Resveratrol metabolite profiling in clinical nutrition research: from diet to uncovering disease risk biomarkers. Epidemiological evidence

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Key words: resveratrol, diet, biomarker, cardiovascular disease, mortality, epidemiology

Abstract

Resveratrol is a bioactive plant compound that has drawn scientist and media attention due to its protective effects against a wide variety of illnesses, including cardiovascular diseases and cancer. In the last two decades, a plethora of preclinical studies have shown these beneficial effects, and some of them have been supported by clinical trials. However, there are few epidemiological studies assessing these relationships, showing mostly inconsistent results between them. This could be partially due to difficulty of accurately estimating dietary resveratrol exposure. The development of Phenol-Explorer, a database containing resveratrol food composition data, will facilitate the estimation of resveratrol intake. Moreover, the discovery and validation of a nutritional biomarker of this exposure, urinary resveratrol metabolite profile, will allow a more accurate assessment of dietary resveratrol exposure. Few epidemiological studies have assessed the potential health effects of resveratrol. Resveratrol was not associated with total mortality, cancer or cardiovascular events; but it was associated with the improvement of serum glucose and triglyceride levels, and the decrease of heart-rate. Together, these findings suggest a potential cardioprotective effect of resveratrol in epidemiological studies, although the evidence is still scarce.

Introduction

Resveratrol is the parent compound of the stilbene family of molecules, which include glycosides (piceid) and polymers (viniferins), found mainly in grape and grape products, such as red wine, and in very low amounts in peanuts, pistachios, some berries, beer, tomato skin, chocolate and apple.[1-5] Interest in resveratrol in nutrition and medicine started in 1992 when it was proposed to explain some of the cardiovascular protective effects of red wine in the French Paradox.[6] Since then, the scientific literature on resveratrol has exponentially increased,[7] and thousands of studies, especially in *in vitro* and in animal models, have investigated its potential activities regarding the prevention or delay of cancer, cardiovascular and neurodegenerative diseases, and aging. Despite the intensive research, few clinical trials on humans have been performed to date. These preclinical and clinical studies have shown that resveratrol exerts several potential beneficial activities such as the improvement of inflammatory and oxidative stress markers, glucose metabolism, and endothelial function, inhibiting platelet aggregation, modulating signaling pathways (e.g., increasing Sirtuin 1 expression),

inducing apoptosis and inhibiting angiogenesis, metastasis, and tumor growth. A recent review extensively summarizes the findings from these investigations.[8]

Experimental studies mostly use pharmaceutical doses of nutrients/compounds, whereas large epidemiological studies, whether observational or interventional, give insight into the effects of dietary doses. The main limitation of these studies is the inaccuracy in the assessment of nutrient/compound exposure leading to discrepancies in the final findings between studies. The main method to assess dietary exposure is dietary questionnaires and/or biomarkers.[9]

The aim of the current article is to review the dietary assessment methodologies for measuring resveratrol exposure and critically evaluate their pros and cons. Furthermore, results of epidemiological studies on resveratrol and health effects obtained by dietary questionnaires or biomarkers are summarized and compared.

Resveratrol and piceid exposure assessment through dietary questionnaires

In nutritional studies, the traditional method to estimate dietary intake of certain nutrient/compound is through dietary questionnaires and a food composition table. Researchers are usually interested in long term exposure and therefore the surveys most commonly used are single food frequency questionnaires (FFQs), food propensity questionnaires, diet histories, multiple 24-h recalls and 3- or 7-day food records.

The advantages of this approach are the relatively low cost, the ease and simplicity of completing the questionnaires and processing the data, and finally, the large amount of dietary data gathered together, including nutrients, foods and even dietary patterns. The main limitation of the questionnaires is that they are mostly self-reported, so they are based on memory of the subjects and their ability to estimate food portion sizes.[10] However, the use of new technologies is facilitating and improving the accuracy of the subjects' responses, such as 'personal digital assistant-', 'mobile-phone-', 'interactive computer-', 'web-', 'camera- and tape recorder-', and 'scan and sensor-based' technologies.[11] The second drawback is related to the limited food composition data available and the high variability of composition, particularly for micronutrients and phytochemicals, between similar foods, and even in the same type of food.

Food composition data on resveratrol and piceid

To the best of our knowledge, Phenol-Explorer is the only food composition database containing data on resveratrol and piceid so far. Phenol-Explorer (www.phenol-explorer.eu) is an online comprehensive food_composition database on all known

polyphenols. It contains data on 502 individual polyphenols from all classes (flavonoids, phenolic acids, lignans, and stilbenes) in 452 foods extracted from the scientific literature.[12] Although limited food composition data on resveratrol is given in a few articles.[1, 2] Resveratrol and piceid are present in high concentrations in grape and grape products, such as wines, and in lower concentrations in peanuts, pistachios, berries, beer, tomato, chocolate and apple (Table 1). It is worth bearing in mind that, concentrations of resveratrol and piceid highly varied in foods, particularly in wine, depending in agronomic and environmental factors, grape varieties, and wine-making technology (e.g. red wine vs. white wine).[13, 14]

Estimation of dietary resveratrol and piceid intakes

Only a few epidemiological studies have estimated the dietary intake of resveratrol and piceid so far using validated dietary questionnaires for polyphenol-rich foods, such as wine. The first one was the European Prospective Investigation into Cancer and Nutrition (EPIC)-Spain cohort, including data on approximately 40,000 men and women aged 35-64 years and recruited in the 1990s from northern and southern regions of Spain.[1] In this study, a validated diet history, covering 600 food items, and ad hoc food composition table were used. The database on *cis*- and *trans*-resveratrol and *cis*- and *trans*-piceid was based on a systematic literature search using data mainly from Spanish foods, analyzed using HPLC diode array or GC/MS and converted into mg/100 g fresh weight. The estimated mean (SD) intake of resveratrol and piceid together was 0.93 (1.67) mg/day and nearly 32% of the individuals were non-consumers. The most abundant of the four stilbenes studied was trans-piceid (53.6%), followed by trans-resveratrol (20.9%), cis-piceid (19.3%) and cis-resveratrol (6.2%). The main food sources were wines (98.4%), grapes and grape juices (1.6%), whereas peanuts, pistachios and berries contributed less than 0.01%. The second study was the SUpplémentation en VItamines et Minéraux AntioXydants (SU.VI.MAX), a French cohort consisting of ~5,000 men and women aged 45-60 years who were recruited between 1995 and 1996.[15] In order to estimate resveratrol and piceid intake, at least six 24-h dietary recalls and the Phenol-Explorer database were used. The mean (SD) intake of resveratrol and piceid were 0.45 (0.45) and 0.94 (1.04) mg/d, respectively. Moreover, the mean (SD) intake of other stilbenes was estimated: piceatannol 3-O-glucoside 1.3 (1.6) mg/d, d-viniferin 0.84 (1.02) mg/d, piceatannol 0.76 (0.93) mg/d, pallidol 0.27 (0.33) mg/d, and e-viniferin 0.2 (0.25) mg/d. Food sources of stilbenes were not presented in the study. The third cohort was the PREvención con DIeta MEDiterránea

(PREDIMED) study.[16] Around 7,000 men and women, aged 55-80 years and at high cardiovascular risk were enrolled from 2003 to 2009 in this large multicenter clinical trial in Spain. At baseline, a validated 137-item FFQ was collected and Phenol-Explorer was used to convert food consumption into resveratrol and piceid intake. Mean (SD) total stilbene intake was 1.84 (3.39) mg/d and the most abundant sources were red wine (94%), white wine (2%), and grapes (1%). The fourth study was the cohort of 304 institutionalized elderly living in Asturias (Spain). In this cross-sectional study, a 372-item FFQ and Phenol-Explorer database were used to estimate resveratrol and piceid intake. The mean (SD) daily consumption of stilbenes was 3.06 (5.56) and 0.62 (1.33) mg/d for men and women, respectively, and red wine was the richest source (99.3%).[17] The fifth study was the Polish arm of the HAPIEE cohort (Health, Alcohol and Psychosocial factors in Eastern Europe). A total of 10,477 men and women, aged 45-69 years, completed a validated 148-item FFQ. In this study, food composition data on stilbenes were also obtained from the Phenol-Explorer database. The mean (SD) stilbenes intake was 0.2 (0.6) mg/d and the main dietary sources were red wine (56%), strawberries (14%) and white wine (12%).[18] Our group has also estimated the intake of resveratrol and piceid in the InCHIANTI (Invecchiare in Chianti, Aging in Chianti) study, a cohort of approximately 1,000 men and women, aged ≥ 65 years, and recruited in two small towns in the Tuscany (Italy).[19] Dietary data was collected using a validated food frequency questionnaire every three years starting from 1998-2000. Phenol-Explorer was also used to estimate the resveratrol and piceid intake. Mean (SD) intakes of resveratrol and piceid were 0.29 (0.38) and 0.76 (1.05) mg/d respectively (unpublished data). Again, red wine was the main food source, accounting for >90% of total resveratrol and piceid.

To date, epidemiological studies assessing dietary resveratrol and piceid intake have shown similar results in Mediterranean countries since they usually have a high consumption of red wine, such as in France, Spain and Italy. However, in the Polish study, the mean intake was at least 9-fold lower than in Mediterranean. Further descriptive analyses are needed to estimate the intake of resveratrol and piceid in non-Mediterranean countries, where greater variability in wine consumption, especially red wine, is found.

Resveratrol and piceid exposure assessment through biomarkers

In modern nutritional epidemiology, there has been intense interest in the discovery, validation and use of nutritional biomarkers. They can be generally defined as a biochemical indicator of recent or long term dietary intake/nutritional status.[20] The main advantage of using nutritional biomarker instead of dietary questionnaires is that they are objective and do not rely on biases or errors of subjects' responses. An 'ideal' dietary biomarker would accurately reflect its dietary intake level and it would be specific, sensitive and applicable to many populations.[21] The disadvantages of nutritional biomarkers are the necessity of biological samples, the complexity of the analytical methodology, and mostly, the high cost.[21] Nutritional biomarkers can be classified into several types: recovery (e.g., doubly labeled water, urinary nitrogen and potassium), predictive (e.g., 24 h urinary sucrose and fructose), concentration and replacement biomarkers (e.g., vitamins, individual fatty acids, and phytoestrogens).[22] Despite the advantages of nutritional biomarkers compare to dietary surveys, few nutritional biomarkers have been validated so far.[22]

In order to discover a biomarker of dietary resveratrol intake it is essential to have a comprehensive knowledge of resveratrol bioavailability and an analytical technique which allows resveratrol and resveratrol metabolites to be measured at low concentrations in biospecimens.

Bioavailability of resveratrol

Piceid, after hydrolysis of the sugar moiety, and resveratrol are usually absorbed in the small intestine. They are almost totally metabolized in the gut mucosa and the liver and conjugated to glucuronide and sulfate groups. Unabsorbed resveratrol reaches the colon and is converted to dihydroresveratrol by the microbiota. Then, it is absorbed, conjugated and found in the systemic circulation.[23, 24] Finally, around 75% of these metabolites are excreted in urine and feces.[25] Pharmacokinetics studies have shown that the maximum concentrations in urine of resveratrol and piceid metabolites are 30-60 min and 6 h after dietary doses in humans, respectively.[26, 27] Resveratrol and piceid standards are recovered in urine at similar percentages.[28] At nutritional doses (<5mg/day), the absorption of resveratrol is higher than at pharmaceutical doses (from one hundred to thousands mg/day).[29]

Methodology for assessment resveratrol in biospecimens

A sensitive, precise, selective and robust analytical method is essential to measure resveratrol in biological samples. Instrumentation is usually gas or liquid

chromatography coupled to a single or a tandem mass spectrometer. Nowadays, an enzymatic or acidic hydrolysis is no longer performed, since we are interested in identifying and quantifying resveratrol metabolites (glucuronides and sulfates). Our group developed a method of characterizing the complete profile of resveratrol in urine using high performance liquid chromatography coupled to electrospray tandem mass spectrometry (LC-ESI-MS/MS) after a sample clean-up with solid-phase extraction (SPE).[30] It was further improved to identify more resveratrol metabolites in a shorter chromatography run-time and was also adapted to 96-well SPE plates to allow a greater daily throughput.[31] Using this methodology, we are able to identify 21 resveratrol derivatives, including resveratrol, dihydroresveratrol, piceid and their glucuronide- and sulfate-conjugated metabolites (Figure 1).[26] Later two microbial metabolites, 3,4'-dihydroxy-trans-stilbene and 3,4'-dihydroxybibenzyl dihydroresveratrol, have been reported using a similar method.[32]

Most analytical techniques used so far quantified resveratrol in biological samples only after an enzymatic hydrolysis,[33, 34] and could not therefore measure the entire profile of resveratrol metabolites. Subsequent analytical methods were developed to measure the resveratrol metabolite profile in biospecimens, but after the intake of pharmaceutical doses and profiling fewer resveratrol metabolites.[27, 35]

Validation studies of resveratrol biomarker

There are several steps for validating a nutritional biomarker. The first one is to get an extensive knowledge of the bioavailability of resveratrol, especially the time- and dose-response curves, after a single dose. This information is mostly provided by pharmacokinetic studies using resveratrol and resveratrol-rich foods (such as wine and grape juice). These studies have been reviewed previously.[8, 36]

The second step is moving from acute to chronic studies. In a double-blind, placebo-controlled study in 40 healthy men and women, after pharmacological doses of resveratrol (six-daily intake of resveratrol capsules [25, 50, 100 or 150 mg] at 4 h intervals) for 13 days, half-lives of plasma resveratrol were 1-3 and 2-5 hours following single-doses and repeated doses, respectively. Maximal plasma concentrations were almost directly proportional to resveratrol intake. High inter-individual variability and circadian variation were also observed.[37] Another study administered capsules containing 0.5, 1, 2.5 or 5 g micronized resveratrol daily for 29 days to healthy men (n=22) and women (n=18) and identified resveratrol-3-O-sulfate, resveratrol-4'- and 3-O-glucuronide as the main plasma metabolites. Moreover, for micronized resveratrol

formulation, resveratrol with reduced particle size, maximal plasma levels of resveratrol and derived-metabolites were 3.6x higher than previously published values for standard resveratrol.[38, 39] Our group has also carried out several randomized, crossover trials assessing the validity of urinary resveratrol metabolite profiles as a biomarker of dietary resveratrol after the consumption 3 different amounts of nutritional resveratrol doses via sparkling wine (300 mL/d, 0.36 mg/d resveratrol), white wine (200 mL/d, 0.40 mg/d resveratrol) and red wine (200 mL/d, 2.56 mg/d resveratrol) for 28 days in 10 healthy subjects. In these trials, the concentrations of total resveratrol metabolites increased upon increased resveratrol intake by 72.4 (95% confidence interval (CI), 48.5-96.2; P=0.005), 211.5 (95% CI, 166.6-256.3; P=0.005) and 560.5 (95% CI, 244.9-876.1; P=0.005) after consumption of sparkling, white, and red wine, respectively, while the concentration of these metabolites did not vary significantly after the washout and the control periods.[40]

The third step is to correlate long-term dietary resveratrol intake using dietary questionnaires and resveratrol concentrations in biological samples in free living populations using large epidemiological studies. To the best of our knowledge, only two observational studies have assessed the usefulness of the resveratrol biomarker so far. In the PREDIMED study, a subsample of 1,000 men and women was evaluated, showing that concentrations of resveratrol metabolites in spot urine were highly correlated with dietary resveratrol intake ($r=0.89$; $P < 0.001$).[38] The measurement of urinary resveratrol metabolites could discriminate wine consumers (resveratrol consumers) from non-wine consumers (sporadic or non-resveratrol consumers) with a sensitivity of 93.3% (95% CI, 91.5-94.7%) and a specificity of 92.1% (95% CI, 90.2-93.7%) using a cut-off of 411.4 nmol/g creatinine.[41] In the InCHIANTI study, 783 men and women, aged 65 years and older, were included in the analysis and the correlation coefficient between dietary intake of resveratrol and total resveratrol metabolites in urine 24-h was 0.67 ($P < 0.001$).[42]

Combining all these studies, we can confirm that the sum of all urinary resveratrol metabolites is a valid concentration biomarker of total dietary resveratrol intake. However, further investigations are needed to assess the potential utility of plasma resveratrol concentrations as a biomarker of resveratrol intake in large observational studies.

Resveratrol and health effects: epidemiological evidence

Although thousands of *in vitro* and *in vivo* animal studies[7] and a few dozen intervention trials in humans[8] evaluating the potential mechanisms and molecular targets of resveratrol action, few data come from large epidemiological studies. In this section, we review the available evidence concerning potential protection by resveratrol exposure, which can be estimated using dietary questionnaires and/or nutritional biomarkers, against mortality and chronic disease risk.

Urinary resveratrol metabolite profiles and total dietary resveratrol intake were not associated with overall mortality after 9 years of follow-up in 783 community-dwelling men and women 65 years or older participating in the InCHIANTI study.[42] However, in the PREDIMED study, high total resveratrol intake decreased 50% all-cause mortality in approximately 7,500 men and women at high cardiovascular risk after a mean follow-up of 4.8 years.[43] In this study, the dose-response effect was borderline significant, but the only significant results were observed with the highest quintile (>5.75mg/day), while in the InChianti study the resveratrol intake was lower and the results were presented in quartiles.

Surprisingly, no association was observed between resveratrol intake and cardiovascular events (nonfatal acute myocardial infarction, nonfatal stroke or death from cardiovascular causes) in the same PREDIMED study.[44] Similar null results were observed in the InCHIANTI study.[42] Indeed in the InCHIANTI study, no association was observed between urinary resveratrol and inflammatory markers (CRP, IL-6, IL-1β, and TNF-α)[42] that are well-known predictors of cardiovascular diseases.[45, 46] Regarding other cardiovascular risk factors, total urinary resveratrol metabolites were inversely associated with glucose and triglyceride levels, and heart rate, but no association was observed with cholesterol levels and blood pressure in a subsample of 1,000 men and women in the PREDIMED study.[47] However, in an Iranian cross-sectional study on 2,618 adult men and women, metabolic syndrome, waist circumference, blood pressure, glucose, triglyceride and HDL levels were not related to dietary resveratrol intake; hypertension was even positively associated with resveratrol intake.[48] Null results in this study could be due to very low intake of resveratrol (0.002mg/day) in this country. In China, a cross-sectional study in 1,393 men and women, aged from 35 to 75 years, dietary resveratrol intake from fruits, vegetables and nuts was not associated with cardiovascular factors (such as carotid intima–media thickness, blood pressure, and serum glucose and lipids).[49] The main limitation of this Chinese study is that resveratrol

from wine and grape juice was not considered, which are by far the major food sources in wine consumer countries.[1]

Few studies have looked for associations between resveratrol exposure and cancer risk In the InCHIANTI study, no association was observed between both urinary and dietary resveratrol and prevalent or incident overall cancer risk after 9 years of follow-up.[42] In the SU.VI.MAX cohort, dietary resveratrol was not related to breast cancer risk in nearly 4,150 women with a median follow-up of 12.6 years.[50] However, in the Swiss Canton of Vaud case control study, including 369 cases and 602 controls, breast cancer risk was inversely associated with dietary resveratrol from grapes but not from wine.[51] This could be explained because alcohol is a well-known risk factor for breast cancer,[52] and it may mask the effects of resveratrol. Furthermore, a study has evaluated the relationship between resveratrol intake and lung function in two large Dutch cohorts.[53] The Doetinchem (n=1,152) and Vlagtwedde-Vlaardingen (n=1,390) studies suggest an inverse association between dietary resveratrol and force vital capacity (FVC), but not with forced expiratory volume in 1 second (FEV1). Similarly, no significant association of resveratrol intake with the presence of airway obstruction, as present in chronic obstructive pulmonary disease, was found.

In general, no associations were observed in most of the epidemiological studies so far, and it is possible that nutritional doses of resveratrol are too low to have the protective effects found at pharmacological doses (hundred milligrams to grams) in preclinical and clinical trials. Measurement error in the assessment of resveratrol exposure may also attenuate the results towards the null results. Furthermore, other compounds more abundant or with stronger effect may mask the effect of resveratrol, such alcohol, and other polyphenols. However, some protective effects against cardiovascular risk factors were suggested, such as the improvement of glucose and triglyceride levels and heart rate, although the evidence is still scarce.

Conclusions

Resveratrol is a fashionable bioactive compound that has been reported to exert a myriad of health benefits through many different mechanisms of action and molecular targets, mostly reported in vitro and in animal (preclinical) studies. In clinical trials, there is growing evidence that resveratrol could exert cardioprotective effects particularly through the improvement of inflammatory markers, atherogenic profile, glucose metabolism and endothelial function. However, a few epidemiological studies

have assessed the potential relationships between dietary resveratrol and the risk of cardiovascular and other chronic diseases. It is mainly due to the limitations for assessing properly dietary exposure of resveratrol. Phenol-Explorer is the first database containing food composition data on resveratrol and its derivatives. It will facilitate the estimation of resveratrol intake in large epidemiological settings. Moreover, the discovery and validation of a nutritional biomarker, urinary resveratrol, will allow resveratrol exposure to be objectively measured. The combination of dietary and urinary data on resveratrol will also improve the exposure estimations and, consequently, increase the strength of the findings.[54]

Results from epidemiological studies are limited and still inconclusive, although some protective effects against cardiovascular diseases were suggested. Further studies are warranted to evaluate the associations with chronic diseases, taking into account current advances in the estimation of dietary resveratrol exposure.

Acknowledgments

The authors are grateful for the support granted by the Spanish government grants from Ministry of Economy and competitiveness (MINECO) and cofounded by FEDER (Fondo Europeo de Desarrollo Regional): the CONSOLIDER-INGENIO 2010 program, FUN-C-FOOD (CSD2007-063), and the JPI HDHL FOODBALL (PCIN-2014-133). We also thank the award of 2014SGR1566 from the Generalitat de Catalunya's Agency AGAUR. Partially funded by the International Nut and Dried Fruit Council Foundation (INC) in collaboration with the Bosch I Gimpera Foundation (FBG307906). M.U-S. thanks the 'Ramón y Cajal' contract (RYC-2011-09677) from MINECO and Fondo Social Europeo.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Zamora-Ros, R., *et al.* 2008. Concentrations of resveratrol and derivatives in foods and estimation of dietary intake in a Spanish population: European Prospective Investigation into Cancer and Nutrition (EPIC)-Spain cohort. *Br. J. Nutr*. **100**: 188-196.
2. Chiva-Blanch, G., *et al.* 2011. Determination of resveratrol and piceid in beer matrices by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A*. **1218**: 698-705.
3. Ragab, A. S., *et al.* 2006. Detection and quantitation of resveratrol in tomato fruit (Lycopersicon esculentum Mill.). *J. Agric. Food. Chem.* **54**: 7175-7179.
4. Hurst, W. J., *et al.* 2008. Survey of the trans-resveratrol and trans-piceid content of cocoa-containing and chocolate products. *J. Agric. Food. Chem.* **56**: 8374-8378.
5. Farneti, B., *et al*. 2015. Is there room for improving the nutraceutical composition of apple? *J. Agric. Food Chem.* **63**: 2750-2759.
6. Renaud, S. & M. de Lorgeril. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*. **339**: 1523-1526.
7. Baur, J. A. & D. A. Sinclair. 2006. Therapeutic potential of resveratrol: the in vivo evidence. *Nat. Rev. Drug. Discov*. **5**: 493-506.
8. Tome-Carneiro, J., *et al.* 2013. Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. *Curr. Pharm. Des*. **19**: 6064-6093.
9. Zamora-Ros, R., *et al.* 2012. Application of Dietary Phenolic Biomarkers in Epidemiology: Past, Present, and Future. *J. Agric. Food. Chem.* **60**: 6648-6657.
10. Shim, J.-S., *et al*. 2014. Dietary assessment methods in epidemiologic studies. *Epidem. Health*. **36**: e2014009.
11. Illner, A. K., *et al.* 2012. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. *Int. J. Epidemiol.* **41**: 1187-1203.
12. Neveu, V., *et al*. 2010. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford).* **2010**:bap024.
13. Gonzalez-Barrio, R., *et al.* 2006. Comparison of ozone and UV-C treatments on the postharvest stilbenoid monomer, dimer, and trimer induction in var. 'Superior' white table grapes. *J. Agric. Food. Chem.* **54**: 4222-4228.
14. Romero-Perez, A. I., *et al.* 2001. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J. Agric. Food. Chem.* **49**: 210-215.
15. Perez-Jimenez, J., *et al.* 2011. Dietary intake of 337 polyphenols in French adults. *Am. J. Clin. Nutr*. **93**: 1220-1228.
16. Tresserra-Rimbau, A., *et al.* 2013. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study. *Nutr. Metab. Cardiovasc. Dis*. **23**: 953-959.
17. Gonzalez, S., *et al.* 2014. Dietary intake of polyphenols and major food sources in an institutionalised elderly population. *J. Hum. Nutr. Diet*. **27**: 176-183.
18. Grosso, G., *et al.* 2014. Estimated dietary intake and major food sources of polyphenols in the Polish arm of the HAPIEE study. *Nutrition*. **30**: 1398-1403.
19. Zamora-Ros, R., *et al.* 2011. Comparison of 24-h volume and creatinine-corrected total urinary polyphenol as a biomarker of total dietary polyphenols in the Invecchiare InCHIANTI study. *Anal. Chim. Acta*. **704**: 110-115.
20. Potischman, N. & J. L. Freudenheim. 2003. Biomarkers of nutritional exposure and nutritional status: an overview. *J. Nutr*. **133** (Suppl. 3): 873S-874S.

21. Potischman, N. 2003. Biologic and methodologic issues for nutritional biomarkers. *J. Nutr*. **133** (Suppl. 3): 875S-880S.
22. Jenab, M., *et al.* 2009. Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum. Genet*. **125**: 507-525.
23. Nicholson, J. K., *et al.* 2012. Host-gut microbiota metabolic interactions. *Science*. **336**: 1262-1267.
24. Blaut, M. & T. Clavel. 2007. Metabolic diversity of the intestinal microbiota: implications for health and disease. *J. Nutr*. **137**: 751S-755S.
25. Wenzel, E. & V. Somoza. 2005. Metabolism and bioavailability of trans-resveratrol. *Mol. Nutr. Food. Res*. **49**: 472-481.
26. Rotches-Ribalta, M., *et al.* 2012. Pharmacokinetics of resveratrol metabolic profile in healthy humans after moderate consumption of red wine and grape extract tablets. *Pharmacol. Res.* **66**: 375-382.
27. Walle, T., *et al.* 2004. High absorption but very low bioavailability of oral resveratrol in humans. *Drug. Metab. Dispos*. **32**: 1377-1382.
28. Burkon, A., Somoza, V. 2008. Quantification of free and protein-bound trans-resveratrol metabolites and identification of trans-resveratrol-C/O-conjugated diglucuronides-Two novel resveratrol metabolites in human plasma. *Mol. Nutr. Food Res.* **52**:549–557.
29. Meng, X., *et al*. 2004. Urinary and plasma levels of resveratrol and quercetin in humans, mice, and rats after ingestion of pure compounds and grape juice. *J. Agric. Food Chem.* **52**:935–942.
30. Urpi-Sarda, M., *et al.* 2005. Uptake of diet resveratrol into the human low-density lipoprotein. Identification and quantification of resveratrol metabolites by liquid chromatography coupled with tandem mass spectrometry. *Anal. Chem.* **77**: 3149-3155.
31. Urpi-Sarda, M., *et al.* 2007. HPLC-tandem mass spectrometric method to characterize resveratrol metabolism in humans. *Clin. Chem*. **53**: 292-299.
32. Bode, L. M., *et al*. 2013. In vivo and in vitro metabolism of trans-resveratrol by human gut microbiota. *Am. J. Clin. Nutr*. **97**: 295-309.
33. Goldberg, D.M., *et al.* 2003. Absorption of three wine-related polyphenols in three different matrices by healthy subjects. *Clin. Biochem.* **36**, 79-87.
34. Ortuño, J., *et al*. 2010. Matrix effects on the availability of resveratrol in humans. *Food Chem.* **120**, 1123-1130.
35. Boockcok, D.J., *et al*. 2007. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. Cancer Epidemiol. Biomarkers Prev. **16**:1246–52.
36. Andres-Lacueva, C., *et al.* 2009. "Bioavailability and Metabolism of Resveratrol." In *Plant Phenolics and Human Health*. Fraga, C. G., Ed.: 265-297. John Wiley & Sons, Inc.
37. Almeida, L., *et al.* 2009. Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. *Mol. Nutr. Food. Res*. **53** (Suppl. 1): S7-15.
38. Brown, V. A., *et al.* 2010. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer. Res*. **70**: 9003-9011.
39. Howells, L.M., *et al*. 2011. Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases-safety, pharmacokinetics, and pharmacodynamics. *Cancer Prev. Res. (Phila).* **4**:1419-1425.

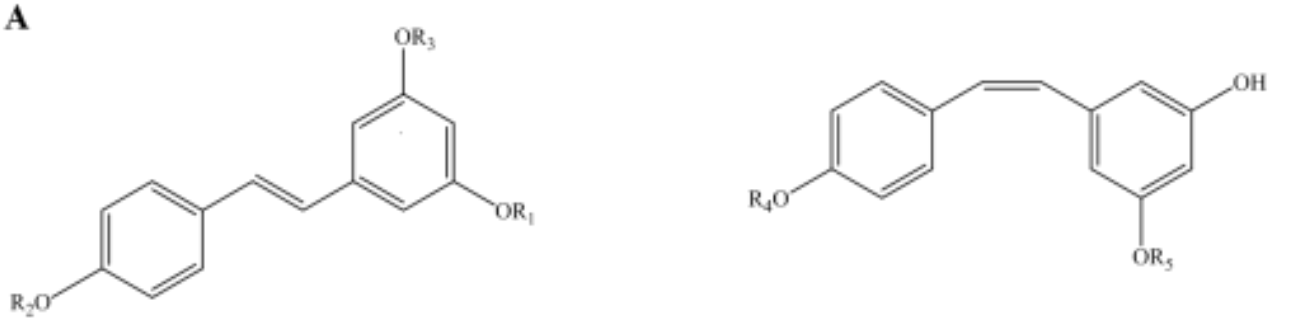
40. Zamora-Ros, R., *et al.* 2006. Diagnostic performance of urinary resveratrol metabolites as a biomarker of moderate wine consumption. *Clin. Chem.* **52**: 1373-1380.
41. Zamora-Ros, R., *et al.* 2009. Resveratrol metabolites in urine as a biomarker of wine intake in free-living subjects: The PREDIMED Study. *Free. Radic. Biol. Med.* **46**: 1562-1566.
42. Semba, R. D., *et al.* 2014. Resveratrol levels and all-cause mortality in older community-dwelling adults. *JAMA. Intern. Med*. **174**: 1077-1084.
43. Tresserra-Rimbau, A., *et al.* 2014. Polyphenol intake and mortality risk: a re-analysis of the PREDIMED trial. *BMC. Med*. **12**: 77.
44. Tresserra-Rimbau, A., *et al.* 2014. Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutr. Metab. Cardiovasc. Dis.* **24**: 639-647.
45. Willerson, J. T. & P. M. Ridker. 2004. Inflammation as a cardiovascular risk factor. *Circulation*. **109**: II2-10.
46. Pearson, T. A., *et al.* 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. **107**: 499-511.
47. Zamora-Ros, R., *et al.* 2012. High urinary levels of resveratrol metabolites are associated with a reduction in the prevalence of cardiovascular risk factors in high-risk patients. *Pharmacol. Res.* **65**: 615-620.
48. Sohrab, G., *et al.* 2013. Dietary polyphenols and metabolic syndrome among Iranian adults. *Int. J. Food. Sci. Nutr*. **64**: 661-667.
49. Li, G., *et al.* 2013. Estimated daily flavonoid and stilbene intake from fruits, vegetables, and nuts and associations with lipid profiles in Chinese adults. *J. Acad. Nutr. Diet.* **113**: 786-794.
50. Touvier, M., *et al.* 2013. Dual association between polyphenol intake and breast cancer risk according to alcohol consumption level: a prospective cohort study. *Breast. Cancer. Res. Treat.* **137**: 225-236.
51. Levi, F., *et al.* 2005. Resveratrol and breast cancer risk. *Eur. J. Cancer. Prev.* **14**: 139-142.
52. Romieu, I., *et al*. Alcohol intake and breast cancer in the European Prospective Investigation into Cancer and Nutrition. *Int. J. Cancer*. In press.
53. Siedlinski, M., *et al.* 2012. Dietary factors and lung function in the general population: wine and resveratrol intake. *Eur. Respir. J.* **39**: 385-391.
54. Freedman, L. S., *et al.* 2010. Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? *Epidemiol. Perspect. Innov.* **7**: 2.

Figure legends:

Figure 1. Chemical structures of resveratrol and its metabolites found in human biological samples.

(A) Intestinal metabolism: *trans*- and *cis*-isomers. **(B)** Microbial metabolism.

A



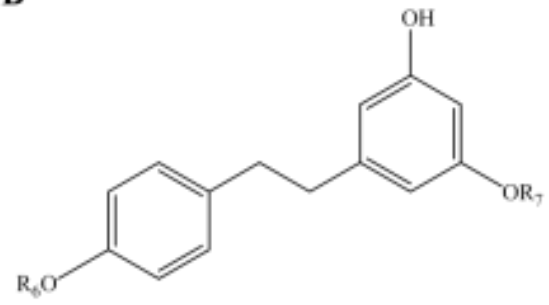
***trans*-Isomers**

trans-Resveratrol-3-*O*-glucuronide: R_1= glucuronic acid, R_2=H, R_3=H
trans-Resveratrol-4'-*O*-glucuronide: R_1= H, R_2=glucuronic acid , R_3=H
trans-Resveratrol-3,4'-*O*-diglucuronide: R_1= glucuronic acid, R_2=glucuronic acid, R_3=H
trans-Resveratrol-3,5-*O*-diglucuronide: R_1= glucuronic acid, R_2=H, R_3=glucuronic acid
trans-Resveratrol-3-*O*-sulfate: R_1= SO_3H, R_2=H , R_3=H
trans-Resveratrol-4'-*O*-sulfate: R_1= H, R_2= SO_3H , R_3=H
trans-Resveratrol-3,4'-*O*-disulfate: R_1= SO_3H, R_2= SO_3H , R_3=H
trans-Resveratrol-3,5-*O*-disulfate: R_1= SO_3H, R_2= H, R_3= SO_3H
trans-Resveratrol-3,5,4'-*O*-trisulfate: R_1= SO_3H, R_2= SO_3H, R_3= SO_3H

***cis*-Isomers**

cis-Resveratrol-3-*O*-glucuronide: R_4= H, R_5= glucuronic acid
cis-Resveratrol-4'-*O*-glucuronide: R_4= glucuronic acid, R_5=H
cis-Resveratrol-3,4'-*O*-diglucuronide: R_4= glucuronic acid, R_5=glucuronic acid
cis-Resveratrol-3-*O*-sulfate: R_4= H, R_5= SO_3H
cis-Resveratrol-4'-*O*-sulfate: R_4= SO_3H, R_5= H
cis-Resveratrol-3,4'-*O*-disulfate: R_4= SO_3H, R_5= SO_3H

B



Dihydroresveratrol: R_6= H, R_7 = H
Dihydroresveratrol-3-O-glucuronide: R_6 = H, R_7 = glucuronic acid
Dihydroresveratrol-4-O-glucuronide: R_6 = glucuronic acid, R_7 = H
Dihydroresveratrol-3-O-sulfate: R_6 = H, R_7 = SO_3H
Dihydroresveratrol-4'-O-sulfate: R_6 = SO_3H , R_7 = H

Table 1. Food composition data sources for resveratrol and piceid content. Values are expressed as mg/100 g and total values per serving.

Food item	*trans*-resveratrol	*cis*-resveratrol	*trans*-piceid	*cis*-piceid	total resveratrol			Reference
	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	serving (g or mL)	mg/serving	
Red wine	0.181	0.044	0.495	0.127	0.847	150	1.271	[1]
Rosé wine	0.041	0.041	0.071	0.154	0.307	150	0.461	[1]
White wine	0.010	0.016	0.260	0.022	0.074	150	0.111	[1]
Sparkling wine	0.005	0.014	0.018	0.055	0.092	150	0.138	[1]
Fortified wine	0.110	0.095	0.141	0.040	0.386	150	0.579	[1]
Grape juice	0.010	tr	0.036	0.043	0.088	250	0.220	[1]
Sangria	0.091	0.022	0.248	0.063	0.424	150	0.636	[1]
Red grapes	0.250	tr	0.060	-	0.310	100	0.310	[1]
White grapes	0.068	tr	0.025	-	0.093	100	0.093	[1]
Peanut butter	0.065	-	0.014	-	0.080	30	0.024	[1]
Peanuts, toasted	0.006	-	-	-	0.006	30	0.002	[1]
Pistachios, toasted	0.007	-	-	-	0.007	30	0.002	[1]
Berries, not specified[a]	0.008	-	-	-	0.008	50	0.004	[1]
Tomatoes[b]	0.001	<0.001	<0.001	<0.001	0.001	100	0.001	[3]
Chocolate[c]	0.069	-	0.263	-	0.332	10-40	0.088	[4]

Beer	0.001	<0.001	<0.001	<0.001	0.001	330	0.005	[2]
Itadori tea (infusion)	0.068	-	0.906	-	0.974	250	2.435	[1]
Apple	-	-	0.030	0.010	0.040	250	0.100	[5]

Abbreviations: tr, traces.

[a]Berries included: blueberry, bilberry, sparkleberry, deerberry, cranberry, lingonberry and partridgeberry.

[b]Skin tomato values (5% of dry weight and 92% humidity) from MicroTom, breafsteak, UglyRipe, Heriloom and PlumTom varieties.

[c]Chocolate included: cocoa powder, unsweetened, semi-sweet, dark and milk chocolate, and chocolate syrup. Serving depends on chocolate product.