Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

Microbial phenolic metabolites are associated with better frontal lobe cognition

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ARTICLE INFO

Article history:
Received 14 July 2022
Received in revised form 15 September 2022
Accepted 14 October 2022

Keywords:
Polyphenols
Neurodegeneration
Microbiota
Gut-brain axis
PREDIMED

ABSTRACT

With increasing life expectancy, neurodegenerative diseases have become one of the leading causes of ill-health in the elderly. Preventive strategies include following healthy diets, such as the Mediterranean diet, which is particularly rich in polyphenols, bioactive compounds with neuroprotective properties. The aim of this study was to assess the association of microbial phenolic metabolites (MPM) with cognition. This cross-sectional analysis was performed with 200 participants of the PREDIMED trial (Barcelona-Clinic recruitment center). A novel method based on liquid chromatography coupled to mass spectrometry was used to identify urinary MPM (protocatechuic acid, enterodiol glucuronide, enterolactone glucuronide, urolithin B glucuronide, and vanillic acid glucuronide), and cognitive function was evaluated with neuropsychological tests. Multivariable-adjusted ordinary least squares regression was used to assess the associations between cognitive function and MPM, and a score was calculated as the weighted sum of MPM. A higher MPM score was associated with better frontal lobe function. Among individual metabolites, vanillic acid glucuronide was correlated with frontal cognitive performance. Participants with higher concentrations of vanillic acid glucuronide and urolithin B glucuronide obtained better scores in the Color Trail Test part 2. A higher score for urinary multi-MPM was associated with better frontal cognitive performance in an older Mediterranean population.

1. Introduction

The prevalence of neurodegenerative diseases has increased considerably in the last years due to population aging[1]. With the growth in life expectancy, dementia has become a leading cause of death in developed countries, most frequently in the form of Alzheimer's disease[2]. The neuronal loss that characterizes Alzheimer's disease particularly affects the frontal and temporal lobes; other associated changes in the brain are the formation of neurofibrillary tangles and amyloid plaques. At present, it is an incurable disease with a late diagnosis, so strategies to prevent or delay its development are urgently needed[3].

Although the precise cause of Alzheimer's disease and related dementias remains unknown, there is evidence that their development is associated with several modifiable risk factors, among them

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Peer review under responsibility of Tsinghua University Press.

Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2023.9250013

education, vascular impairment, physical activity, and dietary habits[4]. Diet in particular, is believed to play a major role in cognitive decline, a pre-dementia stage[5]. In this context, several epidemiological and clinical studies have shown that the Mediterranean diet can both delay age-related cognitive decline[6] and reduce the incidence of neurodegenerative disorders and mortality due to Alzheimer's disease[7]. The Mediterranean diet is a plant-based dietary pattern characterized by a high intake of fruits, vegetables, and seeds rich in bioactive compounds such as polyphenols, which have antioxidant and anti-inflammatory properties[8]. This may partially explain its protective effects against neurodegeneration, which is closely related to oxidative stress and inflammation[9].

Although polyphenols may have beneficial effects on cognitive function[10], they have low bioavailability. Prior to absorption, they are extensively metabolized by colonic microbiota[11], with which they have a bidirectional relationship, as polyphenols can also act as prebiotics and influence microbiota composition. The central nervous system and gut microbiota are deeply connected by biochemical signaling, forming what is known as the gut-brain axis, and polyphenols may be involved by improving cerebral blood flow or directly crossing the blood-brain barrier[12].

Despite growing interest in the gut-brain axis in recent years, to the best of our knowledge no study has evaluated the effect of microbial phenolic metabolites (MPM) detected in urine on neurocognition. Therefore, the aim of the present study was to assess the association of MPM with cognitive function in a subgroup of participants in the PREDIMED trial. A novel methodology previously developed by our group using high precision analytical techniques based on linear ion trap quadrupole-Orbitrap-mass spectrometry (LTQ-Orbitrap-MS) allowed us to identify hitherto scarcely explored compounds derived from the gut microbiota.

2. Materials and methods

2.1 Study design

A cross-sectional analysis was conducted within the PREDIMED (PREvención con DIeta MEDiterránea) study, a large, parallel-group, multicenter, randomized, controlled clinical trial with a mean follow-up of 5 years, designed to assess the effect of the Mediterranean diet enriched in olive oil or nuts on cardiovascular disease (CVD) incidence (http://www.predimed.es)[13]. Participants were recruited in Spain from October 2003 to December 2010 and included 7 447 men (55−80 years old) and women (60−80 years old) at high cardiovascular risk. Eligible participants had type 2 diabetes or at least 3 of the following major risk factors: current smoking, hypertension, dyslipidemia, overweight/obesity or a family history of premature CVD. A detailed description of methods and participants has been published elsewhere[13-14].

The present sub-study was performed in a random subsample of 200 participants from the PREDIMED-Hospital Clinic recruitment center (Barcelona) who participated in a cognitive function study[15]. Participants who reported extreme total energy intakes (> 3 500 or < 500 kcal/day in women or > 4 000 or < 800 kcal/day in men) were excluded from the analysis[16].

The Institutional Review Board (IRB) of the Hospital Clinic (Barcelona, Spain) accredited by the US Department of Health and Human Services (DHHS) update for Federal-wide Assurance for the Protection of Human Subjects for International (Non-US) Institutions #00000738 approved the study protocol on July 16, 2002. All participants provided informed consent and signed a written consent form.

2.2 Covariate assessment

Participants completed a validated, semi-quantitative 137-item food frequency questionnaire with the assistance of trained dietitians, as well as a 14-item questionnaire to assess their adherence to the Mediterranean diet[17]. Trained personnel measured body weight, height, waist circumference and blood pressure. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Physical activity (metabolic equivalent tasks per minutes per day, METs min/day) was assessed with a validated Spanish version of the Minnesota physical activity questionnaire[18].

2.3 Cognitive tests

Cognitive examinations were conducted by an experienced neuropsychologist. The instruments employed for the cognitive assessment were as follows: global cognitive function was evaluated with the Mini-Mental State Examination (MMSE)[19]; intermediate and delayed episodic verbal memory were rated by the Rey Auditory Verbal Learning Test (RAVLT)[20]; episodic verbal memory was assessed with a subtest of the Wechsler Memory Scale (WMS), the verbal paired associates test[21]; semantic fluency was evaluated with an animal fluency test[22]; immediate memory and working memory were assessed with the digit span test of the Wechsler Adult Intelligence Scale (WAIS)[23]; executive function, including attention, visual-motor speed, and cognitive flexibility, were measured with the Color Trail Test (parts 1 and 2)[24]. Three composite scores of cognitive function were constructed for each participant. The frontal function composite was based on the standardized mean results of the Digit Span Backward Test and Color Trail Test (parts 1 and 2), which measured attention, cognitive flexibility and working memory. The memory composite was constructed with the standardized results from the RAVLT and WMS paired associate subtest. Finally, a global cognition composite was obtained by computing the mean standardized results of all the neuropsychological tests, including the MMSE.

2.4 Microbial phenolic metabolites

Fig. 1 shows a schematic representation of the experimental procedure for identifying and quantifying urinary MPM using a solid-phase extraction process followed by a chromatographic and spectrometric analysis in a LTQ-Orbitrap-MS.

2.4.1 Standards and reagents

Protocatechuic acid (PCA), enterodiol, urolithin-A, and urolithin-B were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standard (+) *cis*, *trans*-abscisic acid D6 was obtained from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA). Vanillic acid, enterolactone and creatinine were obtained from Fluka (St. Louis, MO, USA). Standards were stored in powder

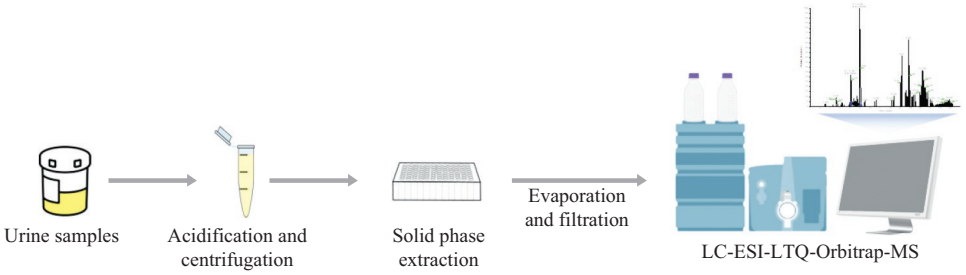

Fig. 1 Schematic representation of the experimental analysis of microbial phenolic metabolites.

form and protected from light. The reagents were purchased from the following commercial suppliers: methanol of LC-MS grade and acetonitrile of HPLC grade from Sigma-Aldrich and formic acid (≥ 98%) from Panreac Química S.A. (Barcelona, Spain). Ultrapure water (Milli-Q) was generated by a Millipore system (Bedford, MA, USA).

2.4.2 Sample preparation

Biological samples were collected after an overnight fast, coded, and stored at −80 ºC until analysis. MPM produced by the gut microbiota were determined using a method previously validated by our group with minor modifications[25]. For the present analyses, we focused only on MPM that are mostly or exclusively produced by the gut microbiota. Briefly, 50 μL of urine samples were diluted 1:20 (*V/V*) with Milli-Q Water and 100 μL of the internal standard abscisic acid D6 was added. The sample dilution was then acidified with 2 μL of formic acid and centrifuged at 15 000 × *g* at 4 ºC for 4 min. To isolate MPM and eliminate undesired compounds, the acidified urines underwent a solid-phase extraction in Water Oasis HLB 96-well plates 30 μm (30 mg) (Water Oasis, Milford, MA, USA). The 96-well plate was activated with methanol and 1.5 mol/L formic acid, and after loading the samples, a clean-up step was performed with 1.5 mol/L formic acid and methanol (0.5%). MPM were eluted with methanol acidified with 1.5 mol/L formic acid, evaporated to dryness with nitrogen gas and reconstituted with 100 μL formic acid (0.05%). After 20 min of vortex mixing, the samples were filtered through 0.22 μm polytetrafluoroethylene 96-well plate filters (Millipore, Massachusetts, USA).

Urinary concentrations of MPM were corrected by urine creatinine, measured according to the Jaffé alkaline picrate method adapted for Thermo microtiter 96-well plates, as described by Medina-Remón et al.[26].

2.4.3 LTQ-Orbitrap ESI analysis

The analysis was performed on an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) equipped with an ESI source working in negative mode as described elsewhere[25]. Chromatographic separation was performed on a Kinetex F5 100A (50 mm × 4.6 mm × 2.6 μm) from Phenomenex (Torrance, CA, USA). Mobile phases A and B were, respectively, 0.05% formic acid in water and 0.05% formic acid in acetonitrile. The following linear gradient was used: held at 98% A for 1.7 min, decreased to 92% A for 3 min, decreased to 80% A for 1.3 min, decreased to 70% A for 1.3 min, decreased to 50% for 0.1 min, decreased to 0% for 1.3 min, then returned to initial conditions for 1.7 min and re-equilibrated for 3 min. The flow rate was set at 0.750 μL/min and the injection volume was 5 μL.

2.4.4 Identification and quantification of MPM

The identification and quantification of MPM was performed using Trace Finder software version 4.1 (Thermo Fisher Scientific, San Jose, CA). As standards for glucuronidated and sulfated MPM were unavailable, these metabolites were quantified with their respective aglycone equivalents.

Due to high inter-individual variability in the metabolism of MPM, the identifiable metabolites were not detected in all the participants. To simplify the analysis and facilitate its comprehension, only metabolites with < 20% of missing values were included in the statistical analyses. Following this criterion, a total of 5 metabolites were included: PCA, enterodiol glucuronide (EDG), enterolactone glucuronide (ELG), urolithin B glucuronide (UBG), and vanillic acid glucuronide (VAG). Missing values of the previously listed metabolites with less than 20% of missing values were replaced by the half of the minimum detectable value.

2.5 Statistical analysis

Baseline characteristics of the participants are described as mean ± standard deviations (SD) for quantitative variables and percentages for categorical variables. The MPM concentrations were normalized and scaled in multiples of 1-SD with Blom's inverse normal transformation to stabilize the variance[27].

The association of MPM with neuropsychological test scores and test composites was assessed with multivariable linear regression adjusted for 2 models of increasing complexity. Model 1 was adjusted for age and sex. Model 2 was further adjusted for smoking (former, never, current), educational level (low, medium and high), APOE e4 genotype, physical activity, BMI, total energy intake, Mediterranean diet adherence (continuous), hypertension, hypercholesterolemia, diabetes, statin treatment, and use of anticholinergic drugs (yes/no). A corrected *P*-value by multiple comparison following the Simes procedure was also considered. In addition, an MPM score was calculated as the weighted sum of concentrations of the target metabolites and modelled the score as a main exposure in the multivariable linear regression model. The weight for each metabolite was the regression coefficient for a 1-SD increment in the plasma concentration estimated from the multivariable linear regression model.

All statistical analyses were performed with Stata 16.0 (Stata-Corp LP, TX. USA). Two-sided significance was set at $P < 0.05$.

3. Results

3.1 General characteristics of participants

The characteristics of all the participants are listed in Table 1 according to quartiles of total urinary MPM concentrations. All the groups were well-balanced in terms of sex, age, and educational level. By study design, all the participants were overweight or obese and had a high prevalence of conventional cardiovascular risk factors: 51% had type-2 diabetes mellitus, 79% had dyslipidemia, and 76% had hypertension. A lower percentage of participants suffered from diabetes in the third quartile of MPM, whereas the second quartile had more participants with hypertension. Only 15% of the study population were current smokers. Physical activity was comparable among groups, although participants in the first quartile tended to be more physically active. Regarding total energy intake, the first quartile displayed the highest values, whereas the last quartile had the lowest.

Table S1 presents the means and SD of urinary metabolite concentrations for the total population and according to quartiles. The predominant metabolites were VAG ((0.222 ± 0.393) μmol/mg of creatinine) and UBG ((0.158 ± 0.605) μmol/mg of creatinine), whereas the least abundant identified metabolite in urine was EDG ((0.003 ± 0.004) μmol/mg of creatinine), followed by PCA ((0.009 ± 0.020) μmol/mg of creatinine) and ELG ((0.020 ± 0.026) μmol/mg of creatinine).

3.2 MPM and cognition composites

The associations between MPM scores and the composite scores for frontal lobe function, memory, and global cognition are presented in Table 2. In the multivariable adjusted model that included sex, age, smoking, education, physical activity, BMI, total energy intake, hypertension, hypercholesterolemia, diabetes, APOE e4 genotype, statin treatment, anticholinergic drugs, and Mediterranean diet adherence, the MPM score was associated with a higher composite score for frontal lobe function (β = 0.69, 95% CI: 0.14, 1.24 per 1-SD increase, P-value = 0.014). On the other hand, no significant association was found with memory or global composite scores. When the MPM scores were modelled as quartiles, we observed that participants in the highest quartile had higher composite scores for frontal function and the relationship was linear (β = 0.51, 95% CI: 0.05, 0.96, P = 0.033 for trend).

Individual polyphenols are described in Table 3. Urine VAG was positively associated per 1-SD increment with the frontal composite score in the fully adjusted model (β = 0.17, 95% CI: 0.03, 0.31 per 1-SD, P-value = 0.018), but it did not remain significant after accounting for multiple testing.

3.3 MPMs and neuropsychological tests

Table S2 describes the associations of individual MPMs modelled continuously (per 1-SD) with neuropsychological tests. Most of the individual MPM did not show any significant association

Table 1
General characteristics of all participants according to quartiles of MPM[a].

Characteristics	All (n = 200)	Q1 (n = 50)	Q2 (n = 50)	Q3 (n = 50)	Q4 (n = 50)	P-value[b]
MPM	0.41 (0.02–4.53)	0.06 (0.02–0.09)	0.12 (0.09–0.15)	0.23 (0.15–0.32)	1.23 (0.34–4.53)	
Women (%)	54.5	46.0	58.0	48.0	66.0	0.156
Age (years)	66.1 ± 5.3	66.8 ± 5.9	65.8 ± 4.3	65.90 ± 5.4	66.0 ± 5.8	0.763
Weight (kg)	76.6 ±11.7	78.4 ± 12.0	77.6 ± 11.4	74.4 ± 11.4	76.0 ± 12.0	0.317
BMI (kg/m^2)	29.5 ± 3.4	29.5 ± 2.9	29.8 ± 3.5	28.6 ± 3.6	30.2 ± 3.6	0.099
Medium & high educational level (%)	33.5	28.0	40.0	28.0	38.0	0.430
Diabetes mellitus (%)	51.0	52.0	70.0	38.0	44.0	0.009
Dyslipidaemia (%)	79.0	80.0	70.0	86.0	80.0	0.264
Hypertension (%)	76.0	74.0	82.0	78.0	70.0	0.533
Current smoker (%)	15.0	12.0	16.0	20.0	12.0	0.881
Physical activity (METS-min/day)	291.0 ± 266.9	332.3 ± 332.6	269.7 ± 250.6	314.0 ± 241.1	247.8 ± 230.2	0.364
Total energy intake (kcal/day)	2 394.1 ± 500.8	2 494.5 ± 487.3	2 367.4 ± 578.0	2 472.2 ± 429.9	2 242.1 ± 470.3	0.045

Note: Q, quartile; MPM, microbial phenolic metabolites; mets, metabolic equivalents. [a] Continuous variables are shown as means ± SDs, and categorical variables are shown as percentages. [b] T-tests were used for continuous variables, and a chi-square test was used for categorical variables.

Table 2
Multivariable adjusted regression between phenolic metabolite scores and composite cognitive scores.

	β (95% CI)[a]	P-value	Q1	Q2	Q3	Q4	P-trend
Frontal lobe function composite							
Model 1[b]	0.88 (0.36, 1.40)	0.001	Ref	0.43 (−0.02, 0.88)	0.30 (−0.15, 0.76)	0.62 (0.14, 1.09)	0.023
Model 2[c]	0.69 (0.14, 1.24)	0.014	Ref	0.47 (0.08, 0.86)	0.46 (0.10, 0.83)	0.51 (0.05, 0.96)	0.033
Memory composite							
Model 1[b]	0.24 (−0.55, 1.03)	0.552	Ref	0.07 (−0.24, 0.38)	−0.07 (−0.37, 0.24)	0.13 (−0.23, 0.49)	0.658
Model 2[c]	0.22 (−0.64, 1.07)	0.620	Ref	0.10 (−0.19, 0.39)	−0.12 (−0.44, 0.19)	0.10 (−0.26, 0.45)	0.908
Global composite							
Model 1[b]	0.73 (−0.74, 1.49)	0.512	Ref	0.06 (−0.17, 0.29)	0.03 (−0.19, 0.25)	0.16 (−0.09, 0.42)	0.198
Model 2[c]	0.37 (−0.74, 1.49)	0.512	Ref	−0.03 (−0.25, 0.20)	−0.01 (−0.25, 0.22)	0.08 (−0.16, 0.32)	0.504

Note: Q, quartile. [a] β, difference between groups; CI, confidence interval. [b] Model 1: sex and age. [c] Model 2: sex, age, smoking habit, educational level, physical activity, BMI, total energy intake, hypertension, hypercholesterolemia, diabetes, APO E genotype, statin treatment, anticholinergic drugs, and Mediterranean diet adherence.

with the cognitive tests, except for UBG and VAG with the Color Trail Test part 2.

As it is shown in Fig. 2, participants with higher urinary UBG and VAG obtained better scores in the Color Trail Test part 2 (β = 0.19, 95% CI: 0.01, 0.36 per 1-SD, P-value = 0.035 and β = 0.19, 95% CI: 0.01, 0.38 per 1-SD, P-value = 0.037, respectively). However, these associations were no longer significant after adjustment for multiple comparisons.

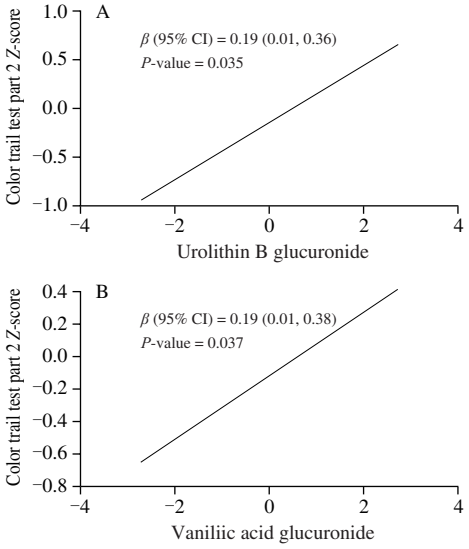

Fig. 2 Multivariable adjusted regression of Color Trail Test Part 2 Z-score of (A) urolithin B glucuronide, and (B) vaniliic acid glucuronide. Metabolites concentrations were normalized and expressed as 1-SD increment. Models adjusted for sex, age, smoking habit, educational level, physical activity, BMI, total energy intake, hypertension, hypercholesterolemia, diabetes, APOE genotype, statin treatment, anticholinergic drugs, and Mediterranean diet adherence.

4. Discussion

In this cross-sectional study conducted in a subsample of participants in the PREDIMED trial, we observed that a score that combined urinary MPMs was associated with better frontal lobe function in a Mediterranean population at high cardiovascular risk. Associations were found between higher composite scores for frontal function and higher concentrations of VAG, and between scores for the Color Trail Test part 2 and both VAG and UBG. Therefore, the findings from the present study suggest that MPMs may be involved in frontal cognition.

The gut microbiota has a potentially high impact on brain function due to its influence on the nervous, endocrine, and immune systems through the gut-brain axis[10]. Thus, maintaining a healthy gut microbiota has emerged as a key factor for the protection of normal brain function[28]. Polyphenols can alter the gut microbial community and, conversely, their metabolism and bioavailability depend on the microbiota composition and associated enzymatic transformations. Thus, polyphenol metabolism differs between individuals, even after the intake of similar dietary sources of phenolic compounds[12]. This would explain why in the present study only 5 urinary MPMs were identified and quantified as widely present among participants.

In the present cross-sectional analysis, we found associations between total MPM scores and frontal functions composite after adjusting for potential confounders, suggesting that a combination of polyphenols derived from the microbiota may have a positive impact on frontal lobe functions. Regarding dietary intakes, studies assessing the consumption of polyphenols using food frequency questionnaires have demonstrated that these molecules have benefits against neurodegeneration. The 2 major classes of polyphenols, flavonoids and phenolic acids, have been shown to exert neuroprotective effects in middle-aged and older adults[29-30]. While total urinary polyphenols have been associated with better cognitive performance in an older Mediterranean population[10], few studies have analyzed the relationship between individual MPMs and cognition. A recent cross-sectional study reported a neuroprotective effect of serum phenolic metabolites, some of which were derived from gut bacteria[29]. Results of animal studies are also consistent with our findings, as they support an inverse association between MPMs and cognitive impairment[30-31].

The frontal lobe of the brain is involved in processes related to executive function, attention and working memory, all of which have been associated with MPMs and are among the cognitive functions

Table 3
Multivariable adjusted regression between individual MPM and composite cognitive test scores.

	Frontal lobe function composite		Memory composite		Global composite	
	β (95% CI)[a]	P-value	β (95% CI)[a]	P-value	β (95% CI)[a]	P-value
Protocatechuic acid						
Model 1[b]	−0.15 (−0.29, −0.01)	0.034	0.02 (−0.09, 0.14)	0.674	−0.03 (−0.11, 0.06)	0.512
Model 2[c]	−0.06 (−0.20, 0.07)	0.343	0.05 (−0.07, 0.17)	0.411	< 0.01 (−0.08, 0.09)	0.953
Enterodiol glucuronide						
Model 1[b]	0.05 (−0.14, 0.23)	0.635	< 0.01 (−0.12, 0.12)	0.984	< −0.01 (−0.09, 0.08)	0.967
Model 2[c]	0.13 (−0.06, 0.32)	0.165	−0.02 (−0.14, 0.09)	0.678	< −0.01 (−0.08, 0.07)	0.905
Enterolactone glucuronide						
Model 1[b]	−0.08 (−0.25, 0.09)	0.367	−0.02 (−0.14, 0.09)	0.674	−0.04 (−0.12, 0.05)	0.386
Model 2[c]	0.01 (−0.15, 0.16)	0.941	−0.06 (−0.17, 0.06)	0.338	−0.04 (0.12, 0.03)	0.274
Urolithin B glucuronide						
Model 1[b]	0.16 (0.02, 0.30)	0.021	0.03 (−0.08, 0.15)	0.583	0.05 (−0.04, 0.12)	0.254
Model 2[c]	0.11 (−0.04, 0.26)	0.139	−0.03 (−0.15, 0.09)	0.588	−0.01 (−0.09, 0.08)	0.892
Vanillic acid glucuronide						
Model 1[b]	0.10 (−0.06, 0.26)	0.214	0.02 (−0.10, 0.14)	0.785	0.01 (−0.09, 0.10)	0.905
Model 2[c]	0.17 (0.03, 0.31)	0.018	0.01 (−0.12, 0.13)	0.889	0.01 (−0.08, 0.11)	0.793

Note: MPM, microbial phenolic metabolites. [a]β, difference between groups; CI, confidence interval. [b] Model 1: sex and age. [c] Model 2: sex, age, smoking habit, educational level, physical activity, BMI, total energy intake, hypertension, hypercholesterolemia, diabetes, APOE genotype, statin treatment, anticholinergic drugs, and Mediterranean diet adherence.

most negatively affected by aging[32-33]. Different studies have shown that polyphenols have a positive impact on cognitive functions related to the frontal lobe, which is consistent with our results. A randomized clinical trial reported an improvement in executive function after consumption of a flavonoid-rich orange juice for 8 weeks[34], and a previous study reported similar benefits 2 h after the intake of cocoa flavonols[35]. In addition, a recent meta-analysis of randomized controlled trials concluded that short- to moderate-term interventions with polyphenols had a significant albeit small positive effect on working and episodic memory[36]. However, previous research evaluated polyphenols' intake have not considered the possible effect of microbiota metabolism. In the present study, we focused on the MPM and, therefore, showed which are the metabolites that are associated with better biological functions that impact on frontal cognition.

Several mechanisms may underlie these beneficial effects of polyphenols, including the counteraction of oxidative stress and neuroinflammation[37]. It has been shown that polyphenols inhibit reactive oxygen species-forming enzymes, prevent metal deposition and neurotoxicity, modulate transcription factors that regulate inflammatory and oxidative pathways, and have an indirect impact on brain function by improving cerebral blood flow[38]. These 3 processes, neuroinflammation, oxidative stress and cerebrovascular function, have been specifically linked to a decline in executive functions[39-40]. These observations provide a biological explanation for our findings of an association between urinary MPMs and frontal lobe function.

Among all the studied metabolites, VAG was the one most strongly associated with frontal cognition, and it also displayed a positive association with the Color Trail Test part 2. VAG is the glucuronidated form of vanillic acid, a phenolic acid metabolized from the anthocyanin cyanidin-3-glucoside by gut microbiota[41]. Consumption of cyanidin glycosides, present in berries, have been widely correlated with better memory response[44]. However, studies assessing the impact of the metabolite VAG or its parent compound vanillic acid on the brain are scarce, a few have evaluated the effect of the aglycone form in animal studies. In experimental models of Alzheimer's disease, vanillic acid appears to attenuate the impact of amyloid plaque accumulation[42-43]. Other studies report that this polyphenol reduced Fe^{2+} and lipopolysaccharide-induced toxicity in the brain[44-45]. Regarding UBG, associations were observed with the Color Trail Test part 2 but not with the frontal function composite score, which suggests it has a lower effect compared to VAG. Previous studies have found positive neurological effects of the parent compound of UBG, urolithin B, an ellagitannin-derived microbial metabolite[46] related to the inhibition of neuronal apoptosis, suppressed microglia activation and suppressed phosphorylation in inflammatory pathways[47-48]. Interestingly, an *in vitro* and *in silico* study demonstrated that polyphenol metabolites can cross the blood brain barrier, and VAG in particular was detected in the apical and basolateral compartments[49]. Therefore, a higher blood brain barrier permeability could promote the neuroprotective effects of these metabolites.

Our study has limitations. First, given that the participants were older Mediterranean individuals at high cardiovascular risk, the results cannot be generalized to other populations. Second, the sample size was relatively small. Third, causality cannot be determined due to the cross-sectional design. The study also has strengths, including the use of biological samples, which provide the most accurate representation of the metabolic state of participants, and administration of a comprehensive battery of neuropsychological tests to assess cognitive function. In addition, the use of high precision equipment such as LTQ-Orbitrap allowed the identification and quantification of MPMs that until now have been scarcely studied in biological samples.

5. Conclusion

The results of the present cross-sectional study using LTQ-Orbitrap-MS suggest that higher concentrations of urinary MPMs, especially VAG, are associated with better frontal lobe function in older Mediterranean adults at high cardiovascular risk. Further studies are needed to elucidate the molecular mechanisms linking these metabolites to cognitive health.

Conflict of interests

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.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of the 11 participating centres. The study was registered with the International Standard Randomized Controlled Trial Number (ISRCTN) 35739639.

Acknowledgement

This research has been supported by the CICYT (AGL2016-75329-R PID2020-114022RB-I00), CIBEROBN from the Instituto de Salud Carlos III, ISCIII from the Ministerio de Ciencia, Innovación y Universidades (AEI/FEDER, UE) and Generalitat de Catalunya (GC) (2017SGR 196). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

I. Domínguez-López thanks the Spanish Ministry of Science Innovation and Universities for the Formación de Profesorado Universitario (FPU20/02478) contract; I. Parilli-Moser is thankful for the predoctoral scholarship FI-SDUR (EMC/2703/2019); A. Vallverdú-Queralt thanks the Ministry of Science Innovation and Universities for the Ramon y Cajal contract (RYC-2016-19355); A. Tresserra-Rimbau is a Serra Húnter Fellow; O. Castañer thanks ISCIII PI20/00012, CP21/00097 for the contract. We thank the CCiT UB facility for the LC-MS/MS equipment support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2023.9250013.

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