for people with severe obesity(133–137).

Results from the manuscript 2 presented in the EPIC cohort showed that dietary intake of various individual compounds, mostly classified as flavonoids, such as quercetin glycosides and catechin, exhibited strong inverse associations with BW changes. The results suggested potential benefits for flavonoids and BW management, aligning with the cross sectional observations in the Fenland population, presented in the manuscript 4. Despite measuring different outcomes, both adiposity parameters and BW changes are closely related with obesity(6). Previous observational studies have reported similar associations in different populations. For example, the MEAL study observed that participants with higher consumption of flavonoids resulted less likely to present obesity (BMI $\geq$ 30 kg/m^2)(107); or in three prospective cohorts from the US, where higher intake of flavonoids contributed to BW maintenance(109). In addition, (poly)phenols have not only been analysed individually, but a recent publication explored total, classes and subclasses using a similar methodology in the EPIC-PANACEA cohort, reaching conclusions in the same direction(111). Despite the cross-sectional design of the Fenland study, the incorporation of diverse adiposity parameters, including the gold standard for body fat measurement (DEXA) (10,11) and assessments of visceral and subcutaneous adipose tissues, lends substantial support

to the hypothesis of flavonoids as potential anti-obesity agents. It opens the way to the longitudinal study of these same associations in the subsequent phases of the Fenland study(130) and aligns with the selected compounds for formulating the supplement designed for the clinical trial.

The associations between plasma (poly)phenols and BW change showed a suggestive trend, though statistical significance was not achieved. Nevertheless, a tendency toward BW loss was observed. Consistent with the earlier assertion, the two biomarkers that exhibited the most pronounced trend, vanillic acid and naringenin, have been investigated in previous studies as metabolites associated with flavonoid intake(138,139). The metabolites present in the blood do not necessarily represent a specific consumed food. As a significant portion of dietary (poly)phenols reaches the colon with minimal alterations to their chemical structure, it has been suggested that the intestinal microbiota may play a crucial role in their metabolism(67,140,141). This is why many of the biomarkers detected in plasma may result from this colonic interaction. Previous research has proposed a link between (poly)phenols, gut microbiota, and obesity management(67,140). The bioactive compounds generated from gut microbiota breakdown of (poly)phenols may exert anti-inflammatory, antioxidant, and metabolic effects, influencing host physiology and potentially interfering in obesity management(104). While the evidence is promising, it is important to note that research in this field is ongoing, and the specific mechanisms underlying the interactions between (poly)phenols, gut microbiota, and obesity management are complex and multifaceted.

A common approach in nutritional epidemiology involves using findings from observational studies to pinpoint dietary compounds that may influence certain outcomes, and test them in interventional trials(142). The work presented in this thesis suggests that the pursuit of comprehensive insights into the potential benefits of (poly)phenols on obesity management would benefit from a strategic shift toward an RCT employing combinations of (poly)phenolic

compounds. Singular (poly)phenols, while individually showcasing promising bioactive properties, often interact with diverse physiological pathways(143). The synergistic effects of combining different compounds may have the potential to amplify their overall impact on obesity-related markers(144). That way, the complex interplay observed in a diverse diet can be mimicked, providing a more holistic understanding of how (poly)phenols collectively influence metabolic and adiposity outcomes. A multimodal strategy may reflect the reality of dietary (poly)phenol intake, offering a more pragmatic and applicable framework for designing interventions that resonate with real-world dietary patterns.

5.2. Strengths and limitations

The present work has major strengths that are highlighted in the following. This thesis encompasses different levels of scientific evidence, and the first strength to be underlined is the design of both EPIC and Fenland studies. In nutritional epidemiology, a key factor in investigating the relationship between diet and disease is the availability of data from large cohort studies as well as the repeatability and generalizability of results(142). The prospective nature of the EPIC study accounts for selection and recall biases. Its extensive sample size along with prolonged follow-up period stand out as major strengths in ensuring the reliability and applicability of findings across different European populations. Similarly, while the Fenland study's sample is smaller compared to EPIC, it remains representative of the Cambridgeshire population, as the difference lies in the population they represent. Another strength is the utilization of validated FFQs for collecting dietary data, and comprehensive, validated databases such as Phenol-Explorer and the USDA, which were employed to estimate the (poly)phenol content. The methodology employed for estimating plasma (poly)phenols enables the measurement of key representatives for most classes of dietary (poly)phenols in blood. It is distinctive for its requirement of a low plasma volume, high sensitivity, and validation across a large number of plasma samples(126). In the Fenland study, the use of DEXA and fat thickness measurements allowed for a more

accurate assessment of BF distribution as markers of central adiposity related to metabolic risk (60,61). Another major strength of this work is the employment of thorough statistical approaches. The use of multilevel mixed linear regression models (EPIC study) incorporating both fixed and random effects, addressed the complexities of real-world data structures and enhanced the precision in the estimations(145). The use of FDR to correct for multiple comparisons enhances the reliability of statistical findings by controlling the rate of false discoveries. The design of both EPIC and Fenland studies allowed adjusting the statistical models for a large number of relevant confounders related to lifestyle, diet, and health status. Finally, a major strength of the work conducted in this thesis is the transition from an observational study to an interventional study. The design of a parallel, double-blinded, placebo-controlled clinical trial allowed for the culmination of the hypothesis with a study situated at one of the highest levels of scientific evidence.

Several limitations are derived from this work. The impact of measurement error and misreporting needs to be considered when interpreting results of observational studies(142). In the case of EPIC, various variables, such as dietary intakes and plasma concentrations of (poly)phenols, along with several confounders, were only assessed at baseline, limiting the ability to account for changes during follow-up. Regarding the Fenland study, the data utilized originated from phase I, introducing a cross-sectional study design that exacerbates the potential for reverse causation. While employing validated FFQs to gather dietary data, it is important to note that these questionnaires were not specifically validated for (poly)phenols, representing a common limitation in observational studies assessing these compounds(87). Due to the observational nature of both EPIC and Fenland studies, the possibility of residual confounding cannot be ruled out. Additionally, these results are not generalizable to diverse populations (e.g., South Asia, South America or Africa), as the majority of the sample comprises White European participants, who overall have Westernized dietary patterns(65). Despite transitioning from observational findings to the design of an intervention, it is important to note that the proposed clinical trial's

duration is much shorter than that of a cohort follow-up. Therefore, within the 12-week monitoring period of the clinical trial, the anticipated outcomes are expected to result from the combination of a hypocaloric diet and the (poly)phenol supplement. Also, unlike EPIC and Fenland population, participants selected for the RCT will present severe obesity, with a BMI equal to or higher than 40 kg/m^2, which limits the generalizability of the findings to people with lower levels of obesity or different demographic profile.

6. Conclusions

The conclusions drawn from the investigations included in this thesis are presented in response to each objective specified at the beginning of this document.

6.1. Conclusion 1

In response to objective 1, higher intakes of the majority of individual dietary (poly)phenols were inversely associated with 5-year body weight change in the EPIC population, highlighting those derived from whole grain cereals, berries, teas, and cocoa. Individual HCAs may have different roles in body weight change depending on their dietary source.

6.2. Conclusion 2

In response to objective 2, higher concentrations of the majority of plasma (poly)phenols showed a tendency towards body weight loss in the EPIC population, though observations were not statistically significant. Among specific (poly)phenols, vanillic acid and naringenin can be highlighted, which may act as biomarkers of flavonoid intake. These findings are preliminary and further studies using (poly)phenol biomarkers are needed to better understand the potential observed trends.

6.3. Conclusion 3

In response to objective 3, higher intakes of total flavonoids and most of the subclasses, were inversely associated with many adiposity parameters including total BF percentage, VAT, BMI, WC, and WHR in the Fenland study. Abdominal adiposity might be a potential pathway for the inverse association between flavonoid-rich foods and adiposity-associated metabolic risk.

6.4. Conclusion 4

In response to objective 4, a (poly)phenol-rich dietary supplement was meticulously formulated. This formulation is grounded in a blend of individual compounds widely used in previous trials (such as green tea and blueberry extract), combined with key compounds derived from

Mediterranean dietary elements (including olives, citrus fruits, grapes, wholegrain cereals, and onions). Noteworthy components encompass catechin, epigallocatechin, anthocyanidins, hydroxytyrosol, ferulic acid, hesperidin, and quercetin. A resultant 400 mg capsule will be recommended at a daily intake of 3 capsules, amounting to 1,200 mg of combined extracts (approximately 830 mg of (poly)phenols). This dosage mirrors the average daily intake of dietary (poly)phenols observed in the European population, providing a promising intervention for improving obesity markers in adults with severe obesity.

6.5. Conclusion 5

In response to objective 5, a double-blinded, randomised, placebo-controlled clinical trial was designed and conducted. This intervention study is expected to provide evidence on the effects of a combination of (poly)phenols on several well-established obesity and cardiometabolic markers, and to unravel possible underlying mechanisms by metabolomic analyses.

7. Future Perspectives

The global obesity epidemic and its associated cardiometabolic risks can be mitigated through lifestyle changes, with dietary choices serving as a crucial pillar in prevention and treatment strategies. The findings from this thesis contribute valuable scientific evidence, shedding light on the role of (poly)phenols as bioactive compounds present in plant-based foods. Specifically, their impact on preventing obesity and influencing adiposity-related parameters in European adults is explored. This evidence not only adds to our understanding but also holds potential for guiding future studies across diverse populations. Moreover, it has implications for shaping (poly)phenol dietary recommendations aimed at preventing or treating health risks associated with obesity.

Based on the results presented in this thesis, several important aspects can be taken into consideration for future research:

- The various investigations conducted primarily involved participants of White-European descent. To generalise findings to the broader population, it is essential to conduct similar large observational studies in diverse cohorts, encompassing participants from different ethnicities, geographical areas, and dietary patterns.
- To assess the potential benefits of (poly)phenols in people with diverse health statuses, the design of longer randomised, controlled trials is needed to complement shorter pilot studies conducted in specific populations.
- To advance the understanding of obesity management and associated health risks, future mechanistic studies are needed. A particularly promising pathway is the exploration of the microbiota-(poly)phenol relationship, a novel area of research. Unravelling the specifics of this relationship holds the potential to elucidate key associations. Such understanding is important in designing innovative preventive and therapeutic measures to address chronic diet-related diseases and enhance human health.
- To overcome the limitations of subjective dietary measurement methods, the inclusion of metabolomics emerges as a valuable tool. Metabolomics not only facilitates the identification of the metabolic fingerprint associated with varied (poly)phenol consumption but also

strengthens the capacity to obtain a comprehensive and objective representation of dietary patterns.

8. Take Home Message

Take Home Message

The findings of this research bear several implications for public health, offering valuable insights into preventive and therapeutic strategies for obesity-associated health risks. (Poly)phenols found in plant-based foods emerge as promising agents in these strategies, presenting opportunities for dietary recommendations aimed at obesity management. The development of a (poly)phenol-rich dietary supplement further supports evidence-based obesity treatment. The incorporation of metabolomics enhances our understanding of the metabolic impact of (poly)phenols, paving the way for personalized health strategies. Advocating for studies in diverse populations acknowledges the importance of global representation, ensuring the generalizability of findings. The emphasis on a holistic approach in randomised control trials, utilizing combinations of (poly)phenolic compounds, reflects the complexity of real-world dietary patterns. Overall, these specific, detailed insights contribute to public health by suggesting ways for future research and intervention strategies, ultimately promoting healthier lifestyles and addressing the challenges of obesity on a broader scale.

9. References

References

1. Controlling the global obesity epidemic [Internet]. [cited 2023 Feb 8]. Available from: https://www.who.int/activities/controlling-the-global-obesity-epidemic

2. A healthy lifestyle - WHO recommendations [Internet]. [cited 2023 Oct 11]. Available from: https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations

3. Abdelaal M, le Roux CW, Docherty NG. Morbidity and mortality associated with obesity. Ann Transl Med. 2017 Apr;5(7):161.

4. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, et al. General and abdominal adiposity and risk of death in Europe. N Engl J Med. 2008 Nov 13;359(20):2105–20.

5. Baioumi AYAA. Chapter 3 - Comparing Measures of Obesity: Waist Circumference, Waist-Hip, and Waist-Height Ratios. In: Watson RR, editor. Nutrition in the Prevention and Treatment of Abdominal Obesity (Second Edition) [Internet]. Academic Press; 2019 [cited 2023 Oct 11]. p. 29–40. Available from: https://www.sciencedirect.com/science/article/pii/B9780128160930000033

6. McGee S. Chapter 13 - Obesity. In: McGee S, editor. Evidence-Based Physical Diagnosis (Fourth Edition) [Internet]. Philadelphia: Elsevier; 2018 [cited 2023 Oct 11]. p. 85-88.e1. Available from: https://www.sciencedirect.com/science/article/pii/B9780323392761000135

7. Waist circumference and waist-hip ratio: report of a WHO expert consultation [Internet]. [cited 2023 Oct 11]. Available from: https://www.who.int/publications-detail-redirect/9789241501491

8. Björntorp P. Obesity. The Lancet. 1997 Aug 9;350(9075):423–6.

9. Zhu S, Heo M, Plankey M, Faith MS, Allison DB. Associations of body mass index and anthropometric indicators of fat mass and fat free mass with all-cause mortality among women in the first and second National Health and Nutrition Examination Surveys follow-up studies. Ann Epidemiol. 2003 Apr;13(4):286–93.

10. Branski LK, Norbury WB, Herndon DN, Chinkes DL, Cochran A, Suman O, et al. Measurement of Body Composition: is there a Gold Standard? JPEN J Parenter Enteral Nutr. 2010;34(1):55–63.

11. Hussain Z, Jafar T, Zaman M uz, Parveen R, Saeed F. Correlations of skin fold thickness and validation of prediction equations using DEXA as the gold standard for estimation of body fat composition in Pakistani children. BMJ Open. 2014 Apr 22;4(4):e004194.

12. Hoffmann J, Thiele J, Kwast S, Borger MA, Schröter T, Schmidt J, et al. A new approach to quantify visceral fat via bioelectrical impedance analysis and ultrasound compared to MRI. Int J Obes. 2023 Oct 27;1–9.

13. Frank AP, de Souza Santos R, Palmer BF, Clegg DJ. Determinants of body fat distribution in humans may provide insight about obesity-related health risks. J Lipid Res. 2019 Oct;60(10):1710–9.

14. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev. 2000 Dec;21(6):697–738.

15. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020 Oct 17;396(10258):1204–22.

16. World Obesity Federation [Internet]. [cited 2023 Apr 3]. World Obesity Atlas 2023. Available from: https://www.worldobesity.org/resources/resource-library/world-obesity-atlas-2023

17. INE [Internet]. [cited 2023 Feb 8]. Índice de masa corporal según grupos de edad y periodo. Available from: https://www.ine.es/jaxi/Datos.htm?path=/t00/mujeres_hombres/tablas_1/l0/&file=d06001.px#!tabs-grafico

18. Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, et al. The global obesity pandemic: shaped by global drivers and local environments. The Lancet. 2011 Aug 27;378(9793):804–14.

19. Lancet T. Urgently needed: a framework convention for obesity control. The Lancet. 2011 Aug 27;378(9793):741.

20. Jukaku SA, Williams SRP. The cause of obesity is multifactorial but GPs can do more. BMJ. 2021 Apr 13;373:n956.

21. Popkin BM, Barquera S, Corvalan C, Hofman KJ, Monteiro C, Ng SW, et al. Toward unified and impactful policies for reducing ultraprocessed food consumption and promoting healthier eating globally. The lancet Diabetes & endocrinology. 2021 Jul 1;9(7):462.

22. Khanna D, Welch BS, Rehman A. Pathophysiology of Obesity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Jun 1]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK572076/

23. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation. 2003 Dec 15;112(12):1796–808.

24. Chait A, den Hartigh LJ. Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. Front Cardiovasc Med. 2020 Feb 25;7:22.

25. Davies NM, Holmes MV, Smith GD. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018 Jul 12;362:k601.

26. Harrison S, Dixon P, Jones HE, Davies AR, Howe LD, Davies NM. Long-term cost-effectiveness of interventions for obesity: A mendelian randomisation study. PLoS Med. 2021 Aug 27;18(8):e1003725.

27. Busutil R, Espallardo O, Torres A, Martínez-Galdeano L, Zozaya N, Hidalgo-Vega Á. The impact of obesity on health-related quality of life in Spain. Health Qual Life Outcomes. 2017 Oct 10;15:197.

28. Santos I, Sniehotta FF, Marques MM, Carraça EV, Teixeira PJ. Prevalence of personal weight control attempts in adults: a systematic review and meta-analysis. Obes Rev. 2017 Jan;18(1):32–50.

29. Cannon CP, Kumar A. Treatment of overweight and obesity: Lifestyle, pharmacologic, and surgical options. Clinical Cornerstone. 2009 Jan 1;9(4):55–71.

30. Bray GA, Frühbeck G, Ryan DH, Wilding JPH. Management of obesity. The Lancet. 2016 May 7;387(10031):1947–56.

31. Srivastava G, Apovian CM. Current pharmacotherapy for obesity. Nat Rev Endocrinol. 2018 Jan;14(1):12–24.

32. EMA. European Medicines Agency. 2018 [cited 2023 Jun 7]. Xenical. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/xenical

33. Chao AM, Tronieri JS, Amaro A, Wadden TA. Semaglutide for the treatment of obesity. Trends Cardiovasc Med. 2023 Apr;33(3):159–66.

34. EMA. European Medicines Agency. 2021 [cited 2023 Jun 7]. Wegovy. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/wegovy

35. EMA. European Medicines Agency. 2018 [cited 2023 Jun 7]. Saxenda. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/saxenda

36. Benaiges D, Goday A, Pedro-Botet J, Más A, Chillarón JJ, Flores-Le Roux JA. Bariatric surgery: to whom and when? Minerva Endocrinol. 2015 Jun;40(2):119–28.

37. Teixeira PJ, Marques MM. Health Behavior Change for Obesity Management. Obes Facts. 2017;10(6):666–73.

38. Puhl RM, Heuer CA. Obesity stigma: important considerations for public health. Am J Public Health. 2010 Jun;100(6):1019–28.

39. The Stigma of Obesity: A Review and Update - Puhl - 2009 - Obesity - Wiley Online Library [Internet]. [cited 2023 Jun 7]. Available from: https://onlinelibrary.wiley.com/doi/full/10.1038/oby.2008.636

40. UNDP [Internet]. [cited 2023 Feb 22]. Goal 3: Good health and well-being | Sustainable Development Goals | United Nations Development Programme. Available from: https://www.undp.org/sustainable-development-goals/good-health

41. EAT [Internet]. [cited 2023 Feb 22]. The Planetary Health Diet. Available from: https://eatforum.org/eat-lancet-commission/the-planetary-health-diet-and-you/

42. Gibbs J, Cappuccio FP. Plant-Based Dietary Patterns for Human and Planetary Health. Nutrients. 2022 Apr 13;14(8):1614.

43. Ahmad SR. Plant-based diet for obesity treatment. Frontiers in Nutrition [Internet]. 2022 [cited 2023 Feb 22];9. Available from: https://www.frontiersin.org/articles/10.3389/fnut.2022.952553

44. Greger M. A Whole Food Plant-Based Diet Is Effective for Weight Loss: The Evidence. Am J Lifestyle Med. 2020;14(5):500–10.

45. Langmann F, Ibsen DB, Tjønneland A, Olsen A, Overvad K, Dahm CC. Adherence to the EAT-Lancet diet in midlife and development in weight or waist circumference after five years in a Danish cohort. Dialogues in Health. 2023 Dec 1;3:100151.

46. Gómez-Donoso C, Martínez-González MÁ, Martínez JA, Gea A, Sanz-Serrano J, Perez-Cueto FJA, et al. A Provegetarian Food Pattern Emphasizing Preference for Healthy Plant-Derived Foods Reduces the Risk of Overweight/Obesity in the SUN Cohort. Nutrients [Internet]. 2019 Jul [cited 2023 Feb 22];11(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6683267/

47. Ea S, Pn A, Gk D, Tj K. Diet and body mass index in 38000 EPIC-Oxford meat-eaters, fish-eaters, vegetarians and vegans. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity [Internet]. 2003 Jun [cited 2023 Feb 22];27(6). Available from: https://pubmed.ncbi.nlm.nih.gov/12833118/

48. Mishra S, Xu J, Agarwal U, Gonzales J, Levin S, Barnard ND. A multicenter randomized controlled trial of a plant-based nutrition program to reduce body weight and cardiovascular risk in the corporate setting: the GEICO study. European Journal of Clinical Nutrition. 2013 Jul;67(7):718.

49. Gm TM, Cr D, Ee W, S W, Ea F. Comparative effectiveness of plant-based diets for weight loss: a randomized controlled trial of five different diets. Nutrition (Burbank, Los Angeles County, Calif) [Internet]. 2015 Feb [cited 2023 Feb 22];31(2). Available from: https://pubmed.ncbi.nlm.nih.gov/25592014/

50. Kahleova H, Petersen KF, Shulman GI, Alwarith J, Rembert E, Tura A, et al. Effect of a Low-Fat Vegan Diet on Body Weight, Insulin Sensitivity, Postprandial Metabolism, and Intramyocellular and Hepatocellular Lipid Levels in Overweight Adults: A Randomized Clinical Trial. JAMA Network Open [Internet]. 2020 Nov [cited 2023 Feb 22];3(11). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7705596/

51. Polyphenols - MeSH - NCBI [Internet]. [cited 2023 Feb 22]. Available from: https://www.ncbi.nlm.nih.gov/mesh?sort=date&term=Polyphenols

52. Rana A, Samtiya M, Dhewa T, Mishra V, Aluko RE. Health benefits of polyphenols: A concise review. Journal of Food Biochemistry. 2022;46(10):e14264.

53. Zamora-Ros R, Knaze V, Rothwell JA, Hémon B, Moskal A, Overvad K, et al. Dietary polyphenol intake in Europe: The European Prospective Investigation into Cancer and nutrition (EPIC) study. European Journal of Nutrition. 2016 Jun 17;55(4):1359–75.

54. Carnauba RA, Hassimotto NMA, Lajolo FM. Estimated dietary polyphenol intake and major food sources of the Brazilian population. Br J Nutr. 2021 Aug 14;126(3):441–8.

55. Pérez-Jiménez J, Fezeu L, Touvier M, Arnault N, Manach C, Hercberg S, et al. Dietary intake of 337 polyphenols in French adults. American Journal of Clinical Nutrition. 2011 Jun 1;93(6):1220–8.

56. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. In: Journal of Nutrition [Internet]. American Institute of Nutrition; 2000 [cited 2022 Oct 11]. Available from: https://www.researchgate.net/publication/322611041_Dietary_Intake_and_Bioavailability_of_Polyphenols

57. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270–8.

58. Ross AB, Shepherd MJ, Schüpphaus M, Sinclair V, Alfaro B, Kamal-Eldin A, et al. Alkylresorcinols in cereals and cereal products. J Agric Food Chem. 2003 Jul 2;51(14):4111–8.

59. Knaze V, Rothwell JA, Zamora-Ros R, Moskal A, Kyrø C, Jakszyn P, et al. A new food-composition database for 437 polyphenols in 19,899 raw and prepared foods used to estimate polyphenol intakes in adults from 10 European countries. The American Journal of Clinical Nutrition. 2018 Sep 1;108(3):517–24.

60. Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L, et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database : the journal of biological databases and curation [Internet]. 2010 [cited 2021 Dec 3];2010. Available from: https://pubmed.ncbi.nlm.nih.gov/20428313/

61. Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem Int Ed Engl. 2011 Jan 17;50(3):586–621.

62. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition. 2004 May;79(5):727–47.

63. Crowe-White KM, Evans LW, Kuhnle GGC, Milenkovic D, Stote K, Wallace T, et al. Flavan-3-ols and Cardiometabolic Health: First Ever Dietary Bioactive Guideline. Adv Nutr. 2022 Dec 22;13(6):2070–83.

64. D'Archivio M, Filesi C, Varì R, Scazzocchio B, Masella R. Bioavailability of the polyphenols: Status and controversies. International Journal of Molecular Sciences. 2010;11(4):1321–42.

65. Di Lorenzo C, Colombo F, Biella S, Stockley C, Restani P. Polyphenols and Human Health: The Role of Bioavailability. Nutrients. 2021 Jan 1;13(1):1–30.

66. Teng H, Chen L. Polyphenols and bioavailability: an update. Critical Reviews in Food Science and Nutrition. 2019 Jul 20;59(13):2040–51.

67. Cardona F, Andrés-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuño MI. Benefits of polyphenols on gut microbiota and implications in human health. J Nutr Biochem. 2013 Aug;24(8):1415–22.

68. Xie L, Vance T, Kim B, Lee SG, Caceres C, Wang Y, et al. Aronia berry polyphenol consumption reduces plasma total and low-density lipoprotein cholesterol in former smokers without lowering biomarkers of inflammation and oxidative stress: a randomized controlled trial. Nutrition Research. 2017 Jan 1;37:67–77.

69. Jin Y, Alimbetov D, George T, Gordon MH, Lovegrove JA. A randomised trial to investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular reactivity and bioavailability of anthocyanins in human subjects. Eur J Clin Nutr. 2011 Jul;65(7):849–56.

70. Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, et al. Flavonoid-Rich Dark Chocolate Improves Endothelial Function and Increases Plasma Epicatechin Concentrations in Healthy Adults. Journal of the American College of Nutrition. 2004 Jun 1;23(3):197–204.

71. Bondonno NP, Bondonno CP, Ward NC, Woodman RJ, Hodgson JM, Croft KD. Enzymatically modified isoquercitrin improves endothelial function in volunteers at risk of cardiovascular disease. British Journal of Nutrition. 2020 Jan;123(2):182–9.

72. Schär MY, Curtis PJ, Hazim S, Ostertag LM, Kay CD, Potter JF, et al. Orange juice-derived flavanone and phenolic metabolites do not acutely affect cardiovascular risk biomarkers: a randomized, placebo-controlled, crossover trial in men at moderate risk of cardiovascular disease. Am J Clin Nutr. 2015 May;101(5):931–8.

73. Ward NC, Hodgson JM, Woodman RJ, Zimmermann D, Poquet L, Leveques A, et al. Acute effects of chlorogenic acids on endothelial function and blood pressure in healthy men and women. Food Funct. 2016 May 18;7(5):2197–203.

74. Lara-Guzmán OJ, Álvarez-Quintero R, Osorio E, Naranjo-Cano M, Muñoz-Durango K. GC/MS method to quantify bioavailable phenolic compounds and antioxidant capacity determination of plasma after acute coffee consumption in human volunteers. Food Research International. 2016 Nov 1;89:219–26.

75. Wightman EL, Haskell-Ramsay CF, Reay JL, Williamson G, Dew T, Zhang W, et al. The effects of chronic trans-resveratrol supplementation on aspects of cognitive function, mood, sleep, health and cerebral blood flow in healthy, young humans. Br J Nutr. 2015 Nov 14;114(9):1427–37.

76. Dietary recommendations for fruit and vegetable intake | Knowledge for policy [Internet]. [cited 2023 May 25]. Available from: https://knowledge4policy.ec.europa.eu/health-promotion-knowledge-gateway/fruit-vegetables-4_en

77. Taguchi C, Kishimoto Y, Fukushima Y, Kondo K, Yamakawa M, Wada K, et al. Dietary intake of total polyphenols and the risk of all-cause and specific-cause mortality in Japanese adults: the Takayama study. Eur J Nutr. 2020 Apr 1;59(3):1263–71.

78. Zamora-Ros R, Biessy C, Rothwell JA, Monge A, Lajous M, Scalbert A, et al. Dietary polyphenol intake and their major food sources in the Mexican Teachers' Cohort. Br J Nutr. 2018 Aug;120(3):353–60.

79. EFSA Panel on Dietetic Products N and A (NDA). Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from

oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), mainte. EFSA Journal. 2011 Apr 1;9(4).

80. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to quercetin and protection of DNA, proteins and lipids from oxidative damage (ID 1647), "cardiovascular system" (ID 1844), "mental state and performance" (ID 1845), and "liver, kidneys" (ID 1846) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal. 2011;9(4):2067.

81. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Safety of synthetic trans-resveratrol as a novel food pursuant to Regulation (EC) No 258/97. EFSA Journal. 2016;14(1):4368.

82. Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, et al. Scientific opinion on the safety of green tea catechins. EFSA Journal [Internet]. 2018 Apr 1 [cited 2021 Dec 15];16(4). Available from: https://www.efsa.europa.eu/en/efsajournal/pub/5239

83. Medicines Agency E. Assessment report on Olea europaea L., folium Final. [cited 2021 Dec 15]; Available from: www.ema.europa.eu/contact

84. Compendium of botanicals | EFSA [Internet]. 2021 [cited 2023 Jun 1]. Available from: https://www.efsa.europa.eu/en/data-report/compendium-botanicals

85. Cousyn G, Dalfrà S, Scarpa B, Geelen J, Anton R, Serafinioo M, et al. Project BELFRIT: Harmonizing the Use of Plants in Food Supplements in the European Union: Belgium, France and Italy – A first Step. European Food and Feed Law Review. 2013;8(3):187–96.

86. Bailey RL. Overview of Dietary Assessment Methods for Measuring Intakes of Foods, Beverages, and Dietary Supplements in Research Studies. Curr Opin Biotechnol. 2021 Aug;70:91–6.

87. Xu Y, Li Y, Ma X, Alotaibi W, Sayec ML, Cheok A, et al. Comparison between dietary assessment methods and biomarkers in estimating dietary (poly)phenol intake. Food Funct. 2023 Feb 6;14(3):1369–86.

88. Kroke A, Klipstein-Grobusch K, Voss S, Möseneder J, Thielecke F, Noack R, et al. Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. The American journal of clinical nutrition. 1999;70(4):439–47.

89. Sharma S, Cao X, Harris R, Hennis AJM, Leske MC, Wu SY, et al. Dietary intake and development of a quantitative food-frequency questionnaire for the Barbados National Cancer Study. Public Health Nutrition. 2007 May;10(5):464–70.

90. Bhagwat S, Haytowitz DB, Holden JM. USDA Database for the Flavonoid Content of Selected Foods Release 3.

91. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. Am J Epidemiol. 2003 Jul 1;158(1):1–13.

92. Mendez MA, Popkin BM, Buckland G, Schroder H, Amiano P, Barricarte A, et al. Alternative Methods of Accounting for Underreporting and Overreporting When Measuring Dietary Intake-Obesity Relations. American Journal of Epidemiology. 2011 Feb 15;173(4):448–58.

93. Spencer JPE, Mohsen MMAE, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. British Journal of Nutrition. 2008 Jan;99(1):12–22.

94. Grubesić RJ, Vuković J, Kremer D, Vladimir-Knezević S. Spectrophotometric method for polyphenols analysis: prevalidation and application on Plantago L. species. J Pharm Biomed Anal. 2005 Sep 15;39(3–4):837–42.

95. Matić P, Sabljić M, Jakobek L. Validation of Spectrophotometric Methods for the Determination of Total Polyphenol and Total Flavonoid Content. J AOAC Int. 2017 Nov 1;100(6):1795–803.

96. Domínguez-Rodríguez G, Plaza M, Marina ML. High-performance thin-layer chromatography and direct analysis in real time-high resolution mass spectrometry of non-extractable polyphenols from tropical fruit peels. Food Research International. 2021 Sep 1;147:110455.

97. López-Fernández O, Domínguez R, Pateiro M, Munekata PES, Rocchetti G, Lorenzo JM. Determination of Polyphenols Using Liquid Chromatography–Tandem Mass Spectrometry Technique (LC–MS/MS): A Review. Antioxidants (Basel). 2020 Jun 2;9(6):479.

98. López-Yerena A, Domínguez-López I, Vallverdú-Queralt A, Pérez M, Jáuregui O, Escribano-Ferrer E, et al. Metabolomics Technologies for the Identification and Quantification of Dietary Phenolic Compound Metabolites: An Overview. Antioxidants (Basel). 2021 May 25;10(6):846.

99. Xu Y, Le Sayec M, Roberts C, Hein S, Rodriguez-Mateos A, Gibson R. Dietary Assessment Methods to Estimate (Poly)phenol Intake in Epidemiological Studies: A Systematic Review. Adv Nutr. 2021 Mar 3;12(5):1781–801.

100. Tabacchi G, Amodio E, Di Pasquale M, Bianco A, Jemni M, Mammina C. Validation and reproducibility of dietary assessment methods in adolescents: a systematic literature review. Public Health Nutr. 2014 Dec;17(12):2700–14.

101. Trakman GL, Forsyth A, Hoye R, Belski R. Developing and validating a nutrition knowledge questionnaire: key methods and considerations. Public Health Nutr. 2017 Oct;20(15):2670–9.

102. Hill EB, Kennedy AJ, Roberts KM, Riedl KM, Grainger EM, Clinton SK. Considerations for Use of the Phenol-Explorer Database to Estimate Dietary (Poly)phenol Intake. J Acad Nutr Diet. 2021 May;121(5):833–4.

103. Finicelli M, Squillaro T, Di Cristo F, Di Salle A, Melone MAB, Galderisi U, et al. Metabolic syndrome, Mediterranean diet, and polyphenols: Evidence and perspectives. Journal of Cellular Physiology. 2019;234(5):5807–26.

104. Aloo SO, Ofosu FK, Kim NH, Kilonzi SM, Oh DH. Insights on Dietary Polyphenols as Agents against Metabolic Disorders: Obesity as a Target Disease. Antioxidants (Basel). 2023 Feb 8;12(2):416.

105. Tanabe H, Pervin M, Goto S, Isemura M, Nakamura Y. Beneficial Effects of Plant Polyphenols on Obesity. Obesity & Control Therapies: Open Access [Internet]. 2017 Dec 28 [cited 2023 Jun 19];4(3). Available from: https://symbiosisonlinepublishing.com/obesity-control-therapies/

106. Zhang Y, Balasooriya H, Sirisena S, Ng K. The effectiveness of dietary polyphenols in obesity management: A systematic review and meta-analysis of human clinical trials. Food Chem. 2023 Mar 15;404(Pt B):134668.

107. Marranzano M, Ray S, Godos J, Galvano F. Association between dietary flavonoids intake and obesity in a cohort of adults living in the Mediterranean area. International Journal of Food Sciences and Nutrition. 2018 Nov 17;69(8):1020–9.

108. Adriouch S, Kesse-Guyot E, Feuillet T, Touvier M, Olié V, Andreeva V, et al. Total and specific dietary polyphenol intakes and 6-year anthropometric changes in a middle-aged general population cohort. International Journal of Obesity. 2018 Mar 1;42(3):310–7.

109. Bertoia ML, Rimm EB, Mukamal KJ, Hu FB, Willett WC, Cassidy A. Dietary flavonoid intake and weight maintenance: three prospective cohorts of 124 086 US men and women followed for up to 24 years. BMJ. 2016 Jan 28;352:i17.

110. Mompeo O, Spector TD, Matey Hernandez M, Le Roy C, Istas G, Le Sayec M, et al. Consumption of Stilbenes and Flavonoids is Linked to Reduced Risk of Obesity Independently of Fiber Intake. Nutrients. 2020 Jun 23;12(6):1871.

111. Castañeda J, Gil-Lespinard M, Almanza-Aguilera E, Llaha F, Gómez JH, Bondonno N, et al. Association between classes and subclasses of polyphenol intake and 5-year body weight changes in the EPIC-PANACEA study. Obesity (Silver Spring). 2023 Apr;31(4):1146–58.

112. Guo X, Tresserra-Rimbau A, Estruch R, Martínez-González MA, Medina-Remón A, Fitó M, et al. Polyphenol Levels Are Inversely Correlated with Body Weight and Obesity in an Elderly Population after 5 Years of Follow Up (The Randomised PREDIMED Study). Nutrients. 2017 May 3;9(5):452.

113. Yang R, Xiu H, Zhou Q, Sun L, Mu H, Li H, et al. Application of Urinary Polyphenol Biomarkers Measured by Liquid Chromatography Tandem Mass Spectrometry to Assess Polyphenol Intake and Their Association with Overweight and Obesity in Free-Living Healthy Subjects. Oxidative Medicine and Cellular Longevity. 2019 Jun 16;2019:e4809836.

114. Azzini E, Giacometti J, Russo GL. Antiobesity Effects of Anthocyanins in Preclinical and Clinical Studies. Oxid Med Cell Longev. 2017;2017:2740364.

115. Akhlaghi M, Ghobadi S, Mohammad Hosseini M, Gholami Z, Mohammadian F. Flavanols are potential anti-obesity agents, a systematic review and meta-analysis of controlled clinical trials. Nutrition, Metabolism and Cardiovascular Diseases. 2018 Jul 1;28(7):675–90.

116. Delpino FM, Figueiredo LM, Caputo EL, Mintem GC, Gigante DP. What is the effect of resveratrol on obesity? A systematic review and meta-analysis. Clin Nutr ESPEN. 2021 Feb;41:59–67.

117. Kim KA, Yim JE. Antioxidative Activity of Onion Peel Extract in Obese Women: A Randomized, Double-blind, Placebo Controlled Study. Journal of Cancer Prevention. 2015 Sep 30;20(3):202–7.

118. Fytili C, Nikou T, Tentolouris N, Tseti IK, Dimosthenopoulos C, Sfikakis PP, et al. Effect of Long-Term Hydroxytyrosol Administration on Body Weight, Fat Mass and Urine Metabolomics: A Randomized Double-Blind Prospective Human Study. Nutrients. 2022 Apr 6;14(7):1525.

119. Llaha F, Zamora-Ros R. The Effects of Polyphenol Supplementation in Addition to Calorie Restricted Diets and/or Physical Activity on Body Composition Parameters: A Systematic Review of Randomized Trials. Frontiers in Nutrition. 2020 Jun 3;7:84.

120. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing.; 2022. Available from: https://www.R-project.org/

121. Riboli E, Hunt K, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutrition. 2002 Dec;5(6b):1113–24.

122. Murphy N, Achaintre D, Zamora-Ros R, Jenab M, Boutron-Ruault MC, Carbonnel F, et al. A prospective evaluation of plasma polyphenol levels and colon cancer risk. International Journal of Cancer. 2018 Oct 1;143(7):1620–31.

123. Zamora-Ros R, Lujan-Barroso L, Achaintre D, Franceschi S, Kyrø C, Overvad K, et al. Blood polyphenol concentrations and differentiated thyroid carcinoma in women from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Am J Clin Nutr. 2021 Jan 4;113(1):162–71.

124. Margetts BM, Pietinen P. European Prospective Investigation into Cancer and Nutrition: Validity studies on dietary assessment methods. International Journal of Epidemiology [Internet]. 1997 [cited 2021 Sep 7];26(SUPPL. 1). Available from: https://pubmed.ncbi.nlm.nih.gov/9126528/

125. Slimani N, Deharveng G, Unwin I, Southgate DAT, Vignat J, Skeie G, et al. The EPIC nutrient database project (ENDB): A first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. European Journal of Clinical Nutrition. 2007 Sep;61(9):1037–56.

126. Achaintre D, Gicquiau A, Li L, Rinaldi S, Scalbert A. Quantification of 38 dietary polyphenols in plasma by differential isotope labelling and liquid chromatography electrospray ionization tandem mass spectrometry. J Chromatogr A. 2018 Jul 13;1558:50–8.

127. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. European Journal of Clinical Nutrition. 1991;45(12):569–81.

128. MRC Epidemiology Unit [Internet]. 2023 [cited 2023 Nov 14]. MRC Epidemiology Unit. Available from: https://www.mrc-epid.cam.ac.uk/

129. Fenland Study Phase 1 [Internet]. MRC Epidemiology Unit. [cited 2023 Sep 27]. Available from: https://www.mrc-epid.cam.ac.uk/research/studies/fenland/fenland-study-phase-1/

130. Information for researchers [Internet]. MRC Epidemiology Unit. [cited 2023 Nov 14]. Available from: https://www.mrc-epid.cam.ac.uk/research/studies/fenland/information-for-researchers/

131. Welch AA, Luben R, Khaw KT, Bingham SA. The CAFE computer program for nutritional analysis of the EPIC-Norfolk food frequency questionnaire and identification of extreme nutrient values. J Hum Nutr Diet. 2005 Apr;18(2):99–116.

132. Ruiz A, Pera G, Díez B, Miguel J, Mundet X, Alzamora T, et al. Validación de una versión reducida en español del cuestionario de actividad física en el tiempo de Minnesota (VREM). Revista Española de Salud Pública. 2012;86(5):495–508.

133. Hibi M, Takase H, Iwasaki M, Osaki N, Katsuragi Y. Efficacy of tea catechin-rich beverages to reduce abdominal adiposity and metabolic syndrome risks in obese and overweight subjects: a pooled analysis of 6 human trials. Nutrition research (New York, NY). 2018 Jul 1;55:1–10.

134. Hsu CH, Tsai TH, Kao YH, Hwang KC, Tseng TY, Chou P. Effect of green tea extract on obese women: a randomized, double-blind, placebo-controlled clinical trial. Clinical nutrition (Edinburgh, Scotland). 2008 Jun;27(3):363–70.

135. Li D, Zhang Y, Liu Y, Sun R, Xia M. Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. The Journal of nutrition. 2015;145(4):742–8.

136. Stull AJ, Cash KC, Johnson WD, Champagne CM, Cefalu WT. Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. The Journal of nutrition. 2010 Oct;140(10):1764–8.

137. Castro-Barquero S, Lamuela-Raventós RM, Doménech M, Estruch R. Relationship between Mediterranean Dietary Polyphenol Intake and Obesity. Nutrients. 2018 Oct 17;10(10):1523.

138. De Ferrars RM, Czank C, Zhang Q, Botting NP, Kroon PA, Cassidy A, et al. The pharmacokinetics of anthocyanins and their metabolites in humans. British Journal of Pharmacology. 2014;171(13):3268–82.

139. Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Advances in Nutrition. 2014;5(4):404–17.

140. Corrêa TAF, Rogero MM, Hassimotto NMA, Lajolo FM. The Two-Way Polyphenols-Microbiota Interactions and Their Effects on Obesity and Related Metabolic Diseases. Frontiers in Nutrition. 2019 Dec 20;6:188.

141. Zhao Y, Zhang X. Interactions of tea polyphenols with intestinal microbiota and their implication for anti-obesity. Journal of the Science of Food and Agriculture. 2020 Feb 1;100(3):897–903.

142. Gibson TM, Ferrucci LM, Tangrea JA, Schatzkin A. Epidemiological and Clinical Studies of Nutrition. Semin Oncol. 2010 Jun;37(3):282–96.

143. Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha M, Spencer J. Polyphenols and Human Health: Prevention of Disease and Mechanisms of Action. Nutrients. 2010 Nov 1;2:1106–31.

144. Mitra S, Tareq AM, Das R, Emran TB, Nainu F, Chakraborty AJ, et al. Polyphenols: A first evidence in the synergism and bioactivities. Food Reviews International. 2023 Aug 25;39(7):4419–41.

145. Trutschel D, Palm R, Holle B, Simon M. Methodological approaches in analysing observational data: A practical example on how to address clustering and selection bias. International Journal of Nursing Studies. 2017 Nov 1;76:36–44.

10. Appendix I: Supplementary Material Manuscript 4

Supplementary Table 1. Association of total dietary flavonoids and subclasses with BMI, WC and WHR in 11,568 participants from the Fenland study.

Study variables	Body mass index		Waist circumference, cm		Waist-to-hip ratio	
	β (95% CI)	Q†	β (95% CI)	Q	β (95% CI)	Q
Mean (SD)						
Total Flavonoids	-0.33 (-0.44; -0.22)	<0.001	-0.84 (-1.13; -0.55)	<0.001	-0.004 (-0.006; -0.002)	<0.001
Subclasses						
Flavanols	-0.27 (-0.36; -0.17)	<0.001	-0.70 (-0.95; -0.45)	<0.001	-0.003 (-0.005; -0.002)	<0.001
Flavan-3-ols	-0.17 (-0.23; -0.11)	<0.001	-0.41 (-0.57; -0.26)	<0.001	-0.002 (-0.003; -0.001)	<0.001
Proanthocyanidins	-0.24 (-0.36; -0.13)	<0.001	-0.72 (-1.04; -0.41)	<0.001	-0.005 (-0.007; -0.003)	<0.001
Theaflavins	-0.02 (-0.03; -0.01)	<0.001	-0.05 (-0.08; -0.03)	<0.001	-0.000 (-0.000; -0.000)	0.011
Anthocyanidins	-0.02 (-0.11; 0.07)	0.703	-0.17 (-0.41; 0.08)	0.222	-0.003 (-0.004; -0.002)	<0.001
Flavonols	-0.26 (-0.38; -0.14)	<0.001	-0.61 (-0.92; -0.29)	<0.001	-0.002 (-0.004; -0.000)	0.038
Flavanones	-0.08 (-0.14; -0.03)	<0.001	-0.22 (-0.37; -0.06)	<0.001	-0.002 (-0.003; -0.001)	<0.001
Flavones	-0.43 (-0.54; -0.33)	<0.001	-0.97 (-1.26; -0.68)	<0.001	-0.004 (-0.006; -0.003)	<0.001
Isoflavones	0.04 (-0.02; 0.10)	0.183	0.20 (0.04; 0.35)	0.019	0.001 (-0.000; 0.002)	0.216

Total flavonoids and subclasses were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. †Q value considered statistically significant at <0.05 after false-discovery correction for two-sided alpha=0.05 applied to 11 flavonoid variables and 8 adiposity parameters. Abbreviations: CI, confidence interval; SD, standard deviation.

Supplementary Table 2. Association of total dietary flavonoids and subclasses according to energy density*, with adiposity parameters in 11,568 participants from the Fenland study.

Outcome	Exposure	β (95% CI)	Q†
Total body fat (%)	Total Flavonoids	-0.33 (-0.48; -0.17)	<0.001
	Flavanols	-0.26 (-0.39; -0.13)	<0.001
	Flavan-3-ols	-0.11 (-0.19; -0.02)	0.024
	Proanthocyanidins	-0.47 (-0.63; -0.31)	<0.001
	Theaflavins	-0.01 (-0.03; 0.00)	0.186
	Anthocyanidins	-0.15 (-0.27; -0.02)	0.051
	Flavonols	-0.11 (-0.28; 0.06)	0.277
	Flavanones	-0.16 (-0.24; -0.08)	<0.001
	Flavones	-0.39 (-0.54; -0.24)	<0.001
	Isoflavones	0.24 (0.15; 0.33)	<0.001
VAT (cm)	Total Flavonoids	-0.09 (-0.13; -0.04)	<0.001
	Flavanols	-0.08 (-0.11; -0.04)	0.001
	Flavan-3-ols	-0.04 (-0.06; -0.01)	0.008
	Proanthocyanidins	-0.11 (-0.16; -0.07)	<0.001
	Theaflavins	0.00 (-0.01; 0.00)	0.075
	Anthocyanidins	-0.02 (-0.06; 0.01)	0.309
	Flavonols	-0.05 (-0.10; -0.00)	0.047
	Flavanones	-0.02 (-0.04; 0.00)	0.151
	Flavones	-0.04 (-0.09; 0.00)	0.088
	Isoflavones	0.04 (0.02; 0.07)	0.001
SCAT (cm)	Total Flavonoids	-0.02 (-0.05; 0.01)	0.281
	Flavanols	-0.02 (-0.04; 0.01)	0.277
	Flavan-3-ols	-0.01 (-0.03; 0.00)	0.239
	Proanthocyanidins	-0.01 (-0.04; 0.02)	0.454
	Theaflavins	-0.00 (-0.00; 0.00)	0.338
	Anthocyanidins	0.01 (-0.02; 0.03)	0.622
	Flavonols	-0.01 (-0.04; 0.02)	0.638
	Flavanones	-0.01 (-0.02; 0.01)	0.460
	Flavones	-0.04 (-0.07; -0.01)	0.012
	Isoflavones	0.03 (0.01; 0.04)	0.004
VAT:SCAT ratio	Total Flavonoids	-0.02 (-0.04; 0.00)	0.055
	Flavanols	-0.02 (-0.04; 0.00)	0.053
	Flavan-3-ols	-0.01 (-0.02; 0.00)	0.192
	Proanthocyanidins	-0.03 (-0.05; -0.01)	0.018
	Theaflavins	-0.00 (0.00; 0.00)	0.460
	Anthocyanidins	-0.01 (-0.03; 0.01)	0.316
	Flavonols	-0.02 (-0.04; 0.00)	0.126
	Flavanones	0.01 (0.00; 0.02)	0.261
	Flavones	0.01 (-0.01; 0.03)	0.320
	Isoflavones	0.00 (-0.01; 0.01)	0.920
Body mass index (kg/m^2)	Total Flavonoids	-0.21 (-0.31; -0.10)	0.001
	Flavanols	-0.17 (-0.26; -0.08)	0.002
	Flavan-3-ols	-0.10 (-0.16; -0.05)	0.002
	Proanthocyanidins	-0.15 (-0.27; -0.04)	0.024
	Theaflavins	-0.01 (-0.02; 0.00)	0.069
	Anthocyanidins	0.01 (-0.09; 0.10)	0.92

Outcome	Exposure	β (95% CI)	Q†
	Flavonols	-0.16 (-0.27; -0.04)	0.018
	Flavanones	-0.08 (-0.14; -0.03)	0.012
	Flavones	-0.30 (-0.40; -0.19)	<0.001
	Isoflavones	0.14 (0.08; 0.20)	<0.001
Waist circumference (cm)	Total Flavonoids	-0.50 (-0.79; -0.21)	0.003
	Flavanols	-0.42 (-0.67; -0.17)	0.004
	Flavan-3-ols	-0.24 (-0.40; -0.08)	0.008
	Proanthocyanidins	-0.45 (-0.76; -0.14)	0.012
	Theaflavins	-0.02 (-0.05; 0.00)	0.135
	Anthocyanidins	-0.02 (-0.26; 0.22)	0.915
	Flavonols	-0.31 (-0.63; -0.00)	0.083
	Flavanones	-0.22 (-0.37; -0.06)	0.015
	Flavones	-0.57 (-0.85; -0.29)	<0.001
	Isoflavones	0.37 (0.21; 0.53)	<0.001
ABSI‡ ($\times 10^{-6}$)	Total Flavonoids	-4.79 (-15.01; 5.48)	0.437
	Flavanols	-5.13 (-13.95; 3.68)	0.320
	Flavan-3-ols	-0.29 (-5.99; 5.41)	0.920
	Proanthocyanidins	-18.12 (-28.93; -7.25)	0.004
	Theaflavins	-0.05 (-1.08; 0.96)	0.920
	Anthocyanidins	-10.62 (-18.42; -2.83)	0.018
	Flavonols	3.49 (-7.85; 1.48)	0.607
	Flavanones	-4.39 (-9.64; 0.87)	0.156
	Flavones	3.52 (-6.04; 0.13)	0.530
	Isoflavones	1.63 (-4.41; 7.66)	0.638
Waist-to-hip ratio	Total Flavonoids	-0.002 (-0.004; -0.001)	0.017
	Flavanols	-0.002 (-0.003; -0.001)	0.017
	Flavan-3-ols	-0.001 (-0.002; 0.000)	0.095
	Proanthocyanidins	-0.004 (-0.005; -0.002)	<0.001
	Theaflavins	-0.000 (-0.000; 0.000)	0.313
	Anthocyanidins	-0.002 (-0.003; -0.000)	0.031
	Flavonols	-0.001 (-0.003; 0.001)	0.454
	Flavanones	-0.001 (-0.002; -0.001)	0.006
	Flavones	-0.002 (-0.004; -0.001)	0.018
	Isoflavones	0.001 (0.000; 0.002)	0.037

*Intakes of flavonoids were calculated by energy density, standardizing the energy intake in 2,000 kcal/d (flavonoids×2,000/energy intake) (34). Total flavonoids and subclasses were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. †Q value considered statistically significant at <0.05 after false-discovery correction for two-sided alpha=0.05 applied to 11 flavonoid variables and 8 adiposity parameters. ‡ABSI coefficients are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where m represents waist circumference in metres, (kg/m2)-2/3 is body mass index raised to -2/3, and m-½ is height in metres raised to -1/2; multiplied by 10^{-6} to help improve readability and consistency of the table (23). Abbreviations: ABSI, a body shape index; CI, confidence interval; SD, standard deviation; VAT, visceral abdominal fat thickness; SCAT, subcutaneous abdominal fat thickness.

Supplementary Table 3. Association of total dietary flavonoids and subclasses with adiposity parameters in 11,568 participants from the Fenland study additionally adjusted for BMI.

Outcome	Exposure	β (95% CI)	Q†
Total body fat (%)	Total Flavonoids	-0.15 (-0.25; -0.06)	0.006
	Flavanols	-0.13 (-0.21; -0.05)	0.008
	Flavan-3-ols	-0.00 (-0.07; 0.03)	0.609
	Proanthocyanidins	-0.34 (-0.44; -0.24)	<0.001
	Theaflavins	-0.00 (-0.01; 0.01)	0.682
	Anthocyanidins	-0.18 (-0.26; -0.11)	0.000
	Flavonols	-0.02 (-0.12; 0.09)	0.868
	Flavanones	-0.06 (-0.11; -0.01)	0.060
	Flavones	-0.07 (-0.16; 0.03)	0.310
	Isoflavones	0.09 (0.04; 0.15)	0.003
VAT (cm)	Total Flavonoids	-0.05 (-0.09; -0.02)	0.015
	Flavanols	-0.05 (-0.08; -0.02)	0.013
	Flavan-3-ols	-0.01 (-0.03; 0.01)	0.314
	Proanthocyanidins	-0.11 (-0.14; -0.07)	<0.001
	Theaflavins	-0.00 (-0.01; 0.00)	0.543
	Anthocyanidins	-0.06 (-0.09; -0.03)	<0.001
	Flavonols	-0.02 (-0.06; 0.02)	0.543
	Flavanones	-0.01 (-0.03; 0.01)	0.603
	Flavones	-0.00 (-0.04; 0.03)	0.980
	Isoflavones	0.02 (0.00; 0.04)	0.098
SCAT (cm)	Total Flavonoids	0.01 (-0.01; 0.03)	0.604
	Flavanols	0.01 (-0.01; 0.03)	0.682
	Flavan-3-ols	0.00 (-0.01; 0.01)	0.858
	Proanthocyanidins	0.01 (-0.01; 0.04)	0.496
	Theaflavins	0.00 (-0.00; 0.00)	0.978
	Anthocyanidins	0.01 (-0.01; 0.03)	0.307
	Flavonols	0.00 (-0.02; 0.03)	0.903
	Flavanones	0.01 (-0.00; 0.02)	0.314
	Flavones	0.01 (-0.01; 0.03)	0.478
	Isoflavones	0.00 (-0.01; 0.02)	0.813
VAT:SCAT ratio	Total Flavonoids	-0.03 (-0.05; -0.00)	0.060
	Flavanols	-0.02 (-0.04; -0.00)	0.066
	Flavan-3-ols	-0.01 (-0.02; 0.00)	0.386
	Proanthocyanidins	-0.04 (-0.06; -0.02)	0.001
	Theaflavins	-0.00 (-0.00; 0.00)	0.623
	Anthocyanidins	-0.03 (-0.05; -0.01)	0.003
	Flavonols	-0.01 (-0.04; 0.01)	0.373
	Flavanones	0.00 (-0.01; 0.01)	0.868
	Flavones	-0.01 (-0.03; 0.02)	0.743
	Isoflavones	0.01 (-0.00; 0.02)	0.384
Waist circumference (cm)	Total Flavonoids	-0.06 (-0.19; 0.07)	0.543
	Flavanols	-0.06 (-0.18; 0.05)	0.438
	Flavan-3-ols	-0.01 (-0.08; 0.06)	0.868
	Proanthocyanidins	-0.19 (-0.33; -0.05)	0.030
	Theaflavins	-0.00 (-0.02; 0.01)	0.869
	Anthocyanidins	-0.15 (-0.26; -0.05)	0.013
	Flavonols	0.03 (-0.11; 0.17)	0.805
	Flavanones	-0.05 (-0.11; 0.02)	0.355

Outcome	Exposure	β (95% CI)	Q†
	Flavones	0.05 (-0.08; 0.17)	0.623
	Isoflavones	0.05 (-0.02; 0.13)	0.307
Waist-to-hip ratio	Total Flavonoids	-0.001 (-0.003; 0.000)	0.150
	Flavanols	-0.001 (-0.002; 0.000)	0.126
	Flavan-3-ols	0.000 (-0.001; 0.000)	0.623
	Proanthocyanidins	-0.003 (-0.005; -0.002)	<0.001
	Theaflavins	0.000 (0.000; 0.000)	0.665
	Anthocyanidins	-0.003 (-0.004; -0.002)	<0.001
	Flavonols	0.000 (-0.002; 0.002)	0.996
	Flavanones	-0.001 (-0.002; 0.000)	0.031
	Flavones	-0.001 (-0.002; 0.000)	0.344
	Isoflavones	0.000 (-0.001; 0.001)	0.996

Total flavonoids and subclasses were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages, and BMI. †Q value considered statistically significant at <0.05 after false-discovery correction for two-sided alpha=0.05 applied to 11 flavonoid variables and 8 adiposity parameters. Abbreviations: BMI, body mass index; CI, confidence interval; SD, standard deviation; VAT, visceral abdominal fat thickness; SCAT, subcutaneous abdominal fat thickness.

Supplementary Table 4. Association of total dietary flavonoids and subclasses with adiposity parameters in 11,336 participants* from the Fenland study

Outcome	Exposure	β (95% CI)	Q†
Total body fat (%)	Total Flavonoids	-0.32 (-0.48; -0.17)	<0.001
	Flavanols	-0.26 (-0.39; -0.13)	0.001
	Flavan-3-ols	-0.10 (-0.19; -0.02)	0.032
	Proanthocyanidins	-0.46 (-0.63; -0.30)	<0.001
	Theaflavins	-0.01 (-0.03; 0.00)	0.200
	Anthocyanidins	-0.15 (-0.28; -0.02)	0.048
	Flavonols	-0.11 (-0.29; 0.06)	0.277
	Flavanones	-0.17 (-0.25; -0.09)	<0.001
	Flavones	-0.39 (-0.55; -0.24)	<0.001
	Isoflavones	0.25 (0.16; 0.33)	<0.001
VAT (cm)	Total Flavonoids	-0.08 (-0.12; -0.03)	0.004
	Flavanols	-0.06 (-0.1; -0.03)	0.004
	Flavan-3-ols	-0.03 (-0.06; -0.01)	0.026
	Proanthocyanidins	-0.10 (-0.14; -0.05)	<0.001
	Theaflavins	0.00 (-0.01; 0.00)	0.099
	Anthocyanidins	-0.02 (-0.06; 0.02)	0.413
	Flavonols	-0.04 (-0.08; 0.01)	0.214
	Flavanones	-0.02 (-0.05; 0.00)	0.152
	Flavones	-0.04 (-0.08; 0.00)	0.132
	Isoflavones	0.05 (0.02; 0.07)	<0.001
SCAT (cm)	Total Flavonoids	-0.02 (-0.05; 0.01)	0.227
	Flavanols	-0.02 (-0.04; 0.01)	0.227
	Flavan-3-ols	-0.01 (-0.03; 0.00)	0.216
	Proanthocyanidins	-0.01 (-0.05; 0.02)	0.452
	Theaflavins	-0.00 (-0.00; 0.00)	0.360
	Anthocyanidins	0.01 (-0.02; 0.03)	0.705
	Flavonols	-0.01 (-0.05; 0.02)	0.467
	Flavanones	-0.01 (-0.02; 0.01)	0.560
	Flavones	-0.04 (-0.07; -0.01)	0.016
	Isoflavones	0.03 (0.01; 0.04)	0.004
VAT:SCAT ratio	Total Flavonoids	-0.02 (-0.04; 0.01)	0.208
	Flavanols	-0.01 (-0.03; 0.00)	<0.001
	Flavan-3-ols	-0.01 (-0.02; 0.01)	0.368
	Proanthocyanidins	-0.02 (-0.05; 0.00)	0.070
	Theaflavins	-0.00 (-0.00; 0.00)	0.520
	Anthocyanidins	-0.01 (-0.03; 0.01)	0.437
	Flavonols	-0.01 (-0.04; 0.01)	0.415
	Flavanones	0.01 (-0.01; 0.02)	0.360
	Flavones	0.01 (-0.01; 0.03)	0.355
	Isoflavones	0.00 (-0.01; 0.01)	0.750
Body mass index (kg/m^2)	Total Flavonoids	-0.21 (-0.32; -0.10)	0.001
	Flavanols	-0.17 (-0.26; -0.07)	0.003
	Flavan-3-ols	-0.10 (-0.16; -0.05)	0.003
	Proanthocyanidins	-0.15 (-0.27; -0.03)	0.028
	Theaflavins	-0.01 (-0.02; 0.00)	0.064
	Anthocyanidins	0.00 (-0.10; 0.09)	0.939

Outcome	Exposure	β (95% CI)	Q†
Waist circumference (cm)	Flavonols	-0.16 (-0.28; -0.05)	0.019
	Flavanones	-0.09 (-0.15; -0.03)	0.007
	Flavones	-0.31 (-0.41; -0.20)	<0.001
	Isoflavones	0.14 (0.08; 0.20)	<0.001
	Total Flavonoids	-0.47 (-0.76; -0.18)	0.006
	Flavanols	-0.39 (-0.64; -0.14)	0.008
	Flavan-3-ols	-0.23 (-0.39; -0.08)	0.012
	Proanthocyanidins	-0.41 (-0.72; -0.09)	0.028
	Theaflavins	-0.03 (-0.05; 0.00)	0.122
	Anthocyanidins	-0.03 (-0.28; 0.21)	0.812
	Flavonols	-0.29 (-0.60; 0.03)	0.132
	Flavanones	-0.23 (-0.39; -0.08)	0.011
	Flavones	-0.58 (-0.86; -0.29)	<0.001
	Isoflavones	0.39 (0.23; 0.55)	<0.001
ABSI‡ ($\times 10^{-6}$)	Total Flavonoids	-2.41 (-13.12; 8.03)	0.705
	Flavanols	-3.44 (-12.31; 5.64)	0.520
	Flavan-3-ols	0.38 (-0.54; 6.14)	0.921
	Proanthocyanidins	-15.28 (-27.14; -4.42)	0.018
	Theaflavins	-0.00 (-1.01; 1.01)	0.999
	Anthocyanidins	-10.21 (-18.47; -2.25)	0.028
	Flavonols	6.32 (-5.24; 18.23)	0.368
	Flavanones	-4.24 (-9.52; 1.27)	0.201
	Flavones	4.83 (-4.86; 15.50)	0.413
	Isoflavones	2.93 (-3.21; 9.01)	0.418
Waist-to-hip ratio	Total Flavonoids	-0.002 (-0.004; 0.000)	0.028
	Flavanols	-0.002 (-0.003; 0.000)	0.028
	Flavan-3-ols	-0.001 (-0.002; 0.000)	0.132
	Proanthocyanidins	-0.003 (-0.005; -0.002)	0.001
	Theaflavins	0.000 (0.000; 0.000)	0.345
	Anthocyanidins	-0.002 (-0.003; 0.000)	0.032
	Flavonols	-0.001 (-0.002; 0.001)	0.538
	Flavanones	-0.001 (-0.002; -0.001)	0.006
	Flavones	-0.002 (-0.004; -0.001)	0.022
	Isoflavones	0.001 (0.000; 0.002)	0.022

*Only plausible reporters of energy intake: participants in the top and bottom 1% of energy intake (n=232) were considered misreporters and excluded from this analysis. Total flavonoids and subclasses were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. †Q value considered statistically significant at <0.05 after false-discovery correction for two-sided alpha=0.05 applied to 11 flavonoid variables and 8 adiposity parameters. ‡ ABSI coefficients are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where m represents waist circumference in metres, (kg/m2)-2/3 is body mass index raised to -2/3, and m-½ is height in metres raised to -1/2; multiplied by 10^{-6} to help improve readability and consistency of the table (23). Abbreviations: ABSI, a body shape index; CI, confidence interval; SD, standard deviation; VAT, visceral abdominal fat thickness; SCAT, subcutaneous abdominal fat thickness.

Supplementary Table 5. Association of total dietary flavonoids and subclasses with adiposity parameters excluding participants with altered different metabolic parameters from the Fenland study, UK.

Outcome	Exposure	HbA1c < 48 mmol/mol or no use of oral antidiabetics *n* 7,275		Total cholesterol <5.5 mmol/l or no use of lipid lowering medication *n* 6,313		BP <140 (sys) or <90 (dia) mmHg or no use of antihypertensives *n* 9,871	
		β (95% CI)	Q†	β (95% CI)	Q	β (95% CI)	Q
Total body fat (%)	Total Flavonoids	-0.46 (-0.66; -0.26)	<0.001	-0.55 (-0.77; -0.33)	<0.001	-0.81 (-0.97; -0.70)	<0.001
	Flavanols	-0.38 (-0.55; -0.21)	<0.001	-0.47 (-0.65; -0.28)	<0.001	-0.70 (-0.84; -0.56)	<0.001
	Flavan-3-ols	-0.17 (-0.27; -0.06)	0.003	-0.24 (-0.35; -0.12)	<0.001	-0.26 (-0.36; -0.16)	<0.001
	Proanthocyanidins	-0.66 (-0.88; -0.44)	<0.001	-0.69 (-0.93; -0.45)	<0.001	-1.09 (-1.24; -0.93)	<0.001
	Theaflavins	-0.02 (-0.04; 0.00)	0.035	-0.03 (-0.05; -0.01)	0.009	-0.04 (-0.06; -0.02)	<0.001
	Anthocyanidins	-0.28 (-0.45; -0.10)	0.002	-0.32 (-0.51; -0.12)	0.002	-0.63 (-0.75; -0.51)	<0.001
	Flavonols	-0.18 (-0.39; 0.04)	0.114	-0.26 (-0.50; -0.02)	0.037	-0.58 (-0.77; -0.40)	<0.001
	Flavanones	-0.18 (-0.3; -0.07)	0.001	-0.19 (-0.31; -0.06)	0.003	-0.24 (-0.31; -0.16)	<0.001
	Flavones	-0.53 (-0.73; -0.33)	<0.001	-0.62 (-0.84; -0.39)	<0.001	-0.56 (-0.70; -0.43)	<0.001
	Isoflavones	0.12 (0.01; 0.23)	0.034	0.16 (0.04; 0.28)	0.008	0.08 (-0.01; 0.17)	0.091
VAT (cm)	Total Flavonoids	-0.12 (-0.17; -0.06)	<0.001	-0.10 (-0.152; -0.042)	0.001	-0.13 (-0.17; -0.09)	<0.001
	Flavanols	-0.10 (-0.14; -0.05)	<0.001	-0.08 (-0.130; -0.036)	0.001	-0.11 (-0.15; -0.07)	<0.001
	Flavan-3-ols	-0.05 (-0.08; -0.02)	0.002	-0.04 (-0.069; -0.011)	0.006	-0.04 (-0.06; -0.01)	0.005
	Proanthocyanidins	-0.16 (-0.22; -0.10)	<0.001	-0.13 (-0.189; -0.069)	<0.001	-0.18 (-0.22; -0.14)	<0.001
	Theaflavins	-0.01 (-0.01; 0.00)	0.025	-0.00 (-0.009; 0.001)	0.114	-0.01 (-0.01; -0.00)	0.002
	Anthocyanidins	-0.06 (-0.11; -0.01)	0.019	-0.06 (-0.113; -0.012)	0.015	-0.11 (-0.14; -0.08)	<0.001
	Flavonols	-0.06 (-0.12; 0.00)	0.039	-0.05 (-0.111; 0.009)	0.093	-0.06 (-0.11; -0.02)	0.005
	Flavanones	-0.04 (-0.07; 0.00)	0.027	-0.03 (-0.054; 0.004)	0.093	-0.04 (-0.05; -0.02)	<0.001
	Flavones	-0.10 (-0.16; -0.05)	<0.001	-0.08 (-0.139; -0.027)	0.003	-0.08 (-0.11; -0.04)	<0.001
	Isoflavones	0.03 (0.00; 0.06)	0.068	0.04 (0.013; 0.067)	0.003	0.05 (0.03; 0.07)	<0.001
SCAT (cm)	Total Flavonoids	-0.05 (-0.09; -0.01)	0.016	-0.04 (-0.08; -0.00)	0.036	-0.07 (-0.10; -0.05)	<0.001
	Flavanols	-0.04 (-0.08; -0.01)	0.013	-0.04 (-0.07; -0.00)	0.031	-0.07 (-0.09; -0.04)	<0.001
	Flavan-3-ols	-0.03 (-0.05; -0.01)	0.01	-0.03 (-0.05; -0.01)	0.016	-0.03 (-0.05; -0.01)	0.001
	Proanthocyanidins	-0.04 (-0.08; 0.01)	0.103	-0.04 (-0.08; 0.01)	0.108	-0.10 (-0.12; -0.07)	<0.001
	Theaflavins	0.00 (-0.01; 0.00)	0.031	-0.00 (-0.01; 0.00)	0.058	-0.01 (-0.01; -0.00)	0.002
	Anthocyanidins	0.01 (-0.03; 0.04)	0.673	-0.01 (-0.04; 0.03)	0.627	-0.05 (-0.07; -0.03)	<0.001
	Flavonols	-0.03 (-0.07; 0.01)	0.189	-0.02 (-0.06; 0.03)	0.399	-0.06 (-0.09; -0.03)	<0.001
	Flavanones	-0.01 (-0.03; 0.01)	0.396	-0.01 (-0.03; 0.02)	0.631	-0.03 (-0.04; -0.01)	<0.001
	Flavones	-0.05 (-0.09; -0.01)	0.008	-0.06 (-0.10; -0.03)	0.001	-0.07 (-0.09; -0.05)	<0.001

Outcome	Exposure	HbA1c < 48 mmol/mol or no use of oral antidiabetics *n* 7,275		Total cholesterol <5.5 mmol/l or no use of lipid lowering medication *n* 6,313		BP <140 (sys) or <90 (dia) mmHg or no use of antihypertensives *n* 9,871	
		β (95% CI)	Q†	β (95% CI)	Q	β (95% CI)	Q
	Isoflavones	0.02 (0.00; 0.04)	0.077	0.01 (-0.01; 0.03)	0.501	-0.00 (-0.02; 0.02)	0.931
VAT:SCAT ratio	Total Flavonoids	-0.02 (-0.05; 0.01)	0.169	-0.009 (-0.038; 0.019)	0.528	-0.009 (-0.029; 0.011)	0.372
	Flavanols	-0.02 (-0.04; 0.01)	0.215	-0.007 (-0.032; 0.017)	0.559	-0.009 (-0.026; 0.009)	0.323
	Flavan-3-ols	0.00 (-0.02; 0.01)	0.722	0.003 (-0.012; 0.018)	0.693	-0.002 (-0.013; 0.009)	0.725
	Proanthocyanidins	-0.04 (-0.07; -0.01)	0.006	-0.033 (-0.064; -0.002)	0.039	-0.012 (-0.030; 0.007)	0.213
	Theaflavins	-0.00 (-0.00; 0.00)	0.911	0.001 (-0.002; 0.003)	0.535	-0.001 (-0.003; 0.002)	0.629
	Anthocyanidins	-0.04 (-0.06; -0.01)	0.005	-0.022 (-0.046; 0.003)	0.087	-0.010 (-0.024; 0.005)	0.187
	Flavonols	-0.01 (-0.04; 0.02)	0.371	-0.005 (-0.036; 0.025)	0.738	0.001 (-0.021; 0.023)	0.923
	Flavanones	0.00 (-0.01; 0.02)	0.811	0.002 (-0.013; 0.016)	0.817	0.007 (-0.002; 0.015)	0.110
	Flavones	-0.01 (-0.04; 0.02)	0.565	0.005 (-0.024; 0.034)	0.746	0.015 (-0.001; 0.031)	0.064
	Isoflavones	0.00 (-0.01; 0.01)	0.981	0.012 (-0.002; 0.026)	0.097	0.018 (0.007; 0.028)	0.001
Body mass index (kg/m^2)	Total Flavonoids	-0.26 (-0.40; -0.12)	<0.001	-0.26 (-0.40; -0.11)	0.001	-0.32 (-0.43; -0.22)	<0.001
	Flavanols	-0.21 (-0.33; -0.09)	0.001	-0.22 (-0.34; -0.09)	0.001	-0.28 (-0.38; -0.19)	<0.001
	Flavan-3-ols	-0.13 (-0.20; -0.05)	0.001	-0.16 (-0.23; -0.08)	<0.001	-0.13 (-0.20; -0.07)	<0.001
	Proanthocyanidins	-0.21 (-0.36; -0.06)	0.006	-0.15 (-0.31; 0.00)	0.054	-0.39 (-0.49; -0.29)	<0.001
	Theaflavins	-0.01 (-0.03; 0.00)	0.035	-0.02 (-0.03; -0.00)	0.040	-0.03 (-0.04; -0.01)	<0.001
	Anthocyanidins	0.00 (-0.13; 0.13)	0.969	0.01 (-0.12; 0.14)	0.905	-0.20 (-0.28; -0.12)	<0.001
	Flavonols	-0.16 (-0.31; -0.01)	0.035	-0.21 (-0.37; -0.05)	0.010	-0.23 (-0.35; -0.12)	<0.001
	Flavanones	-0.11 (-0.19; -0.03)	0.007	-0.08 (-0.16; -0.00)	0.042	-0.10 (-0.15; -0.05)	<0.001
	Flavones	-0.38 (-0.52; -0.24)	<0.001	-0.34 (-0.48; -0.20)	<0.001	-0.30 (-0.38; -0.22)	<0.001
	Isoflavones	0.03 (-0.04; 0.11)	0.364	0.05 (-0.03; 0.12)	0.226	0.02 (-0.03; 0.08)	0.422
Waist circumference (cm)	Total Flavonoids	-0.61 (-0.99; -0.24)	0.001	-0.78 (-1.17; -0.40)	<0.001	-0.90 (-1.18; -0.61)	<0.001
	Flavanols	-0.51 (-0.82; -0.19)	0.002	-0.69 (-1.03; -0.36)	<0.001	-0.79 (-1.05; -0.53)	<0.001
	Flavan-3-ols	-0.28 (-0.48; -0.09)	0.005	-0.44 (-0.64; -0.23)	<0.001	-0.32 (-0.49; -0.15)	<0.001
	Proanthocyanidins	-0.63 (-1.05; -0.22)	0.003	-0.66 (-1.09; -0.23)	0.003	-1.14 (-1.42; -0.86)	<0.001
	Theaflavins	-0.03 (-0.07; 0.00)	0.085	-0.05 (-0.07; -0.01)	0.011	-0.06 (-0.09; -0.03)	<0.001
	Anthocyanidins	-0.13 (-0.45; 0.20)	0.452	-0.14 (-0.49; 0.22)	0.450	-0.64 (-0.86; -0.43)	<0.001
	Flavonols	-0.32 (-0.71; 0.08)	0.117	-0.54 (-0.97; -0.12)	0.013	-0.57 (-0.89; -0.26)	<0.001
	Flavanones	-0.29 (-0.49; -0.10)	0.003	-0.18 (-0.39; 0.03)	0.095	-0.27 (-0.40; -0.14)	<0.001
	Flavones	-0.83 (-1.20; -0.47)	<0.001	-0.78 (-1.17; -0.39)	<0.001	-0.72 (-0.94; -0.49)	<0.001
	Isoflavones	0.14 (-0.06; 0.33)	0.178	0.23 (0.02; 0.44)	0.030	0.22 (0.06; 0.38)	0.007
ABSI‡ ($\times 10^{-6}$)	Total Flavonoids	-3.41 (-17.12; 9.82)	0.610	-12.14 (-26.01; 2.12)	0.096	-20.34 (-29.21; -9.61)	<0.001

Outcome	Exposure	HbA1c < 48 mmol/mol or no use of oral antidiabetics *n* 7,275		Total cholesterol <5.5 mmol/l or no use of lipid lowering medication *n* 6,313		BP <140 (sys) or <90 (dia) mmHg or no use of antihypertensives *n* 9,871	
		β (95% CI)	Q†	β (95% CI)	Q	β (95% CI)	Q
	Flavanols	-3.21 (-15.12; 8.02)	0.573	-12.24 (-24.47; -0.51)	0.041	-17.88 (-26.01; -8.07)	<0.001
	Flavan-3-ols	-1.31 (-58.14; 8.45)	0.718	-4.02 (-12.32; 3.57)	0.297	-2.32 (-8.57; 3.82)	0.456
	Proanthocyanidins	-22.25 (-36.14; -7.94)	0.002	-28.14 (-42.19; -1.34)	<0.001	-35.22 (-45.14; -26.17)	<0.001
	Theaflavins	-0.023 (-1.25; 1.31)	0.971	-0.62 (-2.04; 0.76)	0.379	-0.56 (-1.71; 0.57)	0.330
	Anthocyanidins	-21.21 (-31.24; -11.74)	<0.001	-22.45 (-33.14; -12.17)	<0.001	-27.21 (-34.32; -20.02)	<0.001
	Flavonols	4.92 (-9.51; 19.32)	0.504	0.73 (-14.32; 16.47)	0.925	-7.30 (-19.36; 3.92)	0.201
	Flavanones	-9.11 (-16.21; -2.81)	0.005	-3.45 (-11.02; 4.08)	0.371	-8.12 (-13.05; -3.46)	0.001
	Flavones	-6.75 (-19.21; 5.41)	0.275	-0.98 (-14.23; 12.05)	0.884	-12.08 (-20.25; -4.28)	0.002
	Isoflavones	-1.58 (-9.01; 6.05)	0.694	3.36 (-4.48; 11.21)	0.403	6.71 (0.94; 12.21)	0.023
Waist-to-hip ratio	Total Flavonoids	-0.003 (-0.005; -0.001)	0.012	-0.003 (-0.005; -0.001)	0.005	-0.006 (-0.007; -0.004)	<0.001
	Flavanols	-0.002 (-0.004; 0.000)	0.018	-0.003 (-0.005; -0.001)	0.003	-0.005 (-0.006; -0.003)	<0.001
	Flavan-3-ols	-0.001 (-0.002; 0.000)	0.108	-0.002 (-0.003; 0.000)	0.005	-0.002 (-0.003; -0.001)	0.001
	Proanthocyanidins	-0.005 (-0.007; -0.003)	<0.001	-0.004 (-0.006; -0.002)	0.001	-0.008 (-0.010; -0.006)	<0.001
	Theaflavins	0.000 (0.000; 0.000)	0.313	-0.000 (-0.000; -0.000)	0.048	-0.000 (-0.000; -0.000)	0.001
	Anthocyanidins	-0.003 (-0.005; -0.002)	<0.001	-0.003 (-0.005; -0.001)	0.006	-0.006 (-0.007; -0.004)	<0.001
	Flavonols	-0.001 (-0.003; 0.002)	0.524	-0.001 (-0.004; 0.001)	0.271	-0.003 (-0.005; -0.001)	0.001
	Flavanones	-0.002 (-0.003; -0.001)	<0.001	-0.001 (-0.002; 0.000)	0.127	-0.002 (-0.003; -0.001)	<0.001
	Flavones	-0.004 (-0.006; -0.002)	<0.001	-0.003 (-0.005; -0.001)	0.004	-0.005 (-0.006; -0.003)	<0.001
	Isoflavones	0.000 (-0.001; 0.001)	0.755	0.001 (-0.001; 0.002)	0.301	0.001 (0.000; 0.002)	0.048

Total flavonoids and subclasses were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. †Q value considered statistically significant at <0.05 after false-discovery correction for two-sided alpha=0.05 applied to 11 flavonoid variables and 8 adiposity parameters. ‡ ABSI coefficients are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where m represents waist circumference in metres, (kg/m2)-2/3 is body mass index raised to -2/3, and m-½ is height in metres raised to -1/2; multiplied by 10^{-6} to help improve readability and consistency of the table (23). Abbreviations: ABSI, a body shape index; BP, blood pressure; CI, confidence interval; dia, diastolic; SD, standard deviation; sys, systolic; VAT, visceral abdominal fat thickness; SCAT, subcutaneous abdominal fat thickness.

Supplementary Table 6. Association of total dietary flavonoids with adiposity parameters by sex in 11,568 participants from the Fenland study, UK.

Outcome	Male participants *n* 5,357 β (95% CI)	Female participants *n* 6,211 β (95% CI)	p-interaction
Total body fat (%)	-0.12 (-0.19; -0.05)	-0.13 (-0.18; -0.08)	0.287
VAT (cm)	0.03 (-0.01; 0.06)	-0.12 (-0.16; -0.07)	<0.001
SCAT (cm)	-0.00 (-0.01; -0.00)	-0.01 (-0.01; -0.00)	0.394
VAT:SCAT	0.01 (-0.02; 0.02)	-0.07 (-0.12; -0.03)	0.002
Body mass index (kg/m^2)	-0.23 (-0.37; -0.09)	-0.47 (-0.67; -0.30)	0.083
Waist circumference (cm)	-0.52 (-0.91; -0.13)	-1.22 (-1.65; -0.80)	0.105
ABSI‡ (×10^{-6})	-15.70 (-30.92; -1.32)	-20.64 (-36.40; -4.88)	0.509
Waist-to-hip ratio	-0.36 (-0.56; -0.16)	-0.74 (-0.95; -0.52)	0.084

Total flavonoids were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. ‡ ABSI coefficients are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where m represents waist circumference in metres, (kg/m2)-2/3 is body mass index raised to -2/3, and m-½ is height in metres raised to -1/2; multiplied by 10^{-6} to help improve readability and consistency of the table (23). Abbreviations: ABSI, a body shape index; CI, confidence interval; VAT, visceral abdominal fat thickness; SCAT, subcutaneous abdominal fat thickness.

Supplementary Table 7. Association of total dietary flavonoids with adiposity parameters by age in 11,568 participants from the Fenland study, UK.

Outcome	< 48 years *n* 5,338 β (95% CI)	≥ 48 years *n* 6,230 β (95% CI)	p-interaction
Total body fat (%)	-0.53 (-0.76; -0.29)	-0.51 (-0.71; -0.31)	0.754
VAT (cm)	-0.06 (-0.12; -0.01)	-0.17 (-0.24; -0.11)	0.040
SCAT (cm)	-0.05 (-0.10; -0.01)	-0.04 (-0.07; 0.00)	0.256
VAT:SCAT	-0.01 (-0.04; 0.02)	-0.04 (-0.07; -0.01)	0.037
Body mass index (kg/m^2)	-0.28 (-0.44; -0.12)	-0.34 (-0.49; -0.19)	0.812
Waist circumference (cm)	-0.68 (-1.10; -0.26)	-0.88 (-1.28; -0.48)	0.397
ABSI‡ (×10^{-6})	-3.31 (-11.52; 18.47)	-16.14 (-30.04; -1.88)	0.007
Waist-to-hip ratio	-0.00 (-0.00; 0.00)	-0.01 (-0.01; -0.00)	0.015

Total flavonoids were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. ‡ ABSI coefficients are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where m represents waist circumference in metres, (kg/m2)-2/3 is body mass index raised to -2/3, and m-½ is height in metres raised to -1/2; multiplied by 10^{-6} to help improve readability and consistency of the table (23). Abbreviations: ABSI, a body shape index; CI, confidence interval; VAT, visceral abdominal fat thickness; SCAT, subcutaneous abdominal fat thickness.

Supplementary Table 8. Association of continuous total dietary flavonoids and adiposity parameters by smoking status in 11,568 participants from Fenland study, UK.

Outcome	Never smokers *n* 6,394 β (95% CI)	Former smokers *n* 3,821 β (95% CI)	Current smokers *n* 1,353 β (95% CI)	p-interaction
Total body fat (%)	-0.09 (-0.15; -0.03)	-0.20 (-0.28; -0.12)	-0.11 (-0.21; -0.00)	0.515
VAT (cm)	-0.06 (-0.10; -0.03)	-0.07 (-0.12; -0.02)	0.05 (-0.03; 0.12)	0.001
SCAT (cm)	-0.01 (-0.01; -0.00)	-0.01 (-0.01; -0.00)	0.00 (-0.00; 0.01)	0.446
VAT:SCAT	0.00 (-0.03; 0.03)	-0.04 (-0.07; 0.00)	-0.06 (-0.11; -0.01)	0.002
Body mass index (kg/m^2)	-0.40 (-0.55; -0.25)	-0.38 (-0.56; -0.19)	-0.06 (-0.35; 0.24)	0.003
Waist circumference (cm)	-1.00 (-1.39; -0.60)	-1.11 (-1.61; -0.62)	0.10 (-0.68; 0.89)	0.003
ABSI‡ ($\times 10^{-6}$)	-9.03 (-23.24; 5.34)	-17.02 (-34.12; -0.49)	6.32 (-18.22; 30.41)	0.006
Waist-to-hip ratio	-0.67 (-0.88; -0.46)	-0.66 (-0.91; -0.40)	-0.04 (-0.44; 0.36)	0.001

Total flavonoids were log2-transformed and therefore beta coefficients corresponded the effect size per doubling intake levels. Robust linear regression model adjusted for: age (years), sex, site (Cambridge, Ely, Wisbech), physical activity energy expenditure (kJ/kg/d), age at end of full-time education (years), ethnicity (European origin/non-European origin), household income (<20,000/20,000 to 40,000/>40,000 £/year), smoking status (never/former/current), and dietary intakes of total energy, fibre, vitamin C, alcoholic beverages except wine, dairy products, red and processed meat, fats and cream, poultry and egg, sugar, sugar-sweetened beverages, and artificially-sweetened beverages. ‡Mean and standard deviation for ABSI are in units of $m \cdot (kg/m^2)^{-2/3} \cdot m^{-½}$, where m represents waist circumference in metres, (kg/m2)-2/3 is body mass index raised to -2/3, and m-½ is height in metres raised to -1/2; multiplied by 10^{-6} to help improve readability and consistency of the table (23). Abbreviations: ABSI, a body shape index; CI, confidence interval; VAT, visceral abdominal fat thickness; SCAT, subcutaneous abdominal fat thickness.

11. Appendix II: Other Scientific Contributions

11.1. Mediterranean diet and olive oil, microbiota, and obesity-related cancers. From mechanisms to prevention.

Almanza-Aguilera E, Cano A, **Gil-Lespinard M**, Burguera N, Zamora-Ros R, Agudo A, Farràs M. Mediterranean diet and olive oil, microbiota, and obesity-related cancers. From mechanisms to prevention. Seminars in Cancer Biology [Internet]. 2023 Oct 1;95:103–19. Available from: https://doi.org/10.1016/j.semcancer.2023.08.001

Impact Factor: 14.5

Category: Oncology. Q1 17/241

Seminars in Cancer Biology 95 (2023) 103–119

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Mediterranean diet and olive oil, microbiota, and obesity-related cancers. From mechanisms to prevention

Enrique Almanza-Aguilera [a,1], Ainara Cano [b,1], Mercedes Gil-Lespinard [a], Nerea Burguera [b], Raul Zamora-Ros [a,c,*], Antonio Agudo [a], Marta Farràs [a,**]

[a] Unit of Nutrition and Cancer, Epidemiology Research Program, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Spain
[b] Food Research, AZTI, Basque Research and Technology Alliance (BRTA), Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, 48160, Derio, Spain
[c] Department of Nutrition, Food Sciences, and Gastronomy, Food Innovation Network (XIA), Institute for Research on Nutrition and Food Safety (INSA), Faculty of Pharmacy and Food Sciences University of Barcelona, Barcelona, Spain

ARTICLE INFO

Keywords:
Cancer
Mediterranean diet
Microbiota
Obesity
Olive oil

ABSTRACT

Olive oil (OO) is the main source of added fat in the Mediterranean diet (MD). It is a mix of bioactive compounds, including monounsaturated fatty acids, phytosterols, simple phenols, secoiridoids, flavonoids, and terpenoids. There is a growing body of evidence that MD and OO improve obesity-related factors. In addition, obesity has been associated with an increased risk for several cancers: endometrial, oesophageal adenocarcinoma, renal, pancreatic, hepatocellular, gastric cardia, meningioma, multiple myeloma, colorectal, postmenopausal breast, ovarian, gallbladder, and thyroid cancer. However, the epidemiological evidence linking MD and OO with these obesity-related cancers, and their potential mechanisms of action, especially those involving the gut microbiota, are not clearly described or understood. The goals of this review are 1) to update the current epidemiological knowledge on the associations between MD and OO consumption and obesity-related cancers, 2) to identify the gut microbiota mechanisms involved in obesity-related cancers, and 3) to report the effects of MD and OO on these mechanisms.

1. Introduction

The Mediterranean diet (MD) is primarily a plant-based dietary pattern, consisting of a high intake of fruit, vegetables, legumes, nuts and seeds, whole grains, spices, herbs, and olive oil (OO). Seafood, poultry, eggs, wine (during meals), and dairy products preferably in the form of low-fat cheese and yoghurt are consumed in moderation, while red and processed meats, refined grains and sugars are little or occasionally consumed [1]. Owing to its food composition, the MD is a dietary pattern rich in protective nutrients and bioactive compounds able to prevent several diseases, including obesity and cancer [2].

OO is the main source of fat in the MD [3–6]. OO's chemical composition differs according to olive variety, environmental conditions, ripening, and processing methods. OO has both a saponifiable fraction and a phenolic one. Oleic acid is the main component of the saponifiable fraction; phenolic acids, tyrosols, flavonoids, and lignans are the main components of the phenolic part of virgin olive oil (VOO) [7–9]. Depending on the processing methods, OOs can be classified into

Abbreviations: AICR, American Institute of Cancer Research; BMI, Body mass index; CRC, Colorectal cancer; EFSA, European Food Safety Authority; EPIC, European Prospective Investigation into Cancer and Nutrition; EVOO, Extra virgin olive oil; GERD, Gastroesophageal reflux disease; GM, Gut microbiota; HR, Hazard Ratio; HT, Hydroxytyrosol; IARC, International Agency for Research on Cancer; IGF-I, Insulin-like growth factor-I; IL, Interleukin; LPS, Lipopolysaccharides; MAMP, Microorganism-associated molecular patterns; MD, Mediterranean Diet; OO, Olive oil; PC, Phenolic compounds; PREDIMED, Prevention with Mediterranean Diet; RR, Relative Risk; SCFA, Short chain fatty acid; SHBG, Sex hormone-binding globulin; TMAO, Trimethylamine-N-oxide; TNF-α, Tumour necrosis factor-alpha; VOO, Virgin olive oil; WCRF, World Cancer Research Fund.

* Corresponding author at: Unit of Nutrition and Cancer, Epidemiology Research Program, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Spain.
** Corresponding author.
E-mail addresses: rzamora@ub.edu (R. Zamora-Ros), mfarras@iconcologia.net (M. Farràs).
[1] Contributed equally

https://doi.org/10.1016/j.semcancer.2023.08.001
Received 27 December 2022; Received in revised form 2 July 2023; Accepted 1 August 2023
Available online 4 August 2023

refined OO, common OO, VOO, or extra virgin OO (EVOO). EVOO and VOO are obtained by direct pressing or centrifugation of the olives and are rich in phenolic compounds (PC). Hydroxytyrosol (HT), a phenol, is the main component responsible for VOO's antioxidant effect on low-density lipoproteins, as endorsed by a European Food Safety Authority (EFSA) health claim in 2011. The Fatty acid composition and its richness in antioxidants are responsible for VOO's stability upon heating and may counteract the generation of oxidation-derived pro-carcinogenic molecules such as polycyclic aromatic hydrocarbons and heterocyclic amines [10]. As a result, VOO exhibits high resistance against oxidation in comparison with other oils, and during its use for frying, when the oil is frequently reloaded, its chemical composition is less altered.

The relationship between MD and obesity and obesity-related disorders has been extensively investigated over the past two decades [11]. MD is an effective tool in reducing body weight, particularly when energy is restricted and in combination with increased physical activity [12,13]. Reassuringly, even when MD is not energy-restricted, it is not associated with adulthood weight gain in the short or long term [14]. In addition, a growing body of evidence suggests that higher adherence to MD is related to a lower risk of cancer mortality in the general population, and cancer-specific and all-cause mortality among cancer survivors [15,16].

One of the contributing factors of obesity is gut microbiome dysbiosis. Then, microbiota modulation through diet could play a relevant role in obesity and obesity-related cancer prevention and treatment. The holobiont and the symbiotic relationship between microbiota and host must be seriously considered when studying human metabolism and obesity. Concretely, the holobiont is defined as an assemblage of a host and the other species living in or around it, which collectively form a discrete ecological unit [17]. The holobiont includes the host, virome, microbiome, and any other organisms which contribute to functioning as a whole [18]; this concept was initially introduced by Adolf Meyer-Abich and refined by Dr. Lynn Margulis in the early nineties [17].

In this review, we provide an overview of the epidemiological evidence on the associations between obesity and cancer, and between MD and OO and obesity-related cancers. In addition, we describe the microbiota mechanisms involved in the link between obesity and cancer and highlight how MD and olive oil can modulate gut microbiota (GM). The elucidation of these relationships could be relevant for the development of preventive, diagnostic, and therapeutic strategies against obesity-related cancers.

2. Obesity and cancer risk: epidemiological evidence

Obesity is a complex multifactorial disease defined as an excessive body fat accumulation that causes a health risk. A body mass index (BMI) $\geq$ 30 kg/m^2 is considered obese [19]. Among the well-established factors influencing obesity are the increase in the consumption of hypercaloric and nutritionally poor foods and a sedentary lifestyle. These often coexist with distress, hormonal imbalance, gut microbiome dysbiosis, poor sleep quality, or the consumption of certain medications, and can be boosted by genetic conditions [20]. The prevalence of obesity has risen dramatically worldwide in the last decades: in 2014, over 640 million adults had obesity, a six-fold increase since 1975 [19,21]. In addition, in 2016, over 124 million children and adolescents were obese [19]. These increments go hand in hand with the increased morbidity and mortality rate caused by cancer, and may be promoting obesity-related cancers at a younger age [22]. Currently, cancer is a leading cause of death worldwide, just behind cardiovascular diseases, and obesity is a major public health concern [19]. The association between obesity and cancer risk is supported by a large body of epidemiological evidence, which has been reviewed and meta-analysed by both the International Agency for Research on Cancer (IARC) and the World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR) [23]. Adult body fatness has been established by the IARC as a strong risk factor for 13 different cancer types in humans, summarised in Table 1. In 2012, about 3.6% (481,000) of all new cancers (excluding non-melanoma skin cancer) in adults ($\geq$30 years old) were attributable to excess BMI (defined as 25 kg/m^2 or greater) [24]. In women, postmenopausal breast, endometrial and colon cancers accounted for 72.5% of the total attributable cases to high BMI, whereas in men kidney and colon cancers accounted for 66.0% [24]. Body fatness during childhood or early adulthood has also been associated with a higher risk of several malignancies in adulthood, including leukaemia, Hodgkin's disease, and colorectal cancer (CRC) [25,26]. For some cancer types, sex-related differences in association with obesity and cancer risk appear only later in life. An estimation of new cancers in the European Union using population-attributable risks showed that the incidence of new cancers attributable to excess weight (BMI $\geq$ 25 kg/m^2) was 2.5% for men and 4.1% for women, which suggested a higher risk of obesity-related cancers in adult women [27]. The WCRF/AICR made separate conclusions for body fatness in young women for breast cancer, owing to effect modification by menopausal status: they established that a 5 kg/m^2 increment in BMI probably decreases the risk of premenopausal breast cancer, whereas it increases the risk of post-menopausal breast cancer with a convincing level of evidence [23].

Table 1
Strength of evidence of the association between obesity and cancer risk.

Cancer site	Evidence for increased risk		Risk Estimate (95% CI)[a]
	IARC 2020	WCRF/AICR 2018	
Colorectum	Strong	Strong-Convincing	1.05 (1.03 – 1.07)
Endometrium	Strong	Strong-Convincing	1.50 (1.42 – 1.59)
Breast (post-menopausal)	Strong	Strong-Convincing	1.12 (1.09 – 1.15)
Oesophageal adenocarcinoma	Strong	Strong-Convincing	1.48 (1.35 – 1.62)
Kidney	Strong	Strong-Convincing	1.30 (1.25 – 1.35)
Liver	Strong	Strong-Convincing	1.30 (1.16 – 1.46)
Pancreas	Strong	Strong-Convincing	1.10 (1.07 – 1.14)
Thyroid	Strong	-	1.06 (1.02 – 1.10)[c]
Multiple myeloma	Strong	-	1.09 (1.03–1.16)[d]
Meningioma	Strong	-	1.54 (1.32 – 1.79)[b,e]
Gastric cardia	Strong	Strong-Probable	1.23 (1.07 – 1.40)
Ovary	Strong	Strong-Probable	1.06 (1.02 – 1.11)
Gallbladder	Strong	Strong-Probable	1.25 (1.15 – 1.37)
Prostate (advanced)	Moderate	Strong-Probable	1.12 (1.04 – 1.21)
Mouth, pharynx, and larynx	Moderate	Strong-Probable	1.15 (1.06 – 1.24)
Diffuse large B-cell lymphoma	Moderate	-	1.29 (1.16 – 1.43)[b,f]
Male breast	Moderate	-	1.19 (1.10 – 1.30)[g]
Cervix	Limited	Limited-suggestive	1.02 (0.97 – 1.07)

IARC: International Agency for Research on Cancer; WCRF/AICR: World Cancer Research Fund/American Institute for Cancer Research
Adapted from: World Cancer Report IARC 2020 [253] and Continuous Update Project Expert Report WCRF/AICR 2018[2]
[a]Risk Estimate (95%CI) for 5-unit increment in BMI (kg/m^2) adapted from WCRF/AICR [254] if not stated otherwise.
[b]Risk estimate (95%CI) for highest vs lowest category of BMI
[c]Source: Kitahara et al. 2016 [255]
[d]Source: Teras et al. 2014 [256]
[e]Source: Niedermaier et al. 2015 [257]
[f]Source: Castillo et al. 2013. Only in participants with obesity (BMI$\geq$30 kg/m^2) [258]
[g]Source: Brinton et al. 2014. [259]

3. Mediterranean diet and obesity-related cancers: epidemiological evidence

A growing body of evidence reinforces that an overall healthy dietary pattern, characterised by a low consumption of red and processed meats, high consumption of fruit and vegetables, whole grains rather than refined grains, and plant sources of protein and fat is inversely associated with the risk of cardiometabolic diseases and cancer [28]. At this point, there is solid evidence suggesting that a two-point increment of the MD score is associated with a 4% lower risk of cancer [29]. A recent meta-analysis including data from 3202,496 participants belonging to 117 studies (comprising randomised control trials, cohorts, and case-control studies), comprehensively examined the relationships between adherence to the MD and different cancer risks. As results, the authors showed that high adherence to MD is inversely associated with the risk of cancer mortality in the general population (Relative Risk (RR) = 0.87, 95% CI 0.82, 0.92), and all-cause mortality among cancer survivors (RR = 0.75, 95% CI 0.66, 0.86) [16] (Table 2). MD has been studied for its protective capacity against the risk of several obesity-related cancers, such as aerodigestive and gastrointestinal, gynaecological, and other cancers.

3.1. MD and aerodigestive and gastrointestinal cancers

Two different meta-analyses including data from case-control and prospective cohort studies showed that a high MD adherence was inversely associated with a 10–17% total risk of CRC [16,30], but not with total and CRC mortality [30] (Table 2). The protective effect of the MD against CRC was also observed in specific anatomical locations, including the proximal and distal colon, and rectum. Moreover, high

Table 2
Summary of results from last meta-analyses showing associations between Mediterranean diet adherence or olive oil intake and major outcomes of overall cancer and obesity-related cancer types in observational studies.

Cancer type/site	Outcome	Dietary factor (Highest vs Lowest)[a]	Number and design of studies	OR/RR (95% CI)	Reference
Overall	Cancer mortality	MD adherence	18 cohort	RR_{cohort} = 0.87 (0.82, 0.92)	Morze et al.[14]
Overall	All-cause mortality	MD adherence	8 cohort	RR_{cohort} = 0.75 (0.66, 0.86)	Morze et al.[14]
Overall	Cancer mortality	MD adherence	4 cohort	RR_{cohort} = 0.96 (0.82, 1.11)	Morze et al.[14]
Overall	Cancer reoccurrence	MD adherence	1 cohort	RR_{cohort} = 0.61 (0.18, 2.07)	Morze et al.[14]
Overall	Cancer incidence	OO intake	37 case-control 8 cohort	$OR_{case\text{-}control}$ = 0.65 (0.57, 0.74) RR_{cohort} = 0.90 (0.77, 1.05) $RR_{observational}$ = 0.69 (0.62, 0.77)	Markellos et al.[42]
Colorectal	Cancer incidence	MD adherence	7 case-control 10 cohort	$OR_{case\text{-}control}$ = 0.64 (0.52, 0.79) RR_{cohort} = 0.92 (0.87, 0.99) $RR_{observational}$ = 0.83 (0.76, 0.90)	Morze et al.[14]
Colorectal	Cancer incidence	OO intake	6 case-control 1 cohort	$OR_{case\text{-}control}$ = 0.91 (0.78, 1.06) RR_{cohort} = 0.88 (0.68, 1.14) $RR_{observational}$ = 0.90 (0.79, 1.03)	Markellos et al.[42]
Colorectal	All-cause mortality	MD $adherence_{prediagnosis}$ MD $adherence_{postdiagnosis}$	3 cohort 2 cohort	RR_{cohort} = 0.80 (0.62, 1.04) RR_{cohort} = 0.66 (0.37, 1.17)	Zhong et al.[18]
Colorectal	Cancer mortality	MD $adherence_{prediagnosis}$ MD $adherence_{postdiagnosis}$	3 cohort 1 cohort	RR_{cohort} = 0.90 (0.71, 1.14) RR_{cohort} = 0.84 (0.50, 1.42)	Zhong et al.[18]
Breast	Cancer incidence	MD adherence	11 case-control 12 cohort	$OR_{case\text{-}control}$ = 0.87 0.82, 0.93 RR_{cohort} = 0.97 (0.94, 1.00) $RR_{observational}$ = 0.94 (0.90, 0.97)	Morze et al.[14]
Breast	Cancer incidence	OO intake	11 case-control 3 cohort	$OR_{case\text{-}control}$ = 0.63 (0.45, 0.87) RR_{cohort} = 0.67 (0.29, 1.56) $RR_{observational}$ = 0.67 (0.52, 0.86)	Markellos et al.[42]
Breast	Cancer incidence	OO intake	8 case-control 2 cohort	$OR_{case\text{-}control}$ = 0.48 (0.09, 2.70) RR_{cohort} = 0.76 (0.54, 1.06) $RR_{observational}$ = 0.75 (0.56, 1.00)	Sealy et al.[43]
Gastric	Cancer incidence	MD adherence	3 case-control 4 cohort	$OR_{case\text{-}control}$ = 0.63 (0.53, 0.75) RR_{cohort} = 0.77 (0.64, 0.92) $RR_{observational}$ = 0.70 (0.61, 0.80)	Morze et al.[14]
Gastric	Cancer incidence	OO intake	3 case-control 1 cohort	$OR_{case\text{-}control}$ = 0.65 (0.46, 0.93) RR_{cohort} = 1.15 (0.78, 1.69) $RR_{observational}$ = 0.75 (0.53, 1.05)	Markellos et al.[42]
Pancreatic	Cancer incidence	MD adherence	1 case-control 3 cohort	$OR_{case\text{-}control}$ = 0.48 (0.35, 0.66) RR_{cohort} = 0.92 (0.81, 1.05) $RR_{observational}$ = 0.80 (0.60, 1.06)	Morze et al.[14]
Pancreatic	Cancer incidence	OO intake	1 case-control	$OR_{case\text{-}control}$ = 0.58 (0.35, 0.97)	Markellos et al.[42]
Liver	Cancer incidence	MD adherence	1 case-control 3 cohort	$OR_{case\text{-}control}$ = 0.51 (0.34, 0.77) RR_{cohort} = 0.67 (0.56, 0.80) $RR_{observational}$ = 0.64 (0.54, 0.75)	Morze et al.[14]
Esophageal	Cancer incidence	MD adherence	1 case-control 2 cohort	$OR_{case\text{-}control}$ = 0.26 (0.13, 0.52) RR_{cohort} = 0.85 (0.67, 1.09) $RR_{observational}$ = 0.64 (0.35, 1.16)	Morze et al.[14]
Esophageal	Cancer incidence	OO intake	3 case-control	$OR_{case\text{-}control}$ = 0.47 (0.24, 0.93)	Markellos et al.[42]
Head and neck	Cancer incidence	MD adherence	8 case-control 1 cohort	$OR_{case\text{-}control}$ = 0.54 (0.40, 0.72) RR_{cohort} = 0.73 (0.60, 0.89) $RR_{observational}$ = 0.56 (0.44, 0.72)	Morze et al.[14]
Aerodigestive	Cancer incidence	OO intake	6 case-control	$OR_{case\text{-}control}$ = 0.74 (0.60, 0.91)	Markellos et al.[42]
Endometrial	Cancer incidence	MD adherence	3 case-control 1 cohort	$OR_{case\text{-}control}$ = 0.58 (0.35, 0.95) RR_{cohort} = 0.98 (0.82, 1.17) $RR_{observational}$ = 0.67 (0.41, 1.11)	Morze et al.[14]
Ovarian	Cancer incidence	MD adherence	1 case-control	$OR_{case\text{-}control}$ = 0.91 (0.71, 1.17)	Morze et al.[14]

[a] Comparing highest vs lowest adherence or intake to Mediterranean diet or of olive oil, as appropriate. CI, confidence interval; MD, Mediterranean diet; OO, olive oil; OR, odds ratio, RR, relative risk.

adherence to the MD was associated with a lower overall gastric cancer risk [16,31], and by anatomical location (i.e., cardia and non-cardia) and histological subtype (i.e. intestinal and diffuse) [31,32]. A recent meta-analysis pooling data from one case-control and three cohort studies found that MD adherence was not statistically significantly associated with pancreatic cancer risk (RR = 0.80, 95% CI 0.60, 1.06) [16]. However, findings from two more recent prospective studies observed reductions for high MD adherence of between 18% and 43% in the risk of pancreatic cancer [33,34]. A high MD adherence was inversely related to liver cancer risk in a pooled analysis comprising data from one case-control and three cohort studies with a statistically significant risk reduction of 36% [16]. A similar association was observed in a prospective cohort study revealing that higher adherence to the alternate MD score was significantly associated with a lower risk of liver cancer [35]. A recently published meta-analysis encompassing data from one case-control and two cohort studies found no association between MD adherence and oesophageal cancer risk [16] (Table 2). To our knowledge, only one study has examined the association between MD and gallbladder cancer risk. The cohort study by Larsson et al. followed 76,014 subjects for 13.3 years and observed a significant reduction (Hazard Ratio (HR) = 0.42, 95% CI 0.23, 0.79) in gallbladder cancer risk in individuals following a high adherence to MD [36]. The MD has a beneficial role in the risk of head and neck cancer, which encompasses cancers in the oral cavity, pharynx, and larynx (all obesity-related cancers). Concretely, a recent meta-analysis showed that higher adherence to MD was related to a 44% lower risk of head and neck cancer [16] (Table 2). In summary, adhering to MD is associated with a lower risk of gastric, colorectal, liver, and head and neck cancers; while the results with pancreatic, oesophageal, and gallbladder cancer are still inconclusive.

3.2. MD and gynaecological cancers

Higher adherence to MD has been reported to be protective against breast cancer regardless of the menopausal status (i.e., premenopausal and postmenopausal) and hormone receptor expression (i.e., oestrogen, progesterone, human epidermal growth factor receptor (HER), and mixed) [16,37,38]. Likewise, greater adherence to the MD may positively impact on the quality of life of breast cancer survivors, specifically improving physical functioning, sleep, pain, and overall well-being [39]. A meta-analysis of three case-control and one cohort study by Morze et al., found no significant difference in endometrial cancer risk between low and high MD adherence [16] (Table 2). A prospective cohort study by Xie et al., explored the relationship between MD and ovarian cancer development in 82,948 women [40]. The results found that high MD adherence did not modify the risk of ovarian cancer among participants. Overall, the only gynaecological tumour where higher MD adherence is associated with lower risk is breast cancer.

3.3. MD and other obesity-related types of cancer

Only one study reported results on MD in relation to the risk of thyroid cancer. In a large prospective cohort, the European Prospective Investigation into Cancer and Nutrition (EPIC) study, Llaha et al., mainly found no association between MD adherence and thyroid cancer risk [41]. Pooling data from two large prospective studies (N = 2792,257 person-years of follow-up, with 478 incident multiple myeloma cases), Lee et al., observed a suggestive inverse trend with multiple myeloma risk [42]. In another EPIC sub-study, a higher MD adherence was found to be modestly associated with the risk of overall lymphoma but not by subtypes, including diffuse large B-cell lymphoma [43]. To the best of our knowledge, there are no published studies on the relationship between MD adherence and the risk of other obesity-related cancers (namely thyroid, multiple myeloma, kidney, meningioma, and male breast cancer).

4. Olive oil and obesity-related cancers: epidemiological evidence

Olive oil is a key component of the MD and its consumption has largely been investigated concerning its capacity to reduce cancer risk. A recent meta-analysis of 37 case-control (17,369 cases and 28,294 controls) and eight cohort studies (12,461 incident cases in a total cohort of 929,771 subjects) concluded that higher OO consumption is associated with a 31% lower likelihood of any cancer (pooled RR = 0.69, 95%, CI: 0.62–0.77) [44] (Table 2). In the same meta-analysis, high OO consumption was inversely associated with the risk of oesophageal and breast cancers, but not with colorectal and gastric cancers [44].

4.1. Olive oil and aerodigestive and gastrointestinal cancers

Pooled data from observational studies support that high OO consumption may protect against upper aerodigestive (composed of oral cavity, pharynx, and larynx) and total gastrointestinal and oesophageal subtype cancer risk, but not against colorectal and gastric cancers risk [44] (Table 2). An Italian case-control study showed an inverse relationship between OO and pancreatic cancer [45]. To date, there are no previous publications on the relationship between OO consumption and the risk of liver, and gallbladder cancers.

4.2. Olive oil and gynaecological cancers

Breast cancer is by far the most studied gynaecological cancer in relation to the anticancer effects of OO consumption. Two recent meta-analyses of 10 (7030 cases among 81,436 participants) and 14 (29,830 cases among 987,895 participants) observational studies determined that women consuming higher amounts of OO reduced their risk to develop breast cancer between 25% (RR = 0.75, 95%, CI: 0.56, 1.00) and 33% (RR = 0.67, 95%, CI: 0.52, 0.86) compared with those consuming less [44,46] (Table 2). Contrary to breast cancer, evidence on endometrial and ovarian cancer is limited. Results from two case-control studies showed an inverse association between increased OO consumption and endometrial [47] and ovarian cancer [48] risks.

4.3. Olive oil and other types of cancer

As far as we know, there is no evidence regarding a potential relationship between OO consumption and the risk of other obesity-related cancer types, such as kidney, thyroid, meningioma, multiple myeloma, male breast, and diffuse large B-cell lymphoma cancers.

5. Obesity-related cancers' epidemiological evidence: strengths and limitations

Some limitations in methodological aspects may be behind the inconclusive results on the association between MD, OO, and several obesity-related cancers. For example, as stated in various systematic reviews and meta-analyses, two major limitations in most of these studies are inconsistencies in the definition of the MD pattern and the cut-off points used to differentiate high from low MD adherence [2,16,49,50]. To date, up to 34 different scores have been used in the literature to assess the degree of adherence to MD [51]. MD scores vary, especially as regards the inclusion or not of the alcohol component and the intake levels in the population. This variability might affect the results of MD and cancer relationships. Furthermore, the lack of information about the quality and safety (e.g., product treated or not with chemical agents, antibiotics, or hormones) of foods during the food intake assessment for MD scores could modify results, reducing the benefits of the MD [2]. Another common limitation may relate to dietary measurement errors, especially when dietary questionnaires are self-reported. Furthermore, dietary measurements are usually collected at baseline, which makes accounting for changes in diet during the follow-up not possible. On the

other hand, retrospective assessment of the usual diet, especially in subjects with the disease, as occurs in case-control studies, poses a high likelihood of recall bias and, hence, largely questioning its validity. Regarding OO, most studies acknowledge that they do not differentiate between common, virgin, and extra-virgin types. This distinction is important because compared to the refined type, VOO and EVOO have much higher concentrations of bioactive compounds and may, therefore, have more health benefits [52], including greater protection against cancer [53]. Finally, a higher adherence to the MD usually goes with a higher adherence to a Mediterranean lifestyle (e.g., healthier food preparation, eating locally and seasonally, socializing during meals, and even being more physically active and having an adequate rest) [1]. Although epidemiological studies often control for some of these factors, the presence of possible residual confounding cannot be excluded.

6. Obesity and cancer risk: biological mechanisms

The mechanistic pathways by which obesity is linked to carcinogenesis are not yet fully elucidated; current evidence suggests that several biological mechanisms might explain this association [54–56]. Here, we will focus on those related to chronic inflammation, insulin and insulin-like growth factor-I (IGF-I), sex hormone signalling, gut microbiome dysbiosis, and specific localised mechanisms. Fig. 1 summarises the mechanisms proposed and reflects how dysfunctional adipose tissue acts as one of the main triggers of these processes.

6.1. Chronic inflammation

Obesity is characterised by an excess of adipose tissue, an active organ with metabolic and endocrine activity, and is considered a low chronic inflammatory state [57]. During obesity, adipose tissue is characterised by infiltration of monocytes that switch to M1 macrophages, therefore, leading to dysfunctional adipose tissue [58]. These macrophages are often stimulated by cytokines such as interferon-γ (INF-γ) or by microorganism-associated molecular patterns (MAMPs) such as lipopolysaccharides (LPS) [59]. M1 macrophages alter the function of adipocytes, increasing pro-inflammatory cytokine secretion, including tumour necrosis factor-alpha (TNF-α), interleukin (IL)− 10 and IL-6, or monocyte chemoattractant protein (MCP)− 1 [55,58,60,61]. This leads to the production of free radicals and DNA damage, upregulation of proliferative and anti-apoptotic pathways, angiogenesis, and cell migration [61–63].

Adipokines (such as leptin and adiponectin) are adipocyte-derived hormones involved in metabolism regulation, crosstalk with inflammatory pathways, insulin signalling, angiogenesis, and cellular proliferation [57,64]. Leptin is an adipose-derived hormone linked to satiety and energy homeostasis control and its levels are increased in individuals with obesity. Mechanistic studies have shown that it increases angiogenesis, cell proliferation, migration, and invasion responses, as well as inhibition of apoptosis, which promotes cancer initiation and development [65–67]. A recent review of epidemiological and mechanistic studies linked higher levels of circulating leptin to breast, colon, thyroid, and pancreatic cancers [67,68]. Adiponectin, on the contrary, is produced only by mature adipocytes and its secretion is inhibited by insulin, its circulating levels are inversely correlated with the level of adiposity [69–71]. It has a potent anti-inflammatory effect, acting as an insulin sensitiser, which could indirectly prevent tumour development. In addition, a more direct effect is its ability to inhibit growth factor function (e.g., binding and sequestrating heparin-binding epidermal and basic fibroblast growth factors), decreasing cellular growth and proliferation, preventing DNA damage, and increasing apoptosis [55,70]. Several findings suggested a negative correlation between adiponectin levels and cancer risk, particularly via hormone-obesity-insulin resistance and suppression of growth and proliferation pathways [72]. Different mechanistic and epidemiological studies indicated that hypoadiponectinemia may be associated with the risk of different types of cancer, such as breast, endometrial, colon, gastric, pancreatic and haematological malignancies, among others [70]. In addition, chronic inflammation and oxidative stress, and abnormal secretion of adipocytokines have been included between the biological mechanisms that link

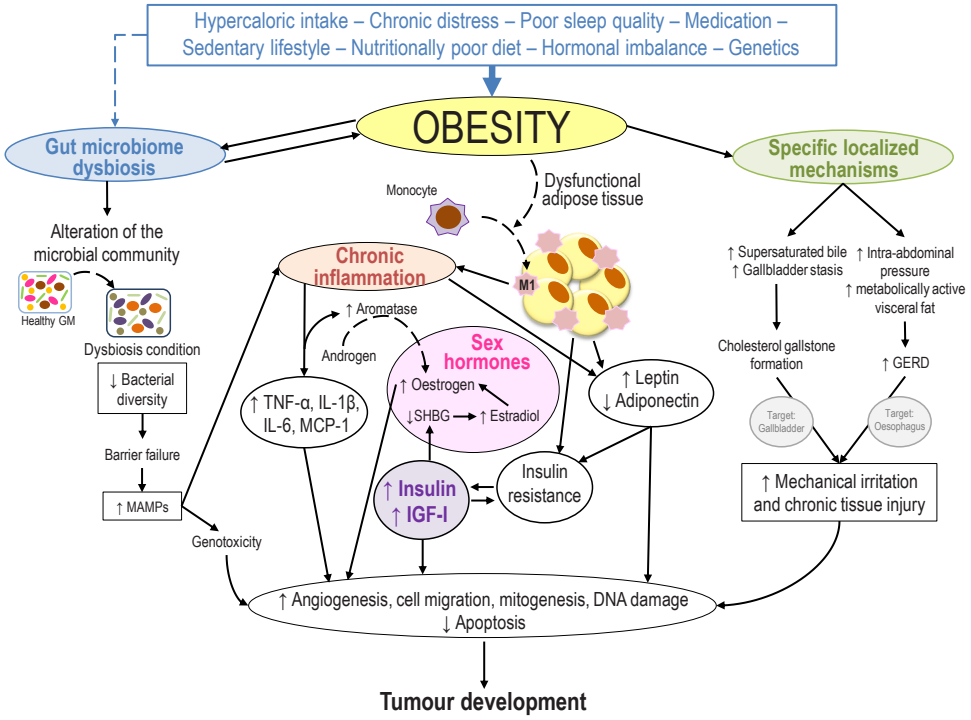


Fig. 1. Proposed mechanisms by which obesity may be linked to cancer risk. GERD, gastroesophageal reflux disease; GM, gut microbiome; IGF, insulin-like growth factor; IL, interleukin; MAMPs, microorganism-associated molecular patterns; MCP, monocyte chemoattractant protein; SHBG, sex hormone binding protein; TNF-α, tumour necrosis factor-alpha.

obesity with urinary cancers [73]. Local or systemic immune inflammation plays a role in the onset and progression of bladder cancer. During inflammation, the activation of inducible nitric oxide synthase (NOS) leads to the generation of nitric oxide, which can have several effects on bladder cancer development. Nitric oxide can impede DNA repair processes and promote angiogenesis, the formation of new blood vessels. N-nitrosamines, which are recognized bladder carcinogens in animals, can form within the bladder as a result of the interaction between oxidative byproducts of nitric oxide and secondary amines. This interaction may directly contribute to the initiation of bladder cancer [74].

6.2. Insulin and insulin-like growth factor I

Greater body fatness and altered adipocyte function is associated with higher circulating levels of insulin, and when body fatness is mainly distributed centrally, in the abdominal area, insulin resistance is more likely to develop [75]. Obesity-associated insulin resistance has been shown to be associated with elevated levels of pro-inflammatory cytokines, as a consequence of dysfunctional adipose tissue [58,76,77]. Chronic hyperinsulinemia may promote abnormal stimulation of multiple cellular signalling cascades, and increase the activity of IGF-I, a hormone primarily produced by the liver [78]. IGF-I binds to insulin receptors in different tissues and promotes cell proliferation, survival, migration, metabolism and angiogenesis, and decreases apoptosis, therefore, increasing the risk of different types of tumours [78–81]. Moreover, insulin decreases sex hormone-binding globulin (SHBG) levels, resulting in higher levels of free oestradiol and, therefore, oestrogen availability, thus increasing the risk of breast cancer [80]. Hyperinsulinemia and higher IGF-I levels have been clearly associated with the risk of breast, endometrial, ovarian, and prostate cancers and have been suggested to be involved in the development of several gastrointestinal cancers, thyroid cancer, and multiple myeloma in epidemiological studies [82–87]. In addition, they have been associated with increased pancreatic and breast cancer mortality [88,89], and overall cancer mortality [81,90,91].

6.3. Sex hormones

An increased dysfunctional adipose tissue leads to an increase in aromatase enzyme (also called oestrogen synthetase) expression and activity. Concretely, aromatase triggering may be induced by increased levels of adipose tissue TNF-α [92,93]. Aromatase is responsible for the conversion of androgens and androgenic precursors to oestrogens in adipose tissue. This production, together with decreased serum SHBG as a consequence of higher insulin and IGF-I plasma levels [80], increases the serum concentration of bioavailable oestradiol [94]. Oestrogens increase cell proliferation and reactive oxygen species and inhibit DNA repair machinery, leading to DNA damage and tumorigenesis [95]; it has been proposed as the mechanistic pathway linking obesity to postmenopausal breast and endometrial cancers [56,96–98]. Higher levels of oestradiol were associated with a higher postmenopausal breast cancer risk in a meta-analysis of eight prospective studies in postmenopausal women [94]. In addition, a review of epidemiological studies concluded that obesity class 1 (BMI>30 and <35 kg/m^2) was associated with a 2.6-fold increase in endometrial cancer risk, while obesity class 2 and 3 (BMI>35 kg/m^2) was associated with a 4.7-fold increase, when compared with women without obesity [99]. Sex hormones have also been implicated in the pathogenesis of urologic obesity-related cancers. Aromatase converts androgens into oestradiol, and, for example, enhanced prostate cancer risk has been associated with an increased oestrogen/testosterone ratio [73].

6.4. Gut microbiome dysbiosis

Recent research has demonstrated that obesity and its metabolic consequences are also related to alterations in GM and intestinal inflammation. GM dysbiosis can be a consequence of several factors, including antibiotic consumption, acute gastrointestinal infections, inflammatory bowel diseases, and diet [100]. Even so, obesity has been previously linked to GM dysbiosis [101]. GM dysbiosis is characterised by an imbalance between pathogens and natural and healthy microbiota, reducing symbionts (health-promoting) and increasing invasive, inflammation-inducing, genotoxic bacteria, and cancer-promoting metabolites [100]. The mechanisms linking obesity-associated GM dysbiosis to cancer development include altered microbial metabolism and generation of pro-carcinogenic metabolites, metabolic dysregulation, and induction of inflammation, as well as host immune response disturbance [102,103]. GM dysbiosis leads to a series of reactions that ultimately result in a cancer-promoting state with increased intestinal permeability. This phenomenon can be physical or at the level of antibacterial defence systems, and favour bacterial translocation [100]. This leads to increased inflammation, mediated by MAMPs, which activate macrophages and promote the secretion of pro-inflammatory cytokines [104,105]. GM dysbiosis can also result in genotoxicity mediated by bacterial genotoxins that induce DNA damage in organs in direct contact with the bacteria, like the gastrointestinal tract [103]. Microbial density is much higher in the gastrointestinal tract than in other organs and the occurrence of gastrointestinal cancers linked to GM dysbiosis is more likely than in other cancers [100,103]. For instance, dysbiosis, intestinal permeability, chronic inflammation, and bacterial genotoxicity were previously linked to CRC in mice [100]. However, the microbiota also mediates other pathways such as bile acids or oestrogen metabolism, and tumorigenic mediators that may exert long-distance effects, triggering tumorigenesis in organs with low or null microbial density, such as breast, liver, lung, or pancreas [100,106]. Dysbiosis of the gastrointestinal and urinary tract microbiome have been linked to higher risk of kidney and bladder cancers. An altered microbiome leads to a dysfunctional modulation of the endogenous anti-tumour immune response, as well as mucosa biofilm formation, pathogenic bacterial colonization, and induction of chronic inflammation via the reactive oxygen species molecular pathway among others [107,108].

6.5. Specific localised mechanisms

Obesity enhances hepatic secretion of cholesterol-supersaturated bile and gallbladder stasis, which may impact on cholesterol gallstone formation, increasing the risk of gallstone-related complications [109]. Gallstones produce mechanical irritation and delayed biliary emptying, resulting in dysplastic changes in the gallbladder [110]. In this sense, results from a recent meta-analysis of observational studies showed that the presence of gallstones is a major risk factor for gallbladder cancer (OR: 7.26; 95% CI: 4.33; 12.18) [111].

Obesity also increases the risk of oesophageal adenocarcinoma, an association that seems to be stronger than for other obesity-related cancers. A potential mechanism explaining this association is the increased occurrence of gastroesophageal reflux disease (GERD) [112]. High abdominal pressure caused by intra-abdominal adiposity relaxes the lower oesophageal sphincter, thus exposing the oesophageal mucosal to gastric content and irritating the mucosa. Recurrent exposure to gastric acid and chronic tissue injury can lead to Barrett's metaplasia and premalignant state [113]. In addition, increased metabolically active visceral fat leads to increased levels of adipokines, including IL-6 and TNF- α, which may also play a role in GERD and the consequent development of oesophageal cancer [114]. A meta-analysis of population-based studies showed that daily GERD symptoms presented a seven-fold increased risk of oesophageal AC (OR: 7.40; 95% CI: 4.94; 11.1) compared with participants without GERD or with less frequent symptoms [115].

6.6. Palmitic acid and tumour growth

Palmitic acid, a saturated fatty acid, has been investigated for its potential role in tumour progression and metastasis formation. Among the profound changes that occur to cells during development of cancer, lipid metabolism experiences a dramatic shift toward enhancement of lipid biosynthesis pathways. Increased lipid uptake, storage, and lipogenesis are strongly up regulated in tumour cells to maintain the structure and fluidity of cell membrane [116]. Studies on the molecular mechanisms underlying the effects of palmitic acid-derived metabolite on cell proliferation have suggested that the fatty acid possesses mitogenic activity upon exposure of fibroblasts to growth factors, even though the biological effect was not attributable to the free fatty acid itself but to a palmitoleic acid-containing inositol phospholipid species that accumulated in the cells upon cell activation. Palmitic acid can activate various signalling pathways within cells that are associated with cell proliferation, survival, and apoptosis resistance. One such pathway is the mammalian target of rapamycin (mTOR) pathway, which regulates cell growth and metabolism. Palmitic acid can stimulate mTOR signalling, leading to increased protein synthesis and cell proliferation. Moreover, excessive levels of palmitic acid can lead to the production of ROS, which can cause cellular damage and DNA mutation. Palmitic acid has been shown to induce epithelial-mesenchymal transition in certain cancer models, such as prostate cancer, where cancer cells lose their epithelial characteristics and acquire mesenchymal properties, facilitating their invasive and migratory capabilities [117].

Palmitic acid has also been linked to metastasis formation. It influences the expression of genes involved in cell adhesion, extracellular matrix remodelling, and metastatic colonization. One study in animal models observed that when oral tumour cells and melanomas from humans were exposed to a palmitic acid rich diet and transplanted into mice, they showed a greater capacity to metastasize, even when this diet was administered for a short period prior to the transfer [118]. Epigenetic modifications of metastatic cells caused by the fatty acid were permanent and cells maintained the most aggressive properties.

7. The effects of the mediterranean diet and olive oil on the biological mechanisms that link obesity and cancer

The Mediterranean diet has been linked to a decreased risk of various cancers associated with obesity. While the precise mechanisms remain incompletely understood, several potential pathways related to obesity have been proposed to mediate the favourable effects of the Mediterranean diet on cancer risk [2]. One such mechanism involves the anti-inflammatory properties of the Mediterranean diet [119]. As previously mentioned, obesity is linked to a condition of persistent low-level inflammation, marked by the secretion of pro-inflammatory cytokines and adipokines like interleukins, TNF-α, and leptin. These substances are produced by adipocytes in white adipose tissue and by inflammatory cells that infiltrate adipose tissue [120]. The Mediterranean diet, abundant in anti-inflammatory foods like fruits, vegetables, whole grains, and healthy fats such as olive oil, contains bioactive compounds such as polyphenols and omega-3 fatty acids that possess anti-inflammatory properties. By mitigating inflammation, the Mediterranean diet may lower the risk of obesity-related cancers. As mentioned, several types of cancer have been specifically related to obesity, where the immune and inflammation response produce cytokines and chemokines that enable cancer development, cellular proliferation, angiogenesis and modify tumour microenvironment [121]. This observation may provide a plausible explanation for the reduced cancer risk associated with the Mediterranean diet, which consists of foods possessing anti-inflammatory properties and other factors with potential anti-cancer effects. Furthermore, the Mediterranean diet's abundant antioxidants contribute to its anti-carcinogenic properties. Oxidative stress, implicated in cancer development, is counteracted by the antioxidants present in the Mediterranean diet. Components such as polyphenols found in fruits like grapes or extra virgin olive oil have been shown to possess anti-carcinogenic effects, including the inhibition of tumour growth and promotion of cancer cell death.

In addition, the Mediterranean diet's positive influence on insulin sensitivity and blood glucose regulation also plays a role in mitigating cancer risk associated with obesity. Through the consumption of fibre-rich foods, low-glycaemic carbohydrates, and healthy fats, the Mediterranean diet stabilizes blood sugar levels, improves insulin sensitivity, and potentially lowers the risk of obesity-related cancers. Higher adherence to a Mediterranean dietary pattern has been linked to improvement of insulin sensitivity and markers of inflammation (lower NF-κβ, higher adiponectin) in participants with overweight and obesity without diabetes [122].

Another significant mechanism that can contribute to the beneficial effects of the Mediterranean diet in obesity and related conditions is the modulation of gut microbiota composition [123]. Obesity alters the gut microbiota, contributing to chronic inflammation and metabolic dysfunction. The Mediterranean diet has been linked to a favourable profile of gut microbiota, primarily attributed to its high content of dietary fibre and bioactive compounds characteristic of a plant-based dietary pattern [123]. It may support a balanced immune system, improved nutrient absorption, and reduced inflammation, thereby impacting cancer risk, by generating metabolites through the fermentation of nutrients, particularly short-chain fatty acids [124,125]. The Mediterranean dietary pattern has been shown to be a major modulator of gut microbiota composition and metabolite production, related to the development of several intestinal and extra-intestinal diseases that may deriver on obesity-associated cancers such as colorectal cancer [126].

In summary, the beneficial effects of the Mediterranean diet on cancer risk, particularly in relation to obesity-related cancers, may be mediated through mechanisms such as anti-inflammatory effects, improved insulin sensitivity and glucose regulation, antioxidant properties, and modulation of gut microbiota composition.

8. Obesity and cancer: microbiota mechanisms

Among the biological mechanisms linking obesity and cancer, in this review, we will focus on those related to microbiota.

The bacterial profile of a "healthy" GM has not been defined yet [127–129], mainly due to the elevated inter- and intraspecific variability. It depends on age, sex, environment, and daily habits (e.g., diet, physical activity, and antibiotics), among others [127,130,131]. Each subject owns a unique fingerprint of microbiota and, perhaps, there is no single "healthy" GM profile. This is why the actual trends of nutritional interventions tend to be personalised [132–134], which explains why individuals following the same diet display very different responses [135,136]. GM, or more specifically, colon microbiota, is the most abundant and, probably, the most relevant in terms of physiological activity. It has been estimated that more than $3.9{\cdot}10^{13}$ microbial cells live in the human colon, which means that their proportion to human eukaryotic cells is 10:1 [137].

Factors that determine our microbiota's fate include some that have an effect even before birth [138]. Some of these, like inherited genetics, are nonmodifiable; however, other factors can be modified, such as the environment and the way we were born and fed during the first 1000 days of life [138]. Interestingly, the bacterial population of our microbiota strongly depends on the community where we live, such as rural, urban, industrialised, and non-industrialised areas [139]. Indeed, when we talk about the mechanisms that lead to the onset of cancer in individuals with obesity, we should consider that the direct cause is not due to alterations occurring in the microbiota or human cells alone, but in the cells and their environment as the holobiont [140]. Besides, the disequilibrium of the complex interactions maintained over time between microbiota and human cells causes health disorders and diseases [141].

The classic definition of obesity does not consider the metabolic

status of individuals. Therefore, when analysing GM in obese individuals, this differentiation should be considered [142]. In individuals with obesity, the GM proportion of the generally most abundant bacterial phyla is often altered, displaying a higher Firmicutes/Bacteroidetes ratio when compared with non-obese individuals. However, the opposite relationship has also been stated [143,144]. Remarkably, no differences were detected in the aforementioned ratio between obese subjects with and without metabolic syndrome [145]. An increase in Firmicutes levels is usually associated with a higher bacterial ability to extract energy from the diet, especially these rich in carbohydrates. This fact is associated with a boosted production of short-chain fatty acids (SCFAs) via saccharolytic bacterial fermentation [146]. Sometimes, this rise in SCFAs may also be due to diets high in fat and, particularly, saturated lipids. Unexpectedly, this could be a part of a compensatory mechanism to eliminate excess energy from the diet [147]. Another explanation of this process could be that the abundance of taxa related to SCFA production, such as the genera *Oscillospira* and *Clostridium*, was increased only in obese subjects without metabolic syndrome [145].

As stated by Crovesy et al., [144], Proteobacteria and Fusobacteria were also increased in subjects with obesity, probably due to a dysbiotic state. During dysbiosis, these phyla are found associated with opportunistic bacteria and low-grade inflammation. In contrast, the phylum Verrucomicrobia, with its best-known member *Akkermansia muciniphila,* tends to have a reduced abundance in obese subjects. Indeed, its high relative abundance is associated with a lower BMI and, therefore, its supplementation may improve some key metabolic parameters [144, 148,149].

8.1. Bacteria, obesity, and cancer… The good, the bad, and the ugly

Tumour development may be triggered either by dysbiotic imbalances of the bacterial community or the bacterial species themselves [150]. Some of these species, also called oncobacteria, are *Helicobacter pylori and hepaticus, Fusobacterium nucleatum, Streptococcus gallotycus* and *bovis, Enterococcus faecalis, Bacteroides fragilis,* and some pathogenic *Escherichia coli* strains [150,151]. The extensively studied type I carcinogen *H. pylori* causes gastric cancer in 3% of individuals in which this species is present [152].

Another concept that requires further research is the fact that microbiota can be found not only in the intestinal lumen and different areas in the body but also in tumours themselves. Even though bacteria can be found in tumours, their microbiota has remained unanalysed until recently [153]. Thanks to one of the pioneering studies regarding the microbiota in various tumour localisations, we know that the microbiota of each tumour type i) tends to be more similar to each other than to other types, iii) has distinct compositions at different taxonomic levels, and iii) can be differentiated based on their different bacterial communities [153]. In fact, using cancer tissues and blood samples, a microbiome-based diagnostic tool capable of discerning between individuals with or without cancer has been developed [154]. While, we currently do not know how to control tumoral microbiota, recent advances in the development of techniques to modulate it have been made, such as the use of genetically modified bacteria [155]. Besides, it is also essential to consider that bacteria are not the only members of the microbiota that have an impact on tumours, as an example, fungal composition is another emerging field. Recently, the finding of distinctive combinations of fungi in 35 cancer types was confirmed [156,157].

It is worth mentioning that GM can connect our digestive system with other parts of the body via both the circulatory and nervous systems, as it is densely vascularised and innervated [158], with a high presence of immune system cells [159]. Both translocation of gut-derived bacteria and MAMPs, which are also relevant to connect the gut lumen back with the rest of the body, are able to generate systemic inflammatory responses [160].

Until recently, GM was almost unexplored in other obesity-related cancers than those of the digestive system, despite the non-invasive nature of stool analysis. There are some cohort-like GM studies, such as the MetaHit European cohort [161], LifeLines-DEEP Dutch cohort [162], Spanish cohort [163], AWI-Gen South-African cohort [164] and Human Microbiome Project USA cohort [165], but they did not include the cancer perspective.

Studies comparing the GM in each type of obesity-related cancer vs. that in controls face the common limitation of non-easy comparability. GM cannot be analysed to draw conclusions without considering certain variables that affect healthy and sick individuals differently. Importantly, not all studies used the same statistical analysis and analytical methodologies. In CRC, GM may play an important role in its development [166–168]. Some cancer types, such as meningioma [169,170], thyroid carcinoma [171,172], kidney tumour [155], and multiple myeloma [173], have been recently analysed from a GM perspective, but the available evidence is still scarce and inconclusive. To our knowledge, there are no studies on other obesity-related cancers, such as oesophageal adenocarcinoma and gallbladder cancer.

8.2. Inflammation and bacterial metabolites

Although there are still many unresolved questions, there is a growing understanding of the impact GM may have on tumour growth and development. Faecal microbiota transplants provide strong evidence that GM can trigger obesity and/or cancer phenotypes [174–176]. Many systems are interconnected with the digestive system, so the mechanisms that are sometimes beneficial in one organ could favour tumour onset in another. In order to classify these mechanisms linking the microbiota to the establishment of obesity-related cancers, we will focus on the one hand, on those processes related to inflammation and, on the other, on those associated with the deregulation of metabolites of bacterial origin.

8.2.1. Inflammation

GM and host cells are in constant cross-talk, allowing the organism to detect any significant change that alters its correct function [177]. Various receptors, such as toll-like (TLRs) and nod-like receptors (NLRs), recognise the molecular patterns associated with the GM, thus maintaining a context-specific immune response [177]. In a healthy situation, a small amount of LPS derived from gram-negative bacteria passes into the bloodstream [178]. But if the LPS concentration in the blood increases, endotoxemia may occur and become serious because it is associated with the development of systemic inflammation [178]. As higher concentrations of LPS are often detected in subjects with obesity, their metabolism tends to be at a pro-inflammatory stage [179]. Endotoxemia also promotes diabetes, underlying the phenomenon of insulin resistance [178].

The increase in LPS concentration may be the consequence of several mechanisms. It could be due to the poor state of the intestinal epithelial barrier, which is covered by a mucous layer. Its regeneration is associated with a sufficient presence of *Akkermansia muciniphila* [180], bacteria usually present in lower concentrations in subjects with obesity. Tight junction proteins, such as zonulin and occludin, which are involved in the control of intestinal permeability (higher protein levels, higher permeability), are often increased in individuals with obesity [181,182]. LPS leakage involves the activation of, among others, TLR-4, triggering the activation of the Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) protein complex, which is usually involved in DNA transcription and whose hyperactivation is associated with the inhibition of apoptosis, promoting the release of various pro-inflammatory cytokines. The other mechanism likely to be behind the increase in blood LPS levels is related to the absorption of dietary lipids since, when chylomicrons, responsible for their transport to the liver, are formed, LPS sneaks in along with fats [178,183]. This mechanism triggers local macrophages and activates an inflammatory response, which, in turn, alters the metabolism of other specialised cells, such as liver Kupffer and stellate cells, activating

progressive processes that may eventually trigger cancer [178].

8.2.2. Dysregulation of bacterial metabolite production

In the microbiota-host relationship, bacteria are known for their ability to metabolise specific compounds from the diet, host cells, and the metabolism of other microorganisms, as well as, for producing essential molecules for health, such as vitamins B and K. However, they can also synthesise some compounds (SCFAs, secondary bile acids and trimethylamine) that can have harmful effects [184].

The GM may contain different bacteria with the ability to metabolise both the same and distinct compounds. Firstly, a number of genera, such as *Clostridium* and *Eubacterium,* are capable of transforming primary bile acids into secondary ones, such as deoxycholic and lithocholic acid, which are usually positively, but in some cases, negatively associated with tumours [185,186]. In obsess individuals, the metabolism of bile acids and GM change concomitantly [187]. Secondly, phosphatidylcholine-, choline-, and carnitine-fermenting bacteria metabolise trimethylamine from dietary origin substrates; trimethylamine is a precursor of trimethylamine-N-oxide (TMAO) and appears to be increased in some cancers, like CRC [188,189]. These bacteria mostly belong to the Firmicutes and Proteobacteria phyla, but not to Bacteroidetes [188]. A recent meta-analysis revealed a positive correlation between TMAO levels and BMI [190]. Thirdly, oestrogen-metabolising bacteria, known as the oestrobolome [191], interact with the inactive form of oestrogen, allowing it to be reabsorbed, increasing its serum levels and the risk of postmenopausal breast cancer [192]. It is worth highlighting that, during menopause, the synthesis of this hormone is transferred to the adipose tissue, which is increased in obese subjects [193]. Fourthly, SCFA-producing bacteria convert dietary fibre into SCFAs. These levels and their impact on health are sometimes controversial but, low SCFA concentrations are associated with higher CRC risk [194,195]. SCFA-producing bacteria are increased in metabolically healthy obese individuals but not in those with metabolic syndrome [145]; Finally, Sulphate-reducing bacteria, which can convert sulphur-containing substances into hydrogen sulphide (H_2S), and nitro-compound-producing bacteria, which synthesise N-nitroso compounds, nitroamides, and nitrosamines. Sulphate-reducing and nitro-compound-producing bacteria produce metabolites that are implicated in carcinogenic processes and are related to obesity since weight loss is associated with lower serum H_2S levels [196–198].

9. The effects of the mediterranean diet and olive oil on the microbiota that link obesity and cancer

In a previous review, our team explored the benefits of OO on the pathophysiology and incidence of cancer, placing special emphasis on the cellular processes involved in its benefits [50]. This current review aims to broaden this picture by the inclusion of the effect of both MD and OO on the GM and microbiota-related mechanisms that link obesity and cancer in humans. Despite not finding any specific studies on the effects of MD/OO on GM in humans with cancer, the evidence presented herein regarding patients with other diseases and healthy volunteers would serve as proof of concept for its potential cancer prevention.

9.1. Preclinical models

The lipid fraction found in OO (mainly monounsaturated fatty acids, but also polyunsaturated fatty acids and saturated fatty acids) and PCs, such as oleuropein or hydroxytyrosol, are noteworthy for cancer prevention. Their potential effects are being studied in vitro and/or in non-human animal models [166]. Among the fatty acids present in OO, oleic acid is the most abundant monounsaturated fatty acid. There is evidence from the 40 s regarding its positive effect on the growth of lactic acid bacteria [199]. More recently, its antibacterial activity has been proven in vitro and in vivo on the opportunistic pathogen *Staphylococcus aureus* [200]. Among polyunsaturated fatty acids, linolenic acid also presented an antibacterial effect in vitro and in vivo against *H. pylori* [201,202]. PCs have a direct prebiotic action, most of them reaching the gut untransformed, where GM enzymes make them partially bioavailable [203,204]. Dietary PCs and their associated metabolites may strongly influence GM composition, inhibiting harmful and stimulating beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus* genera [166,203], and in some cases, also *Faecalibacterium* and *Roseburia* [203–205]. Similarly, in some of the studies summarised in Table 3 with MD and/or OO, the level of these taxa also increased, except for *Faecalibacterium* [206–211]. Conversely, some pathogens decrease in the presence of PC in vitro, such as *Staphylococcus aureus, E. coli* and *Listeria monocytogenes*, and there are beneficial species that also decrease, such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* [143].

9.2. Observational and interventional studies

In recent years, the modulatory potential effect of various food items on GM has been reviewed [212–214]. However, the isolation of the effects of a specific food within different dietary patterns is complex. An increasing number of studies support the benefits of MD in GM [206, 209,210,215,216]. The largest investigations regarding MD were the Prevention with Mediterranean Diet (PREDIMED) and PREDIMED-plus studies, in which the MD effect on GM was studied under particular conditions, such as insulin resistance or weight loss [216–218] (Table 2).

Regarding studies focused on MD, Haro et al., [206] analysed MD's effects in obese men in Spain. GM was found to be modulated by MD by decreasing *Prevotella* and increasing *Roseburia* and *Oscillospira* abundances; and, at the species level, *Parabacteroides distasonis* was increased. In another trial conducted in US healthy volunteers with a high risk of CRC [215], no differences were detected in bacterial abundance or diversity after an MD intervention. In a clinical trial conducted in Italy, after an MD including EVOO, the lactic acid bacteria abundance was higher in the overweight and obese compared with normal weight subjects and with pre-intervention [209] (Table 3). Later, in an observational study in Spain, levels of some beneficial bacteria increased in individuals with high MD adherence, such as *Bifidobacterium animalis,* but also some butyrate-producing ones such as *Roseburia faecis, Ruminococcus bromii*, and *Oscillospira plautii* [210]. In the PREDIMED-Plus study, both interventions, MD and a low-energy MD accompanied by exercise, produced GM changes in individuals with metabolic syndrome (with overweight or obesity) predominantly in the *Lachnospiraceae* and *Ruminococcaceae* families. In both interventions, the abundance of SCFA producers *Lachnospira* and *Lachnospiraceae NK4A136* was increased [216] (Table 3).

Few clinical studies specifically analysed the effect of OO on GM (Table 3) [207,208,211,219], but none were conducted in subjects with cancer. In the crossover study with hypercholesterolemia patients, three VOOs with different phenolic contents were compared. The most remarkable observation of this investigation was the increase in *Bifidobacterium* spp and *Parascardovia denticolens* after the VOO enriched with PC from thyme and OO (500 ppm), compared with the VOO intervention [207]. In another study, an intervention of EVOO consumption in men with undetectable human immunodeficiency virus (HIV) only affected bacterial diversity. In addition, several taxa showed changes at the genus and species level in the overall group and when sex was considered [208] (Table 3). In another trial carried out in overweight women from Brazil who followed an energy-restricted normal fat diet, EVOO consumption did not affect the diversity and relative abundance of GM [219]. In a trial with Chinese hypercholesterolaemia subjects, refined OO increased GM diversity and *Clostridium leptum* [211]. Interestingly, decreased GM diversity is not always an unhealthy sign; for example, a diet rich in EVOO has been linked to a significant reduction in GM diversity, causing a switch to a more protective group of bacteria in animal models [166].

While there is great variability in the results, most bacteria that

Table 3
The effects of MD and OO on GM in observational and interventional studies.

Reference MD	Subjects and Dosage	Methodology	GM changes	Other changes
Haro et al.[188] *CORDIOPREV*Spain	Interventional study with randomised obese adult men (n = 20)MD for one year	16 S rRNA sequencing Stool samples	↓ Prevotella ↑ Roseburia and Oscillospira↑ Parabacteroides distasonis	Changes in the abundance of 7 of 572 stool metabolites (amino acid, peptide, and sphingolipid metabolism associated)
Djuric et al.[197]*Healthy Eating Study*United States of America	Randomised, not-controlled, not-blinded trial with healthy adults with a high risk of colorectal cancer (n = 82)MD for six months	16 S rRNA sequencing Colonic biopsy samples	No significant differences in abundance nor in α-diversity	Bacterial communities differed by several parameters between subjects based on their serum carotenoids levels
Luisi et al.[191]Italy	Interventional study with overweight and obese adults (n = 18) and normal-weight control adults (n = 8) MD enriched with 40 g/day EVOO for three months	qPCR (primers for lactic acid bacteria) Stool samples	↑ Lactic acid bacteria	↓ Markers of inflammationand oxidative stress (subjects after vs. before intervention)↓ Proinflammatory cytokines (overweight and obese vs. controls)↑ IL-10 and adiponectin (overweight and obese vs. controls)
Rosés et al.[192] *Obekit*Spain	Observational study with normal weight, overweight, and obese adults divided by adherence to MD, high (n = 94) or low (n = 128)MD, with different adherence levels, for one year	16 S rRNA sequencing Stool samples	↑ Bifidobacterium animalis↑ Butyrate-producing taxa (Roseburia faecis, Ruminococcus bromii, and Oscillospira plautii)No species related to OO intake in high-adherence MD	
Muralidharan et al.[198] *PREDIMED-Plus*Spain	Randomised, controlled, parallel, not-blinded trial with overweight and obese adults with metabolic syndrome (n = 343)MD or energy restricted-MD with physical activity for one year	16 S rRNA sequencingStool samples	No significant differences in α-diversity↑ Lachnospira and Lachnospiraceae NK4A136↓ Butyricicoccus, Haemophilus, Ruminiclostridium 5, and Eubacterium hallii (in energy restricted MD compared to MD)↑ Coprobacter (in energy restricted MD vs. MD)↓ Haemophilus and Coprococcus 3 (associated with decreased adiposity parameters)↑ Lachnospiraceae NK4A136 (associated with adherence)	↑ in energy-restricted MD vs. MD:- Weight loss- Reduction in some parameters: BMI, fasting glucose, glycated haemoglobin, and triglycerides - HDL-cholesterol
OO				
Martín-Peláez et al.[189, 209]*VOHF*Spain	Randomised, controlled, double-blind, cross-over trial with adults with hypercholesterolemia (n = 12) 25 ml/day VOO (80 mg PCs/kg), FVOO (500 mg PCs/kg), or FVOOT (500 mg PCs/kg from OO and thyme) for three weeks for each oil type	Fluorescence in situ hybridization combined with flow cytometryStool samples	↑ Bifidobacterium and Parascardovia denticolens (in FVOOT compared to VOO)↑ IgA coated bacteria (in FVOO compared to baseline) not significantNo changes in Firmicutes/Bacteroidetes	↓ Oxidised LDL (FVOOT vs. baseline)↑ Protocatechuic acid (FVOOT vs. VOO)↑ Coprostanone (FVOO vs. FVOOT)↑ Faecal hydroxytyrosol and dihydroxyphenylacetic acids (FVOO vs. baseline and VOO)↑ CRP protein (FVOO vs. baseline, VOO, and FVOOT)
Olalla et al.[190]Spain	Interventional study with adults with HIV, aged ≥ 50 years with undetectable viral load (n = 32)50 g/day EVOO for 12 weeks	16 S rRNA sequencing Stool samples	↑ α-diversity (males)↑ Eggerthella, Ruminococcus, Lachnospiraceae, Parabacteroides, and Akkermansia (females)↑ Prevotella, Bacteroidetes, Bifidobacterium, Erysipelotrichaceae, and Eubacterium (males)↑ Gardnerella and Bulleidia moorei ↓ Mogibacterium, Dethiosulfovibrionaceae, Coprococcus, and some Bacilli species	↓ Total cholesterol
Netto Cândido et al.[201]Brazil	Randomised, parallel, double-blind trial with overweight adult women (total n = 52; EVOO n = 19)25 ml/day EVOO for nine weeks inside a breakfast drink with biscuits, inside an energy-restricted and normal fat diet	16 S rRNA sequencingStool samples	No significant differences in α-diversity nor richness (compared to baseline)No significant differences in abundance (in OTU, phyla, and genera levels; compared to baseline)	↑ Paracellular and transcellular permeability LPS concentrations remained unchanged
Lim et al.[193]Haldar et al.[203]China	Randomised, controlled, double-blind trial with adults with borderline hypercholesterolemia (total n = 146; ROO n = 44)30 ml/day ROO for eight weeks	16 S rRNA sequencingStool samples	↑ Clostridium leptum ↑ Veillonella, Clostridium, and Roseburia (negatively associated with pathological blood lipid parameters)	↓ Total and LDL-cholesterol, triglycerides, apolipoprotein B, ApoB/ApoA1 ratio and total cholesterol/HDL-cholesterol ratio

BMI, body mass index; CRP, C reactive protein; EVOO, extra-virgin olive oil; FVOO, functional virgin olive oil, FVOOT, functional virgin olive oil with thyme; LPS, lipopolysaccharide; MD, mediterranean diet; OO, olive oil; PC, phenolic compounds; ROO, refined olive oil; VOO, virgin olive oil;

became more abundant in both MD and OO trials were SCFA producers and particularly, butyrate producers [206–211,216]. From the studies that measured SCFAs in faeces, none detected changes in the fatty acid types tested [207,219]. It is striking that no SCFA changes were found linked to the increase in *Bifidobacterium* spp. [207], despite its known SCFA-producing capacity [220]. This finding underlines, once again, the complexity of GM modulation.

In these OO trials, its consumption could improve several cardiometabolic parameters, such as triglycerides, total cholesterol, cholesterol-associated LDL and HDL, and coprostanol [207,208,216, 221]. However, Luisi et al., found no differences after an MD with EVOO [209]. As cholesterol is the precursor of bile acids, the effects of OO on the above-mentioned metabolites, via the GM, could be beneficial. Interventions with OO in other clinical trials disrupted the levels of bile acid-related bacteria, *e.g.*, *Lachnoclostridium* and *Bilophila* [216], *Oscillospira* [210], and *C. leptum* [211].

There is scant evidence from trials regarding the effects of OO on either intestinal permeability or LPS. In a study with MD in high CRC-

risk subjects, LPS-binding proteins were less abundant in the group with higher serum carotenoid concentrations at baseline (which is negatively associated with CRC) [215]. In a Brazilian trial, paracellular and transcellular permeability were increased after EVOO intervention, although LPS levels remained unchanged in serum [219]. MD with EVOO, and also VOO interventions, can buffer LPS-associated endotoxemia [222,223] and prevent atherosclerosis, which is also linked to cancer [224] and both seem to be interconnected via the GM [225,226].

Both obesity and GM-associated endotoxemia aggravate processes related to systemic inflammation and oxidative stress, but OO has the potential to modulate both. Luisi et al., [209] found that the OO intervention affected both subjects with and without overweight/obesity, promoting a decrease in myeloperoxidase, 8-hydroxy-2-deoxyguanosine, and pro-inflammatory cytokines. IL-10 and adiponectin levels were also increased after OO intervention [209]. In another study by Martin-Peláez [227], the consumption of various VOOs did not cause any improvement in the variables associated with inflammation. They also pointed out that the use of pharmacological doses of a single source of PC increased the concentration of C-reactive protein, but this was not observed when two PC sources were combined. In another study, the decrease in C-reactive protein was associated with lower levels of Dethiosulfovibrionaceae [208]. Some of the GM modulations detected in this review, e.g., increased *Ruminococcus bromii, Roseburia genus, Clostridium leptum, and Bifidobacterium spp,* and decreased *Dethiosulfovibrionaceae*, including the compounds they produce, such as butyrate, have been associated with an anti-inflammatory effect [206–208,210,211]. The modulation of these bacteria is likely to be directed by fatty acids, as they have an antimicrobial effect, which in turn has an anti-inflammatory effect [219].

Insulin resistance is another metabolic complication frequently associated with obesity and inflammation. Interventions with OO and MD have been reported to reduce resistance, favouring sensitivity [206,209,210,215]. There are known negative associations with high serum carotenoid concentration [215], MD high adherence [210], and cytokines TNFα and IL-6 that impair insulin receptor signalling [209]. MD adherence increased insulin sensitivity and the *Roseburia* genus abundance in parallel, which is usually low in subjects with type 2 diabetes, suggesting a role in its prevention [206]. Some GM taxa associated with insulin resistance in non-diabetic subjects were reported in the PREDIMED-Plus study [218]. It is notable that the GM reported as beneficial for the insulin resistance stage, like *Oscillospiraceae*, had the same trend in some of the aforementioned studies [206,210].

10. Conclusions and future perspectives

We conclude that the current epidemiological knowledge shows associations between MD and OO consumption and most obesity-related cancers. In addition, the GM is involved in obesity-related cancers, and there are in vitro but also clinical studies that demonstrate that MD and OO can modify this microbiota. This microbiota modulation could play a role in the prevention and treatment of obesity-related cancers.

Primary cancer prevention involves adopting healthier lifestyle patterns, promoting greater physical activity and healthier food choices, and maintaining optimum body weight. In this context, the concept of "healthy lifestyle score" is gaining interest among researchers because the combination of various modifiable factors (i.e., smoking, BMI, physical activity, and diet), instead of dietary patterns alone, could lead to a greater reduction in the risk of many chronic diseases [228,229], including cancer [230]. It is important to bear in mind that MD has been included in most of the healthy lifestyle scores used today. We are convinced, therefore, that future research examining the relationship between the MD and olive oil adherence and cancer risk should integrate other factors of a traditional Mediterranean lifestyle, such as tobacco and alcohol consumption, physical activity, resting, and social activities.

In addition, microbiota modulation via the diet may play a role in cancer prevention and treatment. The symbiotic relationship between microbiota and the host must be seriously considered when studying human metabolism and obesity. Indeed, bacteria can be friend or foe since dysbiosis forms a part of carcinogenesis and, if we could achieve its correct management, we could decrease the cancer risk.

Given the unique characteristics of the microbiota and host, continued commitment is required for the personalisation of cancer management, both at the individual level and for different population clusters. Microbiota-based tools are being developed to facilitate the detection of different cancers [154,157]. Moreover, there is growing evidence that there is some inter-individual variability in the efficacy of anticancer treatments, and the consumption of probiotics [231] may increase their effectiveness [232–235].

Besides probiotics, there are others gaining importance: prebiotics, substrates selectively utilised by colonic microorganisms; synbiotics, a mixture of probiotics and prebiotics; and postbiotics, non-viable microorganisms and/or their microbial metabolites. Postbiotics have been around in Europe for some time; however, in the European Union, no specific regulation covers probiotics, prebiotics, synbiotics, or postbiotics [236,237]. Recently, in 2021, the use of *Akkermansia muciniphila*, known for its positive effects against obesity, was approved by the EFSA, as a novel food pursuant to Regulation (EU) 2015/2283 [238].

The classic, and often overlooked, way of modulating our microorganisms through diet and other habits also deserves a mention. As we have reviewed, both the MD and one of its main fats, OO, are involved in the relationship between GM and cancer. Furthermore, there are other dietary patterns, such as fasting-mimicking diets, ketogenic diets, and higher fibre diets, with this capacity [233]. A non-sedentary lifestyle and healthy lifestyle patterns associated with higher resting and less stress are other habits that should be considered [239–242]. It is also likely that, over time, other microbiota members, such as fungi (mycobiome) and even viruses (virome), will gain prominence and be analysed together in all those areas where the GM currently has a predominant role [243–245]. Therapies based on bacteriophage viruses are becoming increasingly more known [246,247].

Faecal microbiota transplants could become a fruitful modulatory tool of GM as some trials have already demonstrated [248,249]; indeed, they have usually been used to treat *Clostridium difficile*-resistant infections [250]. Another potential option that might seem like science fiction right now, is the use of models based on organ-on-a-chip, gut--microbiota-on-a-chip, and tumour-on-a-chip, which allows the design of prototypes more like humans, making them more applicable. Something yet more innovative about these chips is that they can be interconnected [251]. But if there is one thing that all these approaches have in common is that they will require the handling of a vast quantity of GM data combined with host metadata. This challenge can be tackled by omics technologies, such as microbiomics, nutrigenomics, and metabolomics. Collaterally, another indispensable tool for handling such complex data will be the use of artificial intelligence and machine learning techniques [252]. It is critical that both data and analytical tools be of open access so that researchers from any part of the world can benefit and contribute to these advances.

Funding source

This research was funded by the Instituto de Salud Carlos III through the project PI22/00412 (Co-funded by European Regional Development Fund, ERDF, a way to build Europe). EA-A, MG-L, and RZ-R were supported by the "Sara Borrell" (CD20/00071), the PFIS (FI19/00185), and the "Miguel Servet II" (CPII20/00009), programs from the Institute of Health Carlos III (Co-funded by European Social Fund (ESF) investing in your future). This work was also financially supported by the Centre for the Development of Industrial Technology (CDTI) of the Spanish Ministry of Science and Innovation under the grant agreement: TECNOMI-FOOD project (CER-20191010).

CRediT authorship contribution statement

Conceptualisation, M.F.; methodology, E.A.-A., A.C., M.G.-L., N.B., R.Z.R., A.A., M.F.; software, E.A.-A., A.C., M.G.-L., N.B., M.F.; validation, M.F., A.C.; formal analysis, E.A.-A., A.C., M.G.-L., N.B., M.F.; investigation, E.A.-A., A.C., M.G.-L., N.B., M.F.; resources, R.Z.R., A.A., M.F.; data curation, E.A.-A., M.G.-L., N.B.; writing—original draft preparation, E.A.-A., A.C., M.G.-L., N.B., M.F.; writing—review and editing, R.Z.R., A.A., M.F.; visualisation, A.C., M.G.-L., N.B.; supervision, R.Z.R., A.A., M.F.; project administration, M.F.; funding acquisition, R.Z.R., A.A., M.F. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

Acknowledgements

We thank Helena Kruyer for her help in editing the text. The authors gratefully thank graphical designer Andrea Deibe Varela for her work and dedication to the graphical abstract. This paper is contribution no 1140 from AZTI, Food Research, Basque Research and Technology Alliance (BRTA). We are grateful to the CERCA Program/Generalitat de Catalunya for institutional support.

References

[1] A. Bach-Faig, E.M. Berry, D. Lairon, J. Reguant, A. Trichopoulou, S. Dernini, Mediterranean diet pyramid today. Science and cultural updates, Public Heal Nutr. 14 (12A) (2011) 2274–2284, https://doi.org/10.1017/S1368980011002515.

[2] M.C. Mentella, F. Scaldaferri, C. Ricci, A. Gasbarrini, G.A.D. Miggiano, Cancer and mediterranean diet: a review, Nutrients 11 (9) (2019) 2059, https://doi.org/10.3390/nu11092059.

[3] A. Trichopoulou, Traditional mediterranean diet and longevity in the elderly: a review, Public Health Nutr. 7 (7) (2004) 943–947, https://doi.org/10.1079/phn2004558.

[4] A. Trichopoulou, T. Costacou, C. Bamia, D. Trichopoulos, Adherence to a Mediterranean diet and survival in a Greek population, N. Engl. J. Med 348 (26) (2003) 2599–2608, https://doi.org/10.1056/NEJMoa025039.

[5] C. Féart, C. Samieri, V. Rondeau, H. Amieva, F. Portet, J.-F. Dartigues, et al., Adherence to a Mediterranean diet, cognitive decline, and risk of dementia, JAMA 302 (6) (2009) 638–648, https://doi.org/10.1001/jama.2009.1146.

[6] N. Scarmeas, Y. Stern, M.-X. Tang, R. Mayeux, J.A. Luchsinger, Mediterranean diet and risk for Alzheimer's disease, Ann. Neurol. 59 (6) (2006) 912–921, https://doi.org/10.1002/ana.20854.

[7] M. Brenes, A. García, P. García, J.J. Rios, A. Garrido, Phenolic compounds in Spanish olive oils, J. Agric. Food Chem. 47 (9) (1999) 3535–3540, https://doi.org/10.1021/jf990009o.

[8] R.W. Owen, A. Giacosa, W.E. Hull, R. Haubner, B. Spiegelhalder, H. Bartsch, The antioxidant/anticancer potential of phenolic compounds isolated from olive oil, Eur. J. Cancer 36 (10) (2000) 1235–1247, https://doi.org/10.1016/s0959-8049(00)00103-9.

[9] M. Servili, R. Selvaggini, S. Esposto, A. Taticchi, G. Montedoro, G. Morozzi, Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil, J. Chromatogr. A 1054 (1) (2004) 113–127.

[10] Y. Allouche, A. Jiménez, J.J. Gaforio, M. Uceda, G. Beltrán, How heating affects extra virgin olive oil quality indexes and chemical composition, J. Agric. Food Chem. 55 (23) (2007) 9646–9654, https://doi.org/10.1021/jf070628u.

[11] R. Estruch, E. Ros, The role of the Mediterranean diet on weight loss and obesity-related diseases, Rev. Endocr. Metab. Disord. 21 (3) (2020) 315–327, https://doi.org/10.1007/s11154-020-09579-0.

[12] J. Salas-Salvadó, A. Díaz-López, M. Ruiz-Canela, J. Basora, M. Fitó, D. Corella, et al., Effect of a lifestyle intervention program with energy-restricted mediterranean diet and exercise on weight loss and cardiovascular risk factors: one-year results of the PREDIMED-plus trial, Diabetes Care 42 (5) (2019) 777–788, https://doi.org/10.2337/dc18-0836.

[13] N. Travier, A. Fonseca-Nunes, C. Javierre, E. Guillamo, L. Arribas, I. Peiró, et al., Effect of a diet and physical activity intervention on body weight and nutritional patterns in overweight and obese breast cancer survivors, Med Oncol. 31 (1) (2014) 783, https://doi.org/10.1007/s12032-013-0783-5.

[14] G. Muscogiuri, L. Verde, C. Sulu, N. Katsiki, M. Hassapidou, E. Frias-Toral, et al., Mediterranean diet and obesity-related disorders: what is the evidence? Curr. Obes. Rep. 11 (4) (2022) 287–304, https://doi.org/10.1007/s13679-022-00481-1.

[15] C. Castro-Espin, A. Agudo, The role of diet in prognosis among cancer survivors: a systematic review and meta-analysis of dietary patterns and diet interventions (Jan), Nutrients 14 (2) (2022) 348, https://doi.org/10.3390/nu14020348.

[16] J. Morze, A. Danielewicz, K. Przybyłowicz, H. Zeng, G. Hoffmann, L. Schwingshackl, An updated systematic review and meta-analysis on adherence to mediterranean diet and risk of cancer, Eur. J. Nutr. 60 (3) (2021) 1561–1586, https://doi.org/10.3390/nu9101063.

[17] J. Baedke, A. Fábregas-Tejeda, A. Nieves Delgado, The holobiont concept before Margulis, J. Exp. Zool. B Mol. Dev. Evol. 334 (3) (2020) 149–155, https://doi.org/10.1002/jez.b.22931.

[18] K.R. Theis, N.M. Dheilly, J.L. Klassen, R.M. Brucker, J.F. Baines, T.C.G. Bosch, et al., Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes, e00028-16, mSystems 1 (2) (2016), https://doi.org/10.1128/mSystems.00028-16.

[19] World Health Organization, Obesity, (n.d.). https://www.who.int/health -topics/obesity#tab=tab_2 (accessed August 15, 2022).

[20] X. Lin, H. Li, Obesity: epidemiology, pathophysiology, and therapeutics, Front. Endocrinol. 12 (2021), 706978, https://doi.org/10.3389/fendo.2021.706978.

[21] B. Lauby-Secretan, C. Scoccianti, D. Loomis, Y. Grosse, F. Bianchini, K. Straif, Body fatness and cancer — viewpoint of the IARC working group, N. Engl. J. Med 375 (8) (2016) 794–798, https://doi.org/10.1056/NEJMsr1606602.

[22] H. Sung, R.L. Siegel, P.S. Rosenberg, A. Jemal, Emerging cancer trends among young adults in the USA: analysis of a population-based cancer registry, Lancet Public Heal 4 (3) (2019) e137–e147, https://doi.org/10.1016/S2468-2667(18)30267-6.

[23] World Cancer Research Fund International/American Institute for Cancer Research. Coninuous Update Project Expert Report 2018. Body fatness and weight gain and the risk of cancer.Available at dietandcancerreport.org.

[24] M. Arnold, N. Pandeya, G. Byrnes, A.G. Renehan, G.A. Stevens, M. Ezzati, et al., Global burden of cancer attributable to high body-mass index in 2012: a population-based study, Lancet Oncol. 16 (1) (2015) 36–46, https://doi.org/10.1016/S1470-2045(14)71123-4.

[25] P. Weihe, J. Spielmann, H. Kielstein, J. Henning-Klusmann, S. Weihrauch-Blüher, Childhood obesity and cancer risk in adulthood, Curr. Obes. Rep. 9 (3) (2020) 204–212, https://doi.org/10.1007/s13679-020-00387-w.

[26] S. Weihrauch-Blüher, P. Schwarz, J.H. Klusmann, Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood, Metabolism 92 (2019) 147–152, https://doi.org/10.1016/j.metabol.2018.12.001.

[27] A.G. Renehan, I. Soerjomataram, M. Tyson, M. Egger, M. Zwahlen, J. W. Coebergh, et al., Incident cancer burden attributable to excess body mass index in 30 European countries, Int J. Cancer 126 (3) (2010) 692–702, https://doi.org/10.1002/ijc.24803.

[28] C.P. Wild, E. Weiderpass, B.W. Stewart (Eds.), World Cancer Report: Cancer Research for Cancer Prevention, International Agency for Research on Cancer, Lyon, France, France, 2020.

[29] F. Sofi, C. Macchi, R. Abbate, G.F. Gensini, A. Casini, Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score, Public Health Nutr. 17 (12) (2014) 2769–2782, https://doi.org/10.1017/S1368980013003169.

[30] Y. Zhong, Y. Zhu, Q. Li, F. Wang, X. Ge, G. Zhou, et al., Association between Mediterranean diet adherence and colorectal cancer: a dose-response meta-analysis, Am. J. Clin. Nutr. 111 (6) (2020) 1214–1225, https://doi.org/10.1093/ajcn/nqaa083.

[31] S. Du, Y. Li, Z. Su, X. Shi, N.L. Johnson, P. Li, et al., Index-based dietary patterns in relation to gastric cancer risk: a systematic review and meta-analysis, Br. J. Nutr. 123 (9) (2020) 964–974, https://doi.org/10.1017/S0007114519002976.

[32] L. Álvarez-Álvarez, F. Vitelli-Storelli, M. Rubín-García, N. Aragonés, E. Ardanaz, G. Castaño-Vinyals, et al., Relationship between the risk of gastric cancer and adherence to the mediterranean diet according to different estimators. MCC-Spain Study, Cancers 13 (21) (2021) 5281, https://doi.org/10.3390/cancers13215281.

[33] S. Julián-Serrano, J. Reedy, K. Robien, R. Stolzenberg-Solomon, Adherence to 5 diet quality indices and pancreatic cancer risk in a large US prospective Cohort, Am. J. Epidemiol. 191 (9) (2022) 1584–1600.

[34] H.N. Luu, P. Paragomi, A. Jin, R. Wang, N. Neelakantan, R.M. van Dam, et al., Quality diet index and risk of pancreatic cancer: findings from the Singapore Chinese health study, Cancer Epidemiol. Biomark. Prev. 30 (11) (2021) 2068–2078, https://doi.org/10.1158/1055-9965.EPI-21-0033.

[35] H.N. Luu, N. Neelakantan, T.-T. Geng, R. Wang, G.B.-B. Goh, J.C. Clemente, et al., Quality diet indexes and risk of hepatocellular carcinoma: Findings from the Singapore Chinese Health Study, Int J. Cancer 148 (9) (2021) 2102–2114, https://doi.org/10.1002/ijc.33367.

[36] S.C. Larsson, N. Hakansson, A. Wolk, Healthy dietary patterns and incidence of biliary tract and gallbladder cancer in a prospective study of women and men, Eur. J. Cancer 70 (2017) 42–47, https://doi.org/10.1016/j.ejca.2016.10.012.

[37] Y. Li, B.-Q. Hu, X.-J. Wu, M.-W. Qi, J. Lang, X. Cui, et al., Adherence to Mediterranean diet and the risk of breast cancer: a meta-analysis, Transl. Cancer Res 7 (5) (2018) 1290–1297, https://doi.org/10.21037/tcr.2018.10.13.

[38] P.A. van den Brandt, M. Schulpen, Mediterranean diet adherence and risk of postmenopausal breast cancer: results of a cohort study and meta-analysis, Int J. Cancer 140 (10) (2017) 2220–2231, https://doi.org/10.1002/ijc.30654.
[39] G. Porciello, C. Montagnese, A. Crispo, M. Grimaldi, M. Libra, S. Vitale, et al., Mediterranean diet and quality of life in women treated for breast cancer: A baseline analysis of DEDiCa multicentre trial, PLoS One 15 (10) (2020), e0239803, https://doi.org/10.1371/journal.pone.0239803.
[40] J. Xie, E.M. Poole, K.L. Terry, T.T. Fung, B.A. Rosner, W.C. Willett, et al., A prospective cohort study of dietary indices and incidence of epithelial ovarian cancer, J. Ovarian Res 7 (2014) 112, https://doi.org/10.1186/s13048-014-0112-4.
[41] F. Llaha, V. Cayssials, M. Farràs, A. Agudo, M. Sandström, A.K. Eriksen, et al., Adherence to mediterranean diet and the risk of differentiated thyroid cancer in a European cohort: The EPIC study, Front Nutr. 9 (2022), 982369, https://doi.org/10.3389/fnut.2022.982369.
[42] D.H. Lee, T.T. Fung, F.K. Tabung, G.A. Colditz, I.M. Ghobrial, B.A. Rosner, et al., Dietary pattern and risk of multiple myeloma in two large prospective US Cohort Studies, JNCI Cancer Spectr. 3 (2) (2019) pkz025, https://doi.org/10.1093/jncics/pkz025.
[43] M. Solans, Y. Benavente, M. Saez, A. Agudo, S. Naudin, F.S. Hosnijeh, et al., Adherence to the mediterranean diet and lymphoma risk in the european prospective investigation into cancer and nutrition, Int J. Cancer 145 (1) (2019) 122–131, https://doi.org/10.1002/ijc.32091.
[44] C. Markellos, M.-E. Ourailidou, M. Gavriatopoulou, P. Halvatsiotis, T. N. Sergentanis, T. Psaltopoulou, Olive oil intake and cancer risk: A systematic review and meta-analysis, PLoS One 17 (1) (2022), e0261649, https://doi.org/10.1371/journal.pone.0261649.
[45] M. Soler, L. Chatenoud, C. La Vecchia, S. Franceschi, E. Negri, Diet, alcohol, coffee and pancreatic cancer: final results from an Italian study, Eur. J. Cancer Prev. 7 (6) (1998) 455–460, https://doi.org/10.1097/00008469-199812000-00005.
[46] N. Sealy, S.E. Hankinson, S.C. Houghton, Olive oil and risk of breast cancer: a systematic review and dose-response meta-analysis of observational studies, Br. J. Nutr. 125 (10) (2021) 1148–1156, https://doi.org/10.1017/S0007114520003499.
[47] A. Tzonou, L. Lipworth, A. Kalandidi, A. Trichopoulou, I. Gamatsi, C.C. Hsieh, et al., Dietary factors and the risk of endometrial cancer: a case–control study in Greece, Br. J. Cancer 73 (10) (1996) 1284–1290, https://doi.org/10.1038/bjc.1996.246.
[48] C. Bosetti, E. Negri, S. Franceschi, R. Talamini, M. Montella, E. Conti, et al., Olive oil, seed oils and other added fats in relation to ovarian cancer (Italy), Cancer Causes Control 13 (5) (2002) 465–470, https://doi.org/10.1023/a:1015760004130.
[49] E. Molina-Montes, E. Salamanca-Fernández, B. Garcia-Villanova, M.J. Sánchez, The impact of plant-based dietary patterns on cancer-related outcomes: a rapid review and meta-analysis, Nutrients 12 (7) (2020) 2010, https://doi.org/10.3390/nu12072010.
[50] M. Farràs, E. Almanza-Aguilera, Á. Hernáez, N. Agustí, J. Julve, M. Fitó, et al., Beneficial effects of olive oil and Mediterranean diet on cancer physio-pathology and incidence, Semin Cancer Biol. 73 (2021) 178–195, https://doi.org/10.1016/j.semcancer.2020.11.011.
[51] C. Galbete, L. Schwingshackl, C. Schwedhelm, H. Boeing, M.B. Schulze, Evaluating Mediterranean diet and risk of chronic disease in cohort studies: an umbrella review of meta-analyses, Eur. J. Epidemiol. 33 (10) (2018) 909–931, https://doi.org/10.1007/s10654-018-0427-3.
[52] L. Schwingshackl, M. Krause, C. Schmucker, G. Hoffmann, G. Rücker, J. J. Meerpohl, Impact of different types of olive oil on cardiovascular risk factors: A systematic review and network meta-analysis, Nutr. Metab. Cardiovasc Dis. 29 (10) (2019) 1030–1039, https://doi.org/10.1016/j.numecd.2019.07.001.
[53] C. Donat-Vargas, Á. Guerrero-Zotano, V. Lope, B. Bermejo, A. Casas, J.M. Baena-Cañada, et al., Type does matter. Use VIRGIN olive oil as your preferred fat to reduce your risk of breast cancer: case-control EpiGEICAM study, Eur. J. Clin. Nutr. 76 (9) (2022) 1343–1346, https://doi.org/10.1038/s41430-022-01101-w.
[54] A.G. Renehan, M. Zwahlen, M. Egger, Adiposity and cancer risk: New mechanistic insights from epidemiology, Nat. Rev. Cancer 15 (8) (2015) 484–498, https://doi.org/10.1038/nrc3967.
[55] A.I. Pérez-Hernández, V. Catalán, J. Gómez-Ambrosi, A. Rodríguez, G. Frühbeck, Mechanisms linking excess adiposity and carcinogenesis promotion, Front Endocrinol. 5 (2014) 65, https://doi.org/10.3389/fendo.2014.00065.
[56] K.I. Avgerinos, N. Spyrou, C.S. Mantzoros, M. Dalamaga, Obesity and cancer risk: Emerging biological mechanisms and perspectives, Metabolism 92 (2019) 121–135, https://doi.org/10.1016/j.metabol.2018.11.001.
[57] N.M. Iyengar, C.A. Hudis, A.J. Dannenberg, Obesity and cancer: local and systemic mechanisms, Annu Rev. Med. 66 (2015) 297–309, https://doi.org/10.1200/JCO.2016.67.4283.
[58] A. Castoldi, C.N. De Souza, N.O. Saraiva Câmara, P.M. Moraes-Vieira, The macrophage switch in obesity development, Front Immunol. 6 (2016) 637, https://doi.org/10.3389/fimmu.2015.00637.
[59] A. Mantovani, A. Sica, S. Sozzani, P. Allavena, A. Vecchi, M. Locati, The chemokine system in diverse forms of macrophage activation and polarization, Trends Immunol. 25 (12) (2004) 677–686, https://doi.org/10.1016/j.it.2004.09.015.
[60] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante, Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Invest 112 (12) (2003) 1796–1808, https://doi.org/10.1172/JCI19246.
[61] F. Zatterale, M. Longo, J. Naderi, G.A. Raciti, A. Desiderio, C. Miele, et al., Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes, Front Physiol. 10 (2020) 1607, https://doi.org/10.3389/fphys.2019.01607.
[62] S.M. Crusz, F.R. Balkwill, Inflammation and cancer: Advances and new agents, Nat. Rev. Clin. Oncol. 12 (10) (2015) 584–596, https://doi.org/10.1038/nrclinonc.2015.105.
[63] C.A. Gilbert, J.M. Slingerland, Cytokines, obesity, and cancer: New insights on mechanisms linking obesity to cancer risk and progression, Annu Rev. Med 64 (2013) 45–57, https://doi.org/10.1146/annurev-med-121211-091527.
[64] S.D. Hursting, J. DiGiovanni, A.J. Dannenberg, M. Azrad, D. LeRoith, W. Demark-Wahnefried, et al., Obesity, energy balance, and cancer: New opportunities for prevention, Cancer Prev. Res 5 (11) (2012) 1260–1272, https://doi.org/10.1158/1940-6207.CAPR-12-0140.
[65] T. Deng, C.J. Lyon, S. Bergin, M.A. Caligiuri, W.A. Hsueh, Obesity, inflammation, and cancer, Annu Rev. Pathol. Mech. Dis. 11 (2016) 421–449, https://doi.org/10.1146/annurev-pathol-012615-044359.
[66] T.-C. Lin, M. Hsiao, V. Sánchez-Margalet, Molecular sciences leptin and cancer: updated functional roles in carcinogenesis, therapeutic niches, and developments, Int J. Mol. Sci. 22 (6) (2021) 2870, https://doi.org/10.3390/ijms22062870.
[67] N. Samad, T. Rao, Role of leptin in cancer: a systematic review (May), Biomed. J. Sci. Tech. Res 18 (1) (2019) 13226–13235, https://doi.org/10.26717/BJSTR.2019.18.003091.
[68] Y.S. Yoon, A.R. Kwon, Y.K. Lee, S.W. Oh, Circulating adipokines and risk of obesity related cancers: A systematic review and meta-analysis, Obes. Res Clin. Pr. 13 (4) (2019) 329–339, https://doi.org/10.1016/j.orcp.2019.03.006.
[69] T. Hajri, H. Tao, J. Wattacheril, P. Marks-Shulman, N.N. Abumrad, Regulation of adiponectin production by insulin: interactions with tumor necrosis factor-α and interleukin-6, Am. J. Physiol. Endocrinol. Metab. 300 (2) (2011) E350–E360, https://doi.org/10.1152/ajpendo.00307.2010.
[70] M. Dalamaga, K.N. Diakopoulos, C.S. Mantzoros, The role of adiponectin in cancer: A review of current evidence, Endocr. Rev. 33 (4) (2012) 547–594, https://doi.org/10.1210/er.2011-1015.
[71] T. Yamauchi, J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, et al., The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity, Nat. Med 7 (8) (2001) 941–946, https://doi.org/10.1038/90984.
[72] V. Izadi, E. Farabad, L. Azadbakht, Serum adiponectin level and different kinds of cancer: a review of recent evidence, ISRN Oncol. 2012 (2012), 982769, https://doi.org/10.5402/2012/982769.
[73] G. Papavasileiou, D. Tsilingiris, N. Spyrou, N.G. Vallianou, I. Karampela, F. Magkos, et al., Obesity and main urologic cancers: Current systematic evidence, novel biological mechanisms, perspectives and challenges, Semin Cancer Biol. 91 (2023) 70–98, https://doi.org/10.1016/j.semcancer.2023.03.002.
[74] D.S. Michaud, Chronic inflammation and bladder cancer, Urol. Oncol. 25 (3) (2007) 260–268, https://doi.org/10.1016/j.urolonc.2006.10.002.
[75] K.G. Seng, A.D. Kriketos, A.M. Poynten, S.M. Furler, C.H. Thompson, E. W. Kraegen, et al., Insulin action, regional fat, and myocyte lipid: Altered relationships with increased adiposity, Obes. Res 11 (11) (2003) 1295–1305, https://doi.org/10.1038/oby.2003.176.
[76] S.E. Borst, The role of TNF-α in insulin resistance, Endocrine 23 (2–3) (2004) 177–182, https://doi.org/10.1385/ENDO:23:2-3:177.
[77] R. Monteiro, I. Azevedo, Chronic inflammation in obesity and the metabolic syndrome, Mediat. Inflamm. 2010 (2010), 289645, https://doi.org/10.1155/2010/289645.
[78] S.M. Louie, L.S. Roberts, D.K. Nomura, Mechanisms linking obesity and cancer, Biochim Biophys. Acta 1831 (10) (2013) 1499–1508, https://doi.org/10.1016/j.bbalip.2013.02.008.
[79] A. Belfiore, The role of insulin receptor isoforms and hybrid insulin/IGF-I receptors in human cancer, Curr. Pharm. Des. 13 (7) (2007) 671–686, https://doi.org/10.2174/138161207780249173.
[80] B. Arcidiacono, S. Iiritano, A. Nocera, K. Possidente, M.T. Nevolo, V. Ventura, et al., Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms, Exp. Diabetes Res 2012 (12) (2012), 789174, https://doi.org/10.1155/2012/789174.
[81] B.P. Leitner, S. Siebel, N.D. Akingbesote, X. Zhang, R.J. Perry, Insulin and cancer: a tangled web, Biochem J. 479 (5) (2022) 583–607, https://doi.org/10.1042/BCJ20210134.
[82] G.C. Kabat, M.Y. Kim, D.S. Lane, O. Zaslavsky, G.Y.F. Ho, J. Luo, et al., Serum glucose and insulin and risk of cancers of the breast, endometrium, and ovary in postmenopausal women, Eur. J. Cancer Prev. 27 (3) (2018) 261–268, https://doi.org/10.1097/CEJ.0000000000000435.
[83] M.J. Gunter, D.R. Hoover, H. Yu, S. Wassertheil-Smoller, J.E. Manson, J. Li, et al., A prospective evaluation of insulin and insulin-like growth factor-i as risk factors for endometrial cancer, Cancer Epidemiol. Biomark. Prev. 17 (4) (2008) 921–929, https://doi.org/10.1158/1055-9965.EPI-07-2686.
[84] D.R. Pandeya, A. Mittal, B. Sathian, B. Bhatta, Role of hyperinsulinemia in increased risk of prostate cancer: A case control study from Kathmandu valley, Asian Pac. J. Cancer Prev. 15 (2) (2014) 1031–1033, https://doi.org/10.7314/apjcp.2014.15.2.1031.
[85] G.A.B. Lima, L.L. Corrêa, R. Gabrich, L.C. Miranda, M.R. Gadelha, IGF-I, insulin and prostate cancer, Arq. Bras. Endocrinol. Metab. 53 (8) (2009) 969–975, https://doi.org/10.1590/s0004-27302009000800010.
[86] B. Jiang, X. Zhang, L.L. Du, Y. Wang, D.B. Liu, C.Z. Han, et al., Possible roles of insulin, IGF-1 and IGFBPs in initiation and progression of colorectal cancer,

World J. Gastroenterol. 20 (6) (2014) 1608–1613, https://doi.org/10.3748/wjg.v20.i6.1608.
[87] A. Knuppel, G.K. Fensom, E.L. Watts, M.J. Gunter, N. Murphy, K. Papier, et al., Circulating insulin-like growth factor-I concentrations and risk of 30 cancers: prospective analyses in UK biobank, Cancer Res 80 (18) (2020) 4014–4021, https://doi.org/10.1158/0008-5472.CAN-20-1281.
[88] N.H. Kim, Y. Chang, S.R. Lee, S. Ryu, H.J. Kim, Glycemic status, insulin resistance, and risk of pancreatic cancer mortality in individuals with and without diabetes, Am. J. Gastroenterol. 115 (11) (2020) 1840–1848, https://doi.org/10.14309/ajg.0000000000000956.
[89] K. Pan, R.T. Chlebowski, J.E. Mortimer, M.J. Gunther, T. Rohan, M.Z. Vitolins, et al., Insulin resistance and breast cancer incidence and mortality in postmenopausal women in the Women's Health Initiative, Cancer 126 (16) (2020) 3638–3647, https://doi.org/10.1002/cncr.33002.
[90] T. Tsujimoto, H. Kajio, T. Sugiyama, Association between hyperinsulinemia and increased risk of cancer death in nonobese and obese people: A population-based observational study, Int J. Cancer 141 (1) (2017) 102–111, https://doi.org/10.1002/ijc.30729.
[91] G. Perseghin, G. Calori, G. Lattuada, F. Ragogna, E. Dugnani, M.P. Garancini, et al., Insulin resistance/hyperinsulinemia and cancer mortality: the Cremona study at the 15th year of follow-up, Acta Diabetol. 49 (6) (2012) 421–428, https://doi.org/10.1007/s00592-011-0361-2.
[92] S.A. Salama, M.W. Kamel, C.R. Diaz-Arrastia, X. Xu, T.D. Veenstra, S. Salih, et al., Effect of tumor necrosis factor-α on estrogen metabolism and endometrial cells: Potential physiological and pathological relevance, J. Clin. Endocrinol. Metab. 94 (1) (2009) 285–293, https://doi.org/10.1210/jc.2008-1389.
[93] S.Q. To, K.C. Knower, C.D. Clyne, Origins and actions of tumor necrosis factor α in postmenopausal breast cancer (Jul), J. Inter. Cytokine Res 33 (7) (2013) 335–345, https://doi.org/10.1089/jir.2012.0155.
[94] T.J. Key, P.N. Appleby, G.K. Reeves, A. Roddam, J.F. Dorgan, C. Longcope, et al., Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women, J. Natl. Cancer Inst. 95 (16) (2003) 1218–1226, https://doi.org/10.1093/jnci/djg022.
[95] P. Bhardwaj, C.M.C. Au, A. Benito-Martin, H. Ladumor, S. Oshchepkova, R. Moges, et al., Estrogens and breast cancer: Mechanisms involved in obesity-related development, growth and progression, J. Steroid Biochem Mol. Biol. 189 (2019) 161–170, https://doi.org/10.1016/j.jsbmb.2019.03.002.
[96] T. Byers, R.L. Sedjo, Body fatness as a cause of cancer: Epidemiologic clues to biologic mechanisms, Endocr. Relat. Cancer 22 (3) (2015) R125–R134, https://doi.org/10.1530/ERC-14-0580.
[97] S.R. César, C. Ibáñez, J. Klaassen, Obesidad y cáncer: la tormenta perfecta, Rev. Med Chil. 142 (2) (2014) 211–221, https://doi.org/10.4067/S0034-98872014000200010.
[98] X. Wang, E.R. Simpson, K.A. Brown, Aromatase overexpression in dysfunctional adipose tissue links obesity to postmenopausal breast cancer, J. Steroid Biochem Mol. Biol. 153 (2015) 35–44, https://doi.org/10.1016/j.jsbmb.2015.07.008.
[99] E. Shaw, M. Farris, J. McNeil, C. Friedenreich, Obesity and endometrial cancer, Recent Results Cancer Res 208 (2016) 107–136, https://doi.org/10.1007/978-3-319-42542-9_7.
[100] R.F. Schwabe, C. Jobin, The microbiome and cancer, Nat. Rev. Cancer 13 (11) (2013) 800–812, https://doi.org/10.1038/nrc3610.
[101] E. Amabebe, F.O. Robert, T. Agbalalah, E.S.F. Orubu, Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism (May), Br. J. Nutr. 123 (10) (2020) 1127–1137, https://doi.org/10.1017/S0007114520000380.
[102] C.J. Rogers, K.S. Prabhu, M. Vijay-Kumar, The microbiome and obesity-An established risk for certain types of cancer, Cancer J. 20 (3) (2014) 176–180, https://doi.org/10.1097/PPO.0000000000000049.
[103] M.T. Weng, Y.T. Chiu, P.Y. Wei, C.W. Chiang, H.L. Fang, S.C. Wei, Microbiota and gastrointestinal cancer, J. Formos. Med Assoc. 118 (Suppl 1) (2019) S32–S41, https://doi.org/10.1016/j.jfma.2019.01.002.
[104] J.C. Arthur, E. Perez-Chanona, M. Mühlbauer, S. Tomkovich, J.M. Uronis, T. J. Fan, et al., Intestinal inflammation targets cancer-inducing activity of the microbiota, Science 338 (6103) (2012) 120–123, https://doi.org/10.1126/science.1224820.
[105] B. Hu, E. Elinav, S. Huber, T. Strowig, L. Hao, A. Hafemann, et al., Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation, Proc. Natl. Acad. Sci. USA 110 (24) (2013) 9862–9867, https://doi.org/10.1073/pnas.1307575110.
[106] Y. Tong, H. Gao, Q. Qi, X. Liu, J. Li, J. Gao, et al., High fat diet, gut microbiome and gastrointestinal cancer, Apr 3, Theranostics 11 (12) (2021) 5889–5910, https://doi.org/10.7150/thno.56157.
[107] C. Andolfi, J.C. Bloodworth, A. Papachristos, R.F. Sweis, The urinary microbiome and bladder cancer: susceptibility and immune responsiveness, Bladder Cancer 6 (3) (2020) 225–235, https://doi.org/10.3233/BLC-200277.
[108] M. Alfano, F. Canducci, M. Nebuloni, M. Clementi, F. Montorsi, A. Salonia, The interplay of extracellular matrix and microbiome in urothelial bladder cancer, Nat. Rev. Urol. 13 (2) (2016) 77–90, https://doi.org/10.1038/nrurol.2015.292.
[109] L. Bonfrate, D.Q.H. Wang, G. Garruti, P. Portincasa, Obesity and the risk and prognosis of gallstone disease and pancreatitis, Best. Pr. Res Clin. Gastroenterol. 28 (4) (2014) 623–635, https://doi.org/10.1016/j.bpg.2014.07.013.
[110] A. Venniyoor, Cholesterol gallstones and cancer of gallbladder (CAGB): molecular links, Med Hypotheses 70 (3) (2008) 646–653, https://doi.org/10.1016/j.mehy.2007.06.040.
[111] D. Huang, H. Joo, N. Song, S. Cho, W. Kim, A. Shin, Association between gallstones and the risk of biliary tract cancer: a systematic review and meta-analysis, Epidemiol. Health 43 (2021), e2021011, https://doi.org/10.4178/epih.e2021011.
[112] J. Lagergren, Influence of obesity on the risk of esophageal disorders, Nat. Rev. Gastroenterol. Hepatol. 8 (6) (2011) 340–347, https://doi.org/10.1038/nrgastro.2011.73.
[113] S.J. Spechler, R.F. Souza, Barrett's Esophagus, N. Engl. J. Med 371 (9) (2014) 836–845, https://doi.org/10.1056/NEJMra1314704.
[114] S. Emerenziani, M.P. Rescio, M.P.L. Guarino, M. Cicala, Gastro-esophageal reflux disease and obesity, where is the link? World J. Gastroenterol. 19 (39) (2013) 6536–6539, https://doi.org/10.3748/wjg.v19.i39.6536.
[115] J.H. Rubenstein, J.B. Taylor, Meta-analysis: The association of oesophageal adenocarcinoma with symptoms of gastro-oesophageal reflux, Aliment Pharm. Ther. 32 (10) (2010) 1222–1227, https://doi.org/10.1111/j.1365-2036.2010.04471.x.
[116] M.A. Bermúdez, L. Pereira, C. Fraile, L. Valerio, M.A. Balboa, J. Balsinde, Roles of palmitoleic acid and its positional isomers, hypogeic and sapienic acids, in inflammation, metabolic diseases and cancer, Cells 11 (14) (2022) 2146.
[117] A. Koeberle, H. Shindou, T. Harayama, T. Shimizu, Palmitoleate is a mitogen, formed upon stimulation with growth factors, and converted to palmitoleoyl-phosphatidylinositol, J. Biol. Chem. 287 (32) (2012) 27244–27254 (Aug).
[118] G. Pascual, D. Domínguez, M. Elosúa-Bayes, F. Beckedorff, C. Laudanna, C. Bigas, et al., Dietary palmitic acid promotes a prometastatic memory via Schwann cells, Nature 599 (7885) (2021) 485–490, https://doi.org/10.1038/s41586-021-04075-0.
[119] C. Tsigalou, T. Konstantinidis, A. Paraschaki, E. Stavropoulou, C. Voidarou, E. Bezirtzoglou, Mediterranean diet as a tool to combat inflammation and chronic diseases. an overview, Biomedicines 8 (7) (2020) 201, https://doi.org/10.3390/biomedicines8070201.
[120] M.F. Landecho, C. Tuero, V. Valentí, I. Bilbao, M. de la Higuera, G. Frühbeck, Relevance of leptin and other adipokines in obesity-associated cardiovascular risk, Nutrients 11 (11) (2019) 2664, https://doi.org/10.3390/nu11112664.
[121] G. Augimeri, F.I. Montalto, C. Giordano, I. Barone, M. Lanzino, S. Catalano, et al., Nutraceuticals in the mediterranean diet: potential avenues for breast cancer treatment, Nutrients 13 (8) (2021) 2557, https://doi.org/10.3390/nu13082557.
[122] S. Sood, J. Feehan, C. Itsiopoulos, K. Wilson, M. Plebanski, D. Scott, et al., Higher adherence to a mediterranean diet is associated with improved insulin sensitivity and selected markers of inflammation in individuals who are overweight and obese without diabetes, Nutrients 14 (20) (2022) 4437, https://doi.org/10.3390/nu14204437.
[123] L.J. Dominguez, N. Veronese, G. Di Bella, C. Cusumano, A. Parisi, F. Tagliaferri, et al., Mediterranean diet in the management and prevention of obesity, Exp. Gerontol. 174 (2023), 112121, https://doi.org/10.1016/j.exger.2023.112121.
[124] F. De Filippis, N. Pellegrini, L. Vannini, I.B. Jeffery, A. La Storia, L. Laghi, et al., High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome, Gut 65 (11) (2016) 1812–1821, https://doi.org/10.1136/gutjnl-2015-309957.
[125] G. Merra, A. Noce, G. Marrone, M. Cintoni, M.G. Tarsitano, A. Capacci, et al., Influence of mediterranean diet on human gut microbiota, Nutrients 13 (1) (2020) 7, https://doi.org/10.3390/nu13010007.
[126] A. Farinetti, V. Zurlo, A. Manenti, F. Coppi, A.V. Mattioli, Mediterranean diet and colorectal cancer: A systematic review, Nutrition 43–44 (2017) 83–88, https://doi.org/10.1016/j.nut.2017.06.008.
[127] E. Rinninella, P. Raoul, M. Cintoni, F. Franceschi, G.A.D. Miggiano, A. Gasbarrini, et al., What is the healthy gut microbiota composition? a changing ecosystem across age, environment, diet, and diseases, Microorganisms 7 (1) (2019) 14, https://doi.org/10.3390/microorganisms7010014.
[128] C.H. King, H. Desai, A.C. Sylvetsky, J. LoTempio, S. Ayanyan, J. Carrie, et al., Baseline human gut microbiota profile in healthy people and standard reporting template, PLoS One 14 (9) (2019), e0206484, https://doi.org/10.1371/journal.pone.0206484.
[129] I. Sharon, N.M. Quijada, E. Pasolli, M. Fabbrini, F. Vitali, V. Agamennone, et al., The core human microbiome: does it exist and how can we find It? A critical review of the concept, Nutrients 14 (14) (2022) 2872, https://doi.org/10.3390/nu14142872.
[130] D. Rothschild, O. Weissbrod, E. Barkan, A. Kurilshikov, T. Korem, D. Zeevi, et al., Environment dominates over host genetics in shaping human gut microbiota, Nature 555 (7695) (2018) 210–215, https://doi.org/10.1038/nature25973.
[131] X. Zhang, H. Zhong, Y. Li, Z. Shi, H. Ren, Z. Zhang, et al., Sex- and age-related trajectories of the adult human gut microbiota shared across populations of different ethnicities, Nat. Aging 1 (1) (2021) 87–100, https://doi.org/10.1038/s43587-020-00014-2.
[132] J.M. Ordovas, L.R. Ferguson, E.S. Tai, J.C. Mathers, Personalised nutrition and health, BMJ 361 (2018), bmj.k2173, https://doi.org/10.1136/bmj.k2173.
[133] I. Jauregibeitia, K. Portune, S. Gaztambide, I. Rica, I. Tueros, O. Velasco, et al., Molecular differences based on erythrocyte fatty acid profile to personalize dietary strategies between adults and children with obesity, Metabolites 11 (1) (2021) 43, https://doi.org/10.3390/metabo11010043.
[134] R. Jinnette, A. Narita, B. Manning, S.A. McNaughton, J.C. Mathers, K. M. Livingstone, Does personalized nutrition advice improve dietary intake in healthy adults? A systematic review of randomized controlled trials, Adv. Nutr. 12 (3) (2021) 657–669, https://doi.org/10.1093/advances/nmaa144.
[135] P. Hernández-Calderón, L. Wiedemann, A. Benítez-Páez, The microbiota composition drives personalized nutrition: Gut microbes as predictive biomarkers for the success of weight loss diets, Front Nutr. 9 (2022) 1006747, https://doi.org/10.3389/fnut.2022.1006747.

[136] A. Leshem, E. Segal, E. Elinav, C. Depommier, A. Everard, C. Druart, et al., The gut microbiome and individual-specific responses to diet, Nat. Med 5 (5) (2020) 1096–1103, https://doi.org/10.1128/mSystems.00665-20.
[137] R. Sender, S. Fuchs, R. Milo, Revised estimates for the number of human and bacteria cells in the body, PLoS Biol. 14 (8) (2016), e1002533, https://doi.org/10.1371/journal.pbio.1002533.
[138] S. Zeng, D. Patangia, A. Almeida, Z. Zhou, D. Mu, R. Paul Ross, et al., A compendium of 32,277 metagenome-assembled genomes and over 80 million genes from the early-life human gut microbiome, Nat. Commun. 13 (1) (2022) 5139, https://doi.org/10.1038/s41467-022-32805-z.
[139] M. Groussin, M. Poyet, A. Sistiaga, S.M. Kearney, K. Moniz, M. Noel, et al., Elevated rates of horizontal gene transfer in the industrialized human microbiome, Cell 184 (8) (2021) 2053–2067.e18, https://doi.org/10.1016/j.cell.2021.02.052.
[140] L. Nyholm, A. Koziol, S. Marcos, A.B. Botnen, O. Aizpurua, S. Gopalakrishnan, et al., Holo-omics: integrated host-microbiota multi-omics for basic and applied biological research, iScience 23 (8) (2020), 101414, https://doi.org/10.1016/j.isci.2020.101414.
[141] Y. Singh, S.E. Hasnain, Holobionts: emerging strategy for interventions against infectious diseases, metabolic disorders & cancer, Indian J. Med Res 140 (1) (2014) 11–14.
[142] A.K. DeGruttola, D. Low, A. Mizoguchi, E. Mizoguchi, Current understanding of dysbiosis in disease in human and animal models, Inflamm. Bowel Dis. 22 (5) (2016) 1137–1150, https://doi.org/10.1097/MIB.0000000000000750.
[143] M. Farràs, L. Martinez-Gili, K. Portune, S. Arranz, G. Frost, M. Tondo, et al., Modulation of the gut microbiota by olive oil phenolic compounds: implications for lipid metabolism, immune system, and obesity, Nutrients 12 (8) (2020) 2200, https://doi.org/10.3390/nu12082200.
[144] L. Crovesy, D. Masterson, E.L. Rosado, Profile of the gut microbiota of adults with obesity: a systematic review, Eur. J. Clin. Nutr. 74 (9) (2020) 1251–1262, https://doi.org/10.1038/s41430-020-0607-6.
[145] M.-H. Kim, K.E. Yun, J. Kim, E. Park, Y. Chang, S. Ryu, et al., Gut microbiota and metabolic health among overweight and obese individuals, Sci. Rep. 10 (1) (2020) 19417, https://doi.org/10.1038/s41598-020-76474-8.
[146] E.F. Murphy, P.D. Cotter, S. Healy, T.M. Marques, O. O'Sullivan, F. Fouhy, et al., Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models, Gut 59 (12) (2010) 1635–1642, https://doi.org/10.1136/gut.2010.215665.
[147] F. Fava, R. Gitau, B.A. Griffin, G.R. Gibson, K.M. Tuohy, J.A. Lovegrove, The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome "at-risk" population, Int J. Obes. 37 (2) (2013) 216–223, https://doi.org/10.1038/ijo.2012.33.
[148] Y. Xu, N. Wang, H.-Y. Tan, S. Li, C. Zhang, Y. Feng, Function of akkermansia muciniphila in obesity: interactions with lipid metabolism, immune response and gut systems, Front Microbiol 11 (2020) 219, https://doi.org/10.3389/fmicb.2020.00219.
[149] C. Depommier, A. Everard, C. Druart, H. Plovier, M. Van Hul, S. Vieira-Silva, et al., Supplementation with akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study, Nat. Med 25 (7) (2019) 1096–1103, https://doi.org/10.1038/s41591-019-0495-2.
[150] M.-H. Lee, Harness the functions of gut microbiome in tumorigenesis for cancer treatment, Cancer Commun. 41 (10) (2021) 937–967, https://doi.org/10.1002/cac2.12200.
[151] D. Rea, G. Coppola, G. Palma, A. Barbieri, A. Luciano, P. Del Prete, et al., Microbiota effects on cancer: from risks to therapies, Oncotarget 9 (25) (2018) 17915–17927, https://doi.org/10.18632/oncotarget.24681.
[152] C.-C. Chen, J.-M. Liou, Y.-C. Lee, T.-C. Hong, E.M. El-Omar, M.-S. Wu, The interplay between Helicobacter pylori and gastrointestinal microbiota, Gut Microbes 13 (1) (2021) 1–22, https://doi.org/10.1080/19490976.2021.1909459.
[153] D. Nejman, I. Livyatan, G. Fuks, N. Gavert, Y. Zwang, L.T. Geller, et al., The human tumor microbiome is composed of tumor type-specific intracellular bacteria, Science 368 (6494) (2020) 973–980, https://doi.org/10.1126/science.aay9189.
[154] G.D. Poore, E. Kopylova, Q. Zhu, C. Carpenter, S. Fraraccio, S. Wandro, et al., Microbiome analyses of blood and tissues suggest cancer diagnostic approach, Nature 579 (7800) (2020) 567–574, https://doi.org/10.1038/s41586-020-2095-1.
[155] Y. Chen, J. Ma, Y. Dong, Z. Yang, N. Zhao, Q. Liu, et al., Characteristics of gut microbiota in patients with clear cell renal cell carcinoma, Front Microbiol 13 (2022), 913718, https://doi.org/10.3389/fmicb.2022.913718.
[156] A.B. Dohlman, J. Klug, M. Mesko, I.H. Gao, S.M. Lipkin, X. Shen, et al., A pan-cancer mycobiome analysis reveals fungal involvement in gastrointestinal and lung tumors, Cell 185 (20) (2022) 3807–3822.e12, https://doi.org/10.1016/j.cell.2022.09.015.
[157] L. Narunsky-Haziza, G.D. Sepich-Poore, I. Livyatan, O. Asraf, C. Martino, D. Nejman, et al., Pan-cancer analyses reveal cancer-type-specific fungal ecologies and bacteriome interactions, Cell 185 (20) (2022) 3789–3806.e17.
[158] Z.-H. Geng, Y. Zhu, Q.-L. Li, C. Zhao, P.-H. Zhou, Enteric nervous system: the bridge between the gut microbiota and neurological disorders, Front Aging Neurosci. 14 (2022), 810483, https://doi.org/10.3389/fnagi.2022.810483.
[159] S.P. Wiertsema, J. van Bergenhenegouwen, J. Garssen, L.M.J. Knippels, The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies, Nutrients 13 (3) (2021) 886, https://doi.org/10.3390/nu13030886.
[160] K. Kouzu, H. Tsujimoto, Y. Kishi, H. Ueno, N. Shinomiya, Bacterial translocation in gastrointestinal cancers and cancer treatment, Biomedicines 10 (2) (2022) 380, https://doi.org/10.3390/biomedicines10020380.
[161] S.D. Ehrlich, MetaHIT: The European Union Project on Metagenomics of the Human Intestinal Tract, in: K.E. Nelson (Ed.), Metagenomics of the Human Body [Internet], Springer New York, New York, NY, 2011, pp. 307–316, https://doi.org/10.1007/978-1-4419-7089-3_15.
[162] A. Zhernakova, A. Kurilshikov, M.J. Bonder, E.F. Tigchelaar, M. Schirmer, T. Vatanen, et al., Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity, Science 352 (6285) (2016) 565–569, https://doi.org/10.1126/science.aad3369.
[163] A. Latorre-Pérez, M. Hernández, J.R. Iglesias, J. Morán, J. Pascual, M. Porcar, et al., The Spanish gut microbiome reveals links between microorganisms and Mediterranean diet, Sci. Rep. 11 (1) (2021) 21602, https://doi.org/10.1038/s41598-021-01002-1.
[164] O.H. Oduaran, F.B. Tamburini, V. Sahibdeen, R. Brewster, F.X. Gómez-Olivé, K. Kahn, et al., Gut microbiome profiling of a rural and urban South African cohort reveals biomarkers of a population in lifestyle transition, BMC Microbiol 20 (1) (2020) 330, https://doi.org/10.1186/s12866-020-02017-w.
[165] Human Microbiome Project Consortium, Structure, function and diversity of the healthy human microbiome, Nature 486 (7402) (2012) 207–214, https://doi.org/10.1038/nature11234.
[166] R. Memmola, A. Petrillo, S. Di Lorenzo, S.C. Altuna, B.S. Habeeb, A. Soggiu, et al., Correlation between olive oil intake and gut microbiota in colorectal cancer prevention, Nutrients 14 (18) (2022) 3749, https://doi.org/10.3390/nu14183749.
[167] M. Rebersek, Gut microbiome and its role in colorectal cancer, BMC Cancer 21 (1) (2021) 1325, https://doi.org/10.1186/s12885-021-09054-2.
[168] T.M. Karpiński, M. Ożarowski, M. Stasiewicz, Carcinogenic microbiota and its role in colorectal cancer development, Semin Cancer Biol. 86 (Pt 3) (2022) 420–430, https://doi.org/10.1016/j.semcancer.2022.01.004.
[169] H. Jiang, W. Zeng, X. Zhang, Y. Pei, H. Zhang, Y. Li, The role of gut microbiota in patients with benign and malignant brain tumors: a pilot study, Bioengineered 13 (3) (2022) 7847–7859, https://doi.org/10.1080/21655979.2022.2049959.
[170] Y. Li, H. Jiang, X. Wang, X. Liu, Y. Huang, Z. Wang, et al., Crosstalk between the gut and brain: importance of the fecal microbiota in patient with brain tumors, Front Cell Infect. Microbiol 12 (2022), 881071, https://doi.org/10.3389/fcimb.2022.881071.
[171] X. Yu, W. Jiang, R.O. Kosik, Y. Song, Q. Luo, T. Qiao, et al., Gut microbiota changes and its potential relations with thyroid carcinoma, J. Adv. Res 35 (2022) 61–70, https://doi.org/10.1016/j.jare.2021.04.001.
[172] J. Zhang, F. Zhang, C. Zhao, Q. Xu, C. Liang, Y. Yang, et al., Dysbiosis of the gut microbiome is associated with thyroid cancer and thyroid nodules and correlated with clinical index of thyroid function, Endocrine 64 (3) (2019) 564–574, https://doi.org/10.1007/s12020-018-1831-x (Epub).
[173] X. Jian, Y. Zhu, J. Ouyang, Y. Wang, Q. Lei, J. Xia, et al., Alterations of gut microbiome accelerate multiple myeloma progression by increasing the relative abundances of nitrogen-recycling bacteria, Microbiome 8 (1) (2020) 74, https://doi.org/10.1186/s40168-020-00854-5.
[174] V.K. Ridaura, J.J. Faith, F.E. Rey, J. Cheng, A.E. Duncan, A.L. Kau, et al., Gut microbiota from twins discordant for obesity modulate metabolism in mice, Science 341 (6150) (2013) 1241214, https://doi.org/10.1126/science.1241214.
[175] N. Alang, C.R. Kelly, Weight gain after fecal microbiota transplantation, Open Forum Infect. Dis. 2 (1) (2015) ofv004, https://doi.org/10.1093/ofid/ofv004.
[176] T. Poutahidis, B.J. Varian, T. Levkovich, J.R. Lakritz, S. Mirabal, C. Kwok, et al., Dietary microbes modulate transgenerational cancer risk, Cancer Res 75 (7) (2015) 1197–1204, https://doi.org/10.1158/0008-5472.CAN-14-2732.
[177] A. Stedman, K. Brunner, G. Nigro, Decrypting the communication between microbes and the intestinal mucosa-A brief review on Pathogénie Microbienne Moléculaire's latest research, Cell Microbiol 21 (11) (2019), e13118, https://doi.org/10.1111/cmi.13118.
[178] F. Di Pierro, Gut microbiota parameters potentially useful in clinical perspective, Microorganisms 9 (11) (2021) 2402, https://doi.org/10.3390/microorganisms9112402.
[179] M. Trøseid, T.K. Nestvold, K. Rudi, H. Thoresen, E.W. Nielsen, K.T. Lappegård, Plasma lipopolysaccharide is closely associated with glycemic control and abdominal obesity: evidence from bariatric surgery, Diabetes Care 36 (11) (2013) 3627–3632, https://doi.org/10.2337/dc13-0451.
[180] S. Kim, Y.-C. Shin, T.-Y. Kim, Y. Kim, Y.-S. Lee, S.-H. Lee, et al., Mucin degrader Akkermansia muciniphila accelerates intestinal stem cell-mediated epithelial development, Gut Microbes 13 (1) (2021) 1–20, https://doi.org/10.1080/19490976.2021.1892441.
[181] B. Ohlsson, M. Orho-Melander, P.M. Nilsson, Higher levels of serum zonulin may rather be associated with increased risk of obesity and hyperlipidemia, than with gastrointestinal symptoms or disease manifestations, Int J. Mol. Sci. 18 (3) (2017), https://doi.org/10.3390/ijms18030582.
[182] L. Genser, D. Aguanno, H.A. Soula, L. Dong, L. Trystram, K. Assmann, et al., Increased jejunal permeability in human obesity is revealed by a lipid challenge and is linked to inflammation and type 2 diabetes, J. Pathol. 246 (2) (2018) 217–230, https://doi.org/10.1002/path.5134.
[183] A.P.B. Moreira, T.F.S. Texeira, A.B. Ferreira, M. Peluzio, C.G. do, R. Alfenas, C. G. de, Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia, Br. J. Nutr. 108 (5) (2012) 801–809, https://doi.org/10.1017/S0007114512001213.

[184] T.S. Postler, S. Ghosh, Understanding the holobiont: how microbial metabolites affect human health and shape the immune system, Cell Metab. 26 (1) (2017) 110–130, https://doi.org/10.1016/j.cmet.2017.05.008.
[185] J.P. Phelan, F.J. Reen, J.A. Caparros-Martin, R. O'Connor, F. O'Gara, Rethinking the bile acid/gut microbiome axis in cancer, Oncotarget 8 (70) (2017) 115736–115747, https://doi.org/10.18632/oncotarget.2280.
[186] R. Yang, L. Qian, Research on gut microbiota-derived secondary bile acids in cancer progression, Integr. Cancer Ther. 21 (2022), 15347354221114100.
[187] R. Li, S. Andreu-Sánchez, F. Kuipers, J. Fu, Gut microbiome and bile acids in obesity-related diseases, Best. Pr. Res Clin. Endocrinol. Metab. 35 (3) (2021), 101493, https://doi.org/10.1016/j.beem.2021.101493.
[188] C.W.H. Chan, B.M.H. Law, M.M.Y. Waye, J.Y.W. Chan, W.K.W. So, K.M. Chow, Trimethylamine-N-oxide as one hypothetical link for the relationship between intestinal microbiota and cancer - where we are and where shall we go? J. Cancer 10 (23) (2019) 5874–5882, https://doi.org/10.7150/jca.31737.
[189] D. Li, Y. Lu, S. Yuan, X. Cai, Y. He, J. Chen, et al., Gut microbiota-derived metabolite trimethylamine-N-oxide and multiple health outcomes: an umbrella review and updated meta-analysis, Am. J. Clin. Nutr. 116 (1) (2022) 230–243, https://doi.org/10.1093/ajcn/nqac074.
[190] P. Dehghan, M.A. Farhangi, L. Nikniaz, Z. Nikniaz, M. Asghari-Jafarabadi, Gut microbiota-derived metabolite trimethylamine N-oxide (TMAO) potentially increases the risk of obesity in adults: An exploratory systematic review and dose-response meta- analysis, Obes. Rev. 21 (5) (2020), e12993, https://doi.org/10.1111/obr.12993.
[191] C.S. Plottel, M.J. Blaser, Microbiome and malignancy, Cell Host Microbe 10 (4) (2011) 324–335, https://doi.org/10.1016/j.chom.2011.10.003.
[192] R. Jarman, S. Ribeiro-Milograna, W. Kalle, Potential of the microbiome as a biomarker for early diagnosis and prognosis of breast cancer, J. Breast Cancer 23 (6) (2020) 579–587, https://doi.org/10.4048/jbc.2020.23.e60.
[193] K.M. Mair, R. Gaw, M.R. MacLean, Obesity, estrogens and adipose tissue dysfunction - implications for pulmonary arterial hypertension, 2045894020952019, Pulm. Circ. 10 (3) (2020), https://doi.org/10.1177/2045894020952023.
[194] E. Alvandi, W.K.M. Wong, M.V. Joglekar, K.J. Spring, A.A. Hardikar, Short-chain fatty acid concentrations in the incidence and risk-stratification of colorectal cancer: a systematic review and meta-analysis, BMC Med 20 (1) (2022) 323, https://doi.org/10.1186/s12916-022-02529-4.
[195] R. Mirzaei, A. Afaghi, S. Babakhani, M.R. Sohrabi, S.R. Hosseini-Fard, K. Babolhavaeji, et al., Role of microbiota-derived short-chain fatty acids in cancer development and prevention, Biomed. Pharm. 139 (2021), 111619, https://doi.org/10.1016/j.biopha.2021.111619 (Epub).
[196] F. Comas, J.M. Moreno-Navarrete, The impact of H(2)S on obesity-associated metabolic disturbances, Antioxidants 10 (5) (2021) 633, https://doi.org/10.3390/antiox10050633.
[197] Y. Cheng, Z. Ling, L. Li, The intestinal microbiota and colorectal cancer, Front Immunol. 11 (2020), 615056, https://doi.org/10.3389/fimmu.2020.615056.
[198] R.E. Shackelford, I.Z. Mohammad, A.T. Meram, D. Kim, F. Alotaibi, S. Patel, et al., Molecular functions of hydrogen sulfide in cancer, Pathophysiol 28 (3) (2021) 437–456, https://doi.org/10.3390/pathophysiology28030028.
[199] W.L. Williams, H.P. Broquist, E.E. Snell, Oleic acid and related compounds as growth factors for lactic acid bacteria, J. Biol. Chem. 170 (2) (1947) 619–630.
[200] C.-H. Chen, Y. Wang, T. Nakatsuji, Y.-T. Liu, C.C. Zouboulis, R.L. Gallo, et al., An innate bactericidal oleic acid effective against skin infection of methicillin-resistant staphylococcus aureus: a therapy concordant with evolutionary medicine, J. Microbiol Biotechnol. 21 (4) (2011) 391–399.
[201] M. Obonyo, L. Zhang, S. Thamphiwatana, D. Pornpattananangkul, V. Fu, L. Zhang, Antibacterial activities of liposomal linolenic acids against antibiotic-resistant helicobacter pylori, Mol. Pharm. 9 (9) (2012) 2677, https://doi.org/10.1021/mp300243w.
[202] S. Thamphiwatana, W. Gao, M. Obonyo, L. Zhang, In vivo treatment of Helicobacter pylori infection with liposomal linolenic acid reduces colonization and ameliorates inflammation, Proc. Natl. Acad. Sci. USA 111 (49) (2014) 17600–17605, https://doi.org/10.1073/pnas.1418230111.
[203] M. D'Archivio, C. Santangelo, A. Silenzi, B. Scazzocchio, R. Varì, R. Masella, Dietary EVOO polyphenols and gut microbiota interaction: are there any sex/gender influences? Antioxidants 11 (9) (2022) 1744, https://doi.org/10.3390/antiox11091744.
[204] G. Rocchetti, M. Luisa Callegari, A. Senizza, G. Giuberti, J. Ruzzolini, A. Romani, et al., Oleuropein from olive leaf extracts and extra-virgin olive oil provides distinctive phenolic profiles and modulation of microbiota in the large intestine, Food Chem. 380 (2022), 132187, https://doi.org/10.1016/j.foodchem.2022.132187.
[205] G. Marcelino, P.A. Hiane, K. de C. Freitas, L.F. Santana, A. Pott, J.R. Donadon, et al., Effects of olive oil and its minor components on cardiovascular diseases, inflammation, and gut microbiota, Nutrients 11 (8) (2019) 1826, https://doi.org/10.3390/nu11081826.
[206] C. Haro, M. Montes-Borrego, O.A. Rangel-Zúñiga, J.F. Alcalá-Díaz, F. Gómez-Delgado, P. Pérez-Martínez, et al., Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population, J. Clin. Endocrinol. Metab. 101 (1) (2016) 233–242, https://doi.org/10.1210/jc.2015-3351.
[207] S. Martín-Peláez, J.I. Mosele, N. Pizarro, M. Farràs, R. de la Torre, I. Subirana, et al., Effect of virgin olive oil and thyme phenolic compounds on blood lipid profile: implications of human gut microbiota, Eur. J. Nutr. 56 (1) (2017) 119–131, https://doi.org/10.1007/s00394-015-1063-2.
[208] J. Olalla, J.M. García de Lomas, N. Chueca, X. Pérez-Stachowski, A. De Salazar, A. Del Arco, et al., Effect of daily consumption of extra virgin olive oil on the lipid profile and microbiota of HIV-infected patients over 50 years of age, Med. (Baltim.) 98 (42) (2019), e17528.
[209] M.L.E. Luisi, L. Lucarini, B. Biffi, E. Rafanelli, G. Pietramellara, M. Durante, et al., Effect of mediterranean diet enriched in high quality extra virgin olive oil on oxidative stress, inflammation and gut microbiota in obese and normal weight adult subjects, Front Pharm. 10 (2019) 1366, https://doi.org/10.3389/fphar.2019.01366.
[210] C. Rosés, A. Cuevas-Sierra, S. Quintana, J.I. Riezu-Boj, J. Alfredo Martínez, F. I. Milagro, et al., Gut microbiota bacterial species associated with mediterranean diet-related food groups in a northern spanish population, Nutrients 13 (2) (2021) 1–17, https://doi.org/10.3390/nu13020636.
[211] R.R.X. Lim, M.A. Park, L.H. Wong, S. Haldar, K.J. Lim, N. Nagarajan, et al., Gut microbiome responses to dietary intervention with hypocholesterolemic vegetable oils, NPJ Biofilms Micro 8 (1) (2022) 24, https://doi.org/10.1038/s41522-022-00287-y.
[212] N. Zmora, J. Suez, E. Elinav, You are what you eat: diet, health and the gut microbiota, Nat. Rev. Gastroenterol. Hepatol. 16 (1) (2019) 35–56, https://doi.org/10.1038/s41575-018-0061-2.
[213] A.S. Wilson, K.R. Koller, M.C. Ramaboli, L.T. Nesengani, S. Ocvirk, C. Chen, et al., Diet and the human gut microbiome: an international review, Dig. Dis. Sci. 65 (3) (2020) 723–740, https://doi.org/10.1007/s10620-020-06112-w.
[214] Rinninella, Cintoni, Raoul, Lopetuso, Scaldaferri, Pulcini, et al., Food components and dietary habits: keys for a healthy gut microbiota composition, Nutrients 11 (10) (2019) 2393, https://doi.org/10.3390/nu11102393.
[215] Z. Djuric, C.M. Bassis, M.A. Plegue, J. Ren, R. Chan, E. Sidahmed, et al., Colonic mucosal bacteria are associated with inter-individual variability in serum carotenoid concentrations, e3, J. Acad. Nutr. Diet. 118 (4) (2018) 606–616, https://doi.org/10.1016/j.jand.2017.09.013.
[216] J. Muralidharan, I. Moreno-Indias, M. Bulló, J.V. Lopez, D. Corella, O. Castañer, et al., Effect on gut microbiota of a 1-y lifestyle intervention with Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus Study, Am. J. Clin. Nutr. 114 (3) (2021) 1148–1158, https://doi.org/10.1093/ajcn/nqab150.
[217] A. Atzeni, S. Galié, J. Muralidharan, N. Babio, F.J. Tinahones, J. Vioque, et al., Gut microbiota profile and changes in body weight in elderly subjects with overweight/obesity and metabolic syndrome, Microorganisms 9 (2) (2021) 346, https://doi.org/10.3390/microorganisms9020346.
[218] A. Atzeni, T.F.S. Bastiaanssen, J.F. Cryan, F.J. Tinahones, J. Vioque, D. Corella, et al., Taxonomic and functional fecal microbiota signatures associated with insulin resistance in non-diabetic subjects with overweight/obesity within the frame of the PREDIMED-plus study, Front Endocrinol. 13 (2022), 804455, https://doi.org/10.3389/fendo.2022.804455.
[219] T.L. Netto Cândido, L.E. da Silva, F.G. Cândido, F.X. Valente, J.S. da Silva, D. R. Gomes Lopes, et al., Effect of the ingestion of vegetable oils associated with energy-restricted normofat diet on intestinal microbiota and permeability in overweight women, Food Res Int 139 (2021), 109951, https://doi.org/10.1016/j.foodres.2020.109951.
[220] B. Usta-Gorgun, L. Yilmaz-Ersan, Short-chain fatty acids production by Bifidobacterium species in the presence of salep, Electron J. Biotechnol. 47 (2020) 29–35, https://doi.org/10.1016/j.ejbt.2020.06.004.
[221] S. Haldar, L.H. Wong, S.L. Tay, J.J. Jacoby, P. He, F. Osman, et al., Two blends of refined rice bran, flaxseed, and sesame seed oils affect the blood lipid profile of chinese adults with borderline hypercholesterolemia to a similar extent as refined olive oil, J. Nutr. 150 (12) (2020) 3141–3151, https://doi.org/10.1093/jn/nxaa274.
[222] E. Sanchez-Rodriguez, E. Lima-Cabello, S. Biel-Glesson, J.R. Fernandez-Navarro, M.A. Calleja, M. Roca, et al., Effects of virgin olive oils differing in their bioactive compound contents on metabolic syndrome and endothelial functional risk biomarkers in healthy adults: a randomized double-blind controlled trial, Nutrients 10 (5) (2018) 626, https://doi.org/10.3390/nu10050626.
[223] S. Bartimoccia, V. Cammisotto, C. Nocella, M. Del Ben, A. D'Amico, V. Castellani, et al., Extra virgin olive oil reduces gut permeability and metabolic endotoxemia in diabetic patients, Nutrients 14 (10) (2022) 2153, https://doi.org/10.3390/nu14102153.
[224] S. Raposeiras Roubín, A. Cordero, The two-way relationship between cancer and atherosclerosis, Rev. Esp. Cardiol. 72 (6) (2019) 487–494, https://doi.org/10.1016/j.rec.2018.12.010.
[225] W.-K. Wu, E.A. Ivanova, A.N. Orekhov, Gut microbiome: A possible common therapeutic target for treatment of atherosclerosis and cancer, Semin Cancer Biol. 70 (2021) 85–97, https://doi.org/10.1016/j.semcancer.2020.06.017.
[226] V. Rovella, G. Rodia, F. Di Daniele, C. Cardillo, U. Campia, A. Noce, et al., Association of gut hormones and microbiota with vascular dysfunction in obesity, Nutrients 13 (2) (2021) 613, https://doi.org/10.3390/nu13020613.
[227] S. Martín-Peláez, O. Castañer, R. Solà, M. Motilva, M. Castell, F. Pérez-Cano, et al., Influence of phenol-enriched olive oils on human intestinal immune function, Nutrients 8 (4) (2016) 213, https://doi.org/10.3390/nu8040213.
[228] E. Mamalaki, S. Charisis, C.A. Anastasiou, E. Ntanasi, K. Georgiadi, V. Balomenos, et al., The longitudinal association of lifestyle with cognitive health and dementia risk: findings from the HELIAD study, Nutrients 14 (14) (2022), https://doi.org/10.3390/nu14142818.
[229] M.-C. Tsai, T.-L. Yeh, H.-Y. Hsu, L.-Y. Hsu, C.-C. Lee, P.-J. Tseng, et al., Comparison of four healthy lifestyle scores for predicting cardiovascular events in a national cohort study, Sci. Rep. 11 (1) (2021) 22146, https://doi.org/10.1038/s41598-021-01213-6.

[230] P.A. van den Brandt, The impact of a healthy lifestyle on the risk of esophageal and gastric cancer subtypes, Eur. J. Epidemiol. 37 (9) (2022) 931–945, https://doi.org/10.1007/s10654-022-00899-w.
[231] C. Hill, F. Guarner, G. Reid, G.R. Gibson, D.J. Merenstein, B. Pot, et al., Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic, Nat. Rev. Gastroenterol. Hepatol. 11 (8) (2014) 506–514, https://doi.org/10.1038/nrgastro.2014.66.
[232] N.L.-N. Ting, H.C.-H. Lau, J. Yu, Cancer pharmacomicrobiomics: targeting microbiota to optimise cancer therapy outcomes, Gut 71 (7) (2022) 1412–1425, https://doi.org/10.1136/gutjnl-2021-326264.
[233] K.L. Greathouse, M. Wyatt, A.J. Johnson, E.P. Toy, J.M. Khan, K. Dunn, et al., Diet-microbiome interactions in cancer treatment: Opportunities and challenges for precision nutrition in cancer, Neoplasia 29 (2022), 100800, https://doi.org/10.1016/j.neo.2022.100800.
[234] A. Sevcikova, N. Izoldova, V. Stevurkova, B. Kasperova, M. Chovanec, S. Ciernikova, et al., The impact of the microbiome on resistance to cancer treatment with chemotherapeutic agents and immunotherapy, Int J. Mol. Sci. 23 (1) (2022) 488, https://doi.org/10.3390/ijms23010488.
[235] W. Ma, Q. Mao, W. Xia, G. Dong, C. Yu, F. Jiang, Gut microbiota shapes the efficiency of cancer therapy, Front Microbiol 10 (2019), 1050, https://doi.org/10.3389/fmicb.2019.01050.
[236] K.S. Swanson, G.R. Gibson, R. Hutkins, R.A. Reimer, G. Reid, K. Verbeke, et al., The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics, Nat. Rev. Gastroenterol. Hepatol. 17 (11) (2020) 687–701, https://doi.org/10.1038/s41575-020-0344-2.
[237] S. Salminen, M.C. Collado, A. Endo, C. Hill, S. Lebeer, E.M.M. Quigley, et al., The international scientific association of probiotics and prebiotics (isapp) consensus statement on the definition and scope of postbiotics, Nat. Rev. Gastroenterol. Hepatol. 18 (9) (2021) 649–667, https://doi.org/10.1038/s41575-021-00440-6.
[238] D. Turck, T. Bohn, J. Castenmiller, S. De Henauw, K.I. Hirsch-Ernst, A. Maciuk, et al., Safety of pasteurised Akkermansia muciniphila as a novel food pursuant to Regulation (EU) 2015/2283, EFSA J. 19 (9) (2021), e06780, https://doi.org/10.2903/j.efsa.2021.6780.
[239] N. Castellanos, G.G. Diez, C. Antúnez-Almagro, M. Bailén, C. Bressa, R. González Soltero, et al., A critical mutualism - competition interplay underlies the loss of microbial diversity in sedentary lifestyle, Front Microbiol 10 (2019) 3142, https://doi.org/10.3389/fmicb.2019.03142.
[240] B. Dorelli, F. Gallè, C. De Vito, G. Duranti, M. Iachini, M. Zaccarin, et al., Can physical activity influence human gut microbiota composition independently of diet? A systematic review, Nutrients 13 (6) (2021) 1890, https://doi.org/10.3390/nu13061890.
[241] M. Han, S. Yuan, J. Zhang, The interplay between sleep and gut microbiota, Brain Res Bull. 180 (2022) 131–146, https://doi.org/10.1016/j.brainresbull.2021.12.016.
[242] C. Delaroque, M. Chervy, A.T. Gewirtz, B. Chassaing, Social overcrowding impacts gut microbiota, promoting stress, inflammation, and dysglycemia, Gut Microbes 13 (1) (2021) 2000275, https://doi.org/10.1080/19490976.2021.2000275.
[243] O.S. Barrera-Vázquez, J.C. Gomez-Verjan, The unexplored world of human virome, mycobiome, and archaeome in aging, J. Gerontol. A Biol. Sci. Med Sci. 75 (10) (2020) 1834–1837, https://doi.org/10.1093/gerona/glz274.
[244] M.L. Richard, H. Sokol, The gut mycobiota: insights into analysis, environmental interactions and role in gastrointestinal diseases, Nat. Rev. Gastroenterol. Hepatol. 16 (6) (2019) 331–345, https://doi.org/10.1038/s41575-019-0121-2.
[245] Z. Cao, N. Sugimura, E. Burgermeister, M.P. Ebert, T. Zuo, P. Lan, The gut virome: A new microbiome component in health and disease, EBioMedicine 81 (2022), 104113, https://doi.org/10.1016/j.ebiom.2022.104113.
[246] Y. Duan, R. Young, B. Schnabl, Bacteriophages and their potential for treatment of gastrointestinal diseases, Nat. Rev. Gastroenterol. Hepatol. 19 (2) (2022) 135–144, https://doi.org/10.1038/s41575-021-00536-z.
[247] G.A. Suh, T.P. Lodise, P.D. Tamma, J.M. Knisely, J. Alexander, S. Aslam, et al., Considerations for the use of phage therapy in clinical practice, Antimicrob. Agents Chemother. 66 (3) (2022), e0207121, https://doi.org/10.1128/AAC.02071-21.
[248] J. Erdmann, How gut bacteria could boost cancer treatments, Nature 607 (7919) (2022) 436–439, https://doi.org/10.1038/d41586-022-01959-7.
[249] M. Bou Zerdan, S. Niforatos, S. Nasr, D. Nasr, M. Ombada, S. John, et al., Fecal microbiota transplant for hematologic and oncologic diseases: principle and practice, Cancers 14 (3) (2022) 691, https://doi.org/10.3390/cancers14030691.
[250] S.M.D. Baunwall, M.M. Lee, M.K. Eriksen, B.H. Mullish, J.R. Marchesi, J. F. Dahlerup, et al., Faecal microbiota transplantation for recurrent Clostridioides difficile infection: An updated systematic review and meta-analysis, 100642, EClinicalMedicine (2020) 29–30, https://doi.org/10.1016/j.eclinm.2020.100642.
[251] S. Moossavi, M.-C. Arrieta, A. Sanati-Nezhad, F. Bishehsari, Gut-on-chip for ecological and causal human gut microbiome research, Trends Microbiol 30 (8) (2022) 710–721, https://doi.org/10.1016/j.tim.2022.01.014.
[252] L.J. Marcos-Zambrano, K. Karaduzovic-Hadziabdic, T. Loncar Turukalo, P. Przymus, V. Trajkovik, O. Aasmets, et al., Applications of machine learning in human microbiome studies: a review on feature selection, biomarker identification, disease prediction and treatment, Front Microbiol 12 (2021), 634511, https://doi.org/10.3389/fmicb.2021.634511.
[253] Wild C.P., Weiderpass E., Stewart B.W. Cancer research for cancer prevention World Cancer Report. (2020) ⟨http://publications.iarc.fr/586⟩. [Accessed August 1, 2022].
[254] World Cancer Research Fund, Diet, nutrition, physical activity and cancer: a global perspective: a summary of the Third Expert Report, World Cancer Res Fund. Int (2018) 1–114. ⟨http://gco.iarc.fr/today⟩ [Accessed August 1, 2022].
[255] C.M. Kitahara, M.L. McCullough, S. Franceschi, S. Rinaldi, A. Wolk, G. Neta, H. Olov Adami, K. Anderson, G. Andreotti, L.E. Beane Freeman, et al., Anthropometric factors and thyroid cancer risk by histological subtype: Pooled analysis of 22 prospective studies, Thyroid 26 (2016) 306–318, https://doi.org/10.1089/thy.2015.0319.
[256] L.R. Teras, C.M. Kitahara, B.M. Birmann, P.A. Hartge, S.S. Wang, K. Robien, A. V. Patel, H.O. Adami, E. Weiderpass, G.G. Giles, et al., Body size and multiple myeloma mortality: A pooled analysis of 20 prospective studies, Br. J. Haematol. 166 (2014) 667–676, https://doi.org/10.1111/bjh.12935.
[257] T. Niedermaier, G. Behrens, D. Schmid, I. Schlecht, B. Fischer, M.F. Leitzmann, Body mass index, physical activity, and risk of adult meningioma and glioma: A meta-analysis, Neurology 85 (2015) 1342–1350, https://doi.org/10.1212/WNL.0000000000002020.
[258] J. Castillo, R.R. Ingham, J.L. Reagan, M. Furman, S. Dalia, J. Mitri, Obesity is associated with increased relative risk of diffuse large B-cell lymphoma: a meta-analysis of observational studies, Clin. Lymphoma Myeloma Leuk. 14 (2013) 122–130, https://doi.org/10.1016/j.clml.2013.10.005.
[259] L.A. Brinton, M.B. Cook, V. McCormack, K.C. Johnson, H. Olsson, J. T. Casagrande, R. Cooke, R.T. Falk, S.M. Gapstur, M.M. Gaudet, et al., Anthropometric and hormonal risk factors for male breast cancer: male breast cancer pooling project results, J. Natl. Cancer Inst. 106 (2014) djt465, https://doi.org/10.1093/jnci/djt465.

11.2. Association between classes and subclasses of polyphenol intake and 5-year body weight changes in the EPIC-PANACEA study.

Castañeda J, **Gil-Lespinard M**, Almanza-Aguilera E, Llaha F, Gómez J, Bondonno NP, et al. Association between classes and subclasses of polyphenol intake and 5-year body weight changes in the EPIC-PANACEA study. Obesity [Internet]. 2023 Jan 24;31(4):1146–58. Available from: https://doi.org/10.1002/oby.23689

Impact Factor: 6.9

Category: Endocrinology & Metabolism. Q1 23/145

Category: Nutrition & Dietetics. Q1 12/88

Received: 29 July 2022 | Revised: 3 November 2022 | Accepted: 29 November 2022

DOI: 10.1002/oby.23689

Obesity A Research Journal | THE OBESITY SOCIETY | WILEY

ORIGINAL ARTICLE

Epidemiology/Genetics

Association between classes and subclasses of polyphenol intake and 5-year body weight changes in the EPIC-PANACEA study

Jazmin Castañeda[1] | Mercedes Gil-Lespinard[1] | Enrique Almanza-Aguilera[1] | Fjorida Llaha[1] | Jesús-Humberto Gómez[2,3] | Nicola Bondonno[4,5] | Anne Tjønneland[4,6] | Kim Overvad[7] | Verena Katzke[8] | Matthias B. Schulze[9,10] | Giovanna Masala[11] | Claudia Agnoli[12] | Maria Santucci de Magistris[13] | Rosario Tumino[14] | Carlotta Sacerdote[15] | Guri Skeie[16] | Magritt Brustad[16,17] | Cristina Lasheras[18] | Esther Molina-Montes[3,19,20,21] | María-Dolores Chirlaque[2,3] | Aurelio Barricarte[3,22,23] | Emily Sonestedt[24] | Marisa da Silva[25] | Ingegerd Johansson[26] | Johan Hultdin[27] | Anne M. May[28] | Nita G. Forouhi[29] | Alicia K. Heath[30] | Heinz Freisling[31] | Elisabete Weiderpass[31] | Augustin Scalbert[31] | Raul Zamora-Ros[1,32]

Correspondence
Raul Zamora-Ros, Department of Nutrition, Food Sciences, and Gastronomy, Faculty of Pharmacy and Food Sciences, University of Barcelona, Avinguda Joan XXIII, 27-31, 08028 Barcelona, Spain.
Email: rzamora@ub.edu and rzamora@idibell.cat

Funding information
Consejo Nacional de Ciencia y Tecnología, Grant/Award Number: ID 693636; Instituto de Salud Carlos III, Grant/Award Numbers: CD20/00071, CPII20/00009, FI19/00185, PI18/00191

Abstract

Objective: The aim of this study was to evaluate the associations among the intake of total polyphenols, polyphenol classes, and polyphenol subclasses and body weight change over 5 years.

Methods: A total of 349,165 men and women aged 25 to 70 years were recruited in the Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating Out of Home and Obesity (PANACEA) project of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort from nine European countries. Body weight was measured at baseline and at follow-up after a median time of 5 years. Polyphenol intake, including four main polyphenol classes and eighteen subclasses, was estimated using validated dietary questionnaires and Phenol-Explorer. Multilevel mixed linear regression models were used to estimate the associations.

Results: Participants gained, on average, 2.6 kg (±5.0 kg) over 5 years. Total flavonoids intake was inversely associated with body weight change (−0.195 kg/5 years, 95% CI: −0.262 to −0.128). However, the intake of total polyphenols (0.205 kg/5 years, 95% CI: 0.138 to 0.272) and intake of hydroxycinnamic acids (0.324 kg/

Jazmin Castañeda and Mercedes Gil-Lespinard contributed equally to this study.

For affiliations, refer to page 1155.

5 years, 95% CI: 0.267 to 0.381) were positively associated with body weight gain. In analyses stratified by coffee consumption, hydroxycinnamic acid intake was positively associated with body weight gain in coffee consumers (0.379 kg/5 years, 95% CI: 0.319 to 0.440), but not in coffee nonconsumers (−0.179 kg/5 years, 95% CI: −0.490 to 0.133).

Conclusions: Higher intakes of flavonoids and their subclasses are inversely associated with a modest body weight change. Results regarding hydroxycinnamic acids in coffee consumers require further investigation.

INTRODUCTION

Overweight and obesity are defined as abnormal or excessive body fat accumulation. Obesity is one of the principal contributors to the global burden of chronic diseases, particularly cardiovascular disease, type 2 diabetes, and certain types of cancers [1]. The prevalence has increased rapidly: the World Health Organization reports that more than 2 billion adults have overweight and obesity worldwide, whereas, in Europe, nearly 60% of adults are classified as having overweight or obesity [2, 3]. Obesity results from a complex interaction of several factors such as diet, lifestyle, socioeconomics, genetics, and environment [4].

Even a moderate weight loss of 5% to 10% has been shown to lead to the significant improvement of several cardiometabolic parameters (e.g., triglycerides, blood pressure, waist circumference, insulin sensitivity, β-cell function) [5] and a lower risk of comorbidities, including cardiovascular disease [6], diabetes mellitus [6], hypertension [7], dyslipidemia [8], and overall mortality [9].

Polyphenols are bioactive phytochemicals found in plant foods and beverages such as fruit, vegetables, seeds, herbs, spices, whole grains, tea, coffee, and wine [10]. They comprise a large variety of chemical structures and they are divided into four main classes: flavonoids, phenolic acids, stilbenes, and lignans [10, 11]. Increasing preclinical and clinical evidence has suggested a role of polyphenols as antiobesity compounds. Indeed, several *in vitro* and *in vivo* studies have shown that polyphenols may stimulate thermogenesis and energy expenditure, inhibit adipocyte differentiation and growth, increase lipolysis, induce β oxidation, and decrease appetite [12, 13]. The antiobesity effects of polyphenols, particularly flavonoids, have also been supported by numerous clinical trials [11, 14].

In contrast, few observational epidemiological studies have examined the role of polyphenol intake in body weight control. The Supplementation with Antioxidant Vitamins and Minerals (Supplementation en Vitamines et Mineraux Antioxydants [SU.VI.MAX]) study observed that a high intake of total polyphenols was associated with lower waist circumference and body mass index (BMI) after 6 years of follow-up [15]. Similarly, an inverse association among total flavonoid intake and body weight, BMI, and waist circumference was observed in the National Health and Nutrition Examination Survey (NHANES) [16]. Likewise, results from the Prevención con Dieta Mediterránea (REDIMED) study indicated that total urinary polyphenol excretion was inversely associated with changes in body weight, BMI, waist circumference, and waist to height ratio over 5 years [17]. Furthermore, the Health, Alcohol and Psychosocial factors in Eastern Europe (HAPPIEE) study reported an inverse association among high total polyphenol intake, particularly from stilbenes and lignans, and BMI and waist circumference [18]. However, these epidemiological studies have some limitations, including a limited number of classes

Study Importance

What is already known?

- Several experimental studies have reported that polyphenols can stimulate different mechanisms in body weight loss, such as thermogenesis, energy expenditure, and induced β oxidation, among others.
- Some clinical trials have described the antiobesity effects of pharmacological doses of some polyphenols.
- There is scarce epidemiological research investigating the associations between classes and subclasses of polyphenols and body weight change, especially with nonflavonoids.

What does this study add?

- This study provides evidence suggesting that the intake of flavonoids is associated with the maintenance of body weight in both men and women.
- Hydroxycinnamic acid intake from coffee is associated with an increase in body weight in coffee consumers, but not in coffee nonconsumers.

How might these results change the direction of research?

- For a better understanding on the influence of polyphenols on body weight loss, future randomized controlled trials using combinations of polyphenols or plant extracts mimicking polyphenol-rich diets are needed.
- Further research evaluating the effect of hydroxycinnamic acids in body weight change is also warranted.

and/or subclasses of polyphenols investigated and a low variability of polyphenol intake due to small geographic variations. Therefore, the present study aimed to examine the associations among intakes of total polyphenols and all polyphenol classes and subclasses estimated using the Phenol-Explorer database and body weight change over 5 years in the large, multicountry European Prospective Investigation into Cancer and Nutrition-Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating Out of Home and Obesity (EPIC-PANACEA) cohort.

METHODS

Study population

EPIC is a prospective cohort study with 521,448 participants, aged from 25 to 70 years, recruited between 1992 and 2000 in 23 centers from 10 Western European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the UK. The EPIC Study was approved by the local ethics committees from the participating centers and the ethical review board of the International Agency for Research on Cancer. All participants signed an informed consent. Further details on the study design and methods have been described previously [19].

The EPIC-PANACEA study is a subcohort of EPIC with follow-up data of anthropometric measurements, and it was originally designed to investigate the determinants of obesity and weight change in EPIC countries. In the present study, we excluded pregnant women and participants with missing diet or lifestyle questionnaires, missing data on weight and height, or unreliable anthropometric values at baseline ($n = 23{,}713$). In addition, we excluded participants with missing data on weight at follow-up ($n = 122{,}154$) and those with unrealistic body weight changes (<−5 or >5 kg/y over several years) or implausible/unusual anthropometry at follow-up (BMI at follow-up < 16 kg/m^2; $n = 2288$). More details regarding the EPIC-PANACEA study design have been described elsewhere [20]. Finally, participants from Greece ($n = 24{,}128$) did not provide data for the present study; therefore, they were also excluded. The final analysis included 93,435 men and 255,730 women.

Anthropometric measures and body weight change

Body weight in EPIC-PANACEA was measured at baseline and at follow-up. The time between the first and second measurements ranged from 2 years (Germany) to 11 years (Italy), with an overall median of 5 years. Standardized methods were used to take anthropometric measurements (body weight and height), except for the centers of France, Norway, and Oxford (UK), where participants self-reported their anthropometric values at baseline. In the follow-up, body weight was self-reported in all centers except in Cambridge (UK) and Doetinchem (the Netherlands), where weight was measured via standardized methods [20]. For the accuracy of self-reported anthropometric measurements (at baseline and follow-up), Oxford correction equations were used to predict measured weight and to calculate the corrected weight change [21]. Our primary outcome was weight change in kilograms per 5 years, calculated as weight at follow-up minus weight at baseline divided by the follow-up time in years to obtain the annual weight change and then multiplied by 5 years.

Dietary assessment and other covariates

Habitual diet was recorded at baseline by validated country- or center-specific dietary questionnaires that captured food and beverage intakes over the previous 12 months [19]. In most centers, these were self-administered food frequency questionnaires, except for Ragusa (Italy), Naples (Italy), and Spain, where face-to-face interviews were conducted and meal-structured questionnaires were used. A combined method of a semiquantitative food frequency questionnaire and a 7-day record was used in the UK and Malmö (Sweden) [19]. Nutrients and total energy intakes were estimated using the standardized EPIC Nutrient Database [22]. Dietary polyphenol intake was estimated using the Phenol-Explorer database, which contains content values for 502 polyphenols in 452 foods and beverages, together with retention factors for cooked and processed food [23]. Dietary polyphenols were divided into four major classes: total flavonoids, phenolic acids, stilbenes, and lignans, plus a minority class of polyphenols and 18 subclasses, specified in Table 2; all classes and subclasses were then summed to calculate total polyphenol intake.

Moreover, validated questionnaires were used at baseline to collect data on tobacco use, education level, menstrual history, physical activity (inactive, moderately inactive, moderately active, active), and clinical data. In addition, information on smoking status (never, former, current) was also collected at follow-up.

Statistical analyses

Polyphenol intake was assessed as a categorical variable based on quintiles distributed throughout the entire EPIC-PANACEA study. In addition, linear trend tests were calculated assigning the median of each quintile as scores. Polyphenol intake was also analyzed as a continuous variable after $\log_2$ transformation to reduce the skewness of intake distributions. Before $\log_2$ transformation, zero values were replaced with half of the nonzero minimum of the polyphenol class or subclass. One-unit increase corresponded to the absolute body weight change (kilograms per 5 years) associated with doubling in intake.

Multilevel mixed linear regression models were used to estimate the association among total, classes, and subclasses of polyphenol intake and body weight change over 5 years, using the EPIC center as a random effect and polyphenol intake and relevant confounders as fixed effects. Missing values (3.5% for educational level, 1.5% for physical activity, 2.1% for education, 4.7% for smoking status) were classified into a separate category (unknown) and included in the models. BMI at baseline and follow-up time in years had a nonlinear association with weight change. Consequently, they were included in the models as restricted cubic splines with three knots (10th, 50th,

and 90th percentiles) according to Harrell [24]. We fitted multivariable models adjusting for potential confounders (as fixed effects) selected a priori. Model 1 was adjusted for sex (male and female), age at baseline (years), and BMI (kilograms per meters squared). In model 2, we also adjusted for lifestyle characteristics: follow-up time (years), alcohol consumption (grams per day), education level (none, primary education, technical or professional school, secondary school, higher education, and unknown), physical activity (inactive, moderately inactive, moderately active, active, and unknown), smoking status at follow-up (never, former, and current), and menopausal status (pre-, post-, and peri-surgically postmenopausal and unknown). Model 3 was further adjusted for variables related to energy: total energy intake (kilocalories per day) and plausibility of energy intake reporting (yes and no) [19]. In addition, for model 3, we replaced total energy (kilocalories per day) with the all-components model (adjusting for all individual components providing energy of the diet) [25]; however, the results remained similar to the previous model; therefore, we did not present them. Finally, model 4 was model 3 additionally adjusted for other dietary factors: vitamin C (milligrams per day) and fiber (grams per day) intake. Furthermore, polyphenol intake was included in the statistical models as energy density (2000 mg/kcal/d). Results from both methods were almost identical, and energy density results were not reported.

The main food source of phenolic acids is coffee, which plays a role in body weight change [26]. Therefore, we performed analyses separately for hydroxycinnamic acid (HCA) class intake in coffee consumers and nonconsumers. Also, because caffeine has been associated with body weight loss [26], we evaluated the associations between total coffee and the type of coffee (caffeinated vs. decaffeinated) and body weight in order to investigate the effect of caffeine in body weight and differentiate the relationships between HCAs and caffeine from coffee intake.

Interaction analyses were performed between classes and subclasses of polyphenol intake (continuous, milligrams per day) and sex, age (<50 and ≥50 years), menopause status (peri-, post-, and premenopause), physical activity (inactive, moderately inactive, moderately active, and active), smoking status at follow-up (never, former, and current smokers), and BMI at baseline (underweight, normal weight, overweight, and obesity) in relation to body weight change. *P* values for the interaction were calculated using the likelihood ratio test. Sex, menopause, smoking status, and BMI had a statistically significant interaction; therefore, models were further fitted separately for each category of the variables. Finally, to assess the robustness of the results, we conducted a sensitivity analysis excluding participants with chronic diseases at baseline (type 2 diabetes, cardiovascular disease, and/or cancer) and participants who either quit smoking or started smoking or had missing data on smoking during follow-up. All p values presented were considered statistically significant at $p < 0.05$. Statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Participants in the highest quintile of polyphenol intake were more likely to be men and older and they had, on average, a lower BMI and a higher education, alcohol consumption, and total energy intake (Table 1). In addition, they were more likely to be more physically active and current smokers. Women with higher total polyphenol intakes were less likely to be premenopausal than those with lower intakes. In all quintiles of total polyphenol intake, phenolic acids were the main contributors, followed by flavonoids, whereas lignans and stilbenes were consumed in low amounts (between 1 and 3 mg/d).

On average, study participants gained 2.6 kg (±5.0 kg) per 5 years of body weight during the follow-up. Body weight changes over 5 years by quintile of total, classes, and subclasses of polyphenol intakes are shown in Table 2 and Supporting Information Table S1. Total polyphenol intakes were positively associated with body weight gain; participants in the highest intake quintile had a 0.205-kg (95% confidence interval [CI]: 0.138 to 0.272) greater 5-year weight gain compared with those in the lowest quintile after multivariable adjustments (model 4; Table 2). Analysis by polyphenol classes showed that higher intakes of flavonoids (β_{Q5} vs. $_{Q1}$ −0.195 kg/5 years, 95% CI: −0.262 to −0.128) and stilbenes (only when modeled continuously, β_{log2} −0.032 kg/5 years, 95% CI: −0.039 to −0.024) were inversely associated with body weight change. Similarly, intakes of individual subclasses of flavonoids, except for isoflavonoids, were statistically, significantly, and inversely associated with body weight change (Table 2). A body weight gain was observed comparing participants in the extreme quintiles of total phenolic acid intake (β_{Q5} vs. $_{Q1}$ 0.328 kg/5 years, 95% CI: 0.268 to 0.386) and its subclass HCAs (β_{Q5} vs. $_{Q1}$ 0.324 kg/5 years, 95% CI: 0.267 to 0.381). However, other subclasses of phenolic acids showed an inverse association with body weight change, such as hydroxybenzoic acid (β_{Q5} vs. $_{Q1}$= −0.244 kg/5 years, 95% CI: −0.317 to −0.170) and hydroxyphenylacetic acid (β_{Q5} vs. $_{Q1}$= −0.204 kg/5 years, 95% CI: −0.275 to −0.132). Minor polyphenol classes such as tyrosols and hydroxycoumarins showed an inverse association with body weight change. Analyses by quintiles of classes, subclasses, and total polyphenol intake were supported by the results using the continuous variable after the $\log_2$ transformation (Figure 1).

Women presented a slightly stronger positive association between total polyphenol intake and body weight gain compared with men (p value interaction < 0.001; Supporting Information Table S2). Total polyphenol and phenolic acid intakes were strongly associated with body weight gain among women in perimenopause (Supporting Information Table S3). For smoking status at follow-up, we found that total polyphenol and phenolic acid class intakes were more strongly associated with weight gain in former smokers, whereas the opposite occurred with total flavonoid intake (Supporting Information Table S4). For BMI, there was an inverse trend between total flavonoid intake and body weight change in participants with underweight, normal weight, and overweight, but a positive trend was detected among participants with obesity (Supporting Information Table S5).

Additional analyses for HCAs were performed by dividing the analysis by coffee consumption, in which we observed a positive association with weight gain in coffee consumers, but not in coffee nonconsumers (Supporting Information Table S6). Subsequently, we separately analyzed the association for total coffee and type of coffee

TABLE 1 Baseline characteristics of the population according to quintiles of total polyphenol intake in the EPIC-PANACEA study ($n = 349,165$)

	Quintile of intake				
Category	**Quintile 1 ($n = 69,832$)**	**Quintile 2 ($n = 69,826$)**	**Quintile 3 ($n = 69,826$)**	**Quintile 4 ($n = 69,841$)**	**Quintile 5 ($n = 69,840$)**
Total polyphenol intake (mg/d)	558.2 (441.2-706.0)	854.8 (788.8-970.7)	1117.5 (1049.4-1248.4)	1427 (1342-1607.3)	1923.8 (1759.7-2856.3)
Follow-up time (y)	5.1 ± 2.4	5.5 ± 2.7	5.3 ± 2.4	4.9 ± 2.0	4.6 ± 1.5
Weight change (kg/5 years)[a]	2.4 ± 5.2	2.5 ± 4.9	2.6 ± 5.0	2.7 ± 4.9	2.8 ± 5.0
Age (y)	50.4 ± 8.9	50.8 ± 9.2	51.7 ± 9.3	52.6 ± 9.2	52.9 ± 8.6
BMI (kg/m^2)	25.6 ± 4.4	25.1 ± 4.1	25.0 ± 4.0	24.8 ± 3.9	24.7 ± 3.8
Alcohol use (g/d)	6.4 ± 12.2	9.5 ± 13.7	12.3 ± 15.6	14.3 ± 17.6	16.7 ± 20.8
Energy intake (kcal/d)	1777 ± 517	1964 ± 544	2092 ± 567	2182 ± 592	2366 ± 634
Women (%)	79.4	74.1	72.0	70.9	69.7
Education level (%)					
None	11.6	4.5	2.4	1.3	0.8
Primary school	28.4	26.9	22.8	19.6	18.3
Technical school	20.2	23.0	23.0	23.2	22.1
Secondary school	20.7	22.1	22.1	21.9	21.9
Higher education	17.5	26.2	26.2	28.3	31.0
Missing	1.4	3.2	3.2	5.4	5.7
Physical activity level (%)					
Inactive	23.6	19.3	17.3	16.8	16.0
Moderately inactive	32.6	35.0	34.9	34.6	33.6
Moderately active	29.5	27.7	26.1	26.1	26.9
Active	12.5	16.2	19.3	20.4	22.3
Missing	1.5	1.6	1.8	1.8	1.0
Smoking at follow-up (%)					
Never	54.1	47.8	47.6	47.8	44.2
Former	25.5	28.4	30.1	32.2	34.3
Current	15.7	17.2	16.8	16.7	20.0
Missing	4.5	6.4	5.3	3.1	1.3
Prevalent diseases at baseline (%)[b]					
No	85.6	86.7	85.3	81	78.7
Yes	8.0	7.5	7.7	7.5	7.2
Missing	6.3	5.6	6.9	11.3	14.0
Menopause (%)					
Premenopausal	29.1	26.3	23.1	21.0	19.9
Postmenopausal	30.6	30.1	32.4	33.8	32.9
Perimenopausal	17.4	15.6	14.1	13.5	14.5
Surgery	2.1	2.0	2.3	2.4	2.3
Classes of polyphenol intake (mg/d)					
Phenolic acids	249.7 (142.4-500.7)	462.0 (328.0-704.5)	585.6 (430.3-917.6)	708.0 (525.7-1215.6)	1025.7 (675.9-1918.3)
Flavonoids	224.8 (150.6-449.6)	340.7 (234.7-649.6)	468.2 (325.5-856.3)	647.8 (424.7-1132.2)	896.6 (548.0-1633.7)
Other polyphenol classes	32.2 (19.9-72.0)	40.1 (26.5-93.5)	45 (28.3-106.4)	48.8 (29.5-117.5)	57 (34.8-134.3)

(Continues)

TABLE 1 (Continued)

Category	Quintile of intake				
	Quintile 1 ($n = 69,832$)	Quintile 2 ($n = 69,826$)	Quintile 3 ($n = 69,826$)	Quintile 4 ($n = 69,841$)	Quintile 5 ($n = 69,840$)
Lignans	1.2 (0.9-3.1)	1.3 (1-4.6.0)	1.4 (1.1-4.5)	1.5 (1.2-3.8)	1.7 (1.4-3.7)
Stilbenes	0.2 (0.05-3.5)	0.4 (0.1-6.5)	0.7 (0.2-8.4)	1.1 (0.2-9.9)	1.4 (0.3-11.2)

Notes: Means ± SD are presented for continuous variables, and percentages are presented for categorical variables. Medians and percentiles (25th to 95th percentile) are presented for polyphenol intake.

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; PANACEA, Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating Out of Home and Obesity.

[a]Calculated as weight at follow-up minus weight at baseline divided by the follow-up time in years and multiplied by 5 years.

[b]Type 2 diabetes, cardiovascular disease, and cancer.

(caffeinated vs. decaffeinated) as exposure variables with body weight changes. Decaffeinated coffee intake was associated with a slightly greater body weight gain than caffeinated coffee (when modeled continuously; Supporting Information Table S6).

In the sensitivity analysis, after excluding participants with chronic disease at baseline ($n = 57,617$) and participants who quit smoking or started smoking during follow-up or with missing values ($n = 35,489$), we observed that the associations among total polyphenol intake and polyphenol classes and body weight change were similar to our main results (Supporting Information Tables S7 and S8).

DISCUSSION

In this large prospective study, a mean body weight gain of 2.6 kg during the 5 years of follow-up was observed. Progressive age-related weight gain in adulthood is a well-observed phenomenon in many nonobese populations such as in the Nurses' Health Study II cohort, which showed a weight change of 4.4 lb (2.0 kg) per 4.4 years of follow-up [27], and in NHANES, the weight change of which was 2.5 kg per 9.8 years of follow-up [28]. In the current study, a positive association among total polyphenol and phenolic acid intakes and body weight gain was observed. Conversely, higher intakes of flavonoids, including anthocyanins, dihydrochalcones, and dihydroflavonols, and other minor polyphenol classes such as tyrosols and stilbenes were inversely associated with body weight change, supporting the evidence from experimental studies that some polyphenol classes and subclasses may play a role in obesity prevention; several mechanisms have been proposed, such as activation of β oxidation processes, stimulation of energy expenditure, and inhibition of adipocyte differentiation [12]. Recently, it has been discussed that polyphenols may modulate type 2 taste receptors responsible for bitter taste recognition, which may play a role in energy/body weight homeostasis [29]. Similar to our findings, a cohort study by Adriouch and colleagues reported that high intakes of flavanones, flavones, and lignans were associated with lower waist circumference and lower BMI. In their study, total polyphenol and phenolic acid intakes were associated with both a lower body weight gain and a lower increase in adiposity over 6 years [15]. Our study showed that total polyphenols were positively related to body weight gain; however, after excluding phenolic acids, we observed an inverse association with body weight change. Such changes were driven by phenolic acids (specifically HCAs), the main contributors to total polyphenols; therefore, the results with total polyphenols need to be interpreted with caution.

Flavonoids are the most-studied polyphenol class in relation to their effects on body weight [13]. In our study, we observed that a higher intake of flavonoids, particularly anthocyanins, flavan-3-ol monomers, theaflavins, flavones, and flavonols, was strongly and inversely associated with body weight change over 5 years. Similarly, in a large prospective cohort, statistically significant inverse associations among subclasses of anthocyanins, flavanols (including proanthocyanidins), and flavonols and body weight change were observed after a 24-year follow-up [27]. Another cohort from the Netherlands observed an association among a higher intake of flavonol/flavone and catechin and a lower increase in BMI in women, but not in men [30]. Similarly, the Mediterranean healthy Eating, Aging and Lifestyle (MEAL) cohort study, with a follow-up over 14 years, reported that women with a high intake of total flavonoids were less likely to have obesity and that flavonol intake was inversely associated with obesity [31]. Although the magnitudes of body weight loss were small, they may contribute to body weight maintenance, which has been reported as a protective factor for diseases such as type 2 diabetes, hypertension, and cardiovascular disease [32]. Some trials have also investigated these body weight effects showing, in general, a body weight reduction after the intervention with supplements rich in polyphenols [11, 14].

Contrary to our expectations, this study found an inverse association among the minor subclasses of phenolic acids (i.e., hydroxybenzoic acids and hydroxyphenylacetic acids) and body weight change, whereas total phenolic acid and HCA intakes were associated with an increase in body weight. Their main food source is coffee [10] and, because other compounds of coffee such as caffeine, trigonelline, and magnesium may possess antiobesogenic properties [33], after we stratified by coffee consumption, non-coffee consumers showed a null association between HCA intake and body weight change, whereas, in coffee consumers, phenolic acid intake

TABLE 2 Associations between intakes of total polyphenols, polyphenol classes, and subclasses (milligrams per day) and body weight change over 5 years in the EPIC-PANACEA study ($n = 349,165$)

	Quintile of intake					
Polyphenol (mg/d)	**Quintile 1, β (95% CI)**	**Quintile 2, β (95% CI)**	**Quintile 3, β (95% CI)**	**Quintile 4, β (95% CI)**	**Quintile 5, β (95% CI)**	***p* value for trend**
Total polyphenols	<720.1	720.1-983.5	983.6-1263.1	1263.2-1630.5	>1630.5	
Model 4	0 (ref)	0.009 (−0.044 to 0.063)	0.011 (−0.046 to 0.069)	0.095 (0.034 to 0.157)	0.205 (0.138 to 0.272)	<0.001
Flavonoids	<231.6	231.6-359.4	359.5-525.3	525.4-786.7	>786.7	
Model 4	0 (ref)	−0.069 (−0.122 to −0.016)	−0.136 (−0.194 to −0.079)	−0.183 (−0.244 to −0.122)	−0.195 (−0.262 to −0.128)	<0.001
Total flavanols	<142.8	142.8-238.4	238.5-369.7	369.8-595.7	>595.7	
Model 4	0 (ref)	−0.023 (−0.076 to 0.03)	−0.072 (−0.128 to −0.015)	−0.100 (−0.16 to −0.041)	−0.141 (−0.207 to −0.075)	<0.001
Flavan-3-ol monomers	<16.0	16.0-30.6	30.7-69.7	69.8-246.9	>246.9	
Model 4	0 (ref)	−0.044 (−0.098 to 0.009)	−0.069 (−0.127 to −0.01)	−0.114 (−0.175 to −0.053)	−0.200 (−0.265 to −0.134)	<0.001
Proanthocyanidins	<113.5	113.5-175.7	175.8-243.2	243.3-345.9	>345.9	
Model 4	0 (ref)	−0.060 (−0.112 to −0.007)	−0.086 (−0.141 to −0.030)	−0.124 (−0.183 to −0.065)	−0.071 (−0.136 to −0.005)	0.081
Theaflavins	0	<3.5	3.5-24.8	24.9-59.1	>59.1	
Model 4	0 (ref)	−0.011 (−0.071 to 0.050)	−0.068 (−0.125 to −0.011)	−0.197 (−0.261 to −0.134)	−0.170 (−0.233 to −0.106)	<0.001
Flavonols	<14.3	14.3-23.6	23.7-36.3	36.4- 63.5	>63.5	
Model 4	0 (ref)	−0.070 (−0.124 to −0.015)	−0.107 (−0.166 to −0.047)	−0.118 (−0.181 to −0.055)	−0.178 (−0.246 to −0.109)	<0.001
Anthocyanins	<10.8	10.8-20.2	20.3-35.7	35.8-64.7	>64.7	
Model 4	0 (ref)	−0.087 (−0.139 to −0.036)	−0.104 (−0.158 to −0.051)	−0.159 (−0.216 to −0.102)	−0.148 (−0.214 to −0.082)	<0.001
Flavanones	<7.7	7.7-17.0	17.1-32.0	32.1-64.7	>64.7	
Model 4	0 (ref)	−0.079 (−0.131 to −0.028)	−0.065 (−0.118 to −0.012)	−0.167 (−0.223 to −0.111)	−0.270 (−0.336 to −0.204)	<0.001
Flavones	<4.9	4.9-7.5	7.6-10.5	10.6-15.3	>15.3	
Model 4	0 (ref)	−0.125 (−0.177 to −0.073)	−0.143 (−0.198 to −0.088)	−0.200 (−0.258 to −0.141)	−0.201 (−0.269 to −0.133)	<0.001
Dihydrochalcones	0	<0.8	0.8-1.8	1.9-3.3	>3.3	
Model 4	0 (ref)	−0.048 (−0.182 to 0.086)	−0.085 (−0.133 to −0.037)	−0.124 (−0.172 to −0.075)	−0.187 (−0.240 to −0.134)	<0.001
Dihydroflavonols	0	<0.16	0.16-1.0	1.1-3.4	>3.4	
Model 4	0 (ref)	0.050 (−0.018 to 0.119)	−0.025 (−0.092 to 0.043)	−0.142 (−0.211 to −0.072)	−0.258 (−0.335 to −0.181)	<0.001
Isoflavonoids	0	>0-0.01	>0.01-0.03	0.04-0.10	>0.10	
Model 4	0 (ref)	−0.019 (−0.115 to 0.078)	−0.038 (−0.134 to 0.057)	0.001 (−0.095 to 0.097)	0.058 (−0.044 to 0.160)	0.004
Total phenolic acids	<298.3	298.3-458.8	458.9-618.9	619.1-881.2	>881.2	
Model 4	0 (ref)	−0.048 (−0.100 to 0.004)	0.006 (−0.047 to 0.059)	0.102 (0.047 to 0.156)	0.328 (0.268 to 0.386)	<0.001
Hydroxycinnamic	<240.6	240.6-416.2	416.3-570.6	570.7-838.2	>838.2	
Model 4	0 (ref)	−0.063 (−0.115 to −0.012)	−0.015 (−0.067 to 0.038)	0.094 (0.04 to 0.147)	0.324 (0.267 to 0.381)	<0.001

(Continues)

TABLE 2 (Continued)

Polyphenol (mg/d)	Quintile of intake					
	Quintile 1, β (95% CI)	Quintile 2, β (95% CI)	Quintile 3, β (95% CI)	Quintile 4, β (95% CI)	Quintile 5, β (95% CI)	*p* value for trend
Hydroxybenzoic	<5.4	5.4-14.9	15.0-33.0	33.1-83.5	>83.5	
Model 4	0 (ref)	−0.083 (−0.141 to −0.025)	−0.104 (−0.171 to −0.037)	−0.147 (−0.218 to −0.077)	−0.244 (−0.317 to −0.170)	<0.001
Hydroxyphenyl acetic	<0.01	0.01-0.05	0.06-0.14	0.15-0.32	>0.32	
Model 4	0 (ref)	−0.037 (−0.088 to 0.014)	−0.176 (−0.228 to −0.124)	−0.219 (−0.274 to −0.163)	−0.204 (−0.275 to −0.132)	<0.001
Lignans	<1.0	1.0-1.2	1.3-1.6	1.7-2.1	>2.1	
Model 4	0 (ref)	−0.0003 (−0.054 to 0.053)	−0.070 (−0.128 to −0.012)	−0.010 (−0.077 to 0.056)	0.093 (0.013 to 0.174)	<0.001
Stilbenes	<0.09	0.09-0.33	0.34-1.1	1.2-2.6	>2.6	
Model 4	0 (ref)	−0.082 (−0.495 to 0.331)	−0.120 (−0.533 to 0.294)	−0.238 (−0.652 to 0.175)	−0.373 (−0.788 to 0.042)	<0.001
Other polyphenol classes	<23.6	23.6-36.3	36.4-50.8	50.9-70.9	>70.9	
Model 4	0 (ref)	0.053 (0.0004 to 0.106)	−0.003 (−0.059 to 0.053)	−0.074 (−0.134 to −0.013)	−0.073 (−0.145 to −0.002)	0.002
Alkylphenols	<5.3	5.3-19.7	19.8-35.2	>35.3-54.7	>54.7	
Model 4	0 (ref)	0.095 (0.037 to 0.152)	−0.005 (−0.071 to 0.060)	−0.112 (−0.183 to −0.041)	−0.167 (−0.247 to −0.086)	<0.001
Hydroxycoumarins	0	>0-0.01	>0.01-0.57	0.58-0.18	>0.18	
Model 4	0 (ref)	−0.052 (−0.134 to 0.030)	−0.083 (−0.172 to 0.006)	−0.214 (−0.303 to −0.124)	−0.270 (−0.367 to −0.174)	<0.001
Tyrosol	<1.1	1.1-2.5	2.6-5.1	5.2-11.4	>11.4	
Model 4	0 (ref)	−0.126 (−0.178 to −0.075)	−0.222 (−0.276 to −0.168)	−0.331 (−0.393 to −0.268)	−0.343 (−0.422 to −0.264)	<0.001
Alkymethoxyphenols	<0.9	0.9-1.9	2.0-2.8	2.9-4.2	>4.2	
Model 4	0 (ref)	−0.146 (−0.356 to 0.064)	−0.201 (−0.412 to 0.011)	−0.071 (−0.284 to 0.141)	0.135 (−0.079 to 0.348)	<0.001

Note: Overall mean 5-year weight gain corresponded to 2.6 kg (SD 5.0), and negative β values indicate less weight gain (kilograms) over the same period.

Model 4: generalized linear mixed models with random effect on the intercept and slope according to center and adjusted for age, sex, and BMI at baseline (three-knot restricted cubic spline), follow-up time in years (three-knot restricted cubic spline), educational level, smoking status, physical activity, alcohol consumption, menopausal status, energy intake, plausibility of dietary energy reporting, vitamin C, and total fiber intakes.

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; PANACEA, Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating Out of Home and Obesity; ref, reference.

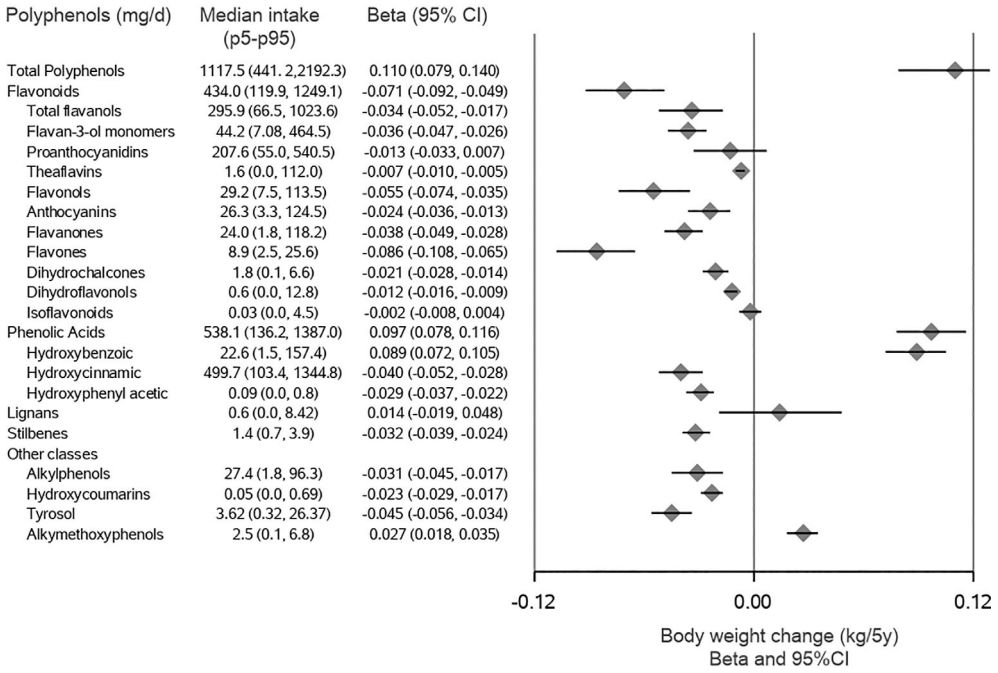


FIGURE 1 The association between intakes of total polyphenols, polyphenol classes, and subclasses (milligrams per day) as continuous variables (after $\log_2$ transformation) and body weight change (kilograms) over 5 years in the EPIC-PANACEA study. Model 4: generalized linear mixed models with random effect on the intercept and slope according to center and adjusted for age, sex, and BMI at baseline (three-knot restricted cubic spline), follow-up time in years (three-knot restricted cubic spline), educational level, smoking status, physical activity, alcohol consumption, menopausal status, total energy intake, plausibility of dietary energy reporting, vitamin C, and total fiber intakes

was positively associated with body weight gain. Some *in vivo* studies have suggested potential mechanisms of HCAs from coffee in weight loss through regulation of lipid and glucose metabolism, e.g., via sterol regulatory element binding transcription factor 1 (SREBP-1C), peroxisome proliferator activated receptor α (PPAR-α), increased fatty acid oxidation, and increased insulin secretion; however, there is not information reported about possible mechanisms of HCAs associated with body weight gain [34]. The direct role of coffee consumption on body composition has been studied in previous cohorts; however, the results are still inconclusive. For instance, two previous cohorts have concluded that higher coffee consumption was associated with lower weight gain, BMI, and waist circumference [26, 35], whereas consumption of more than six cups of coffee per day was associated with higher BMI compared with the group consuming less than two cups per day among Swedish women [35]. Similarly, other studies conducted in Asia have reported that higher coffee consumption (>3 cups/d) was associated with higher risk of obesity compared with lower coffee consumption [36, 37]. However, these results need to be taken with caution, as they considered instant coffee blends that contain sugar and cream. In this sense, the method of coffee preparation, the types of coffee varieties, roasting degree, the size of the serving, and use of milk or cream and sugar added to coffee are factors that may influence the coffee-obesity relationship [38]. For example, the types of coffee have been evaluated (caffeinated vs. decaffeinated), and it was found that decaffeinated coffee was associated with higher body weight gain compared with caffeinated coffee [39]. In our study, we observed an increase in body weight for both types of coffee; however, it is important to highlight that the consumption of caffeinated coffee was much higher (mean = 222.1 mL/d) compared with decaffeinated coffee (mean = 32.3 mL/d). Therefore, phenolic acids and caffeine seem to not have harmful effects on body weight, but it is unclear which potential coffee compounds, if any, might have an obesogenic effect.

The current study found an inverse association of stilbenes with body weight change, which is consistent with that reported in the HAPIEE study for change in waist circumference [18]. However, their results were not statistically significant. Furthermore, it has been previously observed that consumption of stilbenes from berries, red grapes, and wine has antiadipogenic and antilipogenic effects, improving changes in BMI and waist circumference [12, 40]. Future studies on the current findings are still warranted.

Our results also showed that tyrosols are inversely associated with body weight change. The main food sources of tyrosols include olives and olive oil, of which the principal component is hydroxytyrosol [10]. It has been largely studied for its protective effects on low-density lipoprotein oxidation and reduction of oxidative stress [41], and it may be responsible for the antiobesogenic properties of olive oil [41]. Moreover, extra-virgin olive oil is the main source of dressing and cooking fats in the Mediterranean diet, which has been reported to have a protective effect against weight gain, particularly in younger people [42]. The PREDIMED study evaluated the long-term effects of an unrestricted-calorie Mediterranean diet rich in extra-virgin olive oil

on adiposity measures and observed a small reduction in body weight in participants given the Mediterranean diet interventions compared with the control groups [23]. However, more clinical and epidemiological evidence is needed in order to clarify the effect of tyrosols on body weight.

Results of the interaction analyses should be interpreted with caution because even very small body weight differences among subgroups are statistically significant because of our large sample size. We observed that women with perimenopause had a slightly greater weight gain associated with total polyphenol intake. Several polyphenols have estrogenic effects [12], and they would help to prevent body weight gain related to menopause. However, our results pointed out the opposite, probably because of reverse causality. Women with perimenopause might improve their diets and lifestyles [43], but, when they become menopausal, they tend to return to their regular habits [43]. Moreover, analyses by smoking status at follow-up showed that current smokers benefitted more from higher intakes of total polyphenols and phenolic acids, probably because of their ability to reduce tobacco oxidative stress [44]. Finally, interactions by BMI showed that flavonoid intakes were associated with a lower body weight in all groups, except those with obesity. Our hypothesis was that participants with obesity would benefit more from the intake of potential antiobesity compounds [12]. However, our contradictory results could be because participants with obesity tend to underestimate unhealthy foods and overestimate healthy foods more than those without obesity [45].

Strengths of our study include the multicenter prospective design, two measurements of body weight (to calculate body weight change), and a large sample size, which provided sufficient statistical power to perform multiple subgroup and sensitivity analyses. Another strength is the use of Phenol-Explorer, the most comprehensive database on polyphenol content in foods, to measure polyphenol intake. However, our study also had some limitations. First, weight was self-reported at follow-up in most centers; however, we improved the accuracy of these data by using a prediction equation, and the results in the two EPIC centers with measured weight (Cambridge and Doetinchem) were consistent with the rest of the cohort [21]. Second, the use of both self-report diet and lifestyle questionnaires with a single measurement at baseline did not allow us to consider dietary or lifestyle (except tobacco consumption) changes during follow-up. Third, participants diagnosed with severe diseases during follow-up might have changed their dietary and lifestyle habits. However, we performed sensitivity analyses excluding participants with preexisting conditions, and the results remained robust to those of the entire cohort. Fourth, the information regarding the method of preparation and type of coffee was limited, and the quantification of HCAs in coffee was probably underestimated [10]. Fifth, breastfeeding, a part of pregnancy, can also interfere in the standard body weight trajectory. Pregnant women were excluded from our analysis; therefore, most of the breastfeeding women were consequently excluded, except those lactating only at baseline. We assume that this number is very low because the mean average was 50 years old, and, in some centers (such as Spain and Italy), participants were mostly blood donors, and it is not possible to do a blood donation until 6 to 9 months after giving birth. For this reason, we recommend complementing the results from dietary questionnaires with nutritional biomarkers in future studies. Although validated center- and country-specific questionnaires were used to collect polyphenol-rich food data [19], we cannot exclude some measurement error leading to a potential underestimation of any true association. Finally, all models were adjusted for potential confounders; however, some potential residual bias cannot be ruled out.

In conclusion, this study identified a small inverse association between flavonoid intake and body weight change, specifically for anthocyanin, flavan-3-ol monomer, flavanone, flavone, and flavonol subclasses. These results suggest that flavonoids from foods may be promising compounds for weight control. Future randomized controlled trials using combinations of polyphenols or plant extracts mimicking polyphenol-rich diets may provide more definitive evidence to validate these results. In addition, HCAs from coffee showed a positive association with weight gain in coffee consumers. Future research related to coffee constituents, including HCAs, and weight change is warranted.O

AFFILIATIONS

[1]Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet del Llobregat, Spain
[2]Department of Epidemiology, Regional Health Council, IMIB-Arrixaca, Murcia, Spain
[3]CIBER in Epidemiology and Public Health (CIBERESP), Madrid, Spain
[4]Unit of Diet, Genes and Environment, Danish Cancer Society Research Center, Copenhagen, Denmark
[5]Institute for Nutrition Research, School of Medical and Health Sciences, Edith Cowan University, Perth, Western Australia, Australia
[6]Department of Public Health, Section of Environmental Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[7]Department of Public Health, Aarhus University, Aarhus, Denmark
[8]Department of Cancer Epidemiology, German Cancer research Center (DKFZ), Heidelberg, Germany
[9]Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[10]Institute of Nutritional Science, University of Potsdam, Potsdam, Germany
[11]Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for Cancer Research Prevention and Clinical Network (ISPRO), Florence, Italy
[12]Epidemiology and Prevention Unit, Department of Research, IRCCS National Cancer Institute Foundation, Milan, Italy
[13]Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy
[14]Hyblean Association for Epidemiological Research (AIRE-ONLUS), Ragusa, Italy
[15]Unit of Cancer Epidemiology, Città della Salute e della Scienza University-Hospital, Turin, Italy

[16]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway
[17]The Public Dental Health Service Competence Center of Northern Norway, Tromsø, Norway
[18]Department of Functional Biology, University of Oviedo, Oviedo, Spain
[19]Department of Nutrition and Food Science, Campus of Cartuja, University of Granada, Granada, Spain
[20]Biosanitary Research Institute of Granada - ibs.Granada, Granada, Spain
[21]Institute of Nutrition and Food Technology (INYTA) 'José Mataix', Biomedical Research Centre, University of Granada, Granada, Spain
[22]Navarra Public Health Institute, Pamplona, Spain
[23]Navarra Institute for Health Research (IdiSNA), Pamplona, Spain
[24]Nutritional Epidemiology, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden
[25]Register-based Epidemiology, Department of Clinical Sciences Lund, Lund University, Lund, Sweden
[26]Department of Odontology, Umeå University, Umeå, Sweden
[27]Department of Medical Biosciences, Umeå University, Umeå, Sweden
[28]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
[29]MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge, UK
[30]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK
[31]International Agency for Research on Cancer Nutrition (IARC-WHO), Lyon, France
[32]Department of Nutrition, Food Sciences, and Gastronomy, Food Innovation Network (XIA), Institute for Research on Nutrition and Food Safety (INSA), Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain

ACKNOWLEDGMENTS

We thank all EPIC participants and staff for their contribution to the study. We also thank Bertrand Hémon and Catalina Bonet for their valuable help with the EPIC database. We also thank the National Institute for Public Health and the Environment (RIVM; Bilthoven, the Netherlands); the University of Paris-Saclay, the University of Versailles Saint-Quentin-en-Yvelines (UVSQ), National Institute of Health and Medical Research (INSERM), Gustave Roussy Institute, and the Center for Research in Epidemiology and Population Health (CESP; Villejuif, France); the Ministry of Health of the Basque Government, Sub-Directorate for Public Health and Addictions of Gipuzkoa, the Public Health Division of Gipuzkoa, and BioDonostia Research Institute (San Sebastian, Spain); and Oxford University (UK) for their contribution and ongoing support to the EPIC Study.

FUNDING INFORMATION

This study has been funded by Carlos III Health Institute through the project PI18/00191 (co-funded by European Regional Development Fund [ERDF], a way to build Europe). The Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating Out of Home and Obesity (PANACEA) project was funded by the European Union in the framework of the Public Health Programme (project number: 2005328). The coordination of European Prospective Investigation into Cancer and Nutrition (EPIC) is financially supported by International Agency for Research on Cancer (IARC) and also by the Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, which has additional infrastructure support provided by the National Institute for Health and Care Research (NIHR) Imperial Biomedical Research Centre (BRC). The national cohorts are supported by the following: Danish Cancer Society (Denmark); League Against Cancer, Gustave Roussy Institute, General Mutual of National Education, and the National Institute of Health and Medical Research (INSERM; France); German Cancer Aid, German Cancer Research Center (DKFZ), German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), and the Federal Ministry of Education and Research (BMBF) (Germany); Italian Association for Cancer Research (AIRC), Company of Saint Paul, and the National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), and Statistics Netherlands (the Netherlands); Health Research Fund (FIS), Carlos III Institute of Health (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia, and Navarra, and the Catalan Institute of Oncology (ICO; Spain); Swedish Cancer Society, Swedish Research Council, and the County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C8221/A29017 to EPIC-Oxford) and Medical Research Council (MRC; 1000143 to EPIC-Norfolk; MR/M012190/1 to EPIC-Oxford; the UK). Nita G. Forouhi acknowledges support from MRC Epidemiology Unit (MC_UU_0006/3) and from the NIHR Cambridge Biomedical Research Centre diet, nutrition, and lifestyle theme (IS-BRC-1215-20014). She is an NIHR Senior Investigator. The authors thank the Research Centers of Catalonia (CERCA) Program/Government of Catalonia for the institutional support to Bellvitge Biomedical Research Institute (IDIBELL). Jazmin Castañeda thanks the National Council for Science and Technology (CONACYT) predoctoral fellowship (identifier 693636). Mercedes Gil-Lespinard, Enrique Almanza-Aguilera, and Raul Zamora-Ros were supported by the predoctoral contracts for training in research into health (PFIS) (FI19/00185), the "Sara Borrell" (CD20/00071), and the "Miguel Servet II" (CPII20/00009) programs from the Carlos III Institute of Health (cofunded by European Social Fund [ESF], investing in your future).

CONFLICT OF INTEREST

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

For information on how to apply for getting access to EPIC data and/or biospecimens, please follow the instructions at the following link: http://epic.iarc.fr/access/index.php.

DISCLAIMER

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article, and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization.

ORCID

Jazmin Castañeda https://orcid.org/0000-0002-8521-9946
Mercedes Gil-Lespinard https://orcid.org/0000-0002-7387-2139
Enrique Almanza-Aguilera https://orcid.org/0000-0002-4805-0774
Fjorida Llaha https://orcid.org/0000-0003-0534-6484
Jesús-Humberto Gómez https://orcid.org/0000-0001-8442-8327
Nicola Bondonno https://orcid.org/0000-0001-5905-444X
Anne Tjønneland https://orcid.org/0000-0003-4385-2097
Kim Overvad https://orcid.org/0000-0001-6429-7921
Verena Katzke https://orcid.org/0000-0002-6509-6555
Matthias B. Schulze https://orcid.org/0000-0002-0830-5277
Giovanna Masala https://orcid.org/0000-0002-5758-9069
Claudia Agnoli https://orcid.org/0000-0003-4472-1179
Rosario Tumino https://orcid.org/0000-0003-2666-414X
Carlotta Sacerdote https://orcid.org/0000-0002-8008-5096
Guri Skeie https://orcid.org/0000-0003-2476-4251
Magritt Brustad https://orcid.org/0000-0003-0114-5271
Cristina Lasheras https://orcid.org/0000-0003-0482-4229
Esther Molina-Montes https://orcid.org/0000-0002-0428-2426
María-Dolores Chirlaque https://orcid.org/0000-0001-9242-3040
Aurelio Barricarte https://orcid.org/0000-0001-6750-1270
Emily Sonestedt https://orcid.org/0000-0002-0747-4562
Marisa da Silva https://orcid.org/0000-0003-1215-8625
Ingegerd Johansson https://orcid.org/0000-0002-9227-8434
Johan Hultdin https://orcid.org/0000-0002-9599-0961
Nita G. Forouhi https://orcid.org/0000-0002-5041-248X
Alicia K. Heath https://orcid.org/0000-0001-6517-1300
Heinz Freisling https://orcid.org/0000-0001-8648-4998
Elisabete Weiderpass https://orcid.org/0000-0003-2237-0128
Augustin Scalbert https://orcid.org/0000-0001-6651-6710
Raul Zamora-Ros https://orcid.org/0000-0002-6236-6804

REFERENCES

1. Dai H, Alsalhe TA, Chalghaf N, Riccò M, Bragazzi NL, Wu J. The global burden of disease attributable to high body mass index in 195 countries and territories, 1990–2017: an analysis of the Global Burden of Disease Study. *PLoS Med*. 2020;17:e1003198. doi:10.1371/journal.pmed.1003198
2. World Obesity. Prevalence of obesity. Accessed July 15, 2022. https://www.worldobesity.org/about/about-obesity/prevalence-of-obesity.
3. World Health Organization. *WHO European Regional Obesity Report*. WHO Regional Office for Europe; 2022.
4. Albuquerque D, Nóbrega C, Manco L, Padez C. The contribution of genetics and environment to obesity. *Br Med Bull*. 2017;123:159-173.
5. Semlitsch T, Stigler FL, Jeitler K, Horvath K, Siebenhofer A. Management of overweight and obesity in primary care—a systematic overview of international evidence-based guidelines. *Obes Rev*. 2019;20:1218-1230.
6. Wilding JPH, Jacob S. Cardiovascular outcome trials in obesity: a review. *Obes Rev*. 2021;22:e13112. doi:10.1111/obr.13112
7. Fantin F, Giani A, Zoico E, Rossi AP, Mazzali G, Zamboni M. Weight loss and hypertension in obese subjects. *Nutrients*. 2019;11:1667. doi:10.3390/nu11071667
8. Pedersen LR, Olsen RH, Anholm C, et al. Weight loss is superior to exercise in improving the atherogenic lipid profile in a sedentary, overweight population with stable coronary artery disease: a randomized trial. *Atherosclerosis*. 2016;246:221-228.
9. Ma C, Avenell A, Bolland M, et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. *BMJ*. 2017;359:j4849. doi:10.1136/bmj.j4849
10. Zamora-Ros R, Knaze V, Rothwell JA, et al. Dietary polyphenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Eur J Nutr*. 2016;55:1359-1375.
11. Farhat G, Drummond S, Al-Dujaili EAS. Polyphenols and their role in obesity management: a systematic review of randomized clinical trials. *Phytother Res*. 2017;31:1005-1018.
12. Boccellino M, D'Angelo S. Anti-obesity effects of polyphenol intake: current status and future possibilities. *Int J Mol Sci*. 2020;21:5642. doi:10.3390/ijms21165642
13. Most J, Goossens GH, Jocken JW, Blaak EE. Short-term supplementation with a specific combination of dietary polyphenols increases energy expenditure and alters substrate metabolism in overweight subjects. *Int J Obes (Lond)*. 2014;38:698-706.
14. Llaha F, Zamora-Ros R. The effects of polyphenol supplementation in addition to calorie restricted diets and/or physical activity on body composition parameters: a systematic review of randomized trials. *Front Nutr*. 2020;7:84. doi:10.3389/fnut.2020.00084
15. Adriouch S, Kesse-Guyot E, Feuillet T, et al. Total and specific dietary polyphenol intakes and 6-year anthropometric changes in a middle-aged general population cohort. *Int J Obes (Lond)*. 2018;42:310-317.
16. Vernarelli JA, Lambert JD. Flavonoid intake is inversely associated with obesity and C-reactive protein, a marker for inflammation, in US adults. *Nutr Diabetes*. 2017;7:e276. doi:10.1038/nutd.2017.22
17. Guo X, Tresserra-Rimbau A, Estruch R, et al. Polyphenol levels are inversely correlated with body weight and obesity in an elderly population after 5 years of follow up (the randomised PREDIMED study). *Nutrients*. 2017;9:452. doi:10.3390/nu9050452
18. Grosso G, Stepaniak U, Micek A, Stefler D, Bobak M, Pająk A. Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study. *Eur J Nutr*. 2017;56:1409-1420.
19. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 2002;5:1113-1124.
20. May AM, Romaguera D, Travier N, et al. Combined impact of lifestyle factors on prospective change in body weight and waist circumference in participants of the EPIC-PANACEA study. *PLoS One*. 2012;7:e50712. doi:10.1371/journal.pone.0050712
21. Spencer EA, Appleby PN, Davey GK, Key TJ. Validity of self-reported height and weight in 4808 EPIC–Oxford participants. *Public Health Nutr*. 2002;5:561-565.
22. Slimani N, Deharveng G, Unwin I, et al. The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *Eur J Clin Nutr*. 2007;61:1037-1056.

23. Knaze V, Rothwell R, Zamora-Ros R, et al. A new food-composition database for 437 polyphenols in 19,899 raw and prepared foods used to estimate polyphenol intakes in adults from 10 European countries. *Am J Clin Nutr*. 2018;108:517-524.
24. Orsini N, Greenland S. A procedure to tabulate and plot results after flexible modeling of a quantitative covariate. *Stata J*. 2011; 11:1-29.
25. Tomova GD, Arnold KF, Gilthorpe MS, Tennant PWG. Adjustment for energy intake in nutritional research: a causal inference perspective. *Am J Clin Nutr*. 2022;115:189-198.
26. Larsen SC, Mikkelsen ML, Frederiksen P, Heitmann BL. Habitual coffee consumption and changes in measures of adiposity: a comprehensive study of longitudinal associations. *Int J Obes (Lond)*. 2018;42: 880-886.
27. Bertoia ML, Rimm EB, Mukamal KJ, Hu FB, Willett WC, Cassidy A. Dietary flavonoid intake and weight maintenance: three prospective cohorts of 124 086 US men and women followed for up to 24 years. *BMJ*. 2016;352:i17. doi:10.1136/bmj.i17
28. Zhang W, Du J, Wang S, Ma H. Association of weight change patterns across adulthood with incident asthma: a retrospective cohort study. *Sci Rep*. 2022;12:9756. doi:10.1038/s41598-022-13555-w
29. Cui M, Chen B, Xu K, et al. Activation of specific bitter taste receptors by olive oil phenolics and secoiridoids. *Sci Rep*. 2021;11:22340. doi:10.1038/s41598-021-01752-y
30. Hughes LA, Arts IC, Ambergen T, et al. Higher dietary flavone, flavonol, and catechin intakes are associated with less of an increase in BMI over time in women: a longitudinal analysis from the Netherlands Cohort Study. *Am J Clin Nutr*. 2008;88:1341-1352.
31. Marranzano M, Ray S, Godos J, Galvano F. Association between dietary flavonoids intake and obesity in a cohort of adults living in the Mediterranean area. *Int J Food Sci Nutr*. 2018;69:1020-1029.
32. Cercato C, Fonseca FA. Cardiovascular risk and obesity. *Diabetol Metab Syndr*. 2019;11:74. doi:10.1186/s13098-019-0468-0
33. Farias-Pereira R, Park CS, Park Y. Mechanisms of action of coffee bioactive components on lipid metabolism. *Food Sci Biotechnol*. 2019; 28:1287-1296.
34. Alam MA, Subhan N, Hossain H, et al. Hydroxycinnamic acid derivatives: a potential class of natural compounds for the management of lipid metabolism and obesity. *Nutr Metab (Lond)*. 2016;13:27. doi:10.1186/s12986-016-0080-3
35. Rosengren A, Dotevall A, Wilhelmsen L, Thelle D, Johansson S. Coffee and incidence of diabetes in Swedish women: a prospective 18-year follow-up study. *J Intern Med*. 2004;255:89-95.
36. Kim JH, Park YS. Light coffee consumption is protective against sarcopenia, but frequent coffee consumption is associated with obesity in Korean adults. *Nutr Res*. 2017;41:97-102.
37. Lee J, Kim HY, Kim J. Coffee consumption and the risk of obesity in Korean women. *Nutrients*. 2017;9:1340. doi:10.3390/nu9121340
38. Kotyczka C, Boettler U, Lang R, et al. Dark roast coffee is more effective than light roast coffee in reducing body weight, and in restoring red blood cell vitamin E and glutathione concentrations in healthy volunteers. *Mol Nutr Food Res*. 2011;55:1582-1586. doi:10.1002/mnfr.201100248
39. Greenberg JA, Owen DR, Geliebter A. Decaffeinated coffee and glucose metabolism in young men. *Diabetes Care*. 2010;33:278-280.
40. Benbouguerra N, Hornedo-Ortega R, Garcia F, El Khawand T, Saucier C, Richard T. Stilbenes in grape berries and wine and their potential role as anti-obesity agents: a review. *Trends Food Sci Technol*. 2021;112:362-381.
41. Karković Marković A, Torić J, Barbarić M, Jakobušić BC. Hydroxytyrosol, Tyrosol and derivatives and their potential effects on human health. *Molecules*. 2019;24:2001. doi:10.3390/molecules24102001
42. Romaguera D, Norat T, Vergnaud AC, et al. Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. *Am J Clin Nutr*. 2010;92:912-921.
43. Chopra S, Sharma KA, Ranjan P, Malhotra A, Vikram NK, Kumari A. Weight management module for perimenopausal women: a practical guide for gynecologists. *J Midlife Health*. 2019;10:165-172.
44. Sharifi-Rad M, Anil Kumar NV, Zucca P, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Front Physiol*. 2020;11:694. doi:10.3389/fphys.2020.00694
45. Freisling H, Van Bakel MM, Biessy C, et al. Dietary reporting errors on 24 h recalls and dietary questionnaires are associated with BMI across six European countries as evaluated with recovery biomarkers for protein and potassium intake. *Br J Nutr*. 2012;107:910-920.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Castañeda J, Gil-Lespinard M, Almanza-Aguilera E, et al. Association between classes and subclasses of polyphenol intake and 5-year body weight changes in the EPIC-PANACEA study. *Obesity (Silver Spring)*. 2023;31(4):1146-1158. doi:10.1002/oby.23689

11.3. Consumption of sweet beverages and cancer risk. A Systematic Review and Meta-Analysis of Observational Studies.

Llaha F, **Gil-Lespinard M**, Unal P, Villasante I, Castañeda J, Zamora-Ros R. Consumption of sweet beverages and cancer risk. A Systematic Review and Meta-Analysis of Observational Studies. Nutrients [Internet]. 2021 Feb 4;13(2):516. Available from: https://doi.org/10.3390/nu13020516

Impact Factor: 5.9

Category: Nutrition & Dietetics. Q1 17/88

nutrients

MDPI

Review

Consumption of Sweet Beverages and Cancer Risk. A Systematic Review and Meta-Analysis of Observational Studies

Fjorida Llaha †, Mercedes Gil-Lespinard †, Pelin Unal, Izar de Villasante, Jazmín Castañeda and Raul Zamora-Ros *

Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), 08908 Barcelona, Spain; fllaha@idibell.cat (F.L.); mgill@idibell.cat (M.G.-L.); pelin.uenal@dkfz-heidelberg.de (P.U.); idevillasante@idibell.cat (I.d.V.); acastaneda@idibell.cat (J.C.)

* Correspondence: rzamora@idibell.cat; Tel.: +34-932607401

† These authors contributed equally to this work.

Abstract: The consumption of sweet beverages, including sugar-sweetened beverages (SSB), artificial-sweetened beverages (ASB) and fruit juices (FJ), is associated with the risk of different cardiometabolic diseases. It may also be linked to the development of certain types of tumors. We carried out a systematic review and meta-analysis of observational studies aimed at examining the association between sweet beverage intake and cancer risk. Suitable articles published up to June 2020 were sourced through PubMed, Web of Science and SCOPUS databases. Overall, 64 studies were identified, of which 27 were selected for the meta-analysis. This was performed by analyzing the multivariable-adjusted OR, RR or HR of the highest sweet beverage intake categories compared to the lowest one. Random effects showed significant positive association between SSB intake and breast (RR: 1.14, 95% CI: 1.01–1.30) and prostate cancer risk (RR: 1.18, 95% CI: 1.10–1.27) and also between FJs and prostate cancer risk (RR: 1.03, 95% CI: 1.01–1.05). Although the statistically significant threshold was not reached, there tended to be positive associations for the following: SSBs and colorectal and pancreatic cancer risk; FJs and breast, colorectal and pancreatic cancer risk; and ASBs and pancreatic cancer risk. This study recommends limiting sweet beverage consumption. Furthermore, we propose to establish a homogeneous classification of beverages and investigate them separately, to better understand their role in carcinogenesis.

Keywords: systematic review; meta-analysis; cohort; case-control; sugar-sweetened beverages; artificial sweetened beverages; fruit juice; cancer

Citation: Llaha, F.; Gil-Lespinard, M.; Unal, P.; de Villasante, I.; Castañeda, J.; Zamora-Ros, R. Consumption of Sweet Beverages and Cancer Risk. A Systematic Review and Meta-Analysis of Observational Studies. *Nutrients* **2021**, *13*, 516. https://doi.org/10.3390/nu13020516

Academic Editor: Emilio Sacanella
Received: 30 December 2020
Accepted: 30 January 2021
Published: 4 February 2021

1. Introduction

The consumption of sweet beverages has increased in the last decades, with sugar-sweetened beverages (SSB) and artificially sweetened beverages (ASB) among the most widely consumed [1,2]. SSBs contain high levels of sugar that usually come from added sucrose or high fructose corn syrup (HFCS). Another type of sweet beverage is fruit juice (FJ), including fresh and commercial FJs and nectars. Despite their natural and healthy image, they contain high levels of sugar in the form of fructose. Although whole fruit also contains fructose, the fiber present limits the insulin response and increases satiety [3]. High sugar consumption may contribute to excessive energy intake, leading to long-term weight gain [4], higher risk of type 2 diabetes [5] and cardiovascular disease [6].

It has been demonstrated that obesity and type 2 diabetes are well-known risk factors for cancer [7–9]. Diets high in added sugar usually result in weight gain and an increase in adiposity-related metabolic parameters, insulin resistance, bioactivity of steroid hormones, oxidative stress and inflammation, which finally leads to cancer development and progression [9]. The International Agency for Research on Cancer (IARC) reported as strong evidence that excess body fat is a major risk factor for many cancers, including esophageal,

pancreatic, colorectal, post-menopausal breast, endometrial, renal, ovarian, gallbladder, hepatic and gastric cardia, among others [10].

High sugar intake impairs glucose and insulin tolerance and augments insulin and insulin-like growth factor (IGF) levels. Insulin and IGF are major determinants of proliferation and apoptosis, and may therefore influence carcinogenesis [11]. Beverages high in sugar, including SSBs and FJs, have high glycemic indexes [12] which is also suggested to be linked to cancer [13]. Moreover, both caloric and noncaloric sweet palatable substances have been demonstrated to activate the dopaminergic reward system. This can trigger addictive-like behaviors, which might be responsible for increased body fat [14]. ASBs contain low or non-caloric sweeteners (e.g., aspartame) and have been marked as healthier alternatives to SSBs. However, some studies have suggested that ASBs are also deleterious as regards obesity [15] and type 2 diabetes risk [5]. Moreover, it has also been suggested that long-term consumption of aspartame, used in many ASBs, might be carcinogenic [16]. Aspartame in liquids can quickly break down into methanol, and the subsequent metabolized formaldehyde is a documented carcinogenic substance [17].

In light of all this evidence, the association between consumption of sweet beverages and cancer risk has been investigated and reviewed by different studies. A meta-analysis from 2014 studied the association between SSB/ASB consumption and overall and specific cancer but no links were found [18]. Likewise, a 2019 meta-analysis did not find any significant association between SSB/ASB intake and pancreatic cancer risk [19]. However, the two mentioned studies did not perform a separate analysis of SSBs and ASBs which might have elucidated their particular role on cancer. A pooled analysis from 2012 [20] suggested a modest positive association between SSB intake and the risk of pancreatic cancer. Another similar study from 2010 [21] showed no significant association with colon cancer risk. A qualitative review of longitudinal studies from 2018 [22] reported inconsistent results for SSB/FJ intake and cancer risk. A recent French publication [23] reported a positive association between FJs and overall cancer risk. Regarding ASB intake, their results for breast, colorectal and prostate cancer risk were nonsignificant. However, another study [24] showed an increased risk for leukemia in the total population as well as for non-Hodgkin lymphoma and multiple myeloma in men only.

Evidence suggests that the link between sweet beverages consumption and cancer onset is biologically plausible. However, each type of beverage may have different mechanisms of action and different roles in cancer onset. Therefore, our study aimed to investigate these associations, by conducting separate analyses for SSB, ASB and FJ intake and cancer incidence. We analyzed case-control and cohort studies and performed a meta-analysis when feasible. Through this study we intend to update and develop a better understanding of the association between the consumption of sweet beverages and cancer incidence, a disease that caused 9.6 million deaths in 2018, a figure projected to nearly double by 2040 [25].

2. Materials and Methods

2.1. Search Method for Identification of Studies

This study was conducted according to the Preferred Reporting Items for Systematic Reviews Meta-Analysis (PRISMA) guidelines. To identify the suitable articles, we searched in PubMed, Web of Science and SCOPUS databases up to 31 June 2020, using the following keywords: (((((“soft drinks”[All Fields] OR “sugary drinks”[All Fields]) OR “sugary beverages”[All Fields]) OR “fruit juice”[All Fields]) OR “sugar-sweetened beverages”[MeSH Terms]) OR “artificially sweetened beverages”[MeSH Terms]) AND ((((“neoplasms”[MeSH Terms] OR “neoplasm”[All Fields]) OR “cancer”[All Fields]) OR “cancers”[All Fields]) OR “tumor”[All Fields]). We also applied search filters by article type (excluding books, reviews, systematic reviews and meta-analyses) and by species (including only humans). Moreover, reference lists of included manuscripts and relevant reviews were examined for any possible unidentified study. The search process was limited to English and Spanish languages.

2.2. Eligibility Criteria and Data Extraction

Eligible cohort and case-control studies were selected if they met the following criteria: (1) included adult participants free of cancer (if prospective) or with no history of previous cancer (if case-control) at recruitment, except for nonmelanoma skin cancer; (2) overall or site-specific cancer incidence as an outcome; and (3) estimated and reported hazard ratio (HR), risk ratio (RR) or odds ratio (OR) with 95% confidence interval (CI) for the link between any type of sweet beverages and any type of cancer incidence. The exclusion criteria were: (1) participants with previous cancer history or currently undergoing cancer treatment; (2) cancer survival and cancer mortality as an outcome; and (3) duplicated studies. The following data were extracted: first author's name, publication year, study name, country, age and sex of the participants, study sample size, number of cases and controls, follow-up duration, cancer site, type of exposure and amount of intake, dietary assessment methods, confounders' adjustment and HR/RR/OR with 95% CI for the larger degree of adjustment. When time-varying results were reported, those related to baseline data were extracted.

Three review authors independently performed the literature search, study selection and data extraction (FL, MG-L, and PU). Disagreements were discussed between all authors until a consensus was reached.

2.3. Quality Assessment of Included Studies

Two independent review authors (FL and MG-L) examined the methodological quality of the individual studies using the Risk Of Bias In Non-randomized Studies—of Exposures (ROBINS-E) [26] tool for cohort studies and the Newcastle–Ottawa Scale (NOS) [27] adapted for case-control studies. The ROBINS-E tool evaluates the risk of bias by assessing different domains: confounding variables, selection of participants into the study, classification of exposures, departures from intended exposures, missing data, measurement of outcomes and selection of the reported result. Low, moderate or serious risk of bias was established in each study considering all domains. The NOS assesses the selection of groups (0–4 stars), adequacy of comparability between groups (adjustment for confounders) (0–2 stars) and ascertainment of the exposure of interest for case–control studies (0–3 stars). For selection domain, we considered studies with 0–1, 2–3 and 4 stars as serious bias risk, moderate bias risk and high-quality risk, respectively. For comparability between groups, we considered those with 0, 1 and 2 as serious, moderate, and low bias risk, respectively. And finally, for ascertainment of exposure, we considered those 0, 1–2 and 3 as serious risk, moderate risk and low bias risk, respectively. In both tools, when data were not enough for judgment, the domain was classified as 'no information'.

2.4. Data Synthesis and Statistical Analysis

The first obstacle that we had to overcome was the lack of a unique definition for beverages and a variety of other terms. In this text, the following group terms are used to generalize these products: SSB for sugar-sweetened beverages (regular soft drinks/sodas, and non-diet soft drinks/sodas), ASB for artificially sweetened beverages (low and noncaloric soft drinks/sodas, and diet soft drinks/sodas) and FJ for fruit juices. In addition, two other terms are used: SB for sweetened beverages that includes both SSBs and ASBs; SFJ for high-sugar (added or natural) beverages that includes both SSBs and FJs. The quantity of each beverage was provided mostly as categories of frequency of consumption, either in amount (mL or g/day) or serving sizes (cans for SSBs and ASBs, glasses for FJs). To unify the data, we converted the categories to mL/day, based on the study-specific serving size for each beverage. When the serving size was not reported, we referred the national data of each study. Thus, we considered one can equal to 330 mL and one glass equal to 200 mL for European countries [28], one can equal to 360 mL and one glass equal to 240 mL for the United States [29], and one can equal to 375 mL for Australia [30]. One US study [31] expressed consumption as grams of sugar, and we weighed up an average of 10.5 g of sugar per 100 mL of SSB and an average of 9.6 g of sugar per 100 mL of FJ. This

was calculated based on the sugar content of different commercially available products of popular brands [32].

Prior to the analysis, the selected studies were classified by outcome (cancer incidence by site) and exposure (SB, SSB, ASB, FJ and SFJ). Data were summarized in a narrative manner and a meta-analysis was performed only if at least three studies reported data for the same exposure and outcome. In the meta-analysis, results for the total number of participants were considered. Separate analyses were considered (e.g., European-American and African-American women) when the article did not report indices for total population. In the same manner, if studies reported data for specific beverages (e.g., caffeinated and non-caffeinated SSBs), results for the total beverage group (e.g., total SSBs) were weighted up. Despite having extracted data on fruit and vegetables juices together, for the meta-analysis we considered the studies that indicated FJs as the predominant beverage consumed. The meta-analysis was performed by pooling the multivariable-adjusted RR/HR/OR of the highest category of the exposure versus the lowest one, and random effects models were assumed. If statistical outliers were identified, secondary analyses were performed (without outliers) to remove possible sources of heterogeneity. An outlier was considered when its 95% CI lied outside the 95% CI of the pooled effect. To further explain heterogeneity, we performed subgroup and sensitive analyses, dividing studies according to design (cohort/case-control), country (US/non-US, mostly European), level of overall risk of bias (serious/low-moderate) and beverage intake category (high vs. non-consumer/high vs. low). We used Cochran's Q, I^2 and Tau^2 statistics to measure between-study heterogeneity. The statistical analysis was performed with the Metafor package [33] of the R software, version 4.0.1. P values < 0.05 were considered statistically significant.

3. Results

3.1. Literature Search and Study Characteristics

The study selection process according to PRISMA guidelines is reported in Figure 1. In total, 869 potential publications were identified from the databases (PubMed, Web of Science and SCOPUS) and other sources. After removing duplicates, 596 articles were selected, from which 435 were excluded based on titles and 26 on abstracts. Of 135 eligible articles, 71 were excluded due to the following reasons: 59 did not report risk index for sweet beverages and cancer incidence, 3 full-texts were not available, 7 considered other outcomes, 1 case-control study included controls with cancer at recruitment and 1 publication was not in English or Spanish. Finally, 64 studies were included in the systematic review, 27 cohort [23,24,28,31,34–56] and 37 case-control studies [57–93]. Of these, 27 studies were meta-analyzed.

Of the included studies, 29 were performed in the United States (US), 17 in Europe, 6 in Asia, 5 in Canada, 3 in Australia, 2 in Latin-America, 1 in Egypt and 1 was multinational (Italy, Spain, Poland, Northern Ireland, India, Cuba, Canada, Australia and Sudan). They usually included both male and female participants. Ages ranged from 18 to 97 years. The 27 cohort studies were published between 2003 and 2020 and enrolled 4,458,056 participants in total, of which 30,646 developed cancer. Mean duration of the follow-up in cohort studies varied from 2 to 20 years. The 37 case-control studies were published between 1985 and 2019. In total, they enrolled 20,827 cancer cases and 34,315 controls. Most of the controls were selected from the general population.

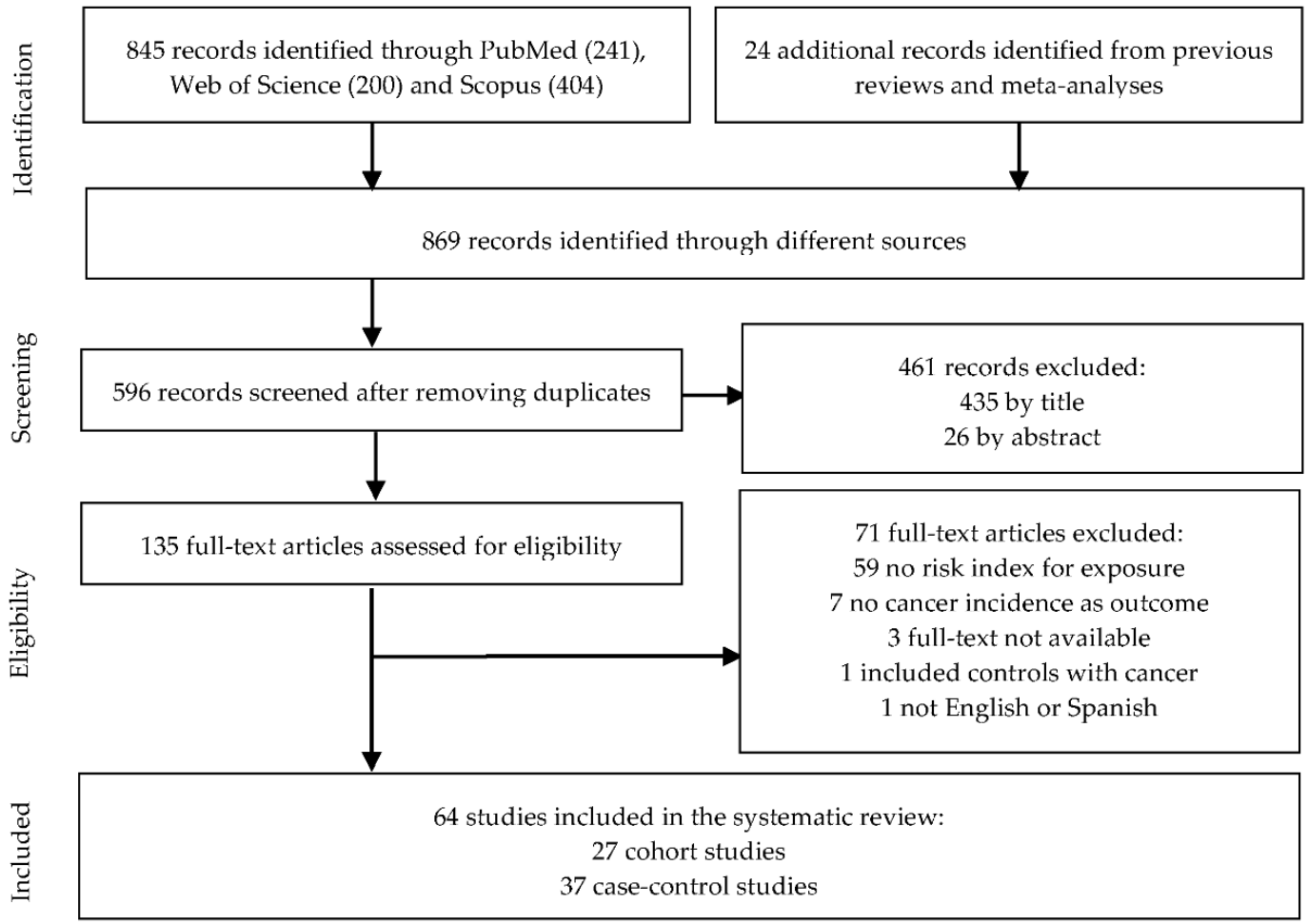


Figure 1. Prisma diagram.

Sweet beverage consumption in both cohort and case-control studies was expressed as categorical or continuous variables. Exposure assessment was collected using food frequency questionnaires (FFQ), 24-h dietary recalls (24-H DR), dietary questionnaires (DQ), interviews, or surveys. Among all the studies, 37 types of cancer were considered as an outcome and 4 cohorts reported data for overall cancer risk, including different types of cancer [23,50,52,54]. In most of the studies, the outcome was confirmed by a medical diagnosis. Overall characteristics of the included studies are summarized in Table 1. Results of the meta-analysis for the random-effect model are summarized in Table 2 and for the subgroup analysis in Table S1.

Table 1. Overall characteristic of the included studies.

Breast Cancer (Breast, Pre- and Post-Menopausal)										
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Chandran et al., 2006 [57]	US, WCHS	Breast Pre-M Post-M	PB case-control	3148	1558 797 761	20–75	F (100)	125-item FFQ	SSB: ≥152 vs. <152 mL/day SSB: ≥152 vs. <152 mL/day SSB: ≥152 vs. <152 mL/day	OR: 0.97 (0.74–1.27) (AA) OR:1.31 (0.91–1.89) (EA) OR: 1.17 (0.79–1.74) (AA) OR: 0.95 (0.58–1.56) (EA) OR: 0.76 (0.51–1.12) (AA) OR: 2.05 (1.13–3.7) (EA)	Age, ethnicity, country, education, age at menarche, menopause and first birth, MS, parity, BF status, history of benign breast disease, family history of BC, HRT, OC use, BMI, and study site.
Chazelas et al., 2019 [23]	France, NNS	Breast Pre-M Post-M	Cohort	101,257 5.1 (median)	693 283 410	42.2/14.4	F (78)	24H-DR	SFJ: >123 vs. <38.1 mL/day (cut-off) SFJ: increase by 100 mL/day SSB: >57.1 vs. <13.6 mL/day (cut-off) SSB: increase by 100 mL/day ASB: >11.6 vs. <4.6 mL/day (cut-off) ASB: increase by 10 mL/day FJ: >81.9 vs. <17.0 mL/day (cut-off) FJ: increase by 100 mL/day SFJ: >123 vs. <38.1 mL/day (cut-off) SFJ: increase by 100 mL/day SSB: >57.1 vs. <13.6 mL/day (cut-off) SSB: increase by 100 mL/day ASB: >11.6 vs. <4.6 mL/day (cut-off) ASB: increase by 10 mL/day FJ: >81.9 vs. <17.0 mL/day (cut-off) FJ: increase by 100 mL/day SFJ: >123 vs. <38.1 mL/day (cut-off) SFJ: increase by 100 mL/day SSB: >57.1 vs. <13.6 mL/day (cut-off) SSB: increase by 100 mL/day ASB: >11.6 vs. <4.6 mL/day (cut-off) ASB: increase by 10 mL/day FJ: >81.9 vs. <17.0 mL/day (cut-off) FJ: increase by 100 mL/day	HR: 1.37 (1.08–1.73) HR: 1.22 (1.07–1.39) HR: 1.10 (0.87–1.39) HR: 1.23 (1.03–1.48) HR: 1.33 (0.98–1.75) HR: 0.97 (0.86–1.09) HR: 1.13 (0.91–1.39) HR: 1.15 (0.97–1.35) HR: 1.28 (1.09–1.83) HR: 1.26 (1.04–1.51) HR: 1.68 (1.45–1.74) HR: 1.34 (1.15–1.70) HR: 1.23 (0.52–2.53) HR: 0.95 (0.81–1.13) HR: 0.98 (0.67–1.43) HR: 1.10 (0.85–1.41) HR: 1.44 (1.05–1.99) HR: 1.19 (0.98–1.44) HR: 0.99 (0.72–1.39) HR: 1.08 (0.79–1.47) HR: 1.10 (0.55–2.12) HR: 1.01 (0.86–1.18) HR: 1.24 (0.95–1.61) HR: 1.19 (0.96–1.48)	Smoking, education, PA, BMI, and height.
Hirvonen et al., 2006 [51]	France, SUVIMAX	Breast	Cohort	4396 6.6	95	35–60	F (100)	24H-DR	FJ: >150 mL/day vs. none	RR: 1.29 (0.80–2.09)	Age, smoking, number of children, OC use, family history of BC, and MS.
Makarem et al., 2018 [52]	US	Breast	Cohort	3184 4	128	54.3	F (53)	FFQ	SFJ: >324 vs. <135 mL/day (cut-off) SSB: >51.4 mL/day vs. none FJ: >180 vs. <38.6 mL/day (cut-off)	HR: 1.00 (0.65–1.57) HR: 1.04 (0.64–1.71) HR: 1.03 (0.67–1.62)	Age, smoking, BMI, EI, alcohol, PA, education, MS, n° of live births, WC, DM and CVD, antioxidant use, energy from fat, and diet soda intake.
Marzbani et al., 2019 [58]	Iran	Breast	HB case-control	620	212	40.2	F (100)	11-item healthcare form	SB [7]: favorable intake vs. ≤1 time/month	OR: 2.8 (1.9–4.3)	Age, education, and BMI
McLaughlin et al., 1992 [69]	US	Breast	PB case-control	3234	1617	56.7	F (100)	SQ-interview	SB [2]: ever vs. never	OR: 1.08 (0.92–1.26)	Age, alcohol, country, race, MS, age at first live birth, diagnosis of benign cancers, and family history of BC.
Potischman et al., 2002 [80]	US	Breast	PB case-control	2019	568	20–44	F (100)	100-item FFQ	SSB: ≥320 mL/day vs. none	OR: 1.09 (0.8–1.5)	Age at diagnosis, study site, race, education, alcohol consumption, years of OC use, smoking, BMI, and EI.

Table 1. *Cont.*

Romanos-Nanclares et al., 2019 [53]	Spain	Breast Pre-M Post-M	Cohort	10,713 2	100 57 43	33.0 (median)	F (100)	FFQ	SSB: >47.1 vs. <11 mL/day SSB: ≥11 mL/day vs. none SSB: >47.1 vs. <11 mL/day	HR: 1.36 (0.74–2.50) HR: 1.16 (0.66–2.07) HR: 2.12 (1.01–4.41)	Age, height, family history of BC, smoking, PA, BMI, age at menarche and menopause, MS, HRT, number of pregnancies >6 month and before 30 years old, months of BF, alcohol, education, DM, GI, EI, U-P food and coffee consumption, and Med-diet adherence.
Hodge et al., 2018 [54]	Australia, MCCS	Post-M	Cohort	35,593 19	946	54.6	F (100)	121-item FFQ	SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. <6.7 mL/day	HR: 1.11 (0.85–1.45) HR: 0.95 (0.73–1.25)	Socioeconomic indexes, country of birth, alcohol intake, smoking, PA, Med-diet score, and sex. ASB also for SSB consumption and WC.
Nomura et al., 2016 [55]	US, BWHS	Breast Pre-M Post-M	Cohort	49,103 13.8	1827 678 826	21–69	F (100)	FFQ	SSB: ≥250 mL/day vs. none SSB: ≥250 mL/day vs. none SSB: ≥250 mL/day vs. none	HR: 0.71 (0.50–1.02) HR: 1.72 (0.91–3.23) HR: 1.11 (0.77–1.61)	Age, geographic region of residence, EI, smoking, family history of BC, education, MS, OC use, parity, HRT, BMI, alcohol, PA, and sedentary time.
Colorectal and Rectal Cancer											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Bener et al., 2010 [88]	Qatar	Colorectal	HB case-control	428	146	53.4	M (58)	DQ	SB: ≥330 vs. ≤47.1 mL/day	OR: 1.62 (1.19–2.17)	Not reported
Chazelas et al., 2019 [23]	France	Colorectal	Cohort	101,257 5.1 (median)	166	42.2 (14.4)	F (78)	24H-DR	SFJ: >123 vs. <38.1 mL/day (F); >141.7 vs. <46.1 mL/day (M) (cut-off) increase by 100 mL/day SSB: >57.1 vs. <13.6 mL/day (F); >65.5 vs. < 14.0 mL/day (M) (cut-off) increase by 100 mL/day ASB: >11.6 vs. <4.6 mL/day (F); >7.9 vs. < 2.7 mL/day (M) (cut-off) increase by 10 mL/day FJ: >81.9 vs. <17.0 mL/day (F); >97.8 vs. <19.9 mL/day (M) (cut-off) increase by 100 mL/day	HR: 1.07 (0.63–1.80) HR: 1.10 (0.84–1.46) HR: 1.01 (0.59–1.71) HR: 1.11 (0.72–1.71) HR: 0.80 (0.44–1.46) HR: 1.02 (0.94–1.10) HR: 1.19 (0.78–1.82) HR: 1.05 (0.75–1.46)	Smoking, education, PA, BMI, and height.
Hodge et al., 2018 [54]	Australia, MCCS	Colorectal	Cohort	35,593 19	1055	54.6	M/F	121-item FFQ	SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. <6.7 mL/day	HR: 1.28 (1.04–1.57) HR: 0.79 (0.60–1.06)	Socioeconomic indexes, country, alcohol, smoking, PA, Med-diet score, and sex. ASB also for SSB consumption and WC.
Makarem et al., 2018 [52]	US	Colorectal	Cohort	3184 4	68	54.3	F (53)	FFQ	SFJ: >362.6 vs. <154.3 mL/day (cut-off) SSB: >180 vs. <25.7 mL/day (cut-off) FJ: >180 vs. < 48.9 mL/day (cut-off)	HR: 1.39 (0.68–2.82) HR: 0.96 (0.51–1.82) HR: 1.66 (0.88–3.12)	Age, smoking, BMI, EI, alcohol, PA, education, MS, nº of live births, WC, DM and CVD, antioxidant use, energy from fat, and diet soda intake.
Mahfouz et al., 2014 [89]	Egypt	Colorectal	HB case-control	450 1	150	<20–>60	F (52)	DQ	SB: daily vs. not daily FJ: daily vs. not daily	OR: 4.6 (1.9–11.01) OR: 0.18 (0.09–0.36)	Not reported

Table 1. *Cont.*

Pacheco et al., 2019 [56]	US	Colorectal	Cohort	99,798 20.1 (median)	1318	52.0 (13.5)	F (100)	FFQ	SSB: ≥60 mL/day vs. never/rare	HR: 1.14 (0.86–1.53)	Age, BMI, EI, smoking, alcohol, family history of CR polyps, multivitamin use, and HT.
Tayyem et al., 2018 [90]	Jordan	Colorectal	HB case-control	501 2	220	52	F (51)	Q-DQ	SB: daily vs. rarely OJ: daily vs. rarely	OR: 1.39 (0.73–2.63) OR: 1.07 (0.45–2.55)	Age, sex, work status, income, PA, marital status, EI, education, other diseases, and history of CR cancer.
Theodoratou et al., 2014 [91]	Scotland	Colorectal	PB case-control	4838 7.0	2062	64.3	M/F	FFQ	SSB: increase by 330 mL/day FJ: increase by 200 mL/day	OR: 1.12 (1.05–1.19) OR: 1.19 (1.11–1.27)	Age, sex, BMI, PA, family history of CR cancer, EI, NSAIDs, eggs, FJ, SSB, white fish, coffee, and magnesium intake.
Murtaugh et al., 2004 [92]	US	Rectal	PB case-control	2157 4	952	30–79	M (57)	Interview	SSB: yes vs. no (M) SSB: yes vs. no (F) ASB: yes vs. no (M) ASB: yes vs. no (F) J: >449 vs. ≤58.3 mL/day (M); J: >596.6 vs. ≤44.6 mL/day (F)	OR: 1.00 (0.80–1.26) OR: 0.96 (0.73–1.27) OR: 1.28 (0.98–1.68) OR: 0.90 (0.67–1.22) OR: 0.92 (0.63–1.34) OR: 1.56 (1.00–2.41)	Age, PA, EI, and dietary fiber and calcium intake.
Esophageal Cancers (Esophagus-Gastric Junction, Esophageal Adenocarcinoma, Squamous Cell Carcinoma)											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Ibiebele et al., 2008 [93]	Australia	AEGJ EAC SCC	PB case-control	2341 4	325 294 238	18–79	M (71)	FF	SB [7]: ≥375 mL/day vs. none SSB [7]: yes vs. no ASB [7]: yes vs. no SB [7]: ≥375 mL/day vs. none SSB [7]: yes vs. no ASB [7]: yes vs. no SB [7]: ≥375 mL/day vs. none SSB [7]: yes vs. no ASB [7]: yes vs. no	OR: 1.07 (0.67–1.73) OR: 0.63 (0.43–0.92) OR: 0.77 (0.46–1.29) OR: 0.94 (0.53–1.66) OR: 1.20 (0.79–1.81) OR: 0.71 (0.37–1.37) OR: 0.40 (0.20–0.78) OR: 0.70 (0.47–1.03) OR: 0.46 (0.25–0.85)	Age, sex, BMI, EI, alcohol, smoking, education, heartburn, and acid reflux symptoms.
Mayne et al., 2006 [59]	US	EAC SCC	PB case-control	1782	228 206	65 Q1, 59.3 Q4	M (78 Q1, 82 Q4)	Proxy and self-interviewed	SSB [7]: ≥355 vs. 10.7 mL/day SSB [7]: ≥355 vs. 10.7 mL/day	OR: 0.47 (0.29–0.76) OR: 0.85 (0.48–1.52)	Age, sex, center, race, proxy interview status, BMI, EI, alcohol and meat intake, cigarettes/day, education, income, and frequency of reflux symptoms.
Ren et al., 2010 [34]	US, NIH-AARP-DHS	EAC SCC	Cohort	481,563 2	305 123	50–71	M (59)	124-item FFQ	SB: ≥355 vs. ≤355 mL/day SB: ≥355 vs. ≤355 mL/day	HR: 1.11 (0.66–1.85) HR: 0.85 (0.46–1.56)	Age, sex, smoking, alcohol, EI, BMI, education, ethnicity, PA, and daily intake of fruit, vegetables, red meat, and white meat.
Stomach Cancers (Gastric Cardia, Gastric Noncardia)											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Hodge et al., 2018 [54]	Australia, MCCS	Gastric cardia	Cohort	35,593 19	165	54.6	M/F	121-item FFQ	SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. 6.7 mL/day	HR: 1.17 (0.73–1.89) HR: 1.03 (0.53–1.98)	Socioeconomic indexes, country, alcohol, smoking, PA, Med-diet score, and sex. ASB also for SSB consumption and WC.

Table 1. *Cont.*

Mayne et al., 2006 [59]	US	Gastric cardia Gastric noncardia	PB case-control	1782	255 352	65 Q1, 59.3 Q4	M (78 Q1, 82 Q4)	Proxy and self-interviewed	SSB [7]: ≥355 vs. <10.7 mL/day SSB [7]: ≥355 vs. <10.7 mL/day	OR: 0.74 (0.46–1.16) OR: 0.65 (0.43–0.98)	Age, sex, center, race, proxy interview status, BMI, EI, alcohol and meat intake cigarettes/day, education, incomes, and frequency of reflux symptoms.
Ren et al., 2010 [34]	US, NIH-AARP-DHS	Gastric cardia Gastric noncardia	Cohort	481,563 2	231 224	50–71	M (59)	124-item FFQ	SB: ≤355 vs. ≥355 mL/day SB: ≥355 vs. ≤355 mL/day	HR: 0.89 (0.55–1.45) HR: 0.75 (0.45–1.24)	Age, sex, smoking, alcohol, EI, BMI, education, ethnicity, PA and daily intake of fruit, vegetables, and white meat.
Pancreatic Cancer											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Bao et al., 2008 [42]	US, NIH-AARP-DHS	Pancreatic	Cohort	487,922 7.2	1258	50–71	F (41)	124-item FFQ	SB: 816.9 mL/day (median) vs. none SSB: 512.8 mL/day (median) vs. none ASB: 816.9 mL/day (median) vs. none	RR: 1.07 (0.86–1.33) RR: 1.01 (0.77–1.31) RR: 1.11 (0.86–1.44)	Age, sex, race, education, BMI, alcohol, smoking, PA, EI, and foliate intake. SSB and ASB were mutually adjusted.
Chan et al., 2009 [76]	US, SFB	Pancreatic	PB case-control	2233	532	21–85	M (53)	131-item FFQ	SB: ≥355 mL/day vs. none SB [7]: ≥355 mL/day vs. none SSB [7]: ≥355 mL/day vs. none ASB [7]: ≥355 mL/day vs. none SSB [4]: ≥355 mL/day vs. none	OR: 1.0 (0.7–1.3) OR: 1.1 (0.8–1.5) OR: 0.9 (0.6–1.3) OR: 1.5 (1.1–2.1) OR: 1.0 (0.6–1.8)	Age, sex, EI, BMI, race, education, smoking, history of DM, PA, red and white meat, fruit and vegetables, eggs, dairy, whole and refine grained, and sweets. SSB and ASB were mutually adjusted.
Gallus et al., 2011 [77]	Italy	Pancreatic	HB case-control	978 7	326	63 (median)	M (53)	FFQ	SB [7]: ≥150 vs. <150 mL/day	OR: 1.02 (0.72–1.44)	Age, sex, study center, education, BMI, smoking, alcohol, EI, family history of pancreatic cancer, and DM.
Gold et al., 1985 [78]	US	Pancreatic	HB, PB case-control	676	274	66.1	F (53)	Interview	ASB: ever vs. never	OR: 0.66 (0.38–1.2)	Religion, occupation, smoking, and alcohol.
Larsson et al., 2006 [41]	Sweden, SMC, COSM	Pancreatic	Cohort	77,797 7.2	131	60.8	F (45)	FFQ	SB: ≥500 mL/day vs. none	HR: 1.93 (1.18–3.14)	Age, sex, education, smoking, BMI, and EI.
Lyon et al., 1992 [79]	US	Pancreatic	PB case-control	512	149	40–79	M/F	DQ	SB (caff): ever vs. never	OR: 1.31 (0.89–1.94)	Unadjusted.
Mack et al., 1986 [81]	US	Pancreatic	PB case-control	980	490	18–65	M (58)	Proxy and direct Interview	SB [7]: ≥1650 vs. <1320 mL/day	RR: 2.6 (0.9–7.4)	Not reported
Mueller et al., 2010 [43]	China and Singapore, SCHS	Pancreatic	Cohort	60,524 14	140	56.5	F (56)	FFQ	SB: ≥67.7 mL/day vs. none J [5]: ≥67.7 mL/day vs. none	HR: 1.87 (1.10–3.15) HR: 1.31 (0.74–2.30)	Age, sex, smoking, BMI, alcohol, EI, PA, DM, education, added sugar, and candy. SB and J were mutually adjusted.
Nothlings et al., 2007 [44]	US	Pancreatic	Cohort	162,150 8	434	59.8	F (55)	FFQ	SSB: ≥151.4 mL/2000 kcal/day vs. none FJ: ≥120 vs. < 9.4 mL/2000 kcal/day	RR: 1.07 (0.82,1.41) RR: 1.08 (0.83,1.41)	Age, sex, smoking, BMI, EI, time on study, race, family history of pancreatic cancer, intake of red, and processed meat.
Navarrete-Muñoz et al., 2016 [45]	10 European countries ‡, EPIC	Pancreatic	Cohort	477,206 11.4	865	51	F (70)	DQ- country specific	SB: >196.4 vs. 0.1–13.1 mL/day SB: increase by 100 mL/day SSB: >121.4 vs. 0.1-4.5 mL/day SSB: increase by 100 mL/day ASB: >92.2 vs. 0.1-2.0 mL/day ASB: increase by 10 mL/day FJ [6]: >123.1 vs. 0.1-8.3 mL/day FJ [6]: increase by 100 mL/day	HR: 0.90 (0.68–1.19) HR: 1.02 (0.98–1.06) HR: 0.90 (0.65–1.25) HR: 1.02 (0.97–1.08) HR: 0.99 (0.61–1.60) HR: 1.02 (0.96–1.08) HR: 0.74 (0.57–0.97) HR: 0.91 (0.84–0.98)	Age, sex, smoking, BMI, alcohol, EI, study center, PA, and DM. FJ and SB were mutually adjusted.
Schernhammer et al., 2005 [46]	US, HPFS, NHS	Pancreatic	Cohort	136,587 14 HPFS, 20 NHS	379	53.7	F (65)	FFQ	SSB: <143.6 vs. > 11.2 mL/day ASB: <143.6 vs. > 11.2 mL/day	RR: 1.13 (0.81–1.58) RR: 1.02 (0.79–1.32)	Age, sex, smoking, BMI, follow-up cycle, PA, DM, and other soft drink intake.

Table 1. *Cont.*

Genitourinary Cancers (Prostate, Renal Cell, Urinary Bladder, Urothelial Cell)											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake [+]**	**HR/RR/OR (95% CI)**	**Adjustments**
Bruemmer et al., 1997 [60]	US	Bladder	PB case-control	620	215	45–65	M (62)	Interview	SSB: >240 vs. < 8 mL/day ASB: >240 < 8 mL/day	OR: 0.4 (0.2–1.1) (M) OR: 5.7 (1.2–26.9) (F) OR: 1.6 (0.7–3.6) (M) OR: 2.3 (0.8–6.3) (F)	Age, country, and smoking.
De Stefani et al., 2007 [61]	Uruguay	Bladder	HB case-control	756	255	30–89	M (88)	64-item FFQ	SB: ≥142 vs. <142 mL/day	OR: 1.1 (0.7–1.7)	Age, sex, residence, education, familiar history of UBC, BMI, occupation, smoking, intake of mate, coffee, tea, and milk.
Hemelt et al., 2010 [62]	China	Bladder	HB case-control	792 3	400	65.8	M (79)	DQ	SB: consumers vs. none FJ: daily vs. none	OR: 2.01 (1.10–3.68) OR: 0.66 (0.26–1.66)	Age, sex, smoking, and frequency and duration of smoking.
Radosavljević et al., 2003 [63]	Serbia	Bladder	HB case-control	260	130	64.9	M (79)	101-item FFQ	SB: >15.7 mL/day (mean) vs. none FJ: >11.6 mL/day (mean) vs. none	OR: 4.73 (2.72–8.18) OR: 0.30 (0.18–0.50)	Smoking
Turati et al., 2015 [64]	Italy	Bladder	HB case-control	1355	665	67 (median)	M (76)	DQ	SB [2]: ≥47 mL/day vs. none	OR: 1.04 (0.73–1.49)	Age, sex, study center, year of interview, smoking, education, alcohol, BMI, and family history of UBC and cystitis.
Wang, 2013 [65]	US	Bladder	HB case-control	2306	1007	64.4	M (78)	FFQ	SB: ≥255.6 mL/day vs. none SSB: ≥126 mL/day vs. none ASB: ≥309.6 mL/day vs. none	OR: 1.34 (1.05–1.70) OR: 1.27 (1.02–1.58) OR: 1.06 (0.85–1.32)	Age, sex, ethnicity, EI, and smoking.
Chazelas et al., 2019 [23]	France	Prostate	Cohort	101,257 5.1 (median)	291	42.2/4.4	M (100)	24H-DR	SFJ: >141.7 vs. <46.1 mL/day (cut-off) SFJ: increase by 100 mL/day SSB: >65.5 vs. <14.0 mL/day (cut-off) SSB: increase by 100 mL/day ASB: >7.9 vs. <2.7 mL/day (cut-off) ASB: increase by 10 mL/day FJ: >97.8 vs. <19.9 mL/day (cut-off) FJ: increase by 100 mL/day	HR: 1.39 (0.96–2.02) HR: 1.10 (0.92–1.31) HR: 1.19 (0.83–1.72) HR: 1.24 (0.95–1.62) HR: 1.33 (1.01–1.75) HR: 0.57 (0.24–1.34) HR: 1.04 (0.76–1.42) HR: 0.97 (0.79–1.2)	Smoking, education, PA, BMI, and height.
Drake et al., 2012 [35]	Sweden, MDC	Prostate	Cohort	8128 14.9	817	45–73	M (100)	168-item FFQ, 7-d menu book Interview	SSB: 297.8 mL/day (median) vs. none FJ: 200 mL/day (median) vs. none	HR: 1.13 (0.92–1.38) HR: 0.99 (0.81–1.22)	Age, year of study entry, time of data collection, EI, height, WC, PA, smoking, education, birth in Sweden, alcohol, calcium and selenium intake, and risk by death from all causes except PC.
Ellison et al., 2000 [36]	Canada, NCSS	Prostate	Cohort	3400 23	201	50–84	M (100)	FFQ	SB [2]: ≥100 mL/day vs. none SB [2]: ≥any vs. none	RR: 1.29 (0.74–2.26) RR:1.09 (0.78–1.35)	Age, alcohol, smoking, BMI, fiber, and EI.
Hodge et al., 2018 [54]	Australia, MCCS	Prostate	Cohort	35,593 19	433	54.6	M (100)	121-item FFQ	SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. <6.7 mL/day	HR: 1.08 (0.78–1.50) HR: 0.81 (0.49–1.33)	Socioeconomic indexes, country of birth, alcohol, smoking, PA, and Med-diet score. ASB also for SSB consumption and WC.
Jain et al., 1998 [66]	Canada	Prostate	PB case-control	1253	617	69.8	M (100)	Q-DH	SB [2]: >200 mL/day vs. none	OR: 0.79 (0.53–1.17)	Age, EI
Makarem et al., 2018 [52]	US	Prostate	Cohort	3184 4	157	54.3	M (100)	FFQ	SFJ: >401 vs. <212.1 mL/day (cut-off) SSB: >180 vs. <25.7 mL/day (cut-off) FJ: >180 vs. <48.9 mL/day (cut-off)	HR: 1.06 (1.03–1.09) HR: 1.38 (0.80–2.38) HR: 1.03 (1.01–1.06)	Age, smoking, BMI, EI, alcohol, PA, education, WC, DM, CVD, antioxidant use, and energy from fat and diet soda intake.

Table 1. *Cont.*

Miles et al., 2018 [31]	US	Prostate	Cohort	22,720 9	1996	65.6 (5.9)	M (100)	FFQ	SSB: >183 vs. <6 mL/day (cut-off) FJ: >190 vs. <24 mL/day (cut-off)	HR: 1.21 (1.06–1.39) HR: 1.07 (0.94–1.22)	Age, sex, smoking, BMI, EI, DM, education, race, family history of PC, and PSA screens.
Sharpe et al., 2002 [67]	Canada	Prostate	PB case-control	875	399	61.5	M (100)	Interviews or DQ	SB [7]: daily drank vs. never drank weekly	OR: 1.0 (0.7–1.4)	Age, ethnicity, socioeconomic status, BMI, cumulative cigarette smoking, and alcohol.
Hodge et al., 2018 [54]	Australia, MCCS	Renal cell	Cohort	35,593 19	146	54.6	M/F	121-item FFQ	SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. <6.7 mL/day	HR: 1.48 (0.87–2.53) HR: 0.92 (0.46–1.84)	Socioeconomic indexes, country of birth, alcohol, smoking, PA, Med-diet score, and sex. ASB also for SSB consumption and WC
Hu et al., 2009 [68]	Canada	Renal cell	PB case-control	6177	1138	20–80	M (51)	FFQ	SB: >230 mL/day vs. none SB: increase by 230 md J: >236 vs. ≤23 mL/day J: increase by 118 mL/day	OR: 1.26 (0.96–1.67) OR: 1.05 (0.97–1.13) OR: 1.53 (1.18–1.99) OR: 1.08 (1.04–1.13)	10-year age groups, province, education, BMI, sex, EI, smoking, intake of alcohol meat, vegetables, and fruits.
Lee et al., 2006 [37]	US	Renal cell	Cohort	136,587 14 HPFS 20 NHS	248	53.7	F (65)	FFQ	SB: ≥670 vs. <47.9 mL/day SSB: increase by 335 mL/day ASB: increase by 335 mL/day FJ: increase by 335 mL/day	RR: 1.03 (0.64–1.68) RR: 0.95 (0.69–1.31) RR: 0.97 (0.82–1.15) RR: 1.06 (0.88–1.28)	BMI, EI, alcohol, smoking, history of HT, DM, multivitamin use, and parity.
Maclure and Willet, 1990 [70]	US	Renal cell	PB case-control	430	203	30–>80	M (67)	FFQ	SB: >480 vs. <68.6 mL/day ASB: >480 vs. <68.6 mL/day FJ: ≥ 480 vs. ≤ 34.3 mL/day	OR: 2.6 (1.4–4.8) OR: 2.7 (1.1–6.5) OR: 0.56 (0.22–1.4)	Age, sex, body weight/height, EI, and education
Ros et al., 2011 [38]	10 European countries [†], EPIC	Urothelial cell	Cohort	233,236 9.3	513	25–70	F (71)	DQ-country specific	SB: ≥99 vs. <8 mL/day (M); ≥20 vs. <8 mL/day (F) FJ: ≥72 vs. <8 mL/day (M); ≥79 vs. 8 mL/day (F)	HR: 1.03 (0.83–1.30) HR: 1.32 (1.05–1.66)	Smoking, EI from fat and nonfat sources. Stratified by age at entry, sex, and center.
Gynecological Cancers (Cervical, Endometrial, Epithelial Ovarian, Ovarian)											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake [+]**	**HR/RR/OR (95% CI)**	**Adjustments**
Herrero et al., 1991 [71]	Colombia, Costa Rica, Mexico and Panama	Cervical	HB, PB case-control	2033	622	46.5	F (100)	FFQ	FJ: >240 vs. <0.8 mL/day	OR: 0.90 (0.7–1.2)	Age, study site, age at 1st intercourse, number of sexual partners and pregnancies, presence of HPV 16/18, interval since last Pap smear, and number of household facilities.
Verreault et al. 1989 [72]	US	Cervical	PB case-control	416	189	20–74	F (100)	66-items FFQ	FJ: ≥ 355 vs. ≤ 48 mL/day	RR: 0.3 (0.2–0.6)	Age, education, smoking, frequency of Pap smears, use of barrier and OC, history of cervical-vaginal infection, age at first intercourse, and number of sexual partners.
Inoue-Choi et al., 2013 [39]	US	Endometrial type I Endometrial type II	Cohort	23,039 14	506 89	61.6	F (100)	FFQ	SFJ: >424.3 vs. ≤55.7 mL/day SSB: >87.4 mL/day vs. none ASB: >144 mL/day vs. none FJ: >288 vs. ≤20.6 mL/day SFJ: >424.3 vs. ≤55.7 mL/day SSB: >87.4 mL/day vs. none ASB: >144 mL/day vs. none FJ: >288 vs. ≤20.6 mL/day	HR: 1.48 (1.09–2.00) HR: 1.78 (1.32–2.40) HR: 0.77 (0.59–1.01) HR: 1.16 (0.87–1.56) HR: 1.09 (0.55–2.15) HR: 1.31 (0.63–2.69) HR: 0.89 (0.48–1.68) HR: 0.97 (0.50–1.88)	Age, smoking, BMI, PA, alcohol, HRT, age at menarche and at menopause, number of live births, DM, and coffee intake.

Table 1. *Cont.*

Hodge et al., 2018 [54]	Australia, MCCS	Endometrial Ovarian	Cohort	35,593 19	167 130	54.6	F (100)	121-item FFQ	SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. <6.7 mL/day SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. <6.7 mL/day	HR: 1.02 (0.54–1.91) HR: 0.81 (0.42–1.55) HR: 1.35 (0.71–2.56) HR: 1.37 (0.72–2.61)	Socioeconomic indexes, country of birth, alcohol, smoking, PA, Med-diet score, and sex. ASB also for SSB consumption and WC.
King et al., 2013 [73]	US	Epithelial ovarian	PB case-control	595 7	205	>21	F (100)	FFQ and Interview	SSB: ≥151.2 vs. <21.6 mL/2000 kcal/day SSB: increase by 360 mL/day	OR: 1.31 (0.77–2.24) OR: 1.63 (0.94–2.83)	Age, education, race, age at menarche, MS, parity, OC use, HRT, BMI, smoking, PA, DM, tubal ligation, intake of fiber, fat, and saturated fat.
Leung et al., 2016 [74]	Canada	Epithelial ovarian	PB case-control	2111 11	524	40–79	F (100)	FFQ and Interview	SB: >9.9 mL/day vs. none	OR: 0.97 (0.72–1.31)	Age, race, education, BMI, smoking, alcohol, history of ovarian/breast cancer, OC use, parity, MS, HRT, and study site.
Song et al., 2008 [75]	US	Epithelial ovarian	PB case-control	2050 3	781	35–74	F (100)	FFQ	SB [3] (caff): ≥720 mL/day vs. none SB [3] (not caff): ≥720 mL/day vs. none	OR: 1.51 (1.03–2.22) OR: 2.60 (1.25–5.39)	Age, BMI, education, smoking, race, country, years of diagnosis, number of pregnancies, OC use, hysterectomy, and family history of breast/ovarian cancer.
Hepatobiliary Cancers (Biliary Tract, Gallbladder, Liver)											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake [+]**	**HR/RR/OR (95% CI)**	**Adjustments**
Stepien et al., 2014 [28]	10 European countries [‡], EPIC	Biliary tract IHBT HCC	Cohort	477,206 11.4	236 66 191	51	F (70)	DQ-country specific	SB: 282.9 mL/day vs. none FJ [1]: 171.7 mL/day vs. none SB: 282.9 mL/day vs. none FJ [1]: 171.7 mL/day vs. none SB: 282.9 mL/day vs. none SB: increase by 300 mL/wk SSB: increase by 330 mL/wk ASB: increase by 330 mL/wk FJ [1]: 171.4 mL/day vs. none FJ [1]: increase by 200 mL/wk	HR: 0.96 (0.90–1.00) HR: 0.99 (0.95–1.03) HR: 0.97 (0.90–1.06) HR: 1.04 (1.00–1.08) HR: 1.83 (1.11–3.02) HR: 1.05 (1.02–1.07) HR: 1.00 (0.95–1.06) HR: 1.06 (1.03–1.09) HR: 1.38 (0.80–2.38) HR: 1.03 (1.01–1.06)	BMI, alcohol, EI, PA, DM, and education.
Larsson et al., 2016 [49]	Sweden, SMC, COSM	IHBT EHBT Gallbladder	Cohort	70,832 13.4	21 127 71	45–83	M (56)	96-item FFQ	SB: ≥400 mL/day vs. none SB: ≥400 mL/day vs. none SB: ≥400 mL/day vs. none	HR: 1.69 (0.41–7.03) HR: 1.79 (1.02–3.13) HR: 2.24 (1.02–4.89)	Age, sex, education, smoking, BMI, dietary protein intake, and EI.
Hematologic Cancers (Leukemia, Lymphoma, Myeloma)											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake [+]**	**HR/RR/OR (95% CI)**	**Adjustments**
Schernhammer et al., 2012 [24]	US, HPFS, NHS	Leukemia Multiple myeloma NHL	Cohort	136,587 14 HPFS 20 NHS	339 285 1324	53.7	F (65)	FFQ	SSB: ≥335 mL/day vs. none ASB: ≥335 mL/day vs. none SSB: ≥335 mL/day vs. none ASB: ≥335 mL/day vs. none SSB: ≥335 mL/day vs. none ASB: ≥335 mL/day vs. none	RR: 1.06 (0.56–2.00) RR: 1.42 (1.00–2.02) RR: 1.47 (0.76–2.83) RR: 1.29 (0.89–1.89) RR: 1.34 (0.98–1.83) RR: 1.13 (0.94–1.34)	Age, BMI, EI, PA, alcohol, race, fruit and vegetables consumption, menopause, and HT. SSB were adjusted for use of ASB and vice-versa.
McCullough et al., 2014 [40]	US, CPS-II NCH	NHL	Cohort	100,442 10	1196	47–95	F (57)	Willett FFQ	ASB: >355 mL/day vs. none SSB: >355 mL/day vs. none	RR: 0.92 (0.73–1.17) RR: 1.10 (0.77–1.58)	Education, race, WC, PA, BMI, EI, DM, family history of cancer, HTR and NSAIDs use, cholesterol-lowering medication, intake of alcohol, read and processed meat, milk, saturated fat, fruits and vegetables, and tea and coffee.

Table 1. *Cont.*

Upper Aerodigestive Cancers (Larynx, Oral Cavity, Oropharyngeal Squamous Cell, Pharynx)											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Zvrko et al., 2008 [82]	Montenegro	Larynx	HB case-control	216 2	108	59.9 (9.7)	M (82)	DQ	SB: yes vs. no	OR: 0.38 (0.16–0.92)	Age, sex, smoking, alcohol, coffee, diet, personal and familiar medical history, education, housing and work conditions, and exposure to toxic components.
Ren et al., 2010 [34]	US, NIH-AARP-DHS	Larynx Pharynx Oral cavity	Cohort	481,563 2	307 178 391	50–71	M (59)	124-item FFQ	SB: ≥355 vs. ≤355 mL/day SB: ≥355 vs. ≤355 mL/day SB: ≥355 vs. ≤355 mL/day	HR: 0.82 (0.55–1.23) HR: 0.76 (0.46–1.25) HR: 0.77 (0.54–1.09)	Age, sex, smoking, alcohol drinking, BMI, EI, education, ethnicity, PA, intake of fruit, vegetables, and red and white meat.
Lissowska et al., 2003 [83]	Poland	Oral cavity	HB case-control	246	122	23–80	M (64)	25-item DQ	FJ: >57 vs. <28.6 mL/day	OR: 0.35 (0.15–0.80)	Age, sex, residence, drinking, and smoking habit.
Kreimer et al., 2006 [84]	9 countries ‡, IARC-MOCS	OOSC	HB case-control	3402	1670	NR	M/F	FFQ	FJ: height vs. low intake	OR: 0.8 (0.6–1.1)	Age, sex, country, education, BMI, smoking, chewing, and alcohol.
Other Cancers											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Vincenti et al., 2008 [85]	Italy	Cutaneous melanoma	PB case-control	118	59	56	F (53)	188-item FFQ	FJ (no OJ): increase by 10 mL/day OJ: increase by 10 mL/day	RR: 0.95 (0.87–1.03) RR: 0.94 (0.88–1.00)	EI, family history of melanoma, skin type, history of sunlight exposure, and sunburns.
Dubrow et al., 2012 [47]	US	Glioma	Cohort	545,771 10	904	62.8 (median)	M (60)	FFQ	SB: >720 mL/day vs. none	HR: 0.87 (0.65–1.15)	Age, sex, race, EI, height, fruit and vegetables intake, and nitrite intake from plants
Luqman et al., 2014 [86]	Pakistan	Lung	HB case-control	1200	400	<40–>70	M (73)	DQ	J: yes vs. no	OR: 0.3 (0.3–0.4)	Not reported
Wu A. et al., 1997 [87]	US	Small intestine	PB case-control	1034	36	30–65	M (69)	Interview	SSB [7]: daily vs. never	OR: 3.6 (1.3–9.8)	Age, ethnicity, and sex.
Zamora-Ros et al., 2018 [48]	10 European countries ‡, EPIC	Thyroid	Cohort	477,206 11.4	748	51	F (70)	DQ- country specific	FJ [1]: > 94 vs. < 1 mL/day FJ [1]: increase by 50 mL/day	HR: 1.23 (0.98–1.53) HR: 1.02 (0.99–1.06)	Age, sex, smoking status, BMI, EI, alcohol, PA, education, center, menopausal status and type, OC use, and infertility problems.
Overall Cancers											
Source	**Country, Study Name**	**Cancer Type**	**Study Design**	**Population Follow-Up (Years)**	**Cases**	**Age (Mean/SD or Range)**	**Sex (%)**	**Dietary Assessment Method**	**Type and Amount of Beverages Intake +**	**HR/RR/OR (95% CI)**	**Adjustments**
Bassett et al., 2020 [50]	Australia, MCCS	Non-obesity related *	Cohort	35,109 19	4789	27–76	F (61)	121-item FFQ	SSB: >375 vs. none or < 12.5 mL/day ASB: >375 vs. none or < 12.5 mL/day	HR: 1.02 (0.86–1.21) HR: 1.23 (1.02–1.48)	Alcohol, country of birth, Med-diet score, PA, socio-economic position, sex, and smoking. ASB also adjusted for SSB intake.

Table 1. *Cont.*

Makarem et al., 2018 [52]	US	Breast, Colorectal, Prostate	Cohort	3184 4	565	54.3	F (53)	FFQ	SFJ: >501 vs. <73.2 mL/day SSB:>180 mL/day vs. none FJ: >216 vs. <23 mL/day (cut-off)	HR: 1.28 (0.97–1.70) HR: 1.00 (0.79–1.27) HR: 1.05 (0.80–1.38)	Age, sex, EI, alcohol, smoking, and BMI.
Hodge et al., 2018 [54]	Australia, MCCS	Obesity-related	Cohort	35,593 19	3283	54.6	F (100)	121-item FFQ	SSB: ≥200 vs. <6.7 mL/day ASB: ≥200 vs. <6.7 mL/day	HR: 1.14 (0.93–1.39) HR: 1.00 (0.79–1.27)	Socioeconomic indexes, country of birth, alcohol, smoking, PA, Med-diet score, and sex. ASB also for SSB consumption and WC.
Chazelas et al., 2019 [23]	France, NNS	Breast, Colorectal, Prostate	Cohort	101,257 5.1 (median)	2193	42.2/14.4	F (78)	24H-DR	SFJ: >141.7 vs. <46.1 mL/day (cut-off) SFJ: increase by 100 mL/day SSB: >65.5 vs. <14.0 mL/day (cut-off) SSB: increase by 100 mL/day ASB: >7.9 vs. <2.7 mL/day (cut-off) ASB: increase by 10 mL/day FJ: >97.8 vs. <19.9 mL/day (cut-off) FJ: increase by 100 mL/day	HR: 1.30 (1.17–1.52) HR: 1.18 (1.10–1.27) HR: 1.06 (1.02–1.21) HR: 1.19 (1.08–1.32) HR: 1.00 (0.84–1.19) HR: 1.02 (0.94–1.10) HR: 1.14 (1.01–1.29) HR: 1.12 (1.03–1.23)	Smoking, education, PA, BMI, and height.

[+] Expressed in milliliter (mL) per day (d) or week (wk) or none (nonconsumers). [†] Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, The Netherlands, and the United Kingdom. [‡] Italy, Spain, Poland, Northern Ireland, India, Cuba, Canada, Australia, and Sudan. * All identified cancers except esophagus (adenocarcinoma), pancreas, colorectum, breast (post-menopausal), endometrium, kidney, ovary, gallbladder, liver, gastric cardia, meningioma, thyroid, multiple myeloma. [1]: Fruit juice and vegetables juice. Vegetables juice <2%. [2]: Colas. [3]: Colas and root beer. [4]: Not carbonated beverages. [5]: Sugarcane juice (20.3%), honeydew melon juice (14.1%), apple juice (12.8%), watermelon juice (9%), carrot juice (9%), pineapple juice (6.4%), star fruit juice (5.1%), and lemon juice drink (5.1%). The remaining canned grape, tomato, and prune juice, along with papaya, plum, and fresh celery juice, each comprised 1.3–2.6% of the total juice consumption reported. [6]: Fruit juice and nectars. [7]: Carbonated beverages. AA: African American; AEGJ: adenocarcinoma of the esophagus-gastric junction; ASB: artificially sweetened beverages; BC: breast cancer; BF: breastfeeding; BMI: body mass index; BWHS: Black Women's Health Study; Caff: caffeinated; CI: confidence interval; COSM: Cohort of Swedish Men; CPS-NCS: Cancer and Prevention Study, Nutrition Cohort Study; CR: colorectal; CVD: cardiovascular disease; EA: European American; EAC: esophageal adenocarcinoma; EI: energy intake; EPIC: European Prospective Investigation into Cancer and nutrition; DH: diet history; DM: diabetes mellitus; DQ: dietary questionnaire; 24H-DR: 24 h dietary recall; F: female; FFQ: food frequency questionnaire; FJ: natural fruit juice; GI: glycemic index; HB: hospital-based; HCC: Hepatocellular Carcinoma; HCS: Hokkaido Cohort Study; HPFS: Health Professionals Follow-up Study; HPV: Human Papilloma Virus; HR: hazard ratio; HRT: hormone replacement therapy; HT: hypertension; IARC-MOCS: International Agency for Research on Cancer, Multicenter Oral Cancer Study; IHBT: intrahepatic biliary tract; J: natural fruit and vegetable juice; M: male; MCCS: Melbourne Collaborative Cohort Study; MDC: Malmö Diet and Cancer; Med: Mediterranean; MS: menopausal status; NCFD: not carbonated fruit drinks; NCSC: Nutrition Canada Survey Study; NHL: non-Hodgkin lymphoma; NNS: Nutri Net-Santé; NIH-AARP-DHS: National Institute of Health-American Association of Retired Persons, Diet and Health Study; NSAIDs: nonsteroidal anti-inflammatory drugs; NHS: Nurses' Health Study; OC: oral contraceptive; OJ: orange juice; OOSC: oral and oropharyngeal squamous cell; OR: odds ratio; PA: physical activity; PB: population-based; PC: prostate cancer; Post-M: post-menopausal breast cancer; PSA: prostate-specific antigen; Pre-M: pre-menopausal breast cancer; Q: quantitative; Q1: first quartile; Q4: quartile four; RR: relative risk; SB: total sweetened beverages, sugar and artificially sweetened beverages; SCC: squamous cell carcinoma SCHS: the Singapore Chinese Health Study; SD: standard deviation; SFQ: structured food questionnaire; SFB: San Francisco Bay Study: SFJ: beverages high in sugar, added or natural, SSB + FJ; SSB: sugar-sweetened beverages; SMC: Swedish Mammography Cohort; SQ: semiqualitative; SUVIMAX: Supplementation en Vitamines et Mineraux Antioxydants Study; UBC: urinary bladder cancer; UP: ultraprocessed; US: the United States; WC: waist circumference; WCHS: Women's Circle of Health Study.

Table 2. Summary of the results of the meta-analysis (random effects model).

Cancer Type	Exposure	N° of Studies		RR (95% CI)	I^2 (%)	Tau^2	*p* within Group +	95% PI
		Cohort	Case-Control					
Breast	SSB	4	3	1.14 (1.01−1.30)	0.0	0.0073	0.69	0.88, 1.47
Breast	FJ	3	0	1.13 (0.93−1.38)	0.0	0.0017	0.79	0.52, 2.46
Breast Pre-M	SSB	3	2	1.37 (0.99−1.88)	55.7	0.0358	0.06	0.68, 2.76
Breast Post-M	SSB	4	2	1.18 (0.79−1.75)	54.8	0.1080	0.05	0.43, 3.23
Colorectal	SSB	4	0	1.18 (0.99−1.41)	0.0	0.0039	0.71	0.82, 1.69
Colorectal	FJ	2	2	0.79 (0.16−3.87)	88.5	0.8629	<0.001	0.008, 73.94
Colorectal *	FJ	2	1	1.29 (0.78−2.12)	0.0	0.0120	0.63	0.17, 9.81
Colorectal	SB	0	3	2.02 (0.45−9.01)	62.9	0.2711	0.07	0.00, 5753.1
Colorectal *	SB	0	2	1.57 (0.74−3.35)	0.0	0.0010	0.67	–
Bladder	SB	0	5	1.66 (0.78−3.56)	83.4	0.3226	<0.001	0.22, 12.37
Bladder *	SB	0	4	1.27 (0.85−1.90)	25.3	0.0425	0.26	0.45, 3.60
Prostate	SSB	5	0	1.18 (1.10−1.27)	0.0	0.0012	0.92	1.03, 1.35
Prostate	FJ	4	0	1.03 (1.01−1.05)	0.0	0.0001	0.93	0.98, 1.09
Prostate	SB	1	2	0.97 (0.56−1.69)	2.9	0.0241	0.36	0.07, 12.7
Renal cell	SB	1	2	1.44 (0.46−4.50)	65.4	0.1559	0.056	0.00, 604.16
Pancreatic	SB	4	4	1.28 (0.95−1.72)	58.6	0.0962	0.02	0.56, 2.90
Pancreatic	SSB	4	2	1.01 (0.92−1.11)	0.0	0.0016	0.92	0.87, 1.17
Pancreatic	ASB	3	2	1.07 (0.77−1.48)	43.6	0.0480	0.13	0.48, 2.36

* Results excluding outliers; + *p* values of Cochran's Q-test heterogeneity. ASB: artificial sweetened beverage; FJ: fruit juice; PI: prediction intervals; Post-M: post-menopausal; Pre-M: pre-menopausal; RR: risk ratio; CI: confidence interval; SB: sweetened beverage (including both SSBs and ASBs); SSB: sugar-sweetened beverage.

3.2. Sweet Beverages and Risk of Breast Cancer

Nine publications reported data on breast cancer, four case-control [57,58,69,80] and five cohort studies [23,51–53,55]. In the meta-analysis with six publications, including four cohort studies [23,52,53,55] and two case-controls [57,80], a significant positive association between high SSB consumption and breast cancer risk was observed (RR: 1.14, 95% CI: 1.0–1.3) (Table 2). No associations were found for FJ intake (Table 2). Marzbani et al. [58] reported a positive association with SBs (OR: 2.8, 95% CI: 1.9–4.3), but no associations were found for ASBs. Subgroup analyses for SSB consumption did not explain further heterogeneity (Table S1).

3.2.1. Sweet Beverages and Risk of Pre-Menopausal Breast Cancer

Three cohort publications [23,53,55] and one case-control (taken as two as indices were separated by ethnicity) [57] were included in the analysis of SSB intake and pre-menopausal breast cancer. Their pooled analysis showed a borderline statistically non-significant positive association (RR: 1.37, 95% CI: 0.99–1.88) (Figure S1), which reached the significance in the subgroup analysis including only cohort studies (RR: 1.60, 95% CI: 1.08–2.37) (Table S1). A cohort study from 2019 [23] also reported data for ASB, FJ and SFJ intake and only indicated a positive association for SFJs (HR: 1.28, 95% CI: 1.09–1.83).

3.2.2. Sweet Beverages and Risk of Post-Menopausal Breast Cancer

A meta-analysis of four cohort studies [23,53–55] and one case-control (taken as two as indices were separated by ethnicity) [57] of SSBs showed non-significant results (Table 2). We performed subgroup analyses based on study design, country, and beverage intake categories. No statistically significant results were found from the heterogeneity test between groups (Table S1). Chazelas et al. [23] investigated the relationship with SFJ consumption and observed a positive association (HR: 1.44, 95% CI: 1.05–1.99). No significant results were reported for ASBs.

3.3. Sweet Beverages and Risk of Intestinal and Colorectal Cancer

Eight publications reported data on colorectal cancer, four case control [88–91] and four cohort studies [23,52,54,56]. A borderline positive association was observed with SSB intake using the random-effect model (RR: 1.18, 95% CI: 0.99−1.41) (Figure S1). No significant results were found either for SBs or for FJs (RR: 2.02, 95% CI: 0.45−9.01 (SB); RR: 0.79, 95% CI: 0.16−3.87 (FJ) (Figure S2). After the exclusion of one outlier, results for the random-effect model remained non-significant. No associations were found for colorectal cancer risk and ASBs. With regard to rectal cancer, no associations were observed with ASBs, SSBs or fruit and vegetables juices [92]. A case-control study on small intestine cancer [65] indicated a significant positive association with SSB consumption (OR: 3.6, 95% CI: 1.3−9.8).

3.4. Sweet Beverages and Risk of Esophageal Cancer

Three publications, one cohort [34] and two case-control studies [59,93] reported data on different types of esophageal cancers, including esophagus-gastric junction, esophageal adenocarcinoma and squamous cell carcinoma. No significant associations were shown between SB, SSB and ASB consumption and esophageal cancers risk.

3.5. Sweet Beverages and Risk of Gastric Cancer

One case-control [59] and two cohort studies [34,54] reported data on different types of gastric cancer (overall, cardia and non-cardia) and SBs, ASBs or SSBs showing no significant associations.

3.6. Sweet Beverages and Risk of Pancreatic Cancer

Eleven publications, six cohort [41–45] and five case-control studies [76–79,81] reported data on pancreatic cancer. No significant results were observed for SBs, SSBs or ASBs (Table 2). Although high heterogeneity was observed for SBs (I^2 = 58.6, p = 0.02) and ASBs (I^2 = 43.6, p = 0.13) (Table 2), after performing subgroup analyses results slightly improved but remained non-significant (Table S1). No association was observed between FJ intake and pancreatic cancer risk.

3.7. Sweet Beverages and Risk of Genitourinary Cancer

3.7.1. Bladder

Six case-control studies [60–65] reported data on bladder cancer. No association between SB consumption and bladder cancer risk was observed in the random-effect meta-analysis including five case-control studies [61–65] (Figure S2). We observed a high heterogeneity in the meta-analysis (I^2 = 83.4%, p = 0.0001). Although heterogeneity was reduced after excluding outliers and doing subgroup analyses, the associations were positive but non-significant (Table S1). A US study suggested a statistically significant relation between SB intake and bladder cancer risk [65]. Two case-control studies [60,65] also considered SSBs and ASBs separately. In a Chinese case-control study [62], SSB intake was suggested as a risk factor for bladder cancer, although no association was found for FJs. Similarly, in a Serbian study [63], no significant association was observed between FJs and bladder cancer risk.

3.7.2. Prostate

Eight publications, six cohorts [23,31,35,36,52,54] and two case-controls [66,67] showed data on prostate cancer. No significant associations were reported for SBs from quantitative analysis. However, positive relations were observed in the random-effect model for SSBs (RR: 1.18, 95% CI: 1.10−1.27) and FJs (RR: 1.03, 95% CI: 1.01−1.05). The results remained the same in a subgroup analysis with 3 non-US (France, Spain, Australia) studies (RR: 1.13, 95% CI: 1.03−1.24) (Table S1). Two cohorts [23,54] reported data on ASB intake and only one [23] found an increased prostate cancer risk of 33% (HR: 1.33, 95% CI: 1.01−1.75).

3.7.3. Renal and Urothelial Cell Cancer

Four publications, two case control [68,70] and two cohort studies [37,54] provided data on renal cell cancer. For our meta-analysis, we selected three publications, two case-control [68,70] and one control study [37] on SBs, but the random-effect meta-analysis showed non-significant results (Table 2). Despite observing a high heterogeneity (I^2 = 65.4%, *p*-value = 0.058), no outliers were found, and the number of studies was too low to perform subgroup analyses (n = 3). One case control study [70] reported a positive association with the intake of ASBs (OR: 2.7, 95% CI: 1.1−6.5) but not the other two [37,54]. No significant results were reported for SSBs or FJs, despite one case-control [68] finding a positive association with the consumption of fruit and vegetable juices taken together (OR: 1.53, 95% CI: 1.18−1.99). The EPIC cohort study [38] reported data on urothelial cell cancer and its association with SBs and FJs. A significant positive association was found only with FJ intake (HR: 1.32, 95% CI: 1.05−1.66).

3.8. Sweet Beverages and Risk of Gynecological Cancers

Two case-control studies [71,72] investigated the relationship between FJ intake and cervical cancer risk. Only one of them [72] found an inverse association (RR: 0.3, 95% CI: 0.2−0.6). Two cohort studies [39,54] reported data on different types of beverages (SSBs, ASBs, FJs and SFJs) and endometrial cancer risk. Only one of them [39] found significant positive associations with both SSBs (HR: 1.78, 95% CI: 1.32−2.40) and SFJs (HR: 1.48, 95% CI: 1.09−2.00). Finally, three case-control studies [69–71] reported data on epithelial ovarian cancer risk. Only one of them [71] found positive associations for caffeinated (OR: 1.51, 95% CI: 1.03−2.22) and non-caffeinated SBs (OR: 2.60, 95% CI: 1.25−5.36). No significant associations were reported for ovarian cancer risk [50].

3.9. Sweet Beverages and Risk of Hepatobiliary Cancers

Two cohort studies [28,49] reported data on different types of sweet beverages and various types of hepatobiliary cancers. The EPIC cohort [28] found no significant results regarding the consumption of either SBs or FJs and biliary tract cancer risk. However, a positive association was observed between both SBs (HR: 1.89, 95% CI: 1.11−3.02) and FJs (RR: 1.03, 95% CI: 1.01−1.06) and hepatocellular carcinoma risk. The Swedish Mammography Cohort and the Cohort of Swedish Men [49] found significant positive associations with both gallbladder (HR: 2.24, 95% CI: 1.02−4.89) and extrahepatic biliary tract cancer risks (HR: 1.79, 95% CI: 1.02−3.13). No significant results were reported for intrahepatic biliary tract cancer risk.

3.10. Sweet Beverages and Risk of Hematologic Cancers

One cohort study [24] reported data on leukemia and multiple myeloma and its association with SSB and ASB intake. Significant associations were found between the consumption of ASBs and leukemia risk (RR: 1.42, 95% CI: 1.00−2.02). No associations were observed in two cohorts [24,40] as regards SSBs or ASBs and non-Hodgkin lymphoma risk.

3.11. Sweet Beverages and Risk of Upper Aerodigestive Cancers

Four studies [34,82–84] reported data on upper aerodigestive cancers. One US-based cohort [34] showed no significant association between SB intake and pharyngeal, laryngeal and oral cavity cancer risks. A case-control study from Montenegro [82] suggested an inverse relation between SBs and larynx cancer risk. The consumption of FJs was inversely associated with oral cavity cancer risk in one case-control study [83] though not in another [84].

3.12. Sweet Beverages and Risk of Other Cancers

Single studies reported data on different types of cancer and their link with sweet beverages. No significant associations were reported for cutaneous melanoma [85], glioma [47] or thyroid cancer risk [48] and any type of sweetened beverages. One case-control study [86] reported an inverse association between natural juices (fruit and vegetables) and lung cancer risk (OR: 0.3, 95% CI: 0.3−0.4).

3.13. Sweet Beverages and Risk of Overall Cancer

An Australian cohort [50] investigated the association between SSBs and ASBs and the risk of non-obesity-related cancers; they reported a positive association only with ASBs (HR: 1.23, 95% CI: 1.02−1.48). Two cohorts [23,52] assessed the relationships between the intake of several types of sweet beverages and obesity-related cancer risk. Only one of them [23] showed positive associations with SSBs (HR: 1.06; 95% CI: 1.02−1.21), FJs (HR: 1.14, 95% CI: 1.01−1.29) and SFJs (HR: 1.30, 95% CI: 1.17−1.52). No association was found for ASBs and obesity-related cancer risk.

3.14. Quality of Included Studies

According to the ROBINS-E tool (Figure 2a, Table S2), 13 of 27 cohort studies presented a moderate overall risk of bias. This is due to some bias being detected mostly in the classification of the exposure domain, deviation from the intended intervention and missing data. Missing data bias was not evaluated for 5 cohorts [36,39,43,51,52], as the publications did not report enough information. All studies fulfilled the criteria of low risk of bias for selection of participants' domain. In addition, 3 [36,37,54] of 27 studies did not adjust the statistical analysis for all potential confounders. Therefore, they were classified at moderate risk of bias. Only one study [50] was classified as moderate risk of bias for outcome measurement, and another [56] for the selection of reported outcomes.

According to the NOS (Figure 2b, Table S3) most of the case-control studies (29 of 37) presented a moderate overall risk of bias; 7 publications presented a serious risk, whereas 1 indicated a low risk. The risk of bias due to the selection of the groups was classified as moderate for 35 studies, high for 2 [58,82] and low for another 2 [59,66]. Most of the case-control studies adjusted their results for relevant and additional confounders and were classified as moderate or low risk of bias for comparability between groups. In addition, 5 were considered as serious risk for this domain, because 4 of them did not adjust for all important confounders [60,63,66,92] and 1 [79] reported results from an unadjusted analysis. Moreover, 5 studies [81,86,88,89] did not report this information and were classified as 'no information' category. The risk of bias due to ascertainment of the exposure was considered moderate in all case-control studies.

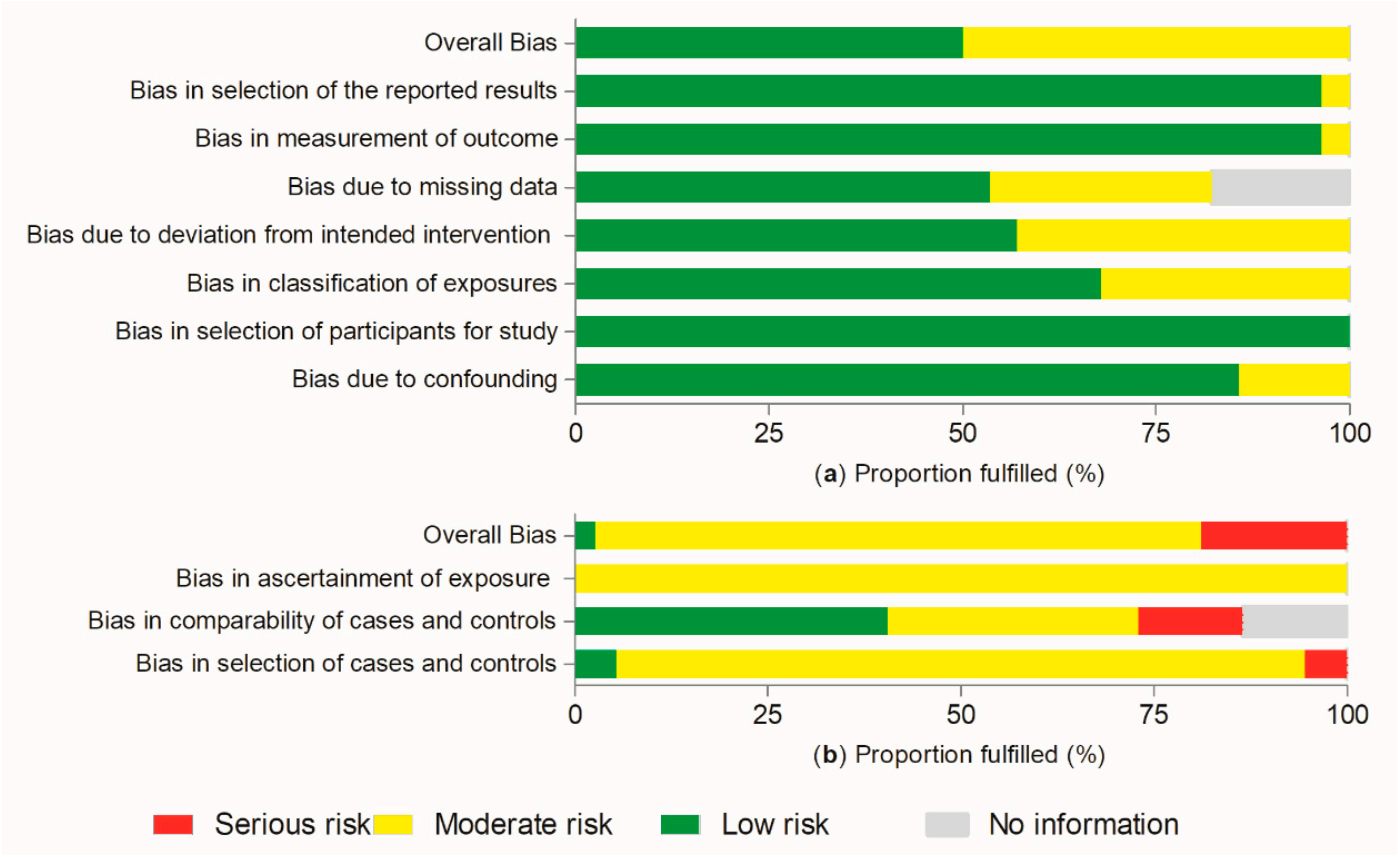


Figure 2. Risk of bias in the included studies. Legend: (**a**) risk of bias in cohort studies according to the Risk of Bias in Non-randomized Studies—of Exposures (ROBINS-E) tool and (**b**) risk of bias in case-control studies according to the Newcastle–Ottawa Scale (NOS).

4. Discussion

4.1. Association between Consumption of Sweet Beverages and Cancer Risk

The aim of this study was to assess the relationships between different groups of sweet beverages and site-specific or overall cancer risk. We conducted a meta-analysis when at least three studies reported data for the same exposure (sweet beverage type) and outcome (cancer site). We found several statistically significant and borderline positive associations between the consumption of SBs, especially SSBs, and in some cases ASBs or FJs, and several cancer risks.

Regarding breast cancer, the meta-analysis showed a positive association using random effects, with a 14% higher risk for SSBs, but non-statistically significant results for pre- and post-menopausal breast cancer. However, after performing subgroup analyses by study type, cohort studies showed significant positive results for pre-menopausal breast cancer and SSBs. Chazelas et al. [23] reported a positive linear trend between SSB intake and breast/pre-menopausal breast cancer risk when SSB consumption increased by 100 mL/day. In line with our results, current evidence supports the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) recommendations of reducing or avoiding SSB intake for breast cancer prevention [94]. One US case-control study [57] conducted a separate analysis for African-American and European-American women. This showed a positive link between SSB intake and post-menopausal breast cancer risk for European-American women only. Likewise, two other cohorts that included mostly Caucasian women [53,54] showed similar results. This evidence suggests that ethnic differences may play a role. However, we could not explore this association as no other studies included women of African descent. In fact, evidence on the role of nutritional factors in breast cancer for this population is limited and inconclusive [95]. Our

meta-analysis did not find significant associations between FJs and breast cancer risk. With regards to the SFJ group, comparing highest versus lowest consumption, Chazelas et al. [23] reported positive relations for SFJs and total, pre- and post-menopausal breast cancer risk. Conversely, Makarem et al. [52] showed no significant associations. A publication from the US [69] found no positive associations for SBs and breast cancer risk; however, a recent case-control study [58] found positive associations.

For colorectal cancer risk, our meta-analysis found no positive results using random effects for SB, SSB or FJ intake. Despite having performed secondary analyses excluding outliers and having explained between-studies heterogeneity, results for the random-effect model remained non-significant. This is in a way consistent with results from a previous meta-analysis, which found no association between SSBs and colon cancer risk using a random-effects model [21]. On the other hand, a cohort study from 2014 found a positive association for an increase in 330 mL/day of SSBs [91]. Likewise, an Australian study that compared extreme categories of SSB intake ($\geq$200 mL/day versus <6.7 mL/day) showed positive results [54]. We included only one study assessing rectal cancer incidence [92]. Here, a separate analysis for women and men was performed. The majority of the results were not significant, and the only positive association was found for juice (fruit and vegetables) consumption in female participants.

In regard to esophageal cancers, publications included in this review were also part of a meta-analysis from 2014 [18]. This meta-analysis reported no association between SBs and esophageal adenocarcinoma and squamous cell carcinoma risk. After extracting separated data for SSB and ASB intake, we found similar results. Despite these observations, positive associations were found in a pooled analysis of US-based case-control studies. This study assessed the association between sugar dietary intake and Barret's esophagus incidence, a precursor for esophageal adenocarcinoma tumor [96]. Even though data from the included studies reported non-significant results for stomach cancer incidence, a Japanese cohort study observed that carbonated drinks and juices appeared to be related to an elevated risk of death from stomach cancer [97].

With respect to pancreatic cancer, we performed a meta-analysis for SBs, SSBs and ASBs. These associations, especially for SBs, tended to be positive but did not reach statistically significant levels using random effect models. These results go along with a recent meta-analysis from 2019 [19] which also showed no association between SB intake and pancreatic cancer risk. Besides that, a pooled analysis from 2012 [20] reported a 56% higher risk of pancreatic cancer for males consuming $\geq$375 mL/day of SSBs compared to non-consumers. Likewise, a Swedish cohort [41] found a 93% higher risk of pancreatic cancer incidence among those who consumed $\geq$500 mL/day of SSBs compared to non-consumers. However, we performed a subgroup analysis taking into account beverage intake category (high vs. non- consumer), but no significant associations were observed (Table S1). In addition, only one study reported separate results for carbonated and noncarbonated SBs, but no significant results were shown [76].

For bladder cancer risk, 3 out of the 6 included case-control studies [62,63,65] showed positive associations for highest versus lowest amounts of SB intake. However, the meta-analysis of these studies together with 2 other case-control studies [61,64] showed no significant associations. Despite performing a second analysis excluding one study that presented some serious bias, the results remained non-significant (Table S1). Hence, our meta-analysis of observational studies reported that SBs appeared to be unrelated to bladder cancer risk. It is not clear how SSBs, ASBs or FJs act in isolation as the evidence is limited.

With reference to prostate cancer, our meta-analysis demonstrated an 18% higher risk for SSBs comparing the highest with the lowest intake. Similarly, we found a small positive association for FJs (a 3% higher risk). No associations were found for SBs, which may suggest that the role of ASBs might not be relevant. However, one study [23] reported a positive association between ASB intake and prostate cancer risk.

Renal cell cancer appeared to be unrelated to SB consumption according to the meta-analysis results. We observed a high between-study heterogeneity (I^2= 65.4%). However, not enough studies (n = 3) were included to perform subgroup analyses. Even so, Maclure and Willet [70] reported a significant positive association between highest versus lowest SB intake and renal cell cancer risk (RR: 2.6, 95% CI: 1.4−4.8). More studies analyzing this association are required for further clarification.

The association between SSB consumption and both endometrial and ovarian cancer risk tended to be positive but did not reach statistically significant levels. One study stratified results by types of endometrial cancer (I and II) [39]. They reported positive associations between highest versus lowest SSB and SFJ consumption and endometrial type I cancer in post-menopausal women, but not in type II. These might be because subtypes may have different risk factors, even though evidence on this etiologic heterogeneity is quite limited [98]. Data from two studies [71,72] suggested that FJ intake might be a protective factor for cervical cancer. FJ consumption is often considered part of a healthy diet and lifestyle [99]. However, none of the mentioned studies [71,72] adjusted for such confounders. Thus, it is not clear if the protective effect was due to FJ intake or other factors. For epithelial ovarian cancer, one US study [75] stratified the results by caffeinated and non-caffeinated colas. Both results were positive statistically significant, but non-caffeinated colas showed a stronger association. Although this might suggest a protective effect of caffeine, a recent meta-analysis of prospective studies found no link between caffeine intake and ovarian cancer risk [100].

In respect of hepatobiliary cancers, data from the included studies showed a positive association with SB consumption, especially for gallbladder cancer, where the risk was doubled [49]. This might be explained by the detrimental association between sucrose/glycemic load and the increased risk of symptomatic gallstone disease [101], which is strongly correlated with gallbladder cancer [102]. Stepien et al. [28] showed slightly positive dose–response associations between SBs, ASBs or FJs and HCC incidence.

As regards hematologic cancers, no associations were found either for sugary or for artificially sweetened beverages, except for leukemia risk, for which one study [24] reported significant positive associations with ASBs. However, a recent review of clinical trials and observational studies observed no association between artificial sweeteners intake and both leukemia and non-Hodgkin lymphoma incidence [103].

The evidence is more limited regarding cancer of the oral cavity, pharynx, larynx, lung, thyroid, glioma and cutaneous myeloma. The available data mainly showed nonsignificant results for SB and FJ intake. Only one study from Montenegro indicated an inverse association between SB intake and laryngeal cancer risk [82]. However, the results from this study should be treated with caution as they presented some methodological inadequacies and its overall risk of bias was classified as 'serious risk' (Table S3). One case-control study from 1997 observed a strong positive association between small intestine cancer risk and SSBs (OR: 3.6, 95% CI: 1.3−9.8), although further high quality evidence is needed [87]. One study that reported incidence of overall non-obesity-related cancers showed no association for SSBs but a positive association for ASBs [50]. Moreover, the largest of 3 studies [23,52,54] on overall obesity-related cancer risk showed positive associations with SFJs, SSBs and FJs, but not with ASB consumption [23]. Similarly, a meta-analysis of clinical trials and observational studies showed no association between artificial sweetener intake, body weight and different types of cancers [103]. Our findings are in accordance and we agree with the previous study [103] upon the uncertainty of the evidence that links artificial sweeteners with different types of cancer.

4.2. Limitations of the Current Data

To the best of our knowledge, this is the first systematic review to evaluate the isolated association between different groups of sweet beverages and cancer risk. Several limitations should be considered while interpreting our findings. Some studies included in this systematic review were difficult to compare due to their design (cohort and case-

control studies), methodology, classification and categories of beverages intake. Therefore, it was a challenge to perform such comparisons. According to the ROBIN-E tool, cohort studies were at low-moderate risk of bias. As per the NOS, the case-control studies were at moderate risk of bias and 6 studies [58,60,66,79,82,86] out of 37 presented serious methodology inadequacies. The number of publications included in most meta-analysis was relatively low (between 3 and 6). On this basis, the pooled effect size was calculated based on risk ratios of cohort and case-control studies together. Not having enough studies was a major limitation to perform subgroup analyses when high between-study heterogeneity was observed. Moreover, the small amount of studies may have been a potential source of unexplained heterogeneity [104]. We did not have enough data to perform subgroup analyses based on different population characteristics (e.g., sex, lifestyle factors or history of cancer). However, we did perform subgroup analyses based on geographical area.

The majority of the included participants were from the US or European countries. Hence, extrapolating our findings to other geographical areas may not be appropriate. We attempted to classify beverages into specific groups. However, some studies did not report precise information on this topic, which might have given rise to misclassifications. Similarly, we attempted to convert original exposure information into amounts of intake (mL/day) based on national data. Nevertheless, this was not possible in all studies which prohibited performance of a dose–response meta-analysis. Another limitation may be the measurement error in collecting dietary data since self-reported questionnaires were used. Moreover, in the longitudinal studies we were limited to the baseline estimation of beverages consumption, and there is a possibility that their consumption changed over time. It is suggested that the link between SSBs or FJs and cancer risk is possible due to their high glycemic indexes [13] and to obesity-inducing pathways [4]. However, these variables were not adequately integrated as confounders in all the studies. Indeed, glycemic index was only considered in one cohort [53]. Despite BMI being a common indicator of obesity and most studies considering it as confounder, only 4 of them [35,40,52,54] adjusted for other obesity indicators such as waist circumference. Most of the studies assessed the association between consumption of SSB and common cancers such as breast, colorectal, prostate and pancreatic cancer. Data were more limited for FJs or ASBs and other types of cancers, especially non-obesity-related ones. FJ consumption may coexist with healthy habits, such as healthy diet or exercise [99]. Therefore, it would have been even better if some studies had adjusted their analysis for such variables. In fact, only 3 publications [52–54] used diet quality as a confounder.

5. Conclusions

The current meta-analysis of cohort and case-control studies indicated a statistically significant positive association between higher consumption of SSB and breast and prostate cancer incidence. As regard pre-menopausal breast cancer, results from cohort studies alone showed a significant association. Likewise, it showed a statistically significant positive link between high intakes of FJs and prostate cancer risk. Although the associations between other sweet beverages and other cancer types were also positive, they did not reach statistically significant levels. The small number of studies and cancer cases might have been a reason why we did not find statistically significant results for several cancer types. Study location (US/non-US, mostly European) did not appear to influence the results. Current evidence indicates that higher incidence of some cancers is related to a high consumption of SSBs. However, the evidence is limited to make recommendations regarding ASBs and FJs. This subject requires further investigation.

We encourage future research in this field to perform more separated analysis on SSB, ASB and FJ consumption. We believe it would be prudent to establish a homogeneous classification of beverages in order to better understand their role in carcinogenesis. We also recommend considering other obesity-related factors besides body mass index, such as waist circumference, glycemic index and quality of diet as confounders. We could not

study the different roles of non-carbonated soft drinks (sport, fruit and tea-based drinks), sometimes used as healthier alternatives to carbonated drinks [105]. Therefore, it would be advisable for future studies to further explore this research area.

This systematic review supports the WCRF/AICR recommendations to limit sugary drinks consumption for cancer prevention [106] and to raise consumers' awareness of their low nutritional quality and high sugar content. We recommend replacement of sweet beverages with plain safe drinking water and infusions without added sugars as the main liquid source for body hydration. Even though some guidelines maintain that moderate consumption of FJs may be part of a healthy diet [107], FJs contain little or no dietary fiber and are positively associated with tooth decay in children [108]. Professional societies have recently recommended limiting children's FJ consumption as means of addressing the obesity epidemic [3]. Whole fruits and plain safe drinking water should also be affirmed as a healthier alternative to sweet beverages in adults. This would aim to promote the appropriate consumption of essential nutrients, to reduce intake of excessive sugars/calories and to therefore lower cardiometabolic disease and cancer incidences [109,110]. The increase in cancer [25], obesity [111] and type 2 diabetes [112] requires policy action. We recommend policymakers worldwide to consider (or continue with) taxation and marketing restriction for sweet beverages, especially SSBs.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/2/516/s1, Table S1: Additional quantitative analysis for the association between sweet beverages and cancer risk; Table S2: Risk of bias in the included cohort studies according to Risk Of Bias In Non-randomized Studies—of Exposures (ROBINS-E); Table S3: Risk of bias in the included case-control studies, according to Newcastle–Ottawa Scale (NOS); Figure S1: Forest plot showing the pooled risk ratios with 95% CI for cancer risk, comparing the highest vs. the lowest sugar-sweetened beverages (SSB) intake category; Figure S2: Forest plot showing pooled risk ratios with 95% CI for cancer risk, comparing the highest vs. the lowest artificially and sugar-sweetened beverages (SB) intake category; Figure S3: Forest plot showing the pooled risk ratios with 95% CI for cancer risk, comparing the highest vs. the lowest fruit juice (FJ) intake category; Figure S4: Forest plot showing the pooled risk ratios with 95% CI for cancer risk, comparing the highest vs. the lowest artificially sweetened beverage (ASB) intake category.

Author Contributions: Conceptualization and design, R.Z.-R.; study selection, F.L., M.G.-L. and P.U.; data extraction, F.L., M.G.-L. and P.U.; data synthesis, F.L., M.G.-L. and P.U.; statistical analysis, I.d.V.; writing—original draft preparation, F.L., M.G.-L. and P.U.; writing—review and editing, J.C. and R.Z.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (cofounded by European Regional Development Fund. ERDF, a way to build Europe) and from the 201943-30-31 project, funded by La Marató de TV-3. We thank CERCA Program/Generalitat de Catalunya for the institutional support. M.G.-L. is grateful for the predoctoral scholarship PFIS (FI19/00185) from the Institute of Health Carlos III. J.C. is thankful for the CONACYT fellowship (ID 693636) from the Mexican Government. R.Z.-R. would like to thank the 'Miguel Servet' program (CP15/00100) from the Institute of Health Carlos III (cofounded by the European Social Fund (ESF)—ESF investing in your future).

Informed Consent Statement: Data was collected from available literature.

Data Availability Statement: Data for doing the meta-analysis is available in the manuscript's tables.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, interpretation of data; or in the writing and publishing of the manuscript.

References

1. Welsh, J.A.; Lundeen, E.A.; Stein, A.D. The sugar-sweetened beverage wars: Public health and the role of the beverage industry. *Curr. Opin. Endocrinol. Diabetes Obes.* **2013**, *20*, 401–406. [CrossRef] [PubMed]
2. Sylvetsky, A.C.; Jin, Y.; Clark, E.J.; Welsh, J.A.; Rother, K.I.; Talegawkar, S.A. Consumption of Low-Calorie Sweeteners among Children and Adults in the United States. *J. Acad. Nutr. Diet.* **2017**, *117*, 441–448. [CrossRef] [PubMed]
3. Wojcicki, J.M.; Heyman, M.B. Reducing childhood obesity by eliminating 100% fruit juice. *Am. J. Public Health* **2012**, *102*, 1630–1633. [CrossRef] [PubMed]
4. Te Morenga, L.; Simonette, M.; Jim, M. Dietary sugars and body weight: Systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ* **2013**, *346*, e7492. [CrossRef] [PubMed]
5. Imamura, F.; O'Connor, L.; Ye, Z.; Mursu, J.; Hayashino, Y.; Bhupathiraju, S.N.; Forouhi, N.G. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: Systematic review, meta-analysis, and estimation of population attributable fraction. *BMJ* **2015**, *351*. [CrossRef]
6. Friedman, F.; Mozaffarian, D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity A Comprehensive Review. *Circulation* **2016**, *133*, 187–225. [CrossRef]
7. Calle, E.E.; Kaaks, R. Overweight, obesity and cancer: Epidemiological evidence and proposed mechanisms. *Nat. Rev. Cancer* **2004**, *4*, 579–591. [CrossRef]
8. Giovannucci, E.; Harlan, D.M.; Archer, M.C.; Bergenstal, R.M.; Gapstur, S.M.; Habel, L.A.; Pollak, M.; Regensteiner, J.G.; Yee, D. Diabetes and Cancer: A consensus report. *Diabetes Care* **2010**, *33*, 1674–1685. [CrossRef] [PubMed]
9. Elliott, S.S.; Keim, N.L.; Stern, J.S.; Teff, K.; Havel, P.J. Fructose, weight gain, and the insulin resistance syndrome. *Am. J. Clin. Nutr.* **2002**, *76*, 911–933. [CrossRef]
10. Lauby-Secretan, B.; Scoccianti, C.; Loomis, D.; Grosse, Y.; Bianchini, F.; Straif, K. Body Fatness and Cancer—Viewpoint of the IARC Working Group. *N. Engl. J. Med.* **2016**, *375*, 794–798. [CrossRef]
11. Giovannucci, E. Insulin, insulin-like growth factors and colon cancer: A review of the evidence. *J. Nutr.* **2001**, *131*, 3109S–3120S. [CrossRef]
12. Atkinson, F.S.; Foster-Powell, K.; Brand-Miller, J.C. International tables of glycemic index and glycemic load values: 2008. *Diabetes Care* **2008**, *31*, 2281–2283. [CrossRef] [PubMed]
13. Sieri, S.; Krogh, V. Dietary glycemic index, glycemic load and cancer: An overview of the literature. *Nutr. Metab. Cardiovasc. Dis.* **2017**, *27*, 18–31. [CrossRef] [PubMed]
14. Lerma-Cabrera, J.M.; Carvajal, F.; Lopez-Legarrea, P. Food addiction as a new piece of the obesity framework. *Nutr. J.* **2016**, 15. [CrossRef]
15. Ruanpeng, D.; Thongprayoon, C.; Cheungpasitporn, W.; Harindhanavudhi, T. Sugar and artificially sweetened beverages linked to obesity: A systematic review and meta-analysis. *QJM Int. J. Med.* **2017**, *110*, 513–520. [CrossRef]
16. Soffritti, M.; Belpoggi, F.; Degli Esposti, D.; Lambertini, L. Aspartame induces lymphomas and leukaemias in rats. *Eur. J. Oncol.* **2005**, *10*, 107–116.
17. International Agency for Research on Cancer Monograph Working Group. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol. *IARC Monogr. Eval. Carcinog. Risks Hum.* **2006**, *88*, 1–478.
18. Boyle, P.; Koechlin, A.; Autier, P. Sweetened carbonated beverage consumption and cancer risk. *Eur. J. Cancer Prev.* **2014**, *23*, 481–490. [CrossRef]
19. Milajerdi, A.; Larijani, B.; Esmaillzadeh, A. Sweetened Beverages Consumption and Pancreatic Cancer: A Meta-Analysis. *Nutr. Cancer* **2019**, *71*, 375–384. [CrossRef]
20. Genkinger, J.M.; Li, R.; Spiegelman, D.; Anderson, K.E.; Albanes, D.; Bergkvist, L.; Bernstein, L.; Black, A.; Van Den Brandt, P.A.; English, D.R.; et al. Coffee, tea, and sugar-sweetened carbonated soft drink intake and pancreatic cancer risk: A pooled analysis of 14 cohort studies. *Cancer Epidemiol. Biomark. Prev.* **2012**, *21*, 305–318. [CrossRef]
21. Zhang, X.; Albanes, D.; Beeson, W.L.; Van Den Brandt, P.A.; Buring, J.E.; Flood, A.; Freudenheim, J.L.; Giovannucci, E.L.; Goldbohm, R.A.; Jaceldo-Siegl, K.; et al. Risk of colon cancer and coffee, tea, and sugar-sweetened soft drink intake: Pooled analysis of prospective cohort studies. *J. Natl. Cancer Inst.* **2010**, *102*, 771–783. [CrossRef] [PubMed]
22. Makarem, N.; Bandera, E.V.; Nicholson, J.M.; Parekh, N. Consumption of Sugars, Sugary Foods, and Sugary Beverages in Relation to Cancer Risk: A Systematic Review of Longitudinal Studies. *Annu. Rev. Nutr.* **2018**, *38*, 17–39. [CrossRef] [PubMed]
23. Chazelas, E.; Srour, B.; Desmetz, E.; Kesse-Guyot, E.; Julia, C.; Deschamps, V.; Druesne-Pecollo, N.; Galan, P.; Hercberg, S.; Latino-Martel, P.; et al. Sugary drink consumption and risk of cancer: Results from NutriNet-Santé prospective cohort. *BMJ* **2019**, *366*, l2408. [CrossRef]
24. Schernhammer, E.S.; Bertrand, K.A.; Birmann, B.M.; Sampson, L.; Willett, W.C.; Feskanich, D. Consumption of artificial sweetener- and sugar-containing soda and risk of lymphoma and leukemia in men and women. *Am. J. Clin. Nutr.* **2012**, *96*, 1419–1428. [CrossRef] [PubMed]
25. World Health Organization (WHO). Report on Cancer: Setting Priorities, Investing Wisely and Providing Care for All. Available online: https://apps.who.int/iris/handle/10665/330745 (accessed on 30 November 2020).
26. Bero, L.; Chartres, N.; Diong, J.; Fabbri, A.; Ghersi, D.; Lam, J.; Lau, A.; McDonald, S.; Mintzes, B.; Sutton, P.; et al. The risk of bias in observational studies of exposures (ROBINS-E) tool: Concerns arising from application to observational studies of exposures. *Syst. Rev.* **2018**, *7*, 242. [CrossRef]

27. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. Available online: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed on 2 November 2020).
28. Stepien, M.; Duarte-Salles, T.; Fedirko, V.; Trichopoulou, A.; Lagiou, P.; Bamia, C.; Overvad, K.; Tjønneland, A.; Hansen, L.; Boutron-Ruault, M.C.; et al. Consumption of soft drinks and juices and risk of liver and biliary tract cancers in a European cohort. *Eur. J. Nutr.* **2014**, *55*, 7–20. [CrossRef] [PubMed]
29. US Food and Drug Administration (FDA), Code of Federal Regulations Title 21. Available online: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.12 (accessed on 30 November 2020).
30. Australian Food Composition Database. Available online: https://www.foodstandards.gov.au/science/monitoringnutrients/afcd/Pages/default.aspx (accessed on 30 November 2020).
31. Miles, F.L.; Neuhouser, M.L.; Zhang, Z.F. Concentrated sugars and incidence of prostate cancer in a prospective cohort. *Proc. Int. Astron. Union* **2018**, *120*, 703–710. [CrossRef] [PubMed]
32. USDA, Food Data Central. Available online: https://ndb.nal.usda.gov/download-datasets.html (accessed on 2 November 2020).
33. Viechtbauer, W. Conducting meta-analyses in R with the metafor. *J. Stat. Softw.* **2010**, *36*, 1–48. [CrossRef]
34. Ren, J.S.; Freedman, N.D.; Kamangar, F.; Dawsey, S.M.; Hollenbeck, A.R.; Schatzkin, A.; Abnet, C.C. Tea, coffee, carbonated soft drinks and upper gastrointestinal tract cancer risk in a large United States prospective cohort study. *Eur. J. Cancer* **2010**, *46*, 1873–1881. [CrossRef]
35. Drake, I.; Sonestedt, E.; Gullberg, B.; Ahlgren, G.; Bjartell, A.; Wallström, P.; Wirfält, E. Dietary intakes of carbohydrates in relation to prostate cancer risk: A prospective study in the Malmö Diet and Cancer cohort. *Am. J. Clin. Nutr.* **2012**, *96*, 1409–1418. [CrossRef]
36. Ellison, L.F. Tea and other beverage consumption and prostate cancer risk: A Canadian retrospective cohort study. *Eur. J. Cancer Prev.* **2000**, *9*, 125–130. [CrossRef] [PubMed]
37. Lee, J.E.; Giovannucci, E.; Smith-Warner, S.A.; Spiegelman, D.; Willett, W.C.; Curhan, G.C. Total fluid intake and use of individual beverages and risk of renal cell cancer in two large cohorts. *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 1204–1211. [CrossRef] [PubMed]
38. Ros, M.M.; Bas Bueno-de-Mesquita, H.B.; Büchner, F.L.; Aben, K.K.H.; Kampman, E.; Egevad, L.; Overvad, K.; Tjønneland, A.; Roswall, N.; Clavel-Chapelon, F.; et al. Fluid intake and the risk of urothelial cell carcinomas in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Int. J. Cancer* **2011**, *128*, 2695–2708. [CrossRef] [PubMed]
39. Inoue-Choi, M.; Robien, K.; Mariani, A.; Cerhan, J.R.; Anderson, K.E. Sugar-sweetened beverage intake and the risk of type I and type II endometrial cancer among postmenopausal women. *Cancer Epidemiol. Biomarkers Prev.* **2013**, *22*, 2384–2394. [CrossRef] [PubMed]
40. McCullough, M.L.; Teras, L.R.; Shah, R.; Diver, W.R.; Gaudet, M.M.; Gapstur, S.M. Artificially and Sugar-Sweetened Carbonated Beverage Consumption Is Not Associated with Risk of Lymphoid Neoplasms in Older Men and Women. *J. Nutr.* **2014**, *144*, 2041–2049. [CrossRef] [PubMed]
41. Larsson, S.C.; Bergkvist, L.; Wolk, A. Consumption of sugar and sugar-sweetened foods and the risk of pancreatic cancer in a prospective study. *Am. J. Clin. Nutr.* **2006**, *84*, 1171–1176. [CrossRef]
42. Bao, Y.; Stolzenberg-Solomon, R.; Jiao, L.; Silverman, D.T.; Subar, A.F.; Park, Y.; Leitzmann, M.F.; Hollenbeck, A.; Schatzkin, A.; Michaud, D.S. Added sugar and sugar-sweetened foods and beverages and the risk of pancreatic cancer in the National Institutes of Health-AARP Diet and Health Study. *Am. J. Clin. Nutr.* **2008**, *88*, 431–440. [CrossRef] [PubMed]
43. Mueller, N.T.; Odegaard, A.; Anderson, K.; Yuan, J.M.; Gross, M.; Koh, W.P.; Pereira, M.A. Soft drink and juice consumption and risk of pancreatic cancer: The singapore chinese health study. *Cancer Epidemiol. Biomarkers Prev.* **2010**, *19*, 447–455. [CrossRef]
44. Nöthlings, U.; Murphy, S.P.; Wilkens, L.R.; Henderson, B.E.; Kolonel, L.N. Dietary glycemic load, added sugars, and carbohydrates as risk factors for pancreatic cancer: The Multiethnic Cohort Study. *Am. J. Clin. Nutr.* **2007**, *86*, 1495–1501. [CrossRef]
45. Navarrete-Muñoz, E.M.; Wark, P.A.; Romaguera, D.; Bhoo-Pathy, N.; Michaud, D.; Molina-Montes, E.; Tjønneland, A.; Olsen, A.; Overvad, K.; Boutron-Ruault, M.C.; et al. Sweet-beverage consumption and risk of pancreatic cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Am. J. Clin. Nutr.* **2016**, *104*, 760–768. [CrossRef]
46. Schernhammer, E.S.; Hu, F.B.; Giovannucci, E.; Michaud, D.S.; Colditz, G.A.; Stampfer, M.J.; Fuchs, C.S. Sugar-sweetened soft drink consumption and risk of pancreatic cancer in two prospective cohorts. *Cancer Epidemiol. Biomarkers Prev.* **2005**, *14*, 2098–2105. [CrossRef] [PubMed]
47. Dubrow, R.; Darefsky, A.S.; Freedman, N.D.; Hollenbeck, A.R.; Sinha, R. Coffee, tea, soda, and caffeine intake in relation to risk of adult glioma in the NIH-AARP Diet and Health Study. *Cancer Causes Control* **2012**, *23*, 757–768. [CrossRef] [PubMed]
48. Zamora-Ros, R.; Béraud, V.; Franceschi, S.; Cayssials, V.; Tsilidis, K.K.; Boutron-Ruault, M.C.; Weiderpass, E.; Overvad, K.; Tjønneland, A.; Eriksen, A.K.; et al. Consumption of fruits, vegetables and fruit juices and differentiated thyroid carcinoma risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Int. J. Cancer* **2018**, *142*, 449–459. [CrossRef] [PubMed]
49. Larsson, S.C.; Giovannucci, E.L.; Wolk, A. Sweetened Beverage Consumption and Risk of Biliary Tract and Gallbladder Cancer in a Prospective Study. *J. Natl. Cancer Inst.* **2016**, *108*. [CrossRef]
50. Bassett, J.K.; Milne, R.L.; English, D.R.; Giles, G.G.; Hodge, A.M. Consumption of sugar-sweetened and artificially sweetened soft drinks and risk of cancers not related to obesity. *Int. J. Cancer* **2020**, *146*, 3329–3334. [CrossRef]

51. Hirvonen, T.; Mennen, L.I.; de Bree, A.; Castetbon, K.; Galan, P.; Bertrais, S.; Arnault, N.; Hercberg, S. Consumption of Antioxidant-Rich Beverages and Risk for Breast Cancer in French Women. *Ann. Epidemiol.* **2006**, *16*, 503–508. [CrossRef]
52. Makarem, N.; Bandera, E.V.; Lin, Y.; Jacques, P.F.; Hayes, R.B.; Parekh, N. Consumption of sugars, sugary foods, and sugary beverages in relation to adiposity-related cancer risk in the framingham offspring cohort (1991–2013). *Cancer Prev. Res.* **2018**, *11*, 347–358. [CrossRef]
53. Romanos-Nanclares, A.; Toledo, E.; Gardeazabal, I.; Jiménez-Moleón, J.J.; Martínez-González, M.A.; Gea, A. Sugar-sweetened beverage consumption and incidence of breast cancer: The Seguimiento Universidad de Navarra (SUN) Project. *Eur. J. Nutr.* **2019**, *58*, 2875–2886. [CrossRef]
54. Hodge, A.M.; Bassett, J.K.; Milne, R.L.; English, D.R.; Giles, G.G. Consumption of sugar-sweetened and artificially sweetened soft drinks and risk of obesity-related cancers. *Public Health Nutr.* **2018**, *21*, 1618–1626. [CrossRef]
55. Nomura, S.J.O.; Dash, C.; Rosenberg, L.; Yu, J.; Palmer, J.R.; Adams-Campbell, L.L. Adherence to diet, physical activity and body weight recommendations and breast cancer incidence in the Black Women's Health Study. *Int. J. Cancer* **2016**, *139*, 2738–2752. [CrossRef]
56. Pacheco, L.S.; Anderson, C.A.M.; Lacey, J.V.; Giovannucci, E.L.; Lemus, H.; Araneta, M.R.G.; Sears, D.D.; Talavera, G.A.; Martinez, M.E. Sugar-sweetened beverages and colorectal cancer risk in the California Teachers Study. *PLoS ONE* **2019**, *14*, e0223638. [CrossRef] [PubMed]
57. Chandran, U.; McCann, S.E.; Zirpoli, G.; Gong, Z.; Lin, Y.; Hong, C.C.; Ciupak, G.; Pawlish, K.; Ambrosone, C.B.; Bandera, E.V. Intake of energy-dense foods, fast foods, sugary drinks, and breast cancer risk in African American and European American women. *Nutr. Cancer* **2014**, *66*, 1187–1199. [CrossRef] [PubMed]
58. Marzbani, B.; Nazari, J.; Najafi, F.; Marzbani, B.; Shahabadi, S.; Amini, M.; Moradinazar, M.; Pasdar, Y.; Shakiba, E.; Amini, S. Dietary patterns, nutrition, and risk of breast cancer: A case-control study in the west of Iran. *Epidemiol. Health* **2019**, *41*, e2019003. [CrossRef] [PubMed]
59. Mayne, S.T.; Risch, H.A.; Dubrow, R.; Chow, W.H.; Gammon, M.D.; Vaughan, T.L.; Borchardt, L.; Schoenberg, J.B.; Stanford, J.L.; West, A.B.; et al. Carbonated soft drink consumption and risk of esophageal adenocarcinoma. *J. Natl. Cancer Inst.* **2006**, *98*, 72–75. [CrossRef]
60. Bruemmer, B.; White, E.; Vaughan, T.L.; Cheney, C.L. Fluid intake and the incidence of bladder cancer among middle-aged men and women in a three-county area of Western Washington. *Nutr. Cancer* **1997**, *29*, 163–168. [CrossRef]
61. De Stefani, E.; Boffetta, P.; Deneo-Pellegrini, H.; Correa, P.; Ronco, A.L.; Brennan, P.; Ferro, G.; Acosta, G.; Mendilaharsu, M. Non-alcoholic beverages and risk of bladder cancer in Uruguay. *BMC Cancer* **2007**, *7*. [CrossRef] [PubMed]
62. Hemelt, M.; Hu, Z.; Zhong, Z.; Xie, L.P.; Wong, Y.C.; Tam, P.C.; Cheng, K.K.; Ye, Z.; Bi, X.; Lu, Q.; et al. Fluid intake and the risk of bladder cancer: Results from the South and East China case-control study on bladder cancer. *Int. J. Cancer* **2010**, *127*, 638–645. [CrossRef] [PubMed]
63. Radosavljević, V.; Janković, S.; Marinković, J.; Djokić, M. Fluid intake and bladder cancer. A case control study. *Neoplasma* **2003**, *50*, 234–238. [PubMed]
64. Turati, F.; Bosetti, C.; Polesel, J.; Zucchetto, A.; Serraino, D.; Montella, M.; Libra, M.; Galfano, A.; La Vecchia, C.; Tavani, A. Coffee, Tea, Cola, and Bladder Cancer Risk: Dose and Time Relationships. *Urology* **2015**, *86*, 1179–1184. [CrossRef]
65. Wang, J.; Wu, X.; Kamat, A.; Grossman, H.B.; Dinney, C.P.; Lin, J. Fluid intake, genetic variants of UDP-glucuronosyltransferases, and bladder cancer risk. *Br. J. Cancer* **2013**, *108*, 2372–2380. [CrossRef]
66. Jain, M.G.; Hislop, G.T.; Howe, G.R.; Burch, J.D.; Ghadirian, P. Alcohol and other beverage use and prostate cancer risk among Canadian men. *Int. J. Cancer* **1998**, *78*, 707–711. [CrossRef]
67. Sharpe, C.R.; Siemiatycki, J. Consumption of non-alcoholic beverages and prostate cancer risk. *Eur. J. Cancer Prev.* **2002**, *11*, 497–501. [CrossRef] [PubMed]
68. Hu, J.; Mao, Y.; DesMeules, M.; Csizmadi, I.; Friedenreich, C.; Mery, L. Total fluid and specific beverage intake and risk of renal cell carcinoma in Canada. *Cancer Epidemiol.* **2009**, *33*, 355–362. [CrossRef]
69. McLaughlin, C.C.; Mahoney, M.C.; Nasca, P.C.; Metzger, B.B.; Baptiste, M.S.; Field, N.A. Breast cancer and methylxanthine consumption. *Cancer Causes Control* **1992**, *3*, 175–178. [CrossRef]
70. Maclure, M.; Willett, W. A case-control study of diet and risk of renal adenocarcinoma. *Epidemiology* **1990**, *1*, 430–440. [CrossRef]
71. Herrero, R.; Potischman, N.; Brinton, L.A.; Reeves, W.C.; Brenes, M.M.; Tenorio, F.; De Britton, R.C.; Gaitan, E. A case-control study of nutrient status and invasive cervical cancer: I. Dietary indicators. *Am. J. Epidemiol.* **1991**, *134*, 1335–1346. [CrossRef] [PubMed]
72. Verreault, R.; Chu, J.; Mandelson, M.; Shy, K. A case-control study of diet and invasive cervical cancer. *Int. J. Cancer* **1989**, *43*, 1050–1054. [CrossRef]
73. King, M.G.; Olson, S.H.; Paddock, L.; Chandran, U.; Demissie, K.; Lu, S.E.; Parekh, N.; Rodriguez-Rodriguez, L.; Bandera, E.V. Sugary food and beverage consumption and epithelial ovarian cancer risk: A population-based case-control study. *BMC Cancer* **2013**, *13*, 94. [CrossRef] [PubMed]
74. Leung, A.C.Y.; Cook, L.S.; Swenerton, K.; Gilks, B.; Gallagher, R.P.; Magliocco, A.; Steed, H.; Köbel, M.; Nation, J.; Brooks-Wilson, A.; et al. Tea, coffee, and caffeinated beverage consumption and risk of epithelial ovarian cancers. *Cancer Epidemiol.* **2016**, *45*, 119–125. [CrossRef]

75. Yoon, J.S.; Kristal, A.R.; Wicklund, K.G.; Cushing-Haugen, K.L.; Rossing, M.A. Coffee, tea, colas, and risk of epithelial ovarian cancer. *Cancer Epidemiol. Biomarkers Prev.* **2008**, *17*, 712–716. [CrossRef]
76. Chan, J.M.; Wang, F.; Holly, E.A. Sweets, sweetened beverages, and risk of pancreatic cancer in a large population-based case-control study. *Cancer Causes Control* **2009**, *20*, 835–846. [CrossRef] [PubMed]
77. Gallus, S.; Turati, F.; Tavani, A.; Polesel, J.; Talamini, R.; Franceschi, S.; La Vecchia, C. Soft drinks, sweetened beverages and risk of pancreatic cancer. *Cancer Causes Control* **2011**, *22*, 33–39. [CrossRef] [PubMed]
78. Gold, E.B.; Gordis, L.; Diener, M.D.; Seltser, R.; Boitnott, J.K.; Bynum, T.E.; Hutcheon, D.F. Diet and other risk factors for cancer of the pancreas. *Cancer* **1985**, *55*, 460–467. [CrossRef]
79. Lyon, J.L.; Mahoney, A.W.; French, T.K.; Moser, R. Coffee consumption and the risk of cancer of the exocrine pancreas: A case-control study in a low-risk population. *Epidemiology* **1992**, *3*, 164–170. [CrossRef]
80. Potischman, N.; Coates, R.J.; Swanson, C.A.; Carroll, R.J.; Daling, J.R.; Brogan, D.R.; Gammon, M.D.; Midthune, D.; Curtin, J.; Brinton, L.A. Increased risk of early-stage breast cancer related to consumption of sweet foods among women less than age 45 in the United States. *Cancer Causes Control* **2002**, *13*, 937–946. [CrossRef] [PubMed]
81. Mack, T.; Yu, M.; Henderson, B. Pancreas cancer and smoking, beverage consumption, and past medical history. *J. Natl. Cancer Inst.* **1986**, *76*, 49–60.
82. Zvrko, E.; Gledović, Z.; Ljaljević, A. Risk factors for laryngeal cancer in Montenegro. *Arh. Hig. Rada Toksikol.* **2008**, *59*, 11–18. [CrossRef]
83. Lissowska, J.; Pilarska, A.; Pilarski, P.; Samolczyk-Wanyura, D.; Piekarczyk, J.; Bardin-Mikolajczak, A.; Zatonski, W.; Herrero, R.; Muñoz, N.; Franceschi, S. Smoking, alcohol, diet, dentition and sexual practices in the epidemiology of oral cancer in Poland. *Eur. J. Cancer Prev.* **2003**, *12*, 25–33. [CrossRef] [PubMed]
84. Kreimer, A.R.; Randi, G.; Herrero, R.; Castellsagué, X.; La Vecchia, C.; Franceschi, S. Diet and body mass, and oral and oropharyngeal squamous cell carcinomas: Analysis from the IARC multinational case-control study. *Int. J. Cancer* **2006**, *118*, 2293–2297. [CrossRef] [PubMed]
85. Vinceti, M.; Bonvicini, F.; Pellacani, G.; Sieri, S.; Malagoli, C.; Giusti, F.; Krogh, V.; Bergomi, M.; Seidenari, S. Food intake and risk of cutaneous melanoma in an Italian population. *Eur. J. Clin. Nutr.* **2008**, *62*, 1351–1354. [CrossRef]
86. Luqman, M.; Javed, M.M.; Daud, S.; Raheem, N.; Ahmad, J.; Khan, A.U.H. Risk factors for lung cancer in the Pakistani population. *Asian Pacific J. Cancer Prev.* **2014**, *15*, 3035–3039. [CrossRef]
87. Wu, A.H.; Yu, M.C.; Mack, T.M. Smoking, alcohol use, dietary factors and risk of small intestinal adenocarcinoma. *Int. J. Cancer* **1997**, *70*, 512–517. [CrossRef]
88. Bener, A.; Moore, M.A.; Ali, R.; El Ayoubi, H.R. Impacts of family history and lifestyle habits on colorectal cancer risk: A case-control study in Qatar. *Asian Pacific J. Cancer Prev.* **2010**, *11*, 963–968.
89. Mahfouz, E.M.; Sadek, R.R.; Abdel-Latief, W.M.; Mosallem, F.A.H.; Hassan, E.E. The role of dietary and lifestyle factors in the development of colorectal cancer: Case control study in Minia, Egypt. *Cent. Eur. J. Public Health* **2014**, *22*, 215–222. [CrossRef] [PubMed]
90. Tayyem, R.F.; Bawadi, H.A.; Shehadah, I.; Bani-Hani, K.E.; Takruri, H.; Al-Jaberi, T.; Heath, D.D. Fast foods, sweets and beverage consumption and risk of colorectal cancer: A case-control study in Jordan. *Asian Pacific J. Cancer Prev.* **2018**, *19*, 261–269. [CrossRef]
91. Theodoratou, E.; Farrington, S.M.; Tenesa, A.; McNeill, G.; Cetnarskyj, R.; Korakakis, E.; Din, F.V.N.; Porteous, M.E.; Dunlop, M.G.; Campbell, H. Associations between dietary and lifestyle risk factors and colorectal cancer in the Scottish population. *Eur. J. Cancer Prev.* **2014**, *23*, 8–17. [CrossRef]
92. Murtaugh, M.A.; Ma, K.N.; Caan, B.J.; Slattery, M.L. Association of fluids from beverages with risk of rectal cancer. *Nutr. Cancer* **2004**, *49*, 25–31. [CrossRef]
93. Ibiebele, T.I.; Hughes, M.C.; O'Rourke, P.; Webb, P.M.; Whiteman, D.C. Cancers of the esophagus and carbonated beverage consumption: A population-based case-control study. *Cancer Causes Control* **2008**, *19*, 577–584. [CrossRef]
94. Turati, F.; Dalmartello, M.; Bravi, F.; Serraino, D.; Augustin, L.; Giacosa, A.; Negri, E.; Levi, F.; Vecchia, C. La Adherence to the world cancer research fund/american institute for cancer research recommendations and the risk of breast cancer. *Nutrients* **2020**, *12*, 607. [CrossRef] [PubMed]
95. Chandran, U.; Hirshfield, K.M.; Bandera, E.V. The role of anthropometric and nutritional factors on breast cancer risk in African-American women. *Public Health Nutr.* **2012**, *15*, 738–748. [CrossRef] [PubMed]
96. Li, N.; Petrick, J.L.; Steck, S.E.; Bradshaw, P.T.; McClain, K.M.; Niehoff, N.M.; Engel, L.S.; Shaheen, N.J.; Corley, D.A.; Vaughan, T.L.; et al. Dietary sugar/starches intake and Barrett's esophagus: A pooled analysis. *Eur. J. Epidemiol.* **2017**, *32*, 1007–1017. [CrossRef] [PubMed]
97. Khan, M.M.H.; Goto, R.; Kobayashi, K.; Suzumura, S.; Nagata, Y.; Sonoda, T.; Sakauchi, F.; Washio, M.; Mori, M. Dietary habits and cancer mortality among middle aged and older Japanese living in Hokkaido, Japan by cancer site and sex. *Asian Pacific J. Cancer Prev.* **2004**, *5*, 58–65.
98. Yang, H.P.; Wentzensen, N.; Trabert, B.; Gierach, G.L.; Felix, A.S.; Gunter, M.J.; Hollenbeck, A.; Park, Y.; Sherman, M.E.; Brinton, L.A. Endometrial cancer risk factors by 2 main histologic subtypes. *Am. J. Epidemiol.* **2013**, *177*, 142–151. [CrossRef]
99. O'Neil, C.E.; Nicklas, T.A.; Rampersaud, G.C.; Fulgoni, V.L. 100% Orange juice consumption is associated with better diet quality, improved nutrient adequacy, decreased risk for obesity, and improved biomarkers of health in adults: National Health and Nutrition Examination Survey, 2003–2006. *Nutr. J.* **2012**, *11*. [CrossRef]

100. Salari-Moghaddam, A.; Milajerdi, A.; Surkan, P.J.; Larijani, B.; Esmaillzadeh, A. Caffeine, Type of Coffee, and Risk of Ovarian Cancer: A Dose-Response Meta-Analysis of Prospective Studies. *J. Clin. Endocrinol. Metab.* **2019**, *104*, 5349–5359. [CrossRef]
101. Tsai, C.J.; Leitzmann, M.F.; Willett, W.C.; Giovannucci, E.L. Glycemic load, glycemic index, and carbohydrate intake in relation to risk of cholecystectomy in women. *Gastroenterology* **2005**, *129*, 105–112. [CrossRef]
102. Randi, G.; Franceschi, S.; La Vecchia, C. Gallbladder cancer worldwide: Geographical distribution and risk factors. *Int. J. Cancer* **2006**, *118*, 1591–1602. [CrossRef] [PubMed]
103. Toews, I.; Lohner, S.; Küllenberg De Gaudry, D.; Sommer, H.; Meerpohl, J.J. Association between intake of non-sugar sweeteners and health outcomes: Systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies. *BMJ* **2019**, *364*. [CrossRef] [PubMed]
104. Ruppar, T. Meta-analysis: How to quantify and explain heterogeneity? *Eur. J. Cardiovasc. Nurs.* **2020**, *19*, 646–652. [CrossRef]
105. Bucher, T.; Siegrist, M. Children's and parents' health perception of different soft drinks. *Br. J. Nutr.* **2015**, *113*, 526–535. [CrossRef]
106. World Cancer Research Fund/American Institute for Cancer Research. Diet, Nutrition, Physical Activity and Cancer: A Global Perspective A Summary of the Third Expert Report. Available online: https://www.wcrf.org/dietandcancer/recommendations/limit-sugar-sweetened-drinks (accessed on 15 December 2020).
107. Heyman, M.B.; Abrams, S.A. Fruit Juice in Infants, Children, and Adolescents: Current Recommendations. *Am. Acad. Pediatrics* **2017**, *139*, 20170967. [CrossRef] [PubMed]
108. Salas, M.M.S.; Nascimento, G.G.; Vargas-Ferreira, F.; Tarquinio, S.B.C.; Huysmans, M.C.D.N.J.M.; Demarco, F.F. Diet influenced tooth erosion prevalence in children and adolescents: Results of a meta-analysis and meta-regression. *J. Dent.* **2015**, *43*, 865–875. [CrossRef] [PubMed]
109. Pan, A.; Malik, V.S.; Schulze, M.B.; Manson, J.A.E.; Willett, W.C.; Hu, F.B. Plain-water intake and risk of type 2 diabetes in young and middle-aged women. *Am. J. Clin. Nutr.* **2012**, *95*, 1454–1460. [CrossRef] [PubMed]
110. Aune, D.; Giovannucci, E.; Boffetta, P.; Fadnes, L.T.; Keum, N.N.; Norat, T.; Greenwood, D.C.; Riboli, E.; Vatten, L.J.; Tonstad, S. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-A systematic review and dose-response meta-analysis of prospective studies. *Int. J. Epidemiol.* **2017**, *46*, 1029–1056. [CrossRef] [PubMed]
111. World Health Organization. Obesity and Overweight. Available online: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed on 23 January 2020).
112. Wild, S.; Bchir, M.B.; Roglic, G.; Green, A.; Sci, M.; Sicree, R.; King, H. Global Prevalence of Diabetes Estimates for the year 2000 and projections for 2030. *Diabetes Care* **2004**, *27*, 1047–1053. [CrossRef] [PubMed]

11.4. Total Antioxidant Capacity Measurements in Food and Beverages.

Poncet G, Castañeda J, Llaha F, **Gil-Lespinard M**, Zamora-Ros R. Chapter 5. Total Antioxidant Capacity Measurements in Food and Beverages. In: Food chemistry, function, and analysis [Internet]. 2021. p. 178–202. Available from: https://doi.org/10.1039/9781839165337-00178

CHAPTER 5

Total Antioxidant Capacity Measurements in Food and Beverages

GABRIELLE PONCET, JAZMIN CASTAÑEDA, FJORIDA LLAHA, MERCEDES GIL-LESPINARD AND RAUL ZAMORA-ROS*

Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain
*Email: rzamora@idibell.cat

5.1 Contribution of Antioxidants to the Total Antioxidant Capacity

Food and beverages contain many individual antioxidants with unique chemical properties, which contribute to the total antioxidant capacity (TAC). There is a large list of foods, with a very high TAC content, such as pecan nut (179.4 μmol Trolox Equivalent (TE)/g), pistachio (79.8 μmol TE/g), plum (62.4 μmol TE/g), blueberry (62.2 μmol TE/g), raspberry (49.3 μmol TE/g), potato (13.2 μmol TE/g), almond (44.5 μmol TE/g), strawberry (35.8 μmol TE/g), broccoli (15.9 μmol TE/g), russet (13.2 μmol TE/g), carrot (12.2 μmol TE/g), coffee with milk (16.3 mmol Fe/100 g), red wine (1.8 to 3.7 mmol Fe/100 g), prepared green tea (0.6 to 2.6 mmol Fe/100 g), grape juice, prune juice and black tea (0.7 to 1.2 mmol Fe/100 g).[1,2] These foods contain a variety of natural antioxidants including phenolic compounds, carotenoids, α-tocopherol and ascorbic acid, among others. These compounds can potentially have health-promoting

Food Chemistry, Function and Analysis No. 28
Handbook of Antioxidant Methodology: Approaches to Activity Determination
Edited by Paul D. Prenzler, Danielle Ryan and Kevin Robards

Published by the Royal Society of Chemistry, www.rsc.org

effects[3,4] and also play an important role in the food industry as natural preservatives.

Natural antioxidants, together with other compounds present in food can interact in additive, synergic or antagonist ways, which also affect the TAC value. Thus, measuring the TAC gives a better overall result than the sum of the antioxidant capacity of each individual compound. TAC data in food do not necessarily indicate the expected health effects in humans, as other aspects such as food matrix and bioavailability/bioactivity of antioxidants need to be considered.[5]

The largest contributor to the TAC is generally polyphenols. They are bioactive compounds exclusively found in plant foods including fruits, vegetables, whole grains and beverages (tea, coffee, wine) and have been shown to possess different biological functions.[6,7] One of their main biological activities is the neutralization of reactive oxygen species, which are compounds that can damage DNA, proteins and lipids.[8] However, dietary polyphenols do not have a direct effect in oxidative damage; they exhibit their antioxidant properties by boosting the body's antioxidant system, consisting of enzymes such as catalase, glutathione peroxidase/reductase, superoxide dismutase, quinone reductase, and Phase I and Phase II enzymes, and low molecular weight antioxidants such as vitamins A, C and E, glutathione, bilirubin, selenium and urate, among others.[9,10]

Polyphenols comprise a wide variety of chemical structures (more than 10 000 compounds have been identified), and these differences affect the TAC. For example, the contribution of catechol (31,41-dihydroxy)-ring-B found in some polyphenols such as flavonoids, has shown a high TAC measured with different assays such as Trolox Equivalent Antioxidant Capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and Ferric Reducing Antioxidant Power (FRAP).[11] Moreover, the temperature, light conditions, food processing and the type and number of polyphenols are other factors that affect their contribution to TAC in foods.

Ascorbic acid (vitamin C) and total carotenoids have been shown to react as mediators in the absorption of different antioxidant components. For example, ascorbic acid is known as a strong water-soluble antioxidant that acts preferentially by hydrogen donation.[12] A study evaluating the TAC content of orange juice, milk and an orange juice milk beverage found that interactions among ascorbic acid, gallic acid, β-carotene, lutein, zeaxanthin and albumin can affect the evaluation of TAC through different assays, due to the different kinetics and reaction mechanisms of those antioxidants.[13]

In summary, it is known that each antioxidant has a variable contribution to the TAC in different foods and beverages. It is difficult to decipher which is the single most effective antioxidant compound in a food, because they are not alone and therefore, they can have complex interactions among them.[14] There are further factors which affect their contribution to TAC, including temperature, food processing and ripening processes that can also affect the type and concentration of natural antioxidants present in food. In addition, not all TAC methods have the same characteristics and measure exactly the same

chemical species. These aspects and their differences in TAC measurement methods will be briefly discussed in the following sections.

5.2 Methods for TAC Measurement in Food and Beverages

To date, many methods for measuring TAC in food and beverages have been developed and are currently used. Methods can be classified into: i) methods based on electron transfer (ET) and ii) methods based on hydrogen atom transfer (HAT). Assays based on ET measure the capability of antioxidants to release an electron. In the HAT-based assays, the goal is to measure the capability of antioxidants to neutralize free radicals, generally peroxyl radicals, by hydrogen atom donation. The HAT-based assays offer more competitive mechanisms. Some methods, referred to as mixed-model assays, combine the mechanisms of ET and HAT. In these assays, the capacity of antioxidants to scavenge a stable radical chromophore or fluorophore has been quantified combining the mechanisms of HAT and ET, playing a different role depending on the reagent present in the solution used.[15,16] Among the wide variety of methods for measuring TAC, the most commonly used are: the oxygen radical absorbance capacity (ORAC), TEAC, FRAP, 2,2′-azino-bis(3-ethylbenzothiazolin-6 sulfonic acid) (ABTS), total radical-trapping antioxidant parameter (TRAP) and DPPH.[17,18]

Some specific methods are better suited to achieve the highest TAC value for certain food and beverages. For example, a recently developed method called Quencher allows the highest measurement of TAC in solids such as meat or nuts. Its procedure consists of direct contact between solid food and the radical reagent solution, in addition there is no extraction and hydrolysis before the measurement of the antioxidant capacity, because in this way the soluble fraction of the sample exerts its antioxidant capacity by quenching the radicals present in the solvent according to the usual liquid–liquid type reaction.[19] In addition, it may provide a better assessment of the TAC due to the possible interactions between the inaccessible antioxidants and the reagent. Although most assays can measure both hydrophilic and lipophilic antioxidants,[20] it is preferable to use different methods for different fractions or foods as they might be more appropriate for one section or another.

For instance, the DPPH assay is more appropriate for lipophilic antioxidants, while the FRAP method is more pertinent for hydrophilic antioxidants.[21] As can be observed in Table 5.1, different assays give different TAC values for the same food, (*e.g.*, the values of strawberry cv. maya or spinach) due to the variabilities between the assays, in particular the different reaction between the antioxidant and the reagent.[22] Consequently, to obtain the most representative TAC value, it is better to combine at least two methods rather than using only one to generate an ''antioxidant score'' including reactivity toward both aqueous and lipid/organic radicals for obtaining a more accurate estimate. There are several options to compute the

Table 5.1 Summary of the most common TAC methods.[a]

	Name of the method	How it works/measurement properties	Example of values for the same food products	References
HAT based assays	Total radical-trapping antioxidant parameter (TRAP)	Based on the protection provided by antioxidants on the fluorescence decay of *R*-phycoerythrin	Strawberry = 8.56 mmol TE/kg FW Spinach = 5.79 mmol TE/kg FW	16, 27
	Oxygen radical absorbance capacity (ORAC)	Ability of the antioxidant to protect the oxidation of β-phycoerythrin or fluorescein by monitoring the fluorescence at a specific wavelength	Strawberry, cv. Maya = 47.14 ± 3.22 mmol TE/kg FW	16, 22
ET based assays	Ferric reducing antioxidant power (FRAP)	Capacity of the sample to reduce iron by following the visible absorbance of the sample at 593 nm	Strawberry, cv. Maya = 7.68 ± 0.55 mmol Fe^{2+}/kg FW Spinach = 26.94 mmol Fe^{2+}/kg FW	15, 16, 22, 27
	Cupric reducing antioxidant capacity (CUPRAC)	Capacity of the sample to reduce copper by following the visible absorbance of the sample at 450 nm		15, 16
[1]ET-HAT mixed assays	Trolox-equivalent antioxidant capacity (TEAC) assay with 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)	Scavenging of the $ABTS^{\bullet+}$ radical cation by antioxidants by monitoring the decrease in absorbance at 645 nm, 734 nm and 815 nm	Strawberry, cv. Maya = 26.92 ± 2.18 mmol TE/kg FW pinach = 8.49 mmol TE/kg FW	16, 22, 27
	2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) assay	Scavenging of the DPPH radicals by antioxidants by monitoring the decrease in absorbance at 515 nm	Strawberry, cv. Maya = 8.29 ± 0.50 mmol TE/kg FW	16, 22
	N,N-dimethyl-p-phenylenediamine dihydrochloride radical scavenging (DMPD) assay	Ability of the antioxidants to scavenge DMPD radical by monitoring the absorbance at 505 nm	Strawberry = 3100 μmol TE/100 g FW Spinach = 500 μmol TE/100 g FW	16, 27
	Trolox Equivalent Antioxidant Capacity (TEAC)	Based on the scavenging of $ABTS^{\bullet+}$ converting it into a colorless product.	Wild strawberry = 33.17 ± 0.05 mmol TE/kg FW pinach 11.9 ± 0.3 μM/g	23–25

[a]Abbreviations: ET (Electron Transfer), HAT (Hydrogen Atom Transfer), FW (fresh weight), TE (Trolox Equivalent).

''antioxidant score'', for example i) averaging data from both methodologies; ii) if the range/size of units of both methods is very different, a standard score (z-score) could be calculated. Each method has its own drawbacks, but combining several TAC methodologies may reduce some of them.[20]

The technical differences between studies lead to changes in the preservation of the product, sample preparation, extraction method, chemicals used and the sample itself.[17] The variety, the degree of ripeness of fruit and vegetables, the geographical area, the terroir and the weather also influence the TAC content.

5.3 TAC Data of Raw Foods

TAC data of raw foods are available in many studies. Most of them measure the TAC of a particular food type, which may not be consistent with what is the intake in the current diet of different populations, *e.g.*, various tropical foods (such as prickly pears, nopal, squash blossoms, mamey, zapote, guava, jicama) are a large part of the Mexican diet, and the information on TAC is lower compared to other foods such as oils or blueberries.[12,26–28] This could eventually lead to misleading TAC values for a real diet in other populations.[29–32] This section is focused in discussing the influence of the varieties of certain foods, the ripeness, the time of harvest and the farming practices on TAC. Several studies that used the same assays to evaluate TAC have reported a wide range of TAC values for some varieties of the same food (such as strawberries, apples, pears and Chinese jujube).[33–35] For example, Nowicka *et al.*[36] reported a TAC value from 390.7 μmol TE/100 g to 1287.7 μmol TE/100 g using the DPPH assay in 90 different cultivars of strawberries *Fragaria x ananassa* Duch. Kevers *et al.*[34] measured the TAC in apples and pears using the ORAC assay and obtained values in the range of 1101 ± 105 μmol TE/100 g fresh weight (FW) to 4917 ± 249 μmol TE/100 g FW for apples, and from 1468 ± 121 μmol TE/100 g FW to 4251 ± 221 μmol TE/100g FW for pears. In addition, Kim *et al.*[35] reported a range between 266 ± 14.5 mg vitamin C equivalent (VCE)/100 g and 559 ± 12.9 mg VCE/100 g for five cultivars of plums using the ABTS assay.

Similarly, beverages produced from different varieties differ in their TAC values. A clear example is wine, produced from different grape varieties and divided into three main types: red, rosé and white wine. There is a clear difference between the TAC value depending on the wine type.[37] Other studies focused on the grape variety also showing large differences. For example, Kondrashov *et al.*[38] analyzed the TAC content of six Cabernet Sauvignon and four Merlot red wines from different countries and reported a range from 8 to 16.6 mmol TE/L for Cabernet Sauvignon and from 7.5 to 11.2 mmol TE/L for Merlot. Similar results were obtained when using the FRAP assay; 7.0 to 15.2 mmol TE/L for Cabernet Sauvignon wines and 6.9 to 9.8 mmol TE/L for Merlot wines. Coletta *et al.*[39] also showed differences between both varieties and viticulture practices, including the training systems and the bud load. They concluded that the variety, the bud load and the

training systems affect the concentration of phenolic compounds in grapes and wine, and consequently, in the TAC.

Agricultural practices, particularly growing conditions, may also influence TAC.[40–42] Gündüz and Özdemir[40] investigated the growth of different types of strawberries under different light conditions and temperatures using a greenhouse, a plastic tunnel and an open field for two years. They found that in the first year of the study the plastic tunnel and the open field significantly influenced the TAC content, but not in the second year. An important finding from this work was that strawberry genotypes have more effect on TAC values than environmental factors; although to confirm this statement, more evidence is needed.

Other studies[41,42] have focused on comparing the impact of organic *vs.* conventional farming on TAC values. These studies were generally similar and concluded that organic strawberries/blueberries provided higher levels of phytonutrients such as anthocyanins, phenolic compounds and TAC, than conventional farming. A positive correlation between the total content of phenolic and anthocyanins compounds and the TAC was found, meaning that both compounds contributed to the TAC and the variation induced by cultivation also caused changes in the TAC content. Overall, it is worth noting that organic farming seems to have many advantages, not only for the environment, but also for the higher concentrations of phytonutrients compared to conventional farming.

Additionally, factors like differences in water stress, availability of mineral nutrients and ultraviolet radiation influence plant growth, consistently influencing phytochemical and TAC values. The environmental conditions, such as the season and time of harvest, can also influence the TAC and total phenolic content.[34,43–45] Large differences in the TAC content can occur depending on the ripening stage.[34,36,44,46–51] Some fruits and vegetables reduce or increase the TAC during ripening, which correlates with phenolic content. Phenolic concentrations and TAC usually increase when fruits are exposed to higher temperatures, such as in summer. However, the total phenolic content (flavonoids and gallotannins) decreases during the ripening of some fruits (*e.g.*, strawberry, pomegranate) due to their oxidation by polyphenol oxidase. However, it has been shown that some polyphenols, such as anthocyanins, can increase their levels during ripening by as much as 100 times compared to the early stages.[44,47] Despite all the current knowledge, the influence of variations induced by the harvest year is not completely understood and deserve further investigation.[52]

Data from a database created by Carlsen *et al.*[2] that includes a large number of foodstuffs suggest that plant products (such as spices, herbs and traditional herbal medicines) have the highest TAC, followed by fruits such as berries and berry products, especially blueberries, blackcurrants, cranberries and goji berries. Among beverages, tea and coffee are at the top of the list. Other foods with a high TAC are nuts, preferably with their pellicle, chocolate and cocoa, dried and fresh fruits such as plums and apricots, vegetables like spinach and red wine.

The evaluation of TAC in nuts, seeds, legumes and cereals can be problematic due to their difficult extraction process. The highest TAC values for nuts, seeds and pulses are reported by Açar *et al.*[53] who used the Quencher approach (useful for solid matrices) for TAC evaluation.

Legumes with dark color (red, bronze and black) have a higher TAC than legumes with a pale color (green, yellow and white).[54] This is due to the fact that the black-colored legumes have significantly higher total phenolic, total flavonoid and condensed tannin contents. In addition, legumes are rich in fiber, which improves antioxidant capacity due to the synergistic physiological process in the gastrointestinal tract.[55]

Even though many researchers have screened a large number of foods and beverages, to date, there is not a standardized database for TAC values. Carlsen *et al.*[2] screened around 3100 food products including fruits, vegetables, spices and animal products with the FRAP assay. Also, the US Department of Agriculture's Nutrient Data Laboratory published a database of hundreds of foodstuffs using the ORAC assay, but it was withdrawn for technical and conceptual reasons.[56] However, all TAC databases have several technical and conceptual drawbacks.[57] Moreover, it is challenging to establish a reference database for TAC as there are several parameters that differ from one study to another, such as the assay used to measure TAC, the extraction process and the varieties measured, among others. For a complete database, it would also be necessary to include TAC data from raw and processed food, considering food processing, storage and preservation methods, which can cause important changes in certain products.

5.4 Effect of Food Processing on TAC Measurements

5.4.1 Cooking Methods

Nowadays, food products can undergo specific processes that influence their composition. It is generally believed that cooking methods lead to a loss of nutrient components, which is normally due to high temperatures and a loss of water and/or fat in the food. Cooking methods such as boiling, frying, microwaving, roasting, steaming and baking, induce chemical changes in the texture and chemical composition of food that can affect the TAC content. In Table 5.2 are summarized some examples of cooking methods and their effect in the TAC value.

Water content in foods is an important factor that influences TAC measurement. Processes using water, such as boiling and steaming, cause changes in the content of some antioxidants, such as the total content of phenolics and carotenoids. The effect of boiling on TAC depends on the food item. On one hand, boiling for 5 minutes might cause structural matrix changes in the cell wall and can increase total carotenoid content (TCC). Moreover, the total phenol content (TPC) in some vegetables (*e.g.*, green beans, broccoli and peppers) increases during cooking or wet heating due to the possible decomposition of complex phenolic components, such as tannins.[58,59]

Table 5.2 Effect of cooking methods on the Total Antioxidant Capacity.[a]

Cooking method	Sample food	Effect in TAC	Raw food reference	Reference
Boiling	Carrot (organic)	↓5.6% RSC	40.4% RSC	60, 61
	Spinach	↓0.47 mmol TE/100 g	0.62 mmol TE/100 g	
	Artichoke	↑0.95 mmol TE/100 g	0.30 mmol TE/100 g	
Steaming	Green beans (purple queen)	↓75.55 ± 1.45 mg GAE/100 g	78.40 ± 1.44 mg GAE/100 g	61, 62
	Potato (organic)	↑32.4% RSC	11% RSC	
	Broccoli (organic)	↑89.0% Radical RSC	73.8% RSC	
Deep-frying	Potato	↑3.45 ± 0.39 μmol TE/g FW	0.53 ± 0.04 μmol TE/g	63
	Tomato	↑13.60 ± 0.24 μmol TE/g FW	2.08 ± 0.07 μmol TE/g	
	Eggplant	↑13.39 ± 0.39 μmol TE/g FW	0.57 ± 0.06 μmol TE/g	
Microwaving	Spinach	↓58.4 ± 1.7 mg GAE/g dry matter	67.8 ± 1 mg GAE/g dry matter	61, 64
	Broccoli	↑89.2% RSC	73.8% RSC	

[a]Abbreviations: RSC Radical Scavenging Capacity, GAE Gallic Acid Equivalent, TE Trolox Equivalent.

On the other hand, the TAC value of spinach, turnip tops, zucchini and asparagus is reduced during boiling, probably due to the instability of the water-soluble vitamins in contact with boiling water.[60] Furthermore, the lixiviation phenomenon[59,60] during boiling needs to be considered, which extracts some components from the food (such as polyphenol, vitamin C or carotenoids content) to the water.

Steaming is the gold standard for cooking vegetables and some fruits due to its capacity to retain most of the natural antioxidants and even increase the TAC value in some foods.[65] Steaming can preserve TPC, particularly in flavonoids as they are sensitive to high temperatures.[66] Heating causes a disruption of cell wall structures that leads to the destruction of complex phenols and releases some components such as high soluble glucuronide derivatives.[58]

The use of the microwave has become a common cooking method, and its effects on natural antioxidants are still under research. Cooking time in a microwave has an impact on the TAC values. Cooking broccoli in a microwave for more than 5 minutes decreases TCC and vitamin C content and consequently the TAC content;[67] while the TAC values of tomatoes and carrots increase after microwaving.[63,64] Similarly to other techniques, microwave causes changes in the food matrix due to the high pressure, high temperature and/or microwave radiation, which may contribute to maintaining or even increasing the TAC.[68]

Roasting in the oven is another method of cooking that can increase TPC and antioxidant capacity of different foods, such as seeds. Roasting increased the antioxidant activity, TPC, oil content and tocopherol content in sesame seeds.[69,70] Red peppers, carrots and cauliflower also increased their TPC during roasting, which could be related to the release of free phenolics from hydrolysable tannins.[68] Additionally, roasting is a very common cooking method used in coffee, a beverage with a high potential antioxidant activity, due to the high content of polyphenols, such as chlorogenic acids. Some antioxidant molecules, such as phenylalanines and heterocyclic compounds, are formed during the roasting process. The roasting process and stage affect the TAC and TPC. The TAC increases in coffees prepared in light roasting conditions, but decreases in medium/dark roasting conditions.[71] Increasing roasting time and darkness and lowering the roasting temperature are suggested to provide the highest TAC of coffee beverages.[72] Lastly, as other methods, a long cooking time and high temperatures during roasting can induce the loss of vitamin C and a significant decrease in the TCC of some foods affecting the TAC value.

Finally, non-water methods such as pan-frying and deep-frying reduce both TCC and TPC. Deep- and pan-frying have a negative effect on the TAC of mushrooms, red peppers and cauliflower, but a remarkably positive effect for potatoes, eggplants and artichokes.[60,73] The positive effect of frying in TAC could be explained by the fact that food immersed in oil at high temperature requires a short cooking time, which increases the retention of the antioxidant compounds in the food matrix.[60] Besides this positive effect, foods absorb and exchange fats and lose water during frying, which also results in a large reduction of TAC during the final frying stage.

Overall, the effect of cooking on the TAC in food depends on the cooking method and the food item used in each method. Steaming can be considered as the most favorable cooking method as it can increase the availability of polyphenols in all vegetables, and avoid important losses of phenolic components that play an important role in the TAC content.[65,67] Steaming is followed by microwaving, boiling and roasting, considering the cooking time and the temperatures as key factors influencing the TAC content, and finally, frying and deep-frying as the culinary techniques with the highest TAC losses.[69] The content of bioactive compounds also depends on the treatment time, size of the sample and the analytical assays. The available databases on TAC generally gather raw food data. Due to the significant changes in TAC content after cooking, it is crucial that TAC databases also include the composition of cooked foods.

5.4.2 Storage and Preservation Methods

Food storage and preservation inhibit the growth of harmful bacteria that speed up decomposition and provide foods with a longer shelf life. They are traditional domestic skills and an industrial activity essential for ensuring food availability and security.

Freezing is currently one of the most important storage methods. For some fruit juices, such as orange juice, freezing reduces the content of flavonoids and hydroxycinnamic derivatives, but it does not affect the total vitamin C content.[74] The TPC can increase in some frozen samples, especially in frozen kale, which exhibits a higher TAC than the fresh sample.[65] In addition, Mazzeo *et al.*[66] observed that steaming for frozen vegetables maintained the TAC content and the bioaccessibility of polyphenols and carotenoids. However, these methods have some limitations, as fresh foods are inherently sensitive to cold, which may cause a reduction of natural antioxidants.

Another common storage method is refrigeration. Temperature and refrigeration time are the two main discussed factors that determine the effect of refrigeration on TAC. Galani *et al.*[75] showed a decrease in TPC, vitamin C and total anthocyanins in fruit and vegetables at 4 °C for 15 days, and an increase in the phenolic acid profile, in particular ellagic, gallic, sinapic and vanillic acids. Interestingly, Kevers *et al.*[76] studied the storage of a variety of food samples with different days and temperatures, and the results showed a temporary increase in TAC content in yellow peppers (34 days, 4 °C), broccoli (27 days, 4 °C) and plums (30 days, room temperature); and a temporary decrease of TAC in leeks (23 days 4 °C) and lettuces (8 days, 4 °C); and finally, a significant decrease of 20% in spinach (19 days, 4 °C) and 40% in melons (7 days, room temperature). The TAC value of other fruits and vegetables remained stable during the temporary changes in the refrigeration conditions, indicating that refrigeration did not affect the TAC of these foods. Cold storage at 6 ± 1 °C can increase anthocyanins, flavanones, hydroxycinnamic acids and total phenolics of blood pigmented oranges and in some cases, an increase in the TAC content was observed during prolonged storage of blood pigmented oranges.[77] Refrigeration is usually a stable method for preserving foods and their TAC values.

To maintain the quality of the harvest, foods can be stored in a controlled or modified atmosphere, with a reduced concentration of oxygen and an increased concentration of carbon dioxide. A controlled atmosphere is a method of storage where the concentration of carbon dioxide, nitrogen, oxygen, temperature and humidity is controlled.[78] Modified atmosphere storage refers to an initial and continuous change in the gas composition due to the respiration rate of food products and the permeability of food packaging.[79] For some fruits, such as oranges and apples, TAC increases from 36 to 50% during a two-month period in air storage.[75] The mechanisms that induce this increase in polyphenols are unknown, but it is clear that these conditions may influence the release of bioactive compounds, in particular polyphenols. In a study with blueberries, an increase of 15–30% of CO_2 in controlled atmosphere storage makes fruits softer than CO_2-free fruits. Also, a high concentration of CO_2 (30%) and variable of O_2 (2% and 21%) preserved polyphenol concentrations during storage, also preserving the TAC content. TAC content can increase when the storage temperature is between 0° and 3° C, which demonstrates that blueberries stored in a controlled atmosphere can significantly extend their shelf life.[80] A controlled atmosphere of nitrogen also maintains the total phenolic and total flavonoid content, but the storage is recommended to be at low temperature (1 °C).[81] In summary, to prevent the loss of TAC in a controlled atmosphere, the storage temperature is suggested to be fixed between 0 °C and 5 °C with low O_2 levels.

Lastly, another common preservation method of commercial products (fruit juices, dairy products, confectionery, meat and meat products) consists of adding additives as preservatives, denoted in Europe by an ''E'' followed by at least three numbers. Some of the most common ones are: i) antioxidants such as ascorbic acid (E300), propyl gallate (E310), sodium ascorbate (E301), tocopherols (E306), butylated hydroxyanisole (BHA, E320), butylated hydroxytoluene (BHT, E321), *etc.*, ii) antimicrobial agents such as acetic acid (E260), potassium acetate (E261), calcium acetate (E263), lactic acid (E270), carbon dioxide (E209) and malic acid (E296) and (iii) antimicrobial agents to prevent browning of food which may occur at any time during handling, processing and storage.[82] To date, data on the role of these additives and their effect on the TAC are very limited. In some studies, sodium benzoate was shown to reduce losses in TPC during storage at low temperature compared to foods without additives, *e.g.*, there is a lower decrease of phenolics, flavonoids and ascorbic acid in commercial fruit juices commercialized with additives compared to those without additives.[83,84] Therefore, sodium benzoate may have a protective effect on natural antioxidants. Likewise, there might be a synergistic or combined effect between additives (BHA and BHT, or BHA and propyl gallate), that might be explained by an increase in oxidative stability of the antioxidants by the mutual protection against the antioxidant radicals produced in the food.[85] However, the evidence supporting the influence of this synergistic effect on TAC is unclear. For this reason, the interest of using natural antioxidants as additives has increased over the years, hoping to offer powerful antioxidant activity and reduce free radicals. Some natural

antioxidants can compete with the effect of synthetic antioxidants, but they need to remain in the food during the different phases of the production. The general population usually believes that ''all natural'' is better than ''artificial'' in food processing, but this is not always true. The effect of natural antioxidants on the TAC and their effectiveness in food processing are not totally clear and need further investigation.

In summary, TAC content in foods and beverages can be maintained, increased or decreased during the cooking, preservation and storage processes. TAC variation is caused by the conversion of large compounds into small compounds and their extractability, which are influenced by the temperature, water content, cooking and storage time.

5.5 The Use of TAC Data in Food Science, Nutrition and Health

5.5.1 Screening of Ingredients

TAC data could be very useful in the food and pharmaceutical industry in order to screen ingredients and foods with a high TAC content. By ingredients, we refer to natural products (fruits, vegetables, cereals, nuts, spices and herbs) used to obtain a final transformed product. Screening ingredients will allow us to select the varieties and ingredients with higher TAC values.

Some plant-based foods like berries and green leafy plants, sometimes referred to in the media as ''superfoods'', are very rich in antioxidants and subsequently in TAC. However, in some cases this information is missing or is not complete. For example, the antioxidant potential of the leaves of *Pereskia aculeata* Miller (Barbads gooseberry) is very high, but its complete nutritional analysis is not available yet.[86] As well as for berries and green leaves, multiple articles report very high TAC values in herbs, spices and nuts.[27,87]

Several studies have analyzed local foodstuffs commonly used in specific areas that can be higher in TAC than same foods in other areas or other similar foods. For instance, a Greek study analyzed local herbs which have higher antioxidant capacity than in other areas.[87] Furthermore, local fruits or vegetables that are commonly consumed by a population may have a greater antioxidant capacity, such as the jujube in China or pitaya in Mexico, compared to other similar foods.[26,33] Screening many ingredients and obtaining a database with detailed TAC values are relevant particularly to improve food development and research due to a better characterization of current or potential ingredients. Moreover, it can be helpful to accurately estimate the dietary TAC intake and its relationships with health outcomes. [87]

5.5.2 Application of TAC Data in Quality Control and Food Processes

The quality of a final product is the main goal for all food producers, processors and consumers. Industrial companies, farmers and agronomists are

constantly striving to ensure that the selection of raw products and materials, processing methods, packaging, storage and distribution are adequate in order to achieve a high standard quality in the final product.[88] There are numerous food laws and regulatory procedures to assess the quality control of products. The general quality assurance systems are Hazard Analysis of Critical Control Points (HACCPs), Good Agricultural Practices (GAPs) and International Organization for Standardization (ISO), which cover the relevant laws on food production, labeling, use of food additives and food supplements.[88–90]

It is difficult to apply these systems for quality control in relation to TAC because there is not a standard assay for TAC measurement available, and the TAC content for each food and beverage differs based on the particular antioxidant assay used. For quality control of TAC values in food and beverages, correlations between assays provide more reliable quality control. ORAC, FRAP and TEAC methods have been shown to be correlated or uncorrelated depending on the type of food, *e.g.*, a positive correlation has been shown for various fruits and vegetables, but not for commercial beverages.[28] A recent study recommends FRAP as the TAC method of ref. 91 However, this approach needs to be carefully considered as the different methods affect the TAC content, as mentioned throughout this chapter. As we have discussed previously, a combination of at least two TAC methods should be recommended.

Another factor influencing the TAC content is food processing, which also needs to be taken into account in the quality control of TAC. Thermal food processing such as cooking, blanching, drying, roasting and pasteurization can damage the antioxidant content, including that of vitamin C, lycopene and polyphenols, in particular many flavonoids. Ahmed *et al.*[92] showed that roasting at high temperatures (120–160 °C) for more than 20 minutes reduced the content of flavonoids and peeling and trimming reduced the content of flavonoids to 39% in onions. Non-thermal methods (ultraviolet, pulsed electric field, high hydrostatic pressure, irradiation and combined non-thermal methods) are gaining attention because of the reduced decay of antioxidants in food, including some improvements in their activity and availability.[93] For instance, irradiation (100–700 Gy) of navel orange fruit followed by 3 weeks of storage at 5 °C showed no effect on its color, juiciness, phenolic content and antioxidant capacity.[94] Consequently, the new methods of processing in industry can be useful tools for maintenance of TAC in foods and provide a healthier final product.

Overall, TAC can be used as a quality parameter to monitor the different processes in the food industry, from the control of raw ingredients to the distribution since TAC content is relatively labile to some processes (cooking, storage, *etc.*). The ideal food processing method should be the one that safeguards the highest TAC (*i.e.*, the antioxidant quality and quantity).

5.5.3 Use of TAC Data in Developing New Foods and Pharmaceutical Supplements

Among the multitude of different food products and beverages, antioxidants are presented in two forms: natural or synthetic. The most used synthetic

antioxidants are BHA, BHT, propyl gallate and tertbutylhydroquinone (TBHQ). Evidence from animal research has shown that high doses of BHA and BHT have harmful health effects[95,96] which have led to the European Food Safety Agency (EFSA) and the European Union regulating their use (Regulation EU 1129/2011). In some countries, the use of several synthetic additives is banned due to their toxicity.[97]

Antioxidants are used as food additives in order to prolong the shelf life of a product due to their stability and ability to scavenge reactive oxygen species. This led to the belief that increasing the TAC value of a product can ameliorate its preservation. Studies on different food products such as biscuits and pork frozen patties[98,99] have shown that the addition of natural antioxidants increased the TAC value and also prolonged the shelf life of the products, without any deterioration of their organoleptic properties. Multiple studies on different types of oils also showed that adding an antioxidant to the product increased their oxidative stability while stocking or cooking.[100–102] A recent review by Grosshagauer *et al.*[103] highlighted that the improvement in the oil extraction process increases the quantity of antioxidants like vitamin E and phenolic compounds, which can be helpful to enhance the oxidative stability. The last studies also compared natural and synthetic antioxidants, and they reported that natural antioxidants can be as powerful and efficient as the synthetics. These are important findings for the food industry, considering the increased demand for natural products. Good alternatives to synthetic antioxidants can be spices and aromatic herbs, as they have a very high TAC content.[2] Yanishlieva and Marinova[104] summarized the data about the use of natural instead of synthetic antioxidants in various oils, and they concluded that the addition of natural antioxidants can protect the quality of the oils the same as or even better than synthetic antioxidants. However, the safety of some natural antioxidants still needs to be completely investigated before replacing the currently used synthetic antioxidants.

TAC data can also be useful in food science for the innovation and development of nutraceuticals, supplements and functional foods. Indeed, antioxidants are known to possess potential benefits for diverse health conditions,[105,106] and the supplementation with antioxidants might be helpful in cases of a poor diet in fruits and vegetables. Supplements are often produced as pills or capsules, ensuring safe storage capable of resisting different conditions. However, selecting antioxidants for new products should be carefully assessed since they can interact with each other *via* additive, synergistic or antagonistic mechanisms. The available antioxidant-rich supplements are mostly limited to vitamins or green tea extracts, but there are plenty of antioxidant types and sources such as phenolic derivatives, peptides/protein hydrolysates, phospholipids and polysaccharides that can be considered and transformed into diverse product concepts.[107]

In conclusion, TAC data can bring current knowledge regarding natural and industrial antioxidant potential products to the next level. Taking into account the increasing demand for more natural products, companies may use rich TAC ingredients to produce better market-oriented products.

5.5.4 Food Policies and Marketing

Nutrition and health claims are statements about the relationships between foods/ingredients and health benefits. Indeed, health claims are a valuable marketing tool, and therefore, their use has to be strictly regulated to ensure that consumers are informed and protected from misleading and false information. The EFSA provides the highest scientific opinions regarding nutrition and health claims and works in parallel with the European Commission. In the European Union countries, the nutrition and health claims are regulated by a Regulation (European Commission No 1924/2006). In the United States, the Food and Drugs Administration (FDA) is responsible for food safety and health promotion and evaluates the food claims in three categories; nutrient content claims, health claims, and structure/function claims (Figure 5.1).[108]

To date, there is not an established health claim regarding TAC. However, EFSA and the Panel at Dietetic Products, Nutrition and Allergies have created a guidance document[109] on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health, based on the capacity of foods and ingredients to scavenge free radicals. However, the evidence originated from *in vitro* models and their capacity to produce beneficial effects in humans has not been fully demonstrated yet.[109] To date, the FDA does not provide nutritional and health claims about TAC.

The separate evaluation of food and components can lead to different statements. For example, EFSA has recognized the antioxidant properties of polyphenols, and the Panel at Dietetic Products, Nutrition and Allergies has recognized the antioxidant properties of food components rich in polyphenols (*e.g.*, olive fruit, olive mill effluent or olive oil, *Olea europaea* L. extract and

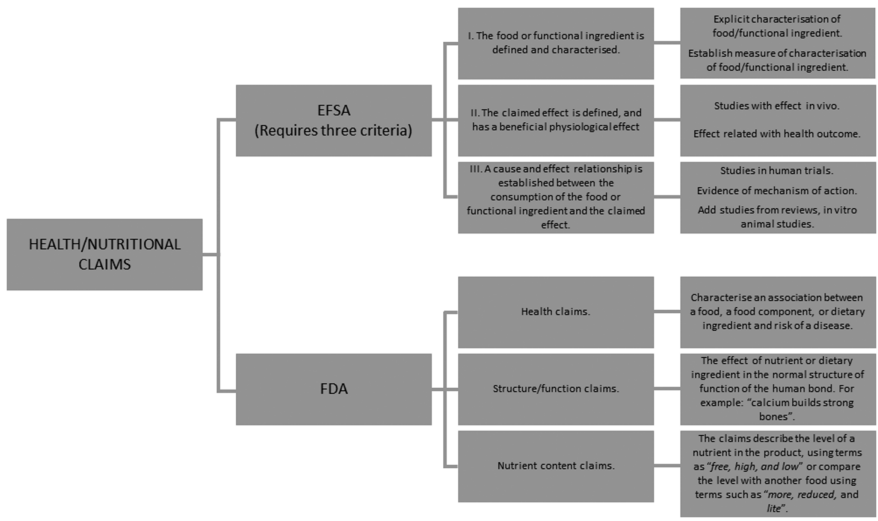


Figure 5.1 Assessment criteria of health/nutritional claims by the European Food Safety Authority (EFSA) and Food and Drugs Administration (FDA).

leaves) and high content of hydroxytyrosol (*e.g.*, oleuropein complex).[110] Nevertheless, in both cases the claims cannot be translated into TAC claims.

For marketing purposes, the TAC is sometimes used as an indicator of food quality and to promote health benefits associated with total antioxidants. Although food antioxidant intake is recognized to be beneficial for health, it is not totally correct to directly correlate the phytochemical content in foods and the body fluid concentration (*e.g.*, serum, plasma, urine) with health effects, since their biological activity also depends on their bioavailability and biofunctionality, that varies between different foods and antioxidant types.[111] Indeed, the bioavailability of antioxidants can be influenced by various factors, such as the molecular structure, the food matrix and processing techniques, enzyme activity, genetic, gut microbiome and hormonal characteristics of the host and the external environmental exposure.[112]

Currently, food labels are used to proclaim the nutritional facts and nutritional/health claims of products, both of which are linked to consumer choice of healthy food.[113] For this reason, it is important to emphasize that, beyond the available evidence, nutrition and health claims on antioxidants cannot be advanced, as the evidence does not yet meet the criteria formulated by EFSA and FDA, described in Figure 5.1. Furthermore, the mechanism of action and bioavailability of some natural antioxidants are not fully understood and require further investigation. In conclusion, nutrition and health claims made for antioxidants and/or TAC for marketing purposes are not scientifically and officially approved, but in the future they could be useful.

5.6 TAC Data, Nutrition and Health

5.6.1 Estimation of the Intake

The importance of natural antioxidants in reducing the risk of chronic diseases has been extensively studied.[114–117] However, the adequate validation of dietary TAC is not yet possible due to the lack of a reference TAC method and database. Attempts to estimate the TAC have been made in several populations worldwide.[111,118–120]

As can be observed in Table 5.3, dietary TAC intake can be expressed in different units and estimated by different TAC assays, making their comparison extremely difficult. Jun *et al.*[29] compared TAC intake between Korean and other populations, reporting 389.2, 345.4 and 772.1 mg VCE per day for Korean, US and Brazilian females respectively. For males, the values were 380.1 mg and 414.6 mg VCE per day for Korea and US populations, respectively. However, a comparison based on food choices may also be feasible, as the studies often described the food contributors to the dietary TAC intake. For example, fruits, vegetables, wine, beer and coffee are the main sources of antioxidants in the Mediterranean diets including those in Spain, Italy and Greece.[114,115,118] In Asian countries, like Korea and Japan, fruits and vegetables, soy and soy products, rice, seaweed, nuts and teas are typically the main contributors to TAC intake.[29,30,116]

Table 5.3 Estimation of dietary Total Antioxidant Capacity intake of different populations.[a]

Population	Method	Dietary TAC	Reference
Korean adults	ABTS	384.7 mg VCE per day	29, 30
	DPPH	20 763 μM TE per day	
	ORAC	54 335 μM TE per day	
	TEAC	876.4 μM TE per day	
US adults	ABTS	503.3 mg VCE per day	32
Spanish adults	FRAP	6014 μmol TE per day	118
	ABTS	3549 μmol TE per day	

[a]Abbreviations: VCE vitamin C equivalent; TE Trolox equivalent; DPPH 2,2-diphenyl-1-picrylhydrazyl; ORAC oxygen radical absorbance capacity; TEAC Trolox Equivalent Antioxidant Capacity; FRAP Ferric Reducing Antioxidant Power; ABTS 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid).

Although Mediterranean and Asian diets are both dietary patterns dominated by a large consumption of plant-based foods, their TAC intakes are highly influenced by the consumption of a few particularly antioxidant-rich products, and minor differences can also be due to variations in geographic characteristics (*e.g.*, food varieties, terroir, climate, *etc.*).

Regarding the diet of North and South American countries, there is a big difference compared to the Mediterranean and Asian diets. After beverages, fruit and vegetables are the main contributors to the Mexican rural dietary TAC, although their consumption is relatively low.[117] In the United States, alcoholic, non-alcoholic beverages and supplements are highlighted as big contributors to dietary TAC.[32] Supplements in the United States provide about 25% of dietary TAC.[32] In a Korean study, supplements represented around 38% of dietary TAC, mainly coming from vitamins and vitamin–mineral supplements.[121] When including supplements in the dietary TAC estimation, a rigorous measure of their TAC content is recommended, since the antioxidant content indicated on the label may be inaccurate.[122]

To obtain dietary data, observational epidemiologic studies mostly use food frequency questionnaires, 24 h dietary recalls and food records.[123] These questionnaires are usually self-administered and are based on the capacity of the population to report/remember their intakes, which may result in biases and measurement errors. In the near future, these questionnaires will be improved using interactive computer and camera-based technologies.[124] A good option can also be the combination of food frequency questionnaires and 24 h dietary recalls or a dietary recall questionnaire longer than one week and shorter than one month, suggested as the period needed to properly evaluate antioxidant content.[125] Questionnaire-based methods have a common limitation in that they do not consider the bioavailability of individual antioxidants in the foodstuffs listed and considered in the study. Biomarkers presented in blood or urine that reflect the intake of antioxidants are an alternative of self-reported dietary intake, although, in large-scale studies they are much more expensive. Measuring biomarkers also have limitations due to the lack of a comprehensive and

validated analytical method and the rapid pharmacokinetics of some antioxidants, like polyphenols.[126]

An accurate estimation of the dietary TAC relies also on the availability of a standardized TAC database, which is not currently available. Additionally, as has been described throughout this chapter, measuring the TAC content in foods is challenging due to many factors that influence TAC values such as plant variety, season of harvest, or food processing and cooking. While investigating the relation between TAC values and health, it is essential to consider the quality of TAC sources, as some unhealthy items such as alcoholic beverages also contribute a lot to dietary TAC.

5.6.2 Total Antioxidant Capacity Intake and its Health Benefits

Evidence that reactive oxygen and nitrogen species and oxidative damage are involved in various inflammatory and degenerative diseases is leading to growing scientific interest in the potential relationships between TAC and various health outcomes.[127,128]

Nutritional TAC is becoming a key measurement in assessing food quality and its link with health, but the lack of a standardized TAC method can lead to discrepancies in the results. For example, the use of FRAP, TRAP and the TEAC test showed that coffee, tea, fruit and vegetables can contribute to 75% of dietary TAC, while the ORAC assay indicates that fruit, vegetables and tea account for the majority of dietary TAC.[129]

Numerous epidemiological studies[120,128,130] and clinical trials[128,131,132] have already shown the positive correlation between the antioxidants contained in foods and the attenuation and prevention of metabolic disorders and the improvement of cognitive function.[133] TAC protects against oxidative damage to lipids and DNA damage, improves the levels of certain biomarkers such as low-density lipoproteins and triglycerides[134–136] and has a protective influence on cardiovascular disease and cancer development.[137,138] Dietary TAC has also shown the potential to reduce pre-diabetes morbidity by lowering glucose tolerance.[129,139] A recent *meta*-analysis of observational studies showed an inverse association between antioxidant consumption and the risk of colon, gastric and endometrial cancer.[140]

Dietary TAC has also been studied in terms of nutritional therapy for obesity, metabolic syndrome, dyslipidemia, cardiovascular diseases and cancer. The randomized controlled trial RESMENAS (MEtabolic Syndrome REduction in NAvarra-Spain) showed a positive correlation between dietary TAC and the reduction of weight, body mass index, waist circumference and fat mass in participants with obesity.[128] Several cohort studies have found a relationship between dietary TAC ($\geq$13.48 mmol per day based on the FRAP assay and $\geq$18 000 mmol per day based on the ORAC assay) and the treatment of cardiovascular disease by lowering blood pressure and concentrations of oxidized LDL[141,142] and reactive protein C, a biomarker of inflammation.[142,143] Nevertheless, the health benefits of TAC have mostly

been demonstrated by observational studies, and its mechanisms are not completely known. More studies are needed to fully understand all the factors involved in their metabolism, bioavailability and the relationship with health biomarkers and outcomes.

In summary, dietary TAC has been shown to play an important role in various health outcomes, and developing TAC data taking into account all the factors mentioned in this chapter can be a good tool to improve dietary TAC, as well as to establish new dietary guidelines.

5.6.3 Future Recommendations and Applications of TAC Data in Biomedical Research

TAC can be considered as a useful tool for clinical and research applications in the health sector. The available information on TAC has increased in recent years. In fact, it has been shown that the sum of antioxidants in the diet has a protective effect against various diseases associated with oxidative stress.[105,144] The current knowledge allows us to identify and classify food sources with high antioxidant capacity in diets, which can be a first step toward healthier diets. In order to standardize and implement the potential benefits of TAC data, the future focus should be on the creation of a single reliable TAC database. Apart from the database, more investigation is needed to understand antioxidant metabolism, bioavailability and their effect on health. It will be helpful to optimize and personalize dietary recommendations and to increase the quality in food production.

Abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzothiazolin-6 Sulfonic Acid)
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
DPPH	2,2-diphenyl-1-picrylhydrazyl
EFSA	European Food Safety Authority
ET	Electron Transfer
FDA	Food and Drugs Administration
FW	Fresh Weight
HAT	hydrogen atom transfer
FRAP	Ferric Reducing Antioxidant Power
ORAC	Oxygen Radical Absorbance Capacity
TAC	Total Antioxidant Capacity
TCC	Total Carotenoid Content
TE	Trolox Equivalent
TEAC	Trolox Equivalent Antioxidant Capacity
TPC	Total Phenol Content (TPC)
TRAP	Total Radical-Trapping Antioxidant Parameter
VCE	Vitamin C Equivalent

Acknowledgements

This research was funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (co-funded by European Regional Development Fund. ERDF, a way to build Europe) and from the 201943-30-31 project, funded by La Marató de TV-3. We thank CERCA Program/Generalitat de Catalunya for the institutional support. J.C. is thankful for the CONACYT fellowship (ID 693636) from the Mexican Government. M.G.-L. is grateful for the predoctoral scholarship PFIS (FI19/00185) from the Instituto de Institute of Health Carlos III. R.Z.-R. would like to thank the ''Miguel Servet'' program (CP15/00100) from the Institute of Health Carlos III (co-funded by the European Social Fund (ESF) – ESF investing in your future).

References

1. X. Wu, L. Gu and J. Holden, *J. Food Compos. Anal.*, 2004, **17**(3–4), 407.
2. M. H. Carlsen, B. L. Halvorsen and K. Holt, *Nutr. J.*, 2010, **9**, 3.
3. D. Huang, *Antioxidants*, 2018, **7**, 9.
4. D. W. Wilson, P. Nasg and H. Singh, *Antioxidants*, 2017, **6**, 1.
5. M. B. Arnao, A. Cano and M. Acosta, *Food Chem.*, 2001, **73**, 239.
6. W. Kalt, A. Cassidy and L. R. Howard, *Adv. Nutr.*, 2020, **11**(2), 224.
7. K. Kawabata, Y. Yoshioka and J. Terao, *Molecules*, 2019, **24**(2), 370.
8. A. A. Dayem, M. K. Hossain, S. B. Lee and K. Kim, *Int. J. Mol. Sci.*, 2017, **18**(1), 1.
9. M. Serafini, D. Villano, G. Spera and N. Pellegrini, *Nutr. Cancer*, 2006, **56**(2), 232.
10. E. B. Kurutas, *Nutr. J.*, 2016, **15**, 1.
11. K. Csepregi, S. Neugart, M. Schreiner and É. Hideg, *Molecules*, 2016, **21**(2), 1.
12. Y. T. Szeto, B. Tomlinson and I. F. F. Benzie, *Br. J. Nutr.*, 2002, **87**(1), 55.
13. A. Zulueta, M. J. Esteve, I. Frasquet and A. Frígola, *Food Chem.*, 2007, **103**(4), 1365.
14. J. Pérez-Jiménez, E. M. Díaz-Rubio and F. Saura-Calixto, *Plant Foods Hum. Nutr.*, 2015, **70**(4), 365.
15. R. Apak, M. Özyürek, K. Güçlü and E. Çapanoğlu, *J. Agric. Food Chem.*, 2016, **64**, 997.
16. R. Apak, E. Capanoglu and F. Shahidi, *Measurement of Antioxidant Activity and Capacity, Recent Trends and Applications*, John Wiley & Sons Ltd, UK, 1st edn, 2017.
17. J. Pérez-Jiménez, S. Arranz, M. Tabernero, M. E. Díaz- Rubio and J. Serrano, *Food Res. Int.*, 2008, **41**(3), 274.
18. S. Im, T. G. Nam, S. G. Lee, Y. J. Kim and O. K. Chun, *Food Sci. Biotechnol.*, 2014, **23**(3), 693.
19. V. Gökmen, A. Serpen and V. Fogliano, *Trends Food Sci. Technol.*, 2009, **20**(6), 278.

20. R. L. Prior, X. Wu and K. Schaich, *J. Agric. Food Chem.*, 2005, **53**(10), 4290.
21. M. D. Rivero-Pérez, P. Muñiz and M. L. González-Sanjosé, *J. Agric. Food Chem.*, 2007, **55**(14), 5476.
22. V. Dragović-Uzelac, B. Levaj, D. Bursać, S. Pedisić, I. Radojčić and A. Biško, *Agric. Conspec. Sci.*, 2007, **72**(4), 279.
23. J. T. J. Mariken, G. R. M. M. Haenen, H. P. Voss and A. Bast, *Food Chem. Toxicol.*, 2004, **42**, 45.
24. Ma. Murcia, Ma. A. Jiménez and M. Martínez-Tomé, *Food Res. Int.*, 2009, **42**(8), 1046.
25. J. Scalzo, A. Politi, N. Pellegrini, B. Mezzetti and M. Battino, *Nutrition*, 2005, **21**(2), 207.
26. R. Zamora-Ros, C. Biessy, J. A. Rothwell, A. Monge and M. Lajous, *Br. J. Nutr.*, 2018, **120**, 353.
27. N. Pellegrini, M. Serafini and B. Colombi, *J. Nutr.*, 2003, **133**, 2812.
28. A. R. Proteggente, A. S. Pannala and G. Paganga, *Free Radical Res.*, 2002, **36**(2), 217.
29. S. Jun, O. K. Chun and H. Joung, *Eur. J. Nutr.*, 2018, **57**(4), 1615.
30. J. H. Han, H.-J. Lee, M. R. Cho, N. Chang, Y. Kim and S.-Y. Oh, *Nutr. Res. Pract.*, 2014, **8**(2), 183.
31. F. Saura-Calixto, *J. Agric. Food Chem.*, 1998, **46**(10), 4303.
32. M. Yang, S. J. Chung, C. E. Chung, D. O. Kim, W. O. Song and S. I. Koo, *Br. J. Nutr.*, 2011, **106**(2), 254.
33. J. W. Li, S. D. Ding and X. L. Ding, *Process Biochem.*, 2005, **40**(11), 3607.
34. C. Kevers, J. Pincemail, J. Tabart, J. O. Defraigne and J. Dommes, *J. Agric. Food Chem.*, 2011, **59**(11), 6165.
35. D. O. Kim, S. W. Jeong and C. Y. Lee, *Food Chem.*, 2003, **81**(3), 321.
36. A. Nowicka, A. Z. Kucharska, A. Sokół-Łętowska and I. Fecka, *Food Chem.*, 2019, **270**, 32.
37. N. Paixão, R. Perestrelo, J. C. Marques and J. S. Câmara, *Food Chem.*, 2007, **105**(1), 204.
38. A. Kondrashov, R. Ševčík, H. Benáková, M. Koštířová and S. Štípek, *E Spen. Eur J Clin Nutr Metab*, 2009, **4**(1), 41.
39. A. Coletta, S. Berto, P. Crupi, M. C. Cravero, P. Tamborra and D. Antonacci, *Food Chem.*, 2014, **152**, 467.
40. K. Gündüz and E. Özdemir, *Food Chem.*, 2014, **155**, 298.
41. P. Jing, S. Y. Wang, C. Y. Wang and Y. Zheng, *Food Chem.*, 2011, **124**, 262.
42. S. Y. Wang, C. T. Chen, W. Sciarappa, C. Y. Wang and M. J. Camp, *J. Agric. Food Chem.*, 2008, **56**(14), 5788.
43. L. R. Howard, N. Pandjaitan, T. Morelock and M. I. Gil, Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season, *J. Agric. Food Chem.*, 2002, **50**(21), 5891.
44. R. M. Ferreyra, S. Z. Viña, A. Mugridge and A. R. Chaves, *Sci. Hortic.*, 2007, **112**(1), 27.
45. R. Tsao, R. Yang and S. Xie, *J. Agric. Food Chem.*, 2005, **53**(12), 4989.

46. M. E. Olsson, J. Ekvall, K. E. Gustavsson, J. Nilsson, D. Pilla and I. Sjöholm, *J. Agric. Food Chem.*, 2004, **52**(9), 2490.
47. O. A. Fawole and U. L. Opara, *S. Afr. J. Bot.*, 2013, **85**, 23.
48. M. Ghasemnezhad, M. Sherafati and G. A. Payvast, *J. Funct. Foods*, 2011, **3**(1), 44.
49. R. Huang, R. Xia, L. Hu, Y. Lu and M. Wang, *Sci. Hortic.*, 2007, **113**(2), 166.
50. M. Schulz, G. D. Borges and L. V. Gonzaga, *Food Res. Int.*, 2015, **77**, 125.
51. Ó. Acosta-Montoya, F. Vaillant, S. Cozzano, C. Mertz, A. M. Pérez and M. V. Castro, *Food Chem.*, 2010, **119**(4), 1497.
52. Z. Nie, C. Wan and C. Chen, *Antioxidants*, 2019, **8**(5), 136.
53. Ö. C. Açar, V. Gökmen, N. Pellegrini and V. Fogliano, *Eur. Food Res. Technol.*, 2009, **229**(6), 961.
54. B. J. Xu, S. H. Yuan and S. K. C. Chang, *J. Food Sci.*, 2007, **72**(2), S167.
55. F. Saura-Calixto, *J. Agric. Food Chem.*, 2011, **59**(1), 43.
56. U.S. Department of Agriculture, Agricultural Research Service, *Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods*, U.S. Department of Agriculture, Agricultural Research Service, Maryland (US), Release 2, 2010.
57. C. G. Fraga, P. I. Oteiza and M. Galleano, *Biochim. Biophys. Acta*, 2014, **1849**(2), 931.
58. K. D. P. P. Gunathilake, K. K. D. Somathilaka Ranaweera and H. P. Vasantha Rupasinghe, *Antioxidants*, 2018, **7**(9), 1.
59. F. J. Kao, Y. S. Chiu and W. D. Chiang, *J. Food Drug Anal.*, 2014, **22**(2), 202.
60. N. Pellegrini, C. Miglio, D. Del Rio, S. Salvatore, M. Serafini and F. Brighenti, *Int. J. Food Sci. Nutr.*, 2009, **60**(2), 12.
61. A. L. K. Faller and E. Fialho, *Food Res. Int.*, 2009, **42**(1), 210.
62. R. Preti, M. Rapa and G. Vinci, *J. Food Qual.*, 2017, 5329070.
63. J. D. P. Ramírez-Anaya, C. Samaniego-Sánchez and M. C. Castañeda-Saucedo, *Food Chem.*, 2015, **18**, 430.
64. F. Natella, F. Belelli, A. Ramberti and C. Scaccini, *J. Food Biochem.*, 2010, **34**(4), 796.
65. H. Ilyasoğlu and N. A. Burnaz, *Int. J. Food Prop.*, 2015, **18**(6), 1298.
66. T. Mazzeo, D. N'Dri, E. Chiavaro, A. Visconti, V. Fogliano and N. Pellegrini, *Food Chem.*, 2011, **128**(3), 627.
67. D. Zhang and Y. Hamauzu, *Food Chem.*, 2004, **88**(4), 503.
68. I. G. Hwang, Y. J. Shin, S. Lee, J. Lee and S. M. Yoo, *Prev. Nutr. Food Sci.*, 2012, **17**(4), 286.
69. I. A. Mohamed Ahmed, M. Musa Özcan, N. Uslu, F. A. L. Juhaimi and M. A. Osman, *J. Food Process. Preserv.*, 2020, **44**(10), 14807.
70. I. A. Mohamed Ahmed, F. Y. Al Juhaimi, M. A. Osman, S. A. Al Maiman, A. B. Hassan and H. A. S. Alqah, *LWT - Food Sci. Technol.*, 2020, **131**, 109825.

71. S. E. W. Opitz, B. A. Goodman, M. Keller, S. Smrke, M. Wellinger and S. Schenker, *Phytochem. Anal.*, 2017, **28**(2), 106.
72. A. Bobková, M. Hudáček, S. Jakabová, Ľ. Belej, M. Capcarová and J. Čurlej, *J. Environ. Sci. Health, Part B*, 2020, **55**(5), 495.
73. E. A. Abourashed, *Antioxidants*, 2013, **2**, 309.
74. I. Klimczak, M. Małecka and M. Szlachta, *J. Food Compos. Anal.*, 2007, **20**, 313.
75. J. H. Y. Galani, J. S. Patel, N. J. Patel and J. G. Talati, *Antioxidants*, 2017, **6**(3), 59.
76. C. Kevers, M. Falkowski, J. Tabart, J. O. Defraigne and J. Dommes, *J. Agric. Food Chem.*, 2007, **55**(21), 8596.
77. P. Rapisarda, M. Lo Bianco, P. Pannuzzo and N. Timpanaro, *Postharvest Biol. Technol.*, 2008, **49**, 348.
78. C. B. Watkins, Transport of Fresh Produce, in *Applied Plant Sciences*, Elsevier, 2nd edn, 2016.
79. M. V. Rama and P. Narasimham, Controlled-atmosphere storage | Effects on Fruit and Vegetables, in *Encyclopedia of Food Sciences and Nutrition*, Academic Press, 2nd edn, 2003.
80. G. Gunes, R. H. Liu and C. B. Watkins, *J. Agric. Food Chem.*, 2002, **50**(21), 5932.
81. E. Tsantili, K. Konstantinidis, M. V. Christopoulos and P. A. Roussos, *Sci. Hortic.*, 2011, **129**(4), 694.
82. V. Emerton and E. Choi, *Essential Guide to Food Additives*, Leatherhead Food International, UK, 1st edn, 2008.
83. A. Kaur, P. P. S. Gill and S. K. Jawandha, *J. Food Sci. Technol.*, 2019, **56**(7), 3391.
84. S. Sarkar, S. Saha, C. Rai and S. Bhattacharyya, *Int. J. Curr. Microbiol. Appl. Sci.*, 2014, **3**(7), 1007.
85. M. Taghvaei and S. M. Jafari, *J. Food Sci. Technol.*, 2015, **52**(3), 1272.
86. J. A. A. Garcia, R. C. G. Corrêa, L. Barros, C. Pereira, R. M. V. Abreu and M. J. Alves, *Food Chem.*, 2019, **294**, 302.
87. A. Roidaki, E. Kollia, E. Panagopoulou, A. Chiou, T. Varzaka and P. Markaki, *Curr Res Nutr Food Sci*, 2016, **4**(2), 138.
88. J. Trienekens and P. Zuurbier, *Int. J. Prod. Econ.*, 2008, **113**(1), 107.
89. J. Qian, M. Li, X. Yang, X. Liu and J. Wang, *Computer And Computing Technologies In Agriculture, Volume II. CCTA*, 2007, 259, 1205.
90. USDA, HACCP Seven Principles, *J. Food Prot. Fed. Regist.*, 1998, **61**(327), 381.
91. R. Queiros, P. R. Tafulo and F. Sales, *Comb. Chem. High Throughput Screening*, 2013, **16**(1), 22.
92. M. Ahmed and J. B. Eun, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**(18), 3159.
93. F. Al-juhaimi, K. Ghafoor, M. M. Özcan, M. H. A. Jahurul, E. E. Babiker and S. Jinap, *J. Food Sci. Technol.*, 2018, **55**(10), 3872.
94. H. McDonald, M. Lu Arpaia, F. Caporaso, D. Obenland, L. Were and C. Rakovski, *Postharvest Biol. Technol.*, 2013, **86**, 91.
95. A. L. Branen, *J. Am. Oil Chem. Soc.*, 1975, **52**(2), 59.

96. N. Ito, S. Fukushima, A. Hagiwara, M. Shibata and T. Ogiso, *J. Natl. Cancer Inst.*, 1983, **70**(2), 343.
97. J. Pokorný, *Trends Food Sci. Technol.*, 1991, **2**, 223.
98. C. Caleja, L. Barros, A. L. Antonio and M. B. P. P. Oliveira, *Food Chem.*, 2017, **216**, 342.
99. A. Sasse, P. Colindres and M. S. Brewer, *J. Food Sci.*, 2009, **74**(1), S30.
100. S. Ching, M. Cheung, Y. T. Szeto and I. F. F. Benzie, *Plant Foods Hum. Nutr.*, 2007, **62**, 39.
101. J. L. Quiles, M. C. Ramírez-Tortosa, J. A. Gómez, J. R. Huertas and J. Mataix, *Food Chem.*, 2002, **76**(4), 461.
102. D. Bera, D. Lahiri and A. Nag, *J. Food Eng.*, 2006, **74**(4), 542.
103. S. Grosshagauer, R. Steinschaden and M. Pignitter, *LWT - Food Sci. Technol.*, 2019, **106**, 72.
104. N. V. Yanishlieva and E. M. Marinova, *Eur. J. Lipid Sci. Technol.*, 2001, **103**(11), 752.
105. B. L. L. Tan, M. E. Norhaizan and W. P. P. Liew, *Eur. J. Lipid Sci. Technol.*, 2001, **103**, 752.
106. D. Aune, N. N. Keum, E. Giovannucci, L. T. Fadnes and P. Boffetta, *Front. Pharmacol.*, 2018, **9**, 1.
107. F. Shahidi and Y. Zhong, *Eur. J. Lipid Sci. Technol.*, 2010, **112**(9), 930.
108. J. C. Rowlands and J. E. Hoadley, *Toxicology*, 2006, **221**(1), 35.
109. C. Agostoni, J. L. Bresson, S. Fairweather-Tait and A. Flynn, *EFSA J.*, 2011, **9**, 1.
110. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), *EFSA J.*, 2011, **9**(4), 2033.
111. A. Pompella, H. Sies, R. Wacker and F. Brouns, *Nutrition*, 2014, **30**(7–8), 791.
112. M. Porrini and P. Riso, *Nutr., Metab. Cardiovasc. Dis.*, 2008, **18**(10), 647.
113. J. Barreiro-Hurlé, A. Gracia and T. de-Magistris, *Food Policy*, 2010, **35**(3), 221.
114. C. Pitsavos, D. B. Panagiotakos, N. Tzima, C. Chrysohoou and M. Economou, *Am. J. Clin. Nutr.*, 2005, **82**(3), 694.
115. C. La Vecchia, A. Decarli, M. Serafini, M. Parpinel and R. Belloccol, *Int. J. Cancer*, 2013, **133**(6), 1447.
116. S. Kobayashi, K. Murakami, S. Sasaki, K. Uenishi, M. Yamasaki and H. Hayabuchi, *Nutr. J.*, 2012, **11**, 91.
117. D. Hervert-Hernández, O. P. García, J. L. Rosado and I. Goñi, *Food Res. Int.*, 2011, **44**(5), 1182.
118. F. Saura-Calixto and I. Goñi, *Food Chem.*, 2006, **94**(3), 442.
119. H. Ye, S. Shen, J. Xu, S. Lin, Y. Yuan and G. S. Jones, *Food Control*, 2013, **34**(2), 619.
120. S. A. Kim, H. Joung and S. Shin, *Nutr. J.*, 2019, **18**(1), 1.
121. S. Kim, Y. Song, J. Lee, S. Jun, S. Shin and G.-A. Wie, *Nutrients*, 2017, **9**(10), 1055.
122. N. P. Seeram, S. M. Henning, Y. Niu, R. Lee and H. S. Scheuller, *J. Agric. Food Chem.*, 2006, **54**(5), 1599.

123. K. L. Tucker, *Nutr., Metab. Cardiovasc. Dis.*, 2007, **17**(2), 74.
124. H. Boeing, *Eur. J. Clin. Nutr.*, 2013, **67**(5), 424.
125. C. D. Ouellette, M. Yang, Y. Wang, T. Vance, M. L. Fernandez and N. Rodriguez, *Nutrition*, 2014, **30**(11–12), 1355.
126. R. Zamora-Ros, M. Touillaud, J. A. Rothwell and I. Romieu, *Am. J. Clin. Nutr.*, 2014, **100**(1), 11.
127. B. Puchau, M. Á. Zulet, A. G. de Echávarri and H. H. M. Hermsdorff, *J. Am. Coll. Nutr.*, 2009, **28**(6), 648.
128. P. Lopez-Legarrea, R. De La Iglesia, I. Abete, I. Bondia-Pons and S. Navas-Carretero, *Nutr. Metab.*, 2013, **10**(1), 1.
129. H. Okubo, H. E. Syddall, D. I. W. Phillips, A. A. Sayer, E. M. Dennison and C. Cooper, *Nutr., Metab. Cardiovasc. Dis.*, 2014, **24**(3), 301.
130. P. Villaverde, M. Lajous, C. J. MacDonald, G. Fagherazzi and F. Bonnet, *Nutr. J.*, 2019, **18**(1), 1.
131. S. Valtueña, N. Pellegrini, L. Franzini, M. A. Bianchi and D. Ardigò, *Am. J. Clin. Nutr.*, 2008, **87**(5), 1290.
132. M. Parohan, J. Anjom-Shoae, M. Nasiri, M. Khodadost and S. R. Khatibi, *Eur. J. Nutr.*, 2019, **58**(6), 2175.
133. L. Goni, M. Fernández-Matarrubia, A. Romanos-Nanclares and C. Razquin, *Br. J. Nutr.*, 2021, **126**(1), 43.
134. B. Alipour, B. Rashidkhani and S. Edalati, *Nutrition*, 2016, **32**(5), 566.
135. T. Bacchetti, I. Turco, A. Urbano, C. Morresi and G. Ferretti, *Nutrition*, 2019, **61**, 164.
136. N. Stedile, R. Canuto, C. D. De Col, J. S. De Sene, A. Stolfo and G. N. D. S. Wisintainer, *Int. J. Food Sci. Nutr.*, 2016, **67**(4), 479.
137. S. Li, M. Xu, Q. Niu, S. Xu, Y. Ding and Y. Yan, *PLoS One*, 2015, **10**(10), 1.
138. M. Serafini and D. Del Rio, *Redox Rep.*, 2004, **9**(3), 145.
139. G. Sotoudeh, M. Abshirini, F. Bagheri, F. Siassi, F. Koohdani and Z. Aslany, *Nutrition*, 2018, **46**, 20.
140. M. Parohan, A. Sadeghi, S. R. Khatibi, M. Nasiri, A. Milajerdi and M. Khodadost, *Crit. Rev. Oncol. Hematol.*, 2019, **138**, 70.
141. S. Rautiainen, E. B. Levitan, N. Orsini, A. Åkesson, R. Morgenstern and M. A. Mittleman, *Am. J. Med.*, 2012, **125**(10), 974.
142. S. Rautiainen, E. B. Levitan, M. A. Mittleman and A. Wolk, *Am. J. Med.*, 2013, **126**(6), 494.
143. F. Brighenti, S. Valtueña, N. Pellegrini, D. Ardigò, D. Del Rio and S. Salvatore, *Br. J. Nutr.*, 2005, **93**(5), 619.
144. E. A. Decker and P. M. Clarkson, *Handbook of Oxidants and Antioxidants in Exercise*, 1st edn, 2000, Elsevier Science, p. 323.