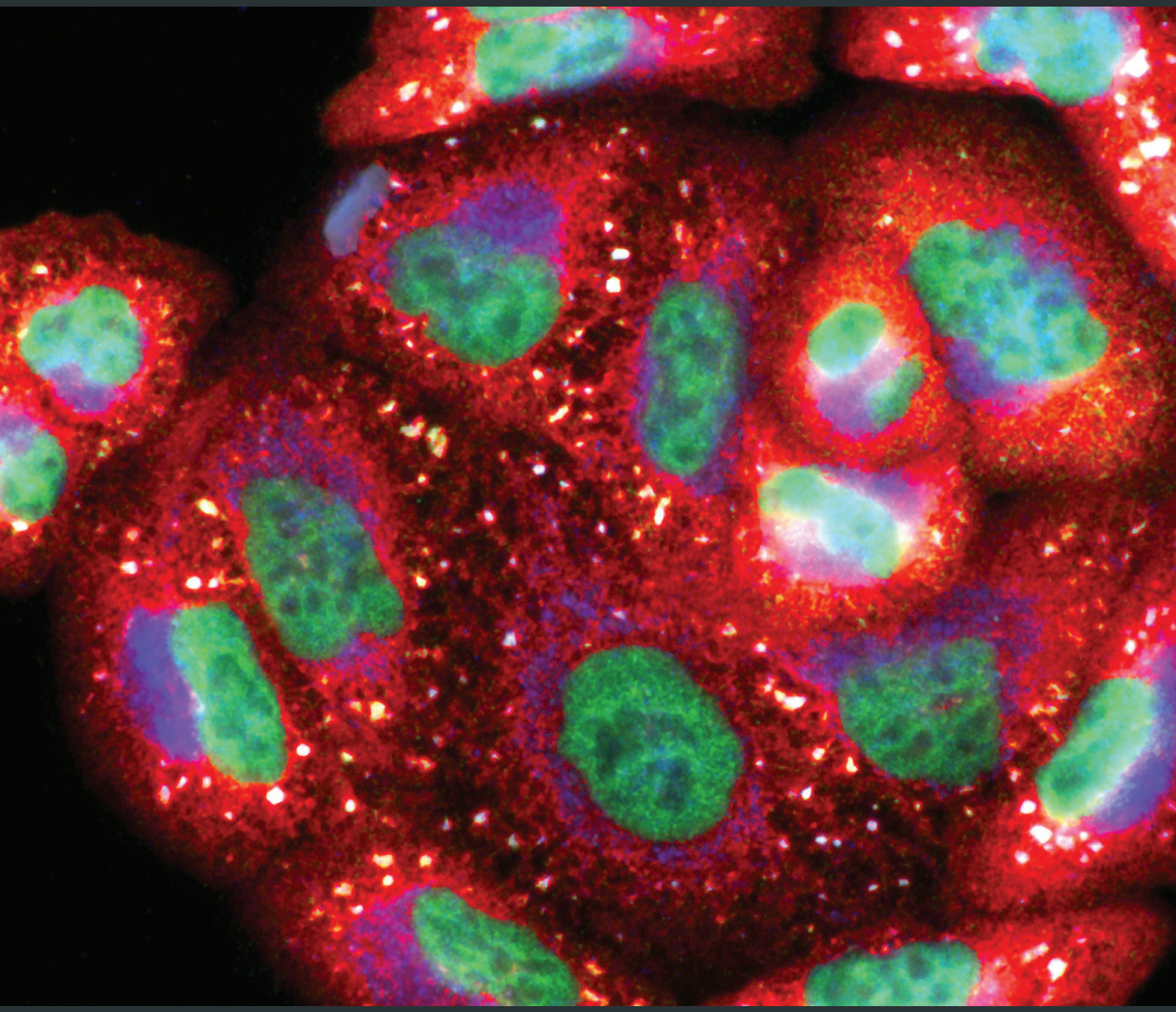
Oxidative Medicine and Cellular Longevity

New Insights into the Benefits of Polyphenols in Chronic Diseases

Lead Guest Editor: Anna V. Queralt
Guest Editors: Anna Tresserra and Sara Arranz



New Insights into the Benefits of Polyphenols in Chronic Diseases

Oxidative Medicine and Cellular Longevity

New Insights into the Benefits of Polyphenols in Chronic Diseases

Lead Guest Editor: Anna V. Queralt
Guest Editors: Anna Tresserra and Sara Arranz

Editorial Board

Contents

Paradoxical Effect of Nonalcoholic Red Wine Polyphenol Extract, Provinols™, in the Regulation of Cyclooxygenases in Vessels from Zucker Fatty Rats (*fa*/*fa*)

Abdelali Agouni, Hadj Ahmed Mostefai, Anne-Hélène Lagrue, Martina Sladkova, Philippe Rouet, Franck Desmoulin, Olga Pechanova, Maria Carmen Martínez, and Ramaroson Andriantsitohaina

Volume 2017, Article ID 8536910, 12 pages

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 1432071, 2 pages
https://doi.org/10.1155/2017/1432071

Editorial

New Insights into the Benefits of Polyphenols in Chronic Diseases

A. Tresserra-Rimbau,[1,2] S. Arranz,[3] and A. Vallverdu-Queralt[4]

[1]*Department of Nutrition, Food Science and Gastronomy, XaRTA, INSA-UB, School of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain*
[2]*CIBEROBN Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain*
[3]*AZTI-Tecnalia, Food Research Division, Derio, Bizkaia, Spain*
[4]*INRA, UMR1083 Sciences pour l'œnologie, 2, Place Viala, 34060 Montpellier Cedex, France*

Correspondence should be addressed to A. Vallverdu-Queralt; avallverdu@ub.edu

Received 26 October 2017; Accepted 26 October 2017; Published 19 November 2017

A wide range of beneficial effects has been attributed to polyphenols. Several studies have shown an inverse association between polyphenols and chronic diseases such as stroke, cardiovascular diseases, metabolic syndrome, type 2 diabetes, and cancer. The Mediterranean diet and its key components, fruits and vegetables, wine, extra virgin olive oil, nuts, and also green tea and chocolate, are well known to contain a large amount of polyphenols. However, the protective effects of polyphenols *in vivo* depend on the extractability from food, intestinal absorption, metabolism, and biological action with target tissues. After the intake of different polyphenols, the metabolites present in plasma and urine vary from one person to another. This may be related to the different gut microbiota composition and/or genetic polymorphisms, and it may be linked to unambiguous health effects.

In this special issue, A. Tresserra-Rimbau et al. summarized the relation between polyphenols and stroke in human intervention studies, animal models, and *in vitro* studies, concluding that dietary polyphenols are inversely associated with morbidity and mortality due to cardio- and cerebrovascular diseases. This is important since the aforementioned diseases are a major concern worldwide.

A. M. Witkowska et al. assessed the link between the intake of dietary polyphenol and the incidence of cardiovascular disease in postmenopausal women. They reported that there was an inverse association between higher dietary polyphenol intake and cardiovascular diseases in this population. In the same line, B. A. Sandoval-Ramírez et al. reported that there is a direct interaction between the phenolic compounds present in beer, such as 8-prenylnaringenin, 6-prenylnaringenin, and isoxanthohumol, and intracellular estrogen receptors, which lead to gene expression modulation, increase in plasma concentrations of sex hormone, and thus modulation of physiological hormone imbalance in menopausal women. Thus, it urges to substitute traditional hormone therapies since they increase health risks. This, again, affirms the potential health benefits of polyphenol-rich food in women.

Metabolic syndrome is a cluster of medical conditions that raises the risk of having cardiovascular diseases and other health problems. According to G. Chiva-Blanch et al., it seems that polyphenols could improve metabolic syndrome components by decreasing body weight, blood pressure, and blood glucose and by improving lipid metabolism. However, evidence from long-term randomized trials is needed to develop public health strategies regarding metabolic syndrome. Based on the results of different studies regarding polyphenols and changes in menopause and metabolic syndrome, S. Kawvised et al. hypothesized that microencapsulated mulberry fruit extract, novel neuroprotectant, and memory-enhancing agent could protect brain damage and improve memory impairment in an animal model of menopause with metabolic syndrome.

Moreover, M. Guasch-Ferré et al. reported that polyphenols and also the Mediterranean diet and its key components are inversely associated to lower glycemia and type 2 diabetes incidence. Different mechanisms have been proposed such as

promotion of glucose uptake in tissues and improvement of insulin sensitivity. Higher intake of flavan-3-ols and their food sources has shown beneficial effects, not only on insulin resistance but also on other cardiometabolic risk factors. Several prospective studies have also reached the same conclusions.

A. Riba et al. reported that resveratrol, a well-known stilbene mainly found in grapes and red wine, was able to prevent cardiac hypertrophy, contractile dysfunction, and remodeling when tested in some animal models of heart failure. A. Riba et al. were able to propose several mechanisms involved in its protective effects (i.e., inhibition of prohypertrophic signaling molecules, improvement of myocardial Ca^{2+} handling, regulation of autophagy, and reduction of oxidative stress and inflammation).

Lastly, A. Agouni et al. studied the vascular effects of dietary supplementation of a nonalcoholic red wine polyphenol extract in Zucker fatty obese rats. They found that the cyclooxygenase (COX) pathway was regulated by the wine polyphenol extract to maintain vascular tone within a physiological range.

In conclusion, this special issue highlights the importance of polyphenols in chronic diseases. Polyphenols from diets or specific foods provide health benefits through different mechanisms involving lipid and glucose metabolism, modifying gene expression or even influencing signaling pathways. However, their biological functions in humans still remain insufficient to claim clear and positive health effects relating to their consumption, particularly with regard to long-term dietary ingestion.

Acknowledgments

The editors thank all authors who submitted their research to this special issue. They also thank the many reviewers for their valuable contribution.

A. Tresserra-Rimbau
S. Arranz
A. Vallverdu-Queralt

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 2867630, 12 pages
https://doi.org/10.1155/2017/2867630

Research Article

An Alginate/Cyclodextrin Spray Drying Matrix to Improve *Shelf Life* and Antioxidant Efficiency of a Blood Orange By-Product Extract Rich in Polyphenols: MMPs Inhibition and Antiglycation Activity in Dysmetabolic Diseases

Maria Rosaria Lauro,[1] Lucia Crascì,[2] Virgilio Giannone,[2,3] Gabriele Ballistreri,[4] Simona Fabroni,[4] Francesca Sansone,[1] Paolo Rapisarda,[4] Anna Maria Panico,[2] and Giovanni Puglisi[2]

[1]*Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 84084 Fisciano, Italy*
[2]*Department of Drug Science, University of Catania, Viale A. Doria, 95100 Catania, Italy*
[3]*Department of Agricultural and Forest Sciences, University of Palermo, Viale delle Scienze Ed.4, 90128 Palermo, Italy*
[4]*Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA) – Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura, Corso Savoia, 190, 95024 Acireale, Italy*

Correspondence should be addressed to Maria Rosaria Lauro; lauro@unisa.it

Received 30 June 2017; Accepted 20 September 2017; Published 2 November 2017

Academic Editor: Anna V. Queralt

Alginate and β-cyclodextrin were used to produce easily dosable and spray-dried microsystems of a dried blood orange extract with antidysmetabolic properties, obtained from a by-product fluid extract. The spray-dried applied conditions were able to obtain a concentrate dried extract without the loss of AOA and with TPC and TMA values of 35–40% higher than that of the starting material. They were also effective in producing microparticles with 80–100% of encapsulation efficiency. The 2% sodium alginate was capable of improving the extract *shelf life*, while the beta-cyclodextrin (1 : 1 molar ratio with dried extract) prolonged the extract antioxidant efficiency by 6 hours. The good inhibition effect of the dried extract on the AGE formation and the MMP-2 and MMP-9 activity is presumably due to a synergic effect exerted by both anthocyanin and bioflavonoid extract compounds and was improved by the use of alginate and cyclodextrin.

1. Introduction

The term "food by-products" indicates that "food wastes" are ultimate substrates for the recovery of functional compounds, as phytochemicals, to develop new products with additional value [1]. Blood orange processing wastes still contain large amounts of anthocyanins, bioflavonoids, and other polyphenols [2]. Many of these compounds are known for their antioxidant effect depending on their concentration [3–5] and their ability to inhibit the metalloproteinase (MMP) activity [6] as MMP-2 and MMP-9, overexpressed during dysmetabolic diseases. In addition, a recent study reported the ability of dietary polyphenols to lower the advanced glycation end product (AGE) formation and protein glycation [7]. For these reasons, phytochemicals may be considered potential bioactive additives for functional food to prevent dysmetabolic pathologies. So, they could be conveniently recovered for nutraceutical purposes.

Our focus was on a Sicilian blood (pigmented or red) orange (cv. "Moro," "Tarocco," and "Sanguinello") by-

product fluid extract (ExF) potentially active on dyslipidemic pathologies [8, 9].

Unfortunately, the fluid extracts are difficult to handle from industry and are more unstable with respect to a dried product. In fact, phytochemical content easily oxidizes/degrades [10]. To overcome this problem, a spray-dried extract (ExMR) was produced. The spray drying technique is able to prevent the appreciable losses of the extract's bioactive compounds without altering the health potential of the extract [11]. To protect phytochemicals from oxidation/degradation phenomena and enhance extract bioavailability, shelf life, and antioxidant efficiency, bioactive ExMR microparticles easily added to common foods of daily diet to enhance the patient compliance were obtained. As carriers, edible biocompatible films and coatings present in the GRAS list and able to deliver several vitamins, antioxidants, and probiotics in food systems [12] were chosen.

In particular, sodium alginate (ALG) is a water-soluble polymer capable of forming a hydrogel polymer matrix which allows a good diffusion of the bioactive compounds. Moreover, several studies showed that treatments with sodium alginate mitigate the glucose excursions, reduce insulin responses, increase satiety, and decrease energy intake and obesity [13, 14]. For its activities, ALG can be used in synergy with the extract in dyslipidemic diseases such as obesity, diabetes, and hypercholesterolemia. Instead, β-cyclodextrin (CD) and its derivatives are able to improve the physicochemical properties of the guest molecules [15], such as degradation and solubility [16], also masking their bitter taste [17].

In our study, solubility, solid state, dissolution properties, and *shelf life* of microparticle-loaded extract were studied and compared to those of the fluid extract to evaluate the effectiveness of the used spray drying technique. The influence of parameters such as the polymer concentration and the extract/polymer ratio on particle yield, distribution, and morphology was also investigated. Furthermore, the extract protection efficacy of the used selected coated polymers has been evaluated. Considering the synergistic involvement of free radicals, AGEs and MMPs on the onset of dysmetabolic diseases [18], the antioxidant activity (ORAC assay), the antiglycation activity, and the inhibition capacity on MMP-2 and MMP-9 (gelatinases involved in vascular remodeling with a consequence of high levels of cholesterol and triglycerides) [6, 19] of both formulated and unformulated extracts have been evaluated.

2. Materials and Methods

2.1. Materials. The fluid aqueous extract obtained from blood orange processing wastes (ExF) was produced by Ortogel SpA (Caltagirone, Sicily, Italy). Beta-cyclodextrin was supplied by Roquette Frères (Lestrem, France). Sodium alginate (ALG), fluorescein (FL), AAPH (2,2′ azobis(2-methylpropionamide) dihydrochloride) 97%, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), aminoguanidine bicarbonate 97% (AMG), bovine serum albumin (BSA), D-(−)-fructose, and sodium azide (NaN_3) were purchased from Sigma-Aldrich Srl (Milan, Italy). Anthocyanins (cyanidin-3-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-rutinoside, and cyanidin-3-sophoroside;delphinidin-3-glucoside and delphinidin-3,5-diglucoside; pelargonidin-3-glucoside and pexlargonidin-3,5-diglucoside; peonidin-3-glucoside; and malvidin-3-glucoside) and flavanones (hesperidin, narirutin, and didymin) were purchased from Extrasynthèse (Genay, France). OmniMMP fluorescent substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2, MMP-9 (refolded) (human) (recombinant) (catalytic domain) and MMP-2 (catalytic domain) (human) (recombinant) were purchased from Vinci-Biochem Srl (Firenze, Italy). Solvents for chromatography were HPLC grade (Merck KGaA, Darmstadt, Germany). All the other chemicals used in the study were of analytical grade and were obtained commercially.

2.2. Manufacture of Spray-Dried ExMR Product. A Buchi Mini Spray Dryer B-191 (Buchi Laboratoriums-Tecnik, Flawil, Switzerland) was used for the drying process: inlet temperature, 120°C; outlet temperature, 68–71°C; spray flow feed rate, 5 ml/min; nozzle diameter, 0.7 mm; drying air flow, 500 l/h; air pressure, 6 atm; and 100% aspirator.

2.3. Qualitative and Quantitative Analyses of Bioactive Compounds in Fluid (ExF) and Spray-Dried Aqueous Extract (ExMR)

2.3.1. Qualitative Analysis. Anthocyanin Purification and HPLC-PDA-ESI/MSn Analysis of Anthocyanins. The extracts were diluted in water for HPLC analysis of anthocyanins. Aqueous extracts were loaded onto C18 Bond Elut SPE cartridges (Varian Inc., Palo Alto, CA, USA) that were previously conditioned with methanol and pure water. The anthocyanins were adsorbed by these cartridges while other soluble compounds such as sugars and acids were removed by washing the columns with pure water. Anthocyanins were eluted with methanol containing 1% formic acid. The acidified methanol solutions were evaporated to dryness; then, the dried fractions were redissolved in 7% aqueous formic acid. Then, the samples were filtered through a 0.45 μm membrane filter (Albet, Barcelona, Spain) and injected into the HPLC-MS^n chromatographic system to identify the individual anthocyanins.

Evaluation of anthocyanins was performed on a Chromolith Performance RP-18 endcapped column (100 × 3.0 mm i.d., monolithic particle size; Merck KGaA, Darmstadt, Germany) using an ultrafast HPLC system coupled to a photodiode array (PDA) detector and Finnigan LXQ ion trap equipped with an electrospray ionization (ESI) interface in series configuration (Thermo Electron, San Jose, CA, USA). HPLC conditions and MS parameters were reported as the same in a previous work [20]. Anthocyanins were identified by using their retention time (tR), MS, and MS^n spectral data in a positive ion mode. In addition, comparison of the MS spectral data with those of pure standards and/or those reported in literature was performed. The relative compositions (%) of the individual anthocyanins were calculated from the peak areas of the

chromatogram detected at 520 nm, using Xcalibur versus 2.0.7 software (Thermo Electron).

HPLC-PDA-ESI/MSn Analysis of Flavanones. Flavanone glycosides, expressed as hesperidin (Hd) equivalents (g/100 g of extract), were determined by HPLC [21] using the HPLC-PDA-ESI/MS^n equipment described above. A sample of the extract was dissolved in dimethyl sulfoxide and diluted with the mobile phase, filtered through a 0.45 μm membrane filter (Albet, Barcelona, Spain), and then injected directly into the column. The eluent was water : acetonitrile : acetic acid (79.5 : 20 : 0.5), and the flow rate was 800 μl/min. The individual flavanones were detected at 280 nm. MS parameters were the same as those described for anthocyanin analysis. Flavanones were identified by using their retention time (tR), MS, and MS^n spectral data in a negative ion mode and also by comparison of the MS data with those of pure standards and/or those reported in literature.

2.3.2. Quantitative Analysis. Total Polyphenol Content (TPC). The polyphenol contents of ExF and ExMR were determined by the Folin-Ciocalteau method according to Aiyegoro and Okoh [22], with slight modifications. 2 mg/ml of both extracts was dissolved in distilled water. Then, 2.5 ml of 10% Folin-Ciocalteau reagent and 2 ml of Na_2CO_3 (2% *w*/*v*) were added to 0.5 ml of each samples. The resulting mixtures were incubated at 45°C with shaking for 15 min. The absorbance of the samples was measured at 765 nm using UV/visible light. The standard curve ($y = 4.292 - 0.0293x$; $R^2 = 0.9951$) was prepared by 0, 0.05, 0.1, 0.15, 0.2, and 0.25 mg·ml^{-1} solutions of gallic acid in water. TPC values are expressed as milligram of gallic acid equivalents (GAE)/g of extract.

Total Monomeric Anthocyanin (TMA) Content. TMA content was assayed by the pH differential method [23] by using a UV/Vis spectrophotometer (Varian Cary 100 Scan, Palo Alto, CA, USA). Total anthocyanin content was expressed as g of cyanidin-3-glucoside equivalents (C3G)/100 g of extract on a dry weight (DW) basis.

2.4. Solubility Studies

2.4.1. ExMR Solubility. Solubility test of dried ExMR was performed in distilled water (3.0 ± 0.2 g/l) and in gastric (GF; pH 1.2) (3.5 ± 0.1 g/l) and intestinal (IF; pH 7.5) (3.8 ± 0.2 g/l) simulated fluids, without enzymes (USP 37) by UV/Vis spectrometry at $\lambda = 310$ nm and expressed as hesperidin equivalents (Hd). Each analysis was made in triplicate.

2.4.2. Phase Solubility. 2.5×10^3 mol of ExMR, expressed as Hd, was suspended in 100 ml of water. Different amounts [24] of CD (1 : 3, 1 : 2, 1 : 1.5, 1 : 1, and 2 : 1 ExMR/CD molar ratio) were added. The samples were shaken, stored at 25°C for 1 hour, and then centrifuged (5 min at 3000 rpm). The supernatants were analyzed in UV apparatus (1 cm cell; $\lambda = 278$ nm).

2.5. Microparticle Preparation. ExMR was suspended (3 : 1 polymer : extract weight ratio) in 1% or 2% (*w*/*v*) ALG aqueous solution, under magnetic stirring [25], to give the ExMR1 (1% ALG) and ExMR2 (2% ALG) microsystems. In the second step, CD (1 : 1 CD : ExMR molar ratio) was dissolved in 2% *w*/*v* ALG water solution (3 : 1 ALG : ExMR weight ratio), to obtain ALGCDExMR microparticles. ALG-free microparticles (CDExMR) were used as control. The spray drying conditions were reported as the same in ExMR preparation. All the spray-dried microparticles were carried out in triplicate, collected, and stored under vacuum (48 h at room temperature).

2.6. Microparticle Properties and Characterization

2.6.1. Particle Size Analyses. Isopropanol was used as a suspending agent for all samples. A Beckman Counter LS 230, Particle Volume Module Plus, UK (instrument obscuration: 8–12%), was used to examine the particle size in triplicate applying the Fraunhofer model. The results were expressed as the median diameter of the particles (d50).

2.6.2. Morphology. The microphotographs of the morphology of all samples were acquired by a confocal (Leica TCS SP2, CF) and a fluorescent microscope (Zeiss Axiophot, FM).

All images were equipped with 63 × 1.4 NA plan apochromat oil immersion objectives (Carl Zeiss Vision, München-Hallbergmoos, Germany) and standard DAPI (4′,6-diamidino-2-phenylindole) optics that adsorb violet radiation (max 372 nm) and emit blue fluorescence (max 456 nm).

2.6.3. Yield of the Process (Y), Encapsulation Efficiency (EE), and Extract Content in Microsystems. *Y* was gravimetrically determined and expressed as the weight percentage of the final product compared to the total amount of the sprayed materials.

EE was calculated according to Sansone et al. [10].

$$\mathrm{EE}\,(\%) = \frac{\text{actual extract content}}{\text{theoretical extract content}}. \quad (1)$$

The actual extract content was calculated as Hd concentration used as a marker and determined in the supernatant solutions of 15 mg of microsystems dissolved in MeOH (15 ml; sonicated for 5 min, centrifuged for 10 min at 300 rpm) by HPLC Agilent equipment (Agilent 1100 series system; model G-1312 pump; Rheodyne Model G-1322A loop (20 μl); DAD G-1315 detector; 150 × 3.9 mm i.d. C18 μ-Bondapack column). The flow rate is 1.0 ml·min^{-1}. The following are the mobile phases: water (solvent A) and methanol (solvent B). The elution gradient was shown as follows: 0 → 5 min (15 → 30%) B, 5 → 10 min (30 → 35%) B, 10 → 20 min (35 → 50%) B, 20 → 30 min (50 → 75%) B, 30 → 35 min (75 → 95%) B, and 35 → 40 min (100%) B. A DAD detector was set at $\lambda = 283$ nm. Hd reference standard solutions were prepared at five concentration levels in the range 1–40 μg/ml. Linear least squares regression equation was derived from the peak area corresponding to Hd ($y = 1798.3x - 54.938$, $R = 0.9996$), where y is the peak area and x the used concentration.

2.6.4. Differential Scanning Calorimetry (DSC) and Fourier-Transform Infrared Spectroscopy (FTIR). DSC. An indium-calibrated Mettler Toledo DSC822e (OH, USA) was used

exposing all the samples to two thermal cycles: a dehydration cycle up to 130°C (heating rate of 20°C/min; temperature maintained at 130°C for 15 min in order to remove the residual solvent); afterwards, the samples were cooled at 25°C and heated up to 350°C (heating rate of 10°/min) [6]. The analyses were carried out in triplicate.

FTIR. A Jasco FT-300 (Tokyo, Japan) Fourier-transform IR spectrometer was used to analyze all samples in two steps: first, the material was dried in a vacuum oven to reduce the presence of water and then analyzed as KBr discs in the spectral region 650–4,000 cm^{-1} at a resolution of 8 cm^{-1}.

2.7. Stability Studies

2.7.1. Accelerated Stability. The stability test was performed according to accelerated stability studies reported in the ICH guidelines (International Conference on Harmonization of Technical Requirements of Pharmaceutical for Human Use, 2003) in a climatic and thermostatic chamber (Mod.CCP37, AMT srl, Milan, Italy), at 40°C ± 2°C/75% RH ± 5% RH for one week and then analyzed by UV and HPLC in terms of extract content and TPC variation. Chromatographic peaks were identified on the basis of the retention times and confirmed by coinjections with an internal standard [14].

2.7.2. Functional Stability (Oxygen Radical Absorbance Capacity: ORAC Assay). In order to determine the *in vitro* antioxidant capacities of both ExF and ExMR, the ORAC method [26] was employed. Also, a comparison between ORAC unprocessed (ExMR) and processed extract by ICH (ExMR ICH) was performed.

The fluorescence probe fluorescein (FL, 10 nM) was used as a reference compound attacked from peroxyl free radicals that are generated from APPH (100 mM) solution. In order to calculate the area under a curve (AUC) of the tested compounds (12.5 μg/ml), the reaction was following at 37°C (pH 7.0) until a fluorescence decay of FL solution in the presence of APPH. Each measurement was repeated at least three times, using a Wallac 1420 Victor 96-well plate reader (PerkinElmer, USA) with a fluorescence filter (excitation 485 nm, emission 520 nm). The Trolox (12.5 μM) was used as an antioxidant control.

The ORAC value refers to the net protection area under the quenching curve of fluorescein in the presence of an antioxidant. The final results (ORAC value) were calculated and expressed in ORAC units (Trolox micromol per microgram of sample (μmol/μg)).

$$\text{ORAC value}(\mu\text{mol}/\mu\text{gram}) = \frac{K(S\,\text{sample}-S\,\text{blank})}{(S\,\text{Trolox}-S\,\text{blank})}, \quad (2)$$

where K is a sample dilution factor and S is the area under the fluorescence decay curve of the sample, Trolox or blank, calculated with Origin®7 (OriginLab Corporation, Northampton, USA).

2.8. In Vitro Dissolution/Release Tests. ExMR (100 mg, sink conditions) or produced formulation corresponding to the same amount of pure dried extract was carried out under sink conditions (corresponding to about) in water using a SOTAX AT smart apparatus (Basel, CH), on a line with a spectrophotometer at $\lambda = 310$ (UV/Vis spectrometer Lambda 25, PerkinElmer Instruments, MA, USA), and USP 37 dissolution test apparatus n.2: paddle, 100 rpm at 37°C. All the dissolution/release tests were made in triplicate; only the mean values are reported in a graph (standard deviations < 5%).

2.9. Antioxidant Efficiency. To determine the antioxidant efficiency of formulated and unformulated samples, a modified ORAC assay was used [6]. Briefly, both 25 μl extracts of all samples were placed in 96-well tissue culture plates. 100 μl FL (10 nM) solution was added to each well to initiate the assay. Then, 25 μl AAPH (100 mM) solution was added to all wells, except for the negative control, to which 25 μl phosphate buffer solution was added. A FL solution without AAPH was used as negative control. A timer was started upon introduction of the free radical generator, and the plate was stored in the dark at 37°C. Unlike the previous method (reported in paragraph 2.7.2.), at each specified time point, the fluorescence of the solution was measured (excitation 492 nm, emission 535 nm) and plotted as a function of time [27, 28], using Origin7 (OriginLab Corporation, Northampton, USA). The y-axis graphs in Figure 1 were split from 6000 to 10000 RFU.

2.10. Antiglycation Activity. According to the method of Derbré et al. [29] with slight modifications, we evaluated the inhibition of fluorescence produced by AGE formation through Maillard reaction. Briefly, as optimum AGE formation, the protein model bovine serum albumin (BSA) (10 mg/ml) was incubated with D-fructose (0.5 M) in phosphate buffer 50 mM pH 7.4 (NaN_3 0.02%) to obtain positive controls. BSA alone was the negative control corresponding to no fluorescence AGE formation. The aminoguanidine (AMG) (400 μg/ml) was used as reference compounds for its AGE inhibition property [30]. The final glycated BSA solutions (300 μL) alone and with the sample (400 μg/mL) were incubated at 37°C in a 96-well microtiter closed with their silicon lids for 7 days. The AGE fluorescence measurement (λexc 370 nm; λem 440 nm) is performed using a VICTOR Wallac 1420 Multilabel Counter fluorimeter (PerkinElmer, USA). The results are reported in relative fluorescence units (RFU), and the percentage of inhibition with respect to the positive control (BSA with fructose) is calculated from the following equation:

$$\%\text{ of inhibition} = \left\{1 - \frac{\text{RFU sample(nm)}}{\text{RFU-positive control(nm)}}\right\} \times 100. \quad (3)$$

2.11. Inhibitory Activity on MMP-2 and MMP-9. The MMP inhibition assay of unformulated and formulated ExMR was based on the inhibition of the hydrolysis of the fluorescence-quenched peptide substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Vinci-Biochem Srl). The assay was performed according to Crascì et al. [31]. The results were plotted with Origin7 (Originlab Corporation, Northampton,

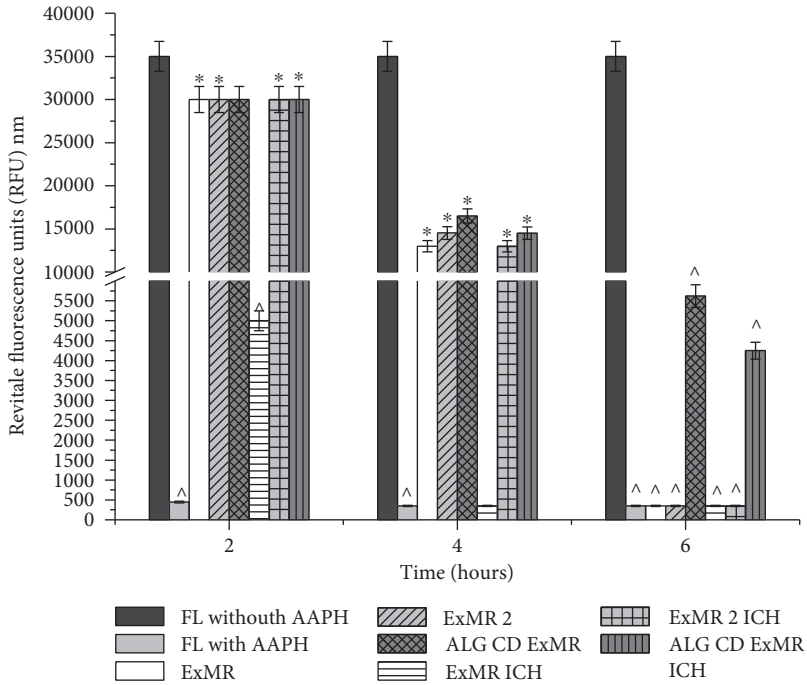


Figure 1: Antioxidant efficiency of unformulated and formulated extracts. Data represent the mean of three independent experiments ± SD. $^{*}p < 0.05$ or $^{\wedge}p < 0.01$ compared with FL without AAPH. ICH: samples processed according to ICH guidelines.

USA) software and are expressed as concentration of inhibitors that reduced of 50% the MMPs activity (IC_{50}).

3. Results and Discussion

3.1. Characterization and Stability of Unprocessed (ExF) and Processed (ExMR) Extracts. Both the extracts (ExF and ExMR) were analyzed by HPLC-MS and showed four major eluted constituents (Tables 1 and 2), two anthocyanins, cyanidin 3-(6″-malonyl) glucoside (CMG, t_R 18.9 min) and cyanidin 3-glucoside (C3G, t_R 15.2 min) and two *O*-glycosylated flavonoids, hesperidin (Hd, t_R 18.5 min) and narirutin (Nr, t_R 13.5 min) (Figure 2).

In agreement with previous data reported in literature [32, 33], the major component Hd was chosen as marker [6]. Both Hd and C3G, as bioflavonoid and anthocyanin markers, respectively, were used as comparison compounds to determine the bioactive content in MMP studies.

The analytic data of ExF showed that the TPC (Table 3) and TMA values were equal to 35% and 40% of the spray-dried ExMR extract, respectively. In fact, in ExF, the levels of TPC (mg gallic acid/g extract) and TMA (%) were 12.0 ± 0.18 mg/g and 0.322 ± 0.02%, respectively. The antioxidant activity (Table 3) expressed in ORAC units (AOA) was 1.9 ± 0.8 ORAC units. The evaluation of the extract properties is essential to obtain spray-dried powders with optimized physicochemical and biological properties.

3.2. Spray Drying Process. ExF has been spray dried to obtain a powder extract which can be easily handled. The spray drying technique is the most commonly used in food and nutrapharmaceutical industries [34]. It is a crucial step that can affect the extract stability [35] and plays an important role in determining the properties and cost of dried products [36]. On this consideration, it is interesting to note that the selected spray dryer conditions were able to obtain a product yield equal to 60% and without loss of AOA activity (2.6 ± 0.3 ORAC units). Furthermore, the TPC and TMA values (33.5 ± 0.21 mg/g and 0.814 ± 0.1%, resp.) were higher than that of ExF. These results are probably due to the spray drying parameters such as the inlet temperature (120°C) which were optimized in order to protect and reduce the loss of polyphenols because of degradation [25].

3.3. Solubility Studies. ExMR has a slight water solubility (3.0 ± 0.1 mg/l) at room temperature that was notably affected by the presence of CD used as enhancer of dissolution rate, limiting factor of *in vivo* bioavailability.

The amount of CD required to increase a sample bioavailability can be evaluated by the phase solubility studies and can affect the various processes occurring during the delivery in the gastrointestinal (GI) environment [37].

The phase solubility (Figure 3) showed a Bs-type profile [24]. CD is capable to enhance the ExMR solubility with a linear increase below the 1 : 2 ExMR/CD molar ratio. The ascending portion indicates a 1 : 1 stoichiometry complex; at higher CD concentrations appears a short plateau indicating the formation of an insoluble or with a different stoichiometry complex in the solution. For this reason, conventionally, we assumed a 1 : 1 ExMR molar ratio to obtain an improvement of water solubility (from 3.0 ± 0.1 mg/l to 6.0 ± 0.5 mg/l) and an enhancement of the

Table 1: Total anthocyanin content of ExMR and ExF and relative percentage of individual anthocyanins.

Peak number[a]	t_R (min)	$(M)^+$ (m/z)	MS^n (m/z)	Anthocyanins	Relative composition[b] (%)	
					ExMR	ExF
1	13.9	611	449/287	Cyanidin 3,5-diglucoside	1.31 ± 0.01	1.72 ± 0.08
2	14.2	465	303	Delphinidin 3-glucoside	2.65 ± 0.02	2.96 ± 0.13
3	14.5	611	287	Cyanidin 3-sophoroside	0.51 ± 0.01	0.16 ± 0.02
4	15.2	449	287	Cyanidin 3-glucoside	39.87 ± 0.17	31.80 ± 0.06
5	15.9	595	287	Cyanidin 3-rutinoside	1.34 ± 0.24	0.43 ± 0.10
6	16.8	479	317	Petunidin 3-glucoside	1.55 ± 0.02	1.53 ± 0.02
7	17.5	551	465/303	Delphinidin 3-(6″-malonyl)glucoside	1.53 ± 0.01	0.67 ± 0.08
8	17.9	463	301	Peonidin 3-glucoside	2.88 ± 0.03	2.90 ± 0.03
9	18.2	565	479/317	Petunidin 3-(6″-malonyl)glucoside	1.48 ± 0.01	0.58 ± 0.05
10	18.9	535	449/287	Cyanidin 3-(6″-malonyl)glucoside	21.73 ± 0.10	36.40 ± 0.05
11	19.9	593	449/287	Cyanidin 3-(6″-dioxalyl)glucoside	5.91 ± 0.03	8.02 ± 0.05
12	20.4	—	271	Pelargonidin derivative	1.23 ± 0.01	5.55 ± 0.03
13	21.7	549	463/301	peonidin 3-(6″-malonyl)glucoside	13.84 ± 0.11	5.81 ± 0.12
14	22.2	—	287	Cyanidin derivative	2.35 ± 0.03	1.01 ± 0.12
15	23.3	—	301	Peonidin derivative	1.82 ± 0.02	0.46 ± 0.08
Total anthocyanins (g C3G/100 g extract)[c]					0.81 ± 0.01	0.32 ± 0.02

[a]The numbering is according to Figures 2(a) and 2(b). [b]Relative percentage of anthocyanins was based on HPLC peak areas recorded at 520 nm. [c]Anthocyanins are expressed as cyanidin 3-glucoside equivalents.

Table 2: Concentration of individual flavanones and total flavanones in ExMR and ExF.

Peak number[a]	t_R (min)	$(M–H)^-$ (m/z)	MS^n (m/z)	Flavanones	g Hd/100 g[b]	
					ExMR	ExF
1	13.5	579	271	Narirutin	5.48 ± 0.05	2.22 ± 0.06
2	18.5	609	301	Hesperidin	5.73 ± 0.02	2.35 ± 0.11
3	59.9	593	285	Didymin	0.79 ± 0.01	0.33 ± 0.02
Total flavanones (g Hd/100 g extract)[b]					12.00 ± 0.07	4.90 ± 1.13

[a]The numbering is according to Figures 2(c) and 2(d). [b]Flavanones are expressed as hesperidin equivalents.

dissolution rate of the extract, as confirmed by the dissolution/release test.

3.4. Spray-Dried Microparticle Properties and Characterization

3.4.1. Alginate Microparticles (ExMR1 and ExMR2). In the first step, in order to achieve stable microsystems, ExMR was spray dried using 1% or 2% ALG water solutions *w*/*v* as coating polymer and 3 : 1 polymer : extract weight ratios. The best formulation was in the presence of 2% of ALG (ExMR2). The low amount (1%) of ALG in ExMR1 produced microparticles with greater particle size (5 ± 0.8 μm) than ExMR2 (1.5 ± 0.5 μm), probably due to aggregate formation and high moisture content [38, 39] (Figure 4(a)). For this reason, 2% of ALG formulation (ExMR2) were selected. The results showed that the selected parameters (spray drying conditions and 2% of ALG) were able to produce well-formed microparticles (Figure 4(b)) and to obtain a good EE (80.0%). These were due to the ALG amount, able to reduce in solid dispersion the molecular mobility of the bioactive compounds avoiding the phase separation, while the spray drying parameters were effective to lower the accumulation on the chamber wall [24, 40–43].

The ExMR2 DSC thermogram (Figure 5) did not show any extract peak, confirming its complete encapsulation, such as that supported by FTIR analysis. Moreover, ALG shows high decomposition temperature. In fact, the ALG thermogram exhibits a first endothermic peak at 100°C (correlated to the release of water) and an exothermic peak at 250°C, due to pyrolysis reaction in the polymer [44]. Since, some food manufacturing processes, especially baked good production, required high cooking temperature (150°–200°C) [45]. The ALG thermal behavior of the produced formulation could be suitable to protect the loaded active ingredient also to prevent the premature crystallization of the sugars [46] present in the extract.

2% ALG water solution is also able to improve the dissolution rate of ExMR (Figure 6). In fact, about 49% of the extract were released from ExMR2 in 5 min with respect to about 16% of pure extract that dissolved at the same time.

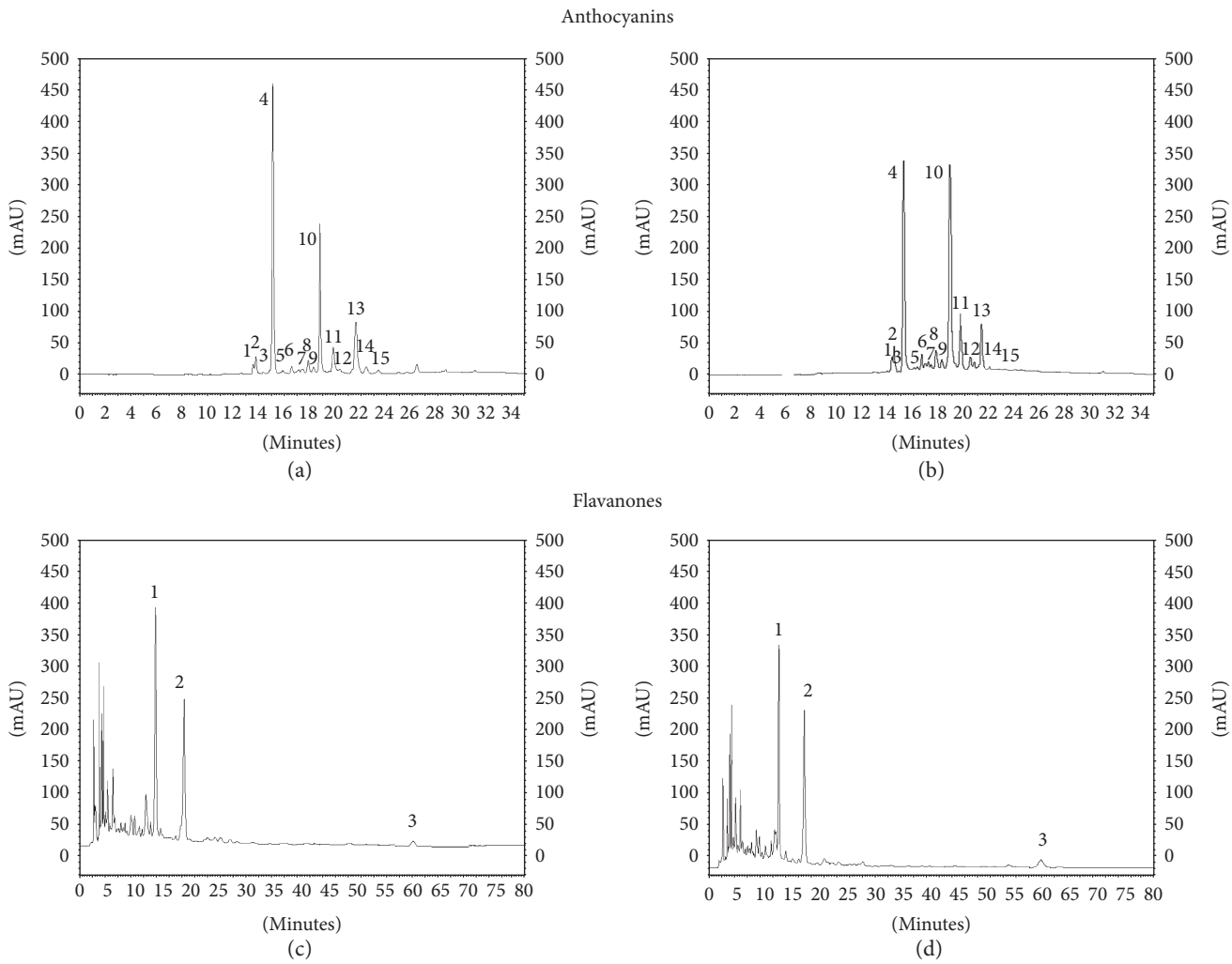


Figure 2: HPLC chromatograms of anthocyanins of ExMR (a) and ExF (b) detected at 520 nm (see Table 1). HPLC chromatograms of flavanones of ExMR (c) and ExF (d) detected at 280 nm (see Table 2).

Table 3: Quantitative antioxidant activity (ORAC assay) and total polyphenol content (TPC) of fluid extract (ExF), spray-dried extract (ExMR), and spray-dried extract processed according to ICH guidelines (ExMR ICH).

Sample	TPC (mg GAE/g of extract)	ORAC units (μmol TE/μg of extract)
ExF	12.00 ± 0.18	$1.90 \pm 0.80^*$
ExMR	33.50 ± 0.21	$2.60 \pm 0.30^*$
ExMR ICH	28.80 ± 0.60	$0.70 \pm 0.08^*$

Reported values are the means ± standard deviation (SD) ($n = 3$). *Significantly different at $p < 0.05$ compared to 1 ORAC unit of Trolox.

This behavior was probably due to the presence of ALG that increase the extract-water interaction due to its high hydrophilic behavior [6, 13]. Because extract-water release was incomplete (Figure 6) (85% in 30 min), in the second step, microparticles with enhancement of the dissolution rate were developed in the presence of CD.

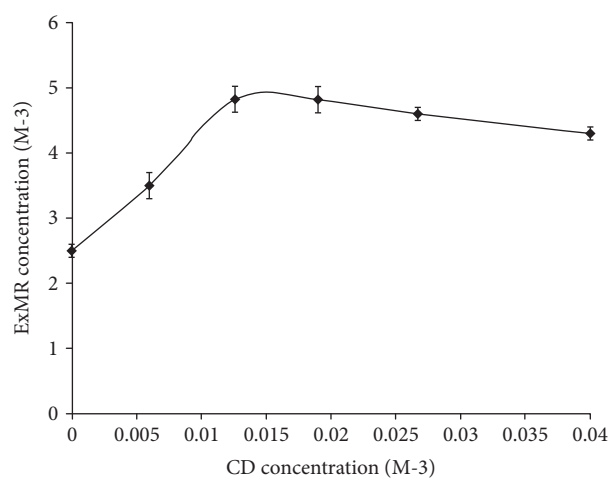


Figure 3: Solubility phase diagram of ExMR in the presence of β-cyclodextrin.

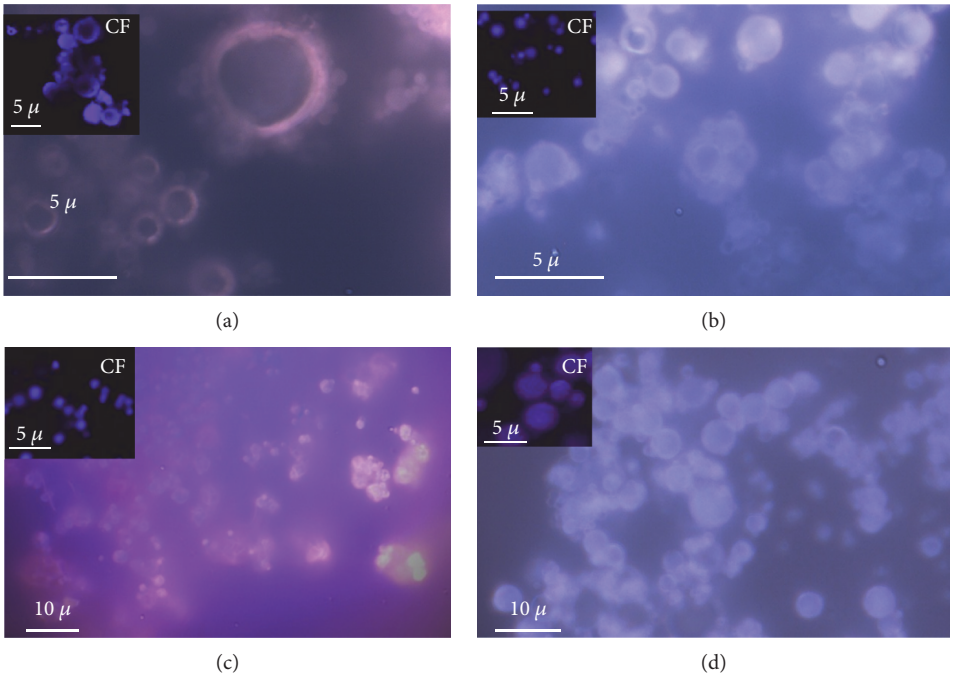


Figure 4: Fluorescence (FM, ×63 and ×40) and confocal (CF) microphotographs of ExMR1 (a), ExMR2 (b) CDExMR (c), and ALGCDExMR (d) microsystems.

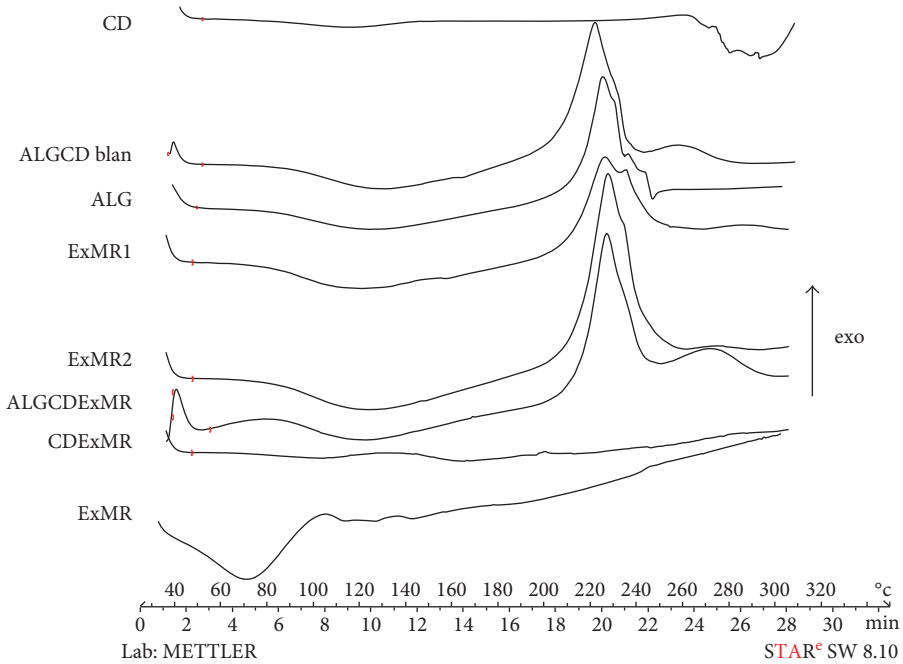


Figure 5: Differential scanning calorimetry of pure materials (ExMR, CD, and ALG), micropowders, blank (ALGCD blank), and loading microsystems (ExMR1, ExMR2, CDExMR, and ALGCDExMR).

3.4.2. Alginate/Cyclodextrin Microparticles (CDExMR and ALGCDExMR). The presence of CD reduces the particle size during the spray drying process and improves the EE (90.0–100.0%). In fact, the micrograph of the batches CDExMR and ALGCDExMR showed the presence of small microparticles (Figures 4(c) and 4(d)) with dimensions of about 0.5 ± 0.02 and 1.0 ± 0.2 μm, respectively.

The CDExMR thermogram shows a series of peaks from 180°C to 280°C which are superimposable with that of pure ExMR, with slight shifts. This behavior confirms that the

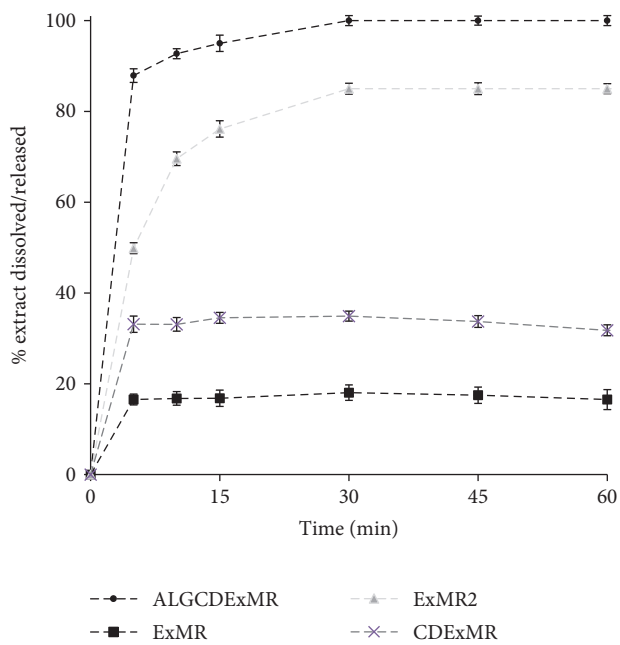


Figure 6: Dissolution/release profile of ExMR2, CDExMR, and ALGCDExMR in comparison with ExMR micropowder dissolution profiles in water.

1 : 1 CD/ExMR molar ratio is unable to form a complex. Instead, the shifts show that microparticle components interact only to form −H bonds, such as confirmed by FTIR spectroscopy (amplification of OH band at 3500 cm^{-1}; data not shown) [47].

The −H bonds between CD and ExMR could be responsible of the enhancement of the extract dissolution rate. About 33.0% of the extract dissolved from CDExMR in water after 5 minutes, with respect to about 16.0% of pure ExMR that dissolved at same time. Moreover, when used together, ALG and CD act synergistically enhancing the dissolution/release of ExMR (about 90% after 5 min) from ALGCDExMR, and all loaded doses were released (100% in 30 min).

3.5. Accelerated and Functional Stability (ICH Guidelines) and ORAC Test. The extract powders obtained from blood orange processing wastes are rich in polyphenols, easily subject to oxidation/degradation phenomena. This behavior is a critical point for their use in food or pharmaceutical field [48]. To examine the *shelf life*, the antioxidant efficiency and the effect of the ALG and CD polymers, and the microencapsulation processes on the stability of the extract in storage conditions, the "real-time" stability was reproduced according to ICH guidelines in a climatic chamber and in a brief time period in extreme conditions. The functional stability of ICH extract was performed by the ORAC assay.

After one week at 40°C, an increase in ExMR weight, determined by the gravimetric method, was observed (28.0%). This is probably due to its hygroscopicity. The quantitative ORAC and TPC also showed a significant decrease in the ExMR AOA (from 2.6 to 0.7 ORAC units) and TPC (about 12.0%) (Table 3). Moreover, while CDExMR

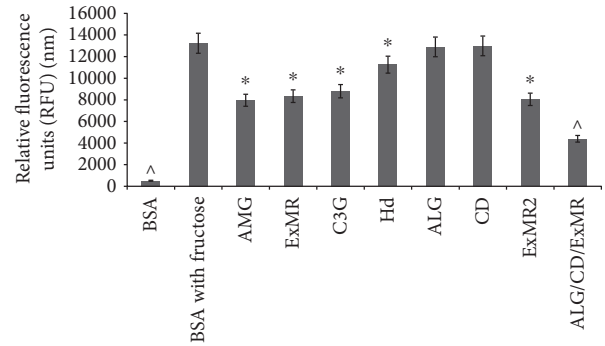


Figure 7: Antiglycation effect of unformulated and formulated extracts, Hd and C3G, major biopolyphenol compounds, in comparison with AMG assay standard control. Results are means ± SD. Significantly different at $^{*}p < 0.05$ or $^{\wedge}p < 0.01$ compared with positive control (BSA with fructose).

values slightly decreased (about 5.0%), ExMR2 and ALGCDExMR remained quite unaltered, showing that this significant result in terms of stability (<1%) is due to ALG that is able to enhance ExMR *shelf life*.

The decrease of ExMR data was probably due to thermal degradation of few polyphenols contained in citrus extract. In fact, literature data report that anthocyanins in blood orange juice presented high-rate constant of degradation in the range 30°–90°C [49] with 69% losses after 90° for 120 min [50]. Instead, for flavanones such as hesperidin, no significant decrease was noticed in the range 70–90°C after 240 min [51]. In fact, it was demonstrated that glycosidic flavonoids are more resistant than aglycone flavonoids to heat treatments [52].

3.6. Antioxidant Efficiency. The ORAC assay has been performed in order to evaluate the qualitative antioxidant efficiency of the best formulation, untreated and treated according to ICH. The data (Figure 1) showed that at 4 hours, ALG and CD slightly improve the ExMR antioxidant efficiency with respect to the unformulated extract, with a major effect for CD polymer. On the contrary, at 6 hours, ExMR and all the formulations without CD (ExMR2 and ExMR ICH) had lost their antioxidant efficiency. Indeed, ALG/CD/ExMR and ALG/CD/ExMR ICH maintain and prolong the extract activity only with a slow further spontaneous decomposition of fluorescein. These results suggest that the presence of CD in the microsystems preserved the antioxidant efficiency of the extract for a longer time and enhanced the stability of the extract.

3.7. Antiglycation Activity. The amount of AGEs is elevated during hyperglycemic and/or oxidative stress conditions [53]. This process induces irreversibly fluorescent macroprotein derivative formation, termed AGEs, via Maillard reaction [54]. Considering that a diet rich in natural antioxidant compounds protect against protein glycation [18], we evaluated the inhibitory effect of both unformulated and formulated extracts on fluorescent AGE formation. The results (Figure 7) showed a good ExMR capability to inhibit AGEs, reducing the max fluorescent value of positive

Table 4: Inhibitory activity on MMP-2 and MMP-9.

Compounds	IC_{50} MMP-2 (μg/ml)	IC_{50} MMP-9 (μg/ml)
ExMR	1.12 ± 0.12	5.52 ± 0.72
ALG	n.a.	n.a.
CD	$4.03 \pm 0.35^{\wedge}$	$2.98 \pm 0.31^{*}$
Hd	n.a.	n.a.
C3G	$10.57 \pm 1.35^{\wedge}$	7.27 ± 1.05
ExMR2	$4.49 \pm 0.85^{\wedge}$	$4.27 \pm 0.46^{*}$
ALGCDExMR	$0.49 \pm 0.09^{\wedge}$	$1.40 \pm 0.19^{\wedge}$

n.a. = not active ($IC_{50} > 100$ μg/ml). Reported values are the means ± standard deviation (SD) ($n = 3$). $^{*}p < 0.05$ compared to ExMR value. $^{\wedge}p < 0.01$ compared to ExMR value.

control (BSA with fructose) from 13,254 nm to 8338 nm. This value corresponded to 37.0% of inhibition, and the observed effect is superimposable to that of the AMG assay standard (40.0% of inhibition). Also, in order to determine which representative compounds influence the most the antiglycation effect, the ExMR activity was compared with those of C3G and Hd, the selected representative standard compounds. Both samples are able to inhibit AGE formation, showing a fluorescence of 8798 nm and 11,249 nm, respectively, corresponding to 36.3% and 15.2% of inhibition. This demonstrates that the ExMR activity is due to a synergic effect of both polyphenol class compounds (bioflavonoids and anthocyanins). Moreover, to evaluate the polymer effect, ExMR2 and ALGCDExMR were also tested. The major activity of formulation was found in ALGCDExMR with a fluorescence value of 4335 nm, corresponding to 69.2% of inhibition, followed by ExMR2 with a fluorescence value of 8040 nm, corresponding to 39.3% of inhibition. The results underline that all the formulations tested showed an AGE direct inhibition, indicating that both CD and ALG polymers are able to protect the extract from degradation and oxidation phenomena.

3.8. Inhibitory Activity on MMP-2 and MMP-9. AGE accumulation has a role in the increase of different metalloproteinase expression [18]. Because many polyphenol compounds inhibit both collagenase and gelatinase activities [55, 56], we evaluated the inhibition ability of the tested samples on MMP-2 and MMP-9.

The results reported in Table 4, expressed as the concentration value (μg/ml) of potential inhibitor that reduces of 50% the MMP activity (IC_{50}), showed that ExMR possesses a high capability to inhibit both MMP-2 and MMP-9. This could be due to the anthocyanin content. In fact, the representative flavonoid Hd is not active, while the anthocyanin C3G has an IC_{50} value of 10.57 ± 1.35 μg/ml and 7.27 ± 1.05 μg/ml on MMP-2 and MMP-9, respectively.

In order to evaluate if the formulation can influence the activity of the pure extract, the inhibitory activity of formulated extract (ExMR2 and ALGCDExMR) and pure materials (ExMR, ALG and CD) were assayed. We observed that the presence of ALG in ExMR2 reduced the inhibition activity on MMP-2 (4.49 ± 0.85 μg/ml), but improved the activity on MMP-9 (4.27 ± 0.46 μg/ml), while the presence of CD in ALGCDExMR improved the inhibitory effect on both MMPs (0.49 ± 0.09 μg/ml and 1.40 ± 0.19 μg/ml on MMP-2 and MMP-9, resp.) with respect to pure ExMR (1.12 ± 0.12 μg/ml and 5.52 ± 0.72 μg/ml, resp.). This could be explained with a high inhibition effect of CD on MMP-2 (4.03 ± 0.35 μg/ml) and MMP-9 (2.98 ± 0.31 μg/ml), probably due to the presence of free hydroxyl groups that support the hydrogen bond with the enzyme-active site [57].

4. Conclusions

An easily spray-dried handle antioxidant extract (ExMR) was produced to formulate dietary supplements for human health as well-formed and stable microparticles of ALG and CD also to make it suitable to be added in baked goods as bioactive food ingredients. The bioactivity on dysmetabolic disease was due to extract polyphenol compounds. In fact, anthocyanins and bioflavonoids both resulted responsible for the dried extract in vitro AGE inhibition activity, while only the anthocyanin content was effective with respect to in vitro MMP inhibition. Also, CD and ALG polymers were able to improve these activities. On one hand, CD improved the MMP inhibitory activity of the extract presumably because of the presence of free hydroxyl groups that support the hydrogen bond with the enzyme-active site; on the other hand, CD acted synergistically with ALG enhancing the dissolution/release of ExMR and improving the in vitro AGE direct inhibition of extract, also protecting it from degradation and oxidation phenomena. Furthermore, CD preserved the extract antioxidant efficiency and stability, while the choice of 2% of ALG as coated polymer (3 : 1 ALG/ExMR weight ratio) was effective to improve the extract wettability and its *shelf life*.

This research represents an advantageous way to re-evaluate a blood orange citrus by-products of the Sicilian industry and to develop human dietary supplement which also acts to be added as "bioactive food ingredients" in functional foods like baked goods.

Conflicts of Interest

The authors declare that there are no competing interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank the Sicily region for the financial support within PO FESR Sicilia 2007–2013, "ALIFUIDEA: Alimenti Funzionali e Integratori Nutraceutici a base di Lupino bianco e derivati di Agrumi" (ALIFUIDEA Project. "Functional Food and Nutraceutical Supplements with Bioactive Compounds from Citrus Fruit and Lupine-Based Ingredients").

References

[1] C. M. Galanakis, "Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications," *Trends in Food Science & Technology*, vol. 26, pp. 68–87, 2012.

[2] P. Rapisarda, S. E. Bellomo, and F. Intrigliolo, "Anthocyanins in blood oranges: composition and biological activity," in *Recent Research Developments in Agricultural and Food Chemistry*, S. G. Pandalai, Ed., vol. 5, pp. 217–230, Research Signpost, Trivandrum, India, 2001.

[3] P. Rapisarda, A. Tomaino, R. Lo Cascio, F. Bonina, A. De Pasquale, and A. Saijia, "Antioxidant effectiveness as influenced by phenolic content of fresh orange juices," *Journal of Agricultural and Food Science*, vol. 47, pp. 4718–4723, 1999.

[4] P. Rapisarda, S. Fabroni, S. Peterek, G. Russo, and H. P. Mock, "Juice of new citrus hybrids (*Citrus clementina* Hort. ex Tan.×*C. sinensis* L. Osbeck) as a source of natural antioxidants," *Food Chemistry*, vol. 117, no. 2, pp. 212–218, 2009.

[5] A. M. Panico, F. Garufi, S. Nitto et al., "Antioxidant activity and phenolic content of strawberry genotypes from *Fragaria* x *ananassa*," *Pharmaceutical Biology*, vol. 47, no. 3, pp. 203–208, 2009.

[6] M. R. Lauro, L. Crasci, C. Carbone, R. P. Aquino, A. M. Panico, and G. Puglisi, "Encapsulation of a citrus by-product extract: development, characterization and stability studies of a nutraceutical with antioxidant and metalloproteinases inhibitory activity," *LWT-Food Science and Technology*, vol. 62, no. 1, pp. 169–176, 2015.

[7] M. R. Lauro, L. Crascí, F. Sansone, V. Cardile, A. M. Panico, and G. Puglisi, "Development and in vitro evaluation of an innovative "dietary flavonoid supplement" on osteoarthritis process," *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 7503240, 10 pages, 2017.

[8] F. Salamone, G. Li Volti, L. Titta et al., "Moro orange juice prevents fatty liver in mice," *World Journal of Gastroenterology*, vol. 18, no. 29, pp. 3862–3868, 2012.

[9] L. Titta, M. Trinei, M. Stendardo et al.I. Berniakovich, K. Petroni, C. Tonelli et al., "Blood orange juice inhibits fat accumulation in mice," *International Journal of Obesity*, vol. 34, no. 3, pp. 578–588, 2010.

[10] F. Sansone, T. Mencherini, P. Picerno, M. d'Amore, R. P. Aquino, and M. R. Lauro, "Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts," *Journal of Food Engineering*, vol. 105, no. 3, pp. 468–476, 2011.

[11] E. B. Rodriguez, M. E. Flavier, D. B. Rodriguez-Amaya, and J. Amaya-Farfán, "Phytochemicals and functional foods. Current situation and prospect for developing countries," *Segurança Alimentar e Nutricional*, vol. 13, no. 1, pp. 1–22, 2006.

[12] C. Soukoulis, L. Yonekura, H. H. Gan, S. Behboudi-Jobbehdar, C. Parmenter, and I. Fisk, "Probiotic edible films as a new strategy for developing functional bakery products: the case of pan bread," *Food Hydrocolloids*, vol. 39, no. 100, pp. 231–242, 2014.

[13] J. R. Paxman, J. C. Richardson, P. W. Dettmar, and B. M. Corfe, "Daily ingestion of alginate reduces energy intake in free-living subjects," *Appetite*, vol. 51, no. 3, pp. 713–719, 2008.

[14] D. Houghton, M. D. Wilcox, P. I. Chater, I. A. Brownlee, C. J. Seal, and J. P. Pearson, "Biological activity of alginate and its effect on pancreatic lipase inhibition as a potential treatment for obesity," *Food Hydrocolloids*, vol. 49, pp. 18–24, 2015.

[15] M. R. Lauro, C. Carbone, F. Sansone et al., "Innovative oral spray-dried idebenone systems to improve patient compliance," *Drug Development and Industrial Pharmacy*, vol. 42, no. 7, pp. 1127–1136, 2016.

[16] M. R. Lauro, C. Carbone, R. Auditore et al., "A new inclusion complex of amlodipine besylate and soluble β-cyclodextrin polymer: preparation, characterization and dissolution profile," *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, vol. 76, no. 1-2, pp. 19–28, 2013.

[17] A. Nasir, S. L. Harikumar, and K. Amanpreet, "Cyclodextrins: an excipient tool in drug delivery: a review," *International Research Journal of Pharmacy*, vol. 3, no. 4, pp. 44–50, 2012.

[18] L. Crascì, M. R. Lauro, G. Puglisi, and A. Panico, "Natural antioxidant polyphenols on inflammation management: anti-glycation activity vs metalloproteinases inhibition," *Critical Reviews in Food Science and Nutrition*, pp. 1–12, 2016.

[19] G. Derosa, P. Maffioli, A. D'Angelo et al., "Evaluation of metalloproteinase 2 and 9 levels and their inhibitors in combined dyslipidemia," *Clinical and Investigative Medicine*, vol. 32, no. 2, pp. 124–132, 2009.

[20] F. V. Romeo, G. Ballistreri, S. Fabroni et al., "Chemical characterization of different sumac and pomegranate extracts effective against *Botrytis cinerea* rots," *Molecules*, vol. 20, no. 7, pp. 11941–11958, 2015.

[21] R. L. Rouseff, S. F. Martin, and C. O. Youtsey, "Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus," *Journal of Agricultural and Food Chemistry*, vol. 35, no. 6, pp. 1027–1030, 1987.

[22] O. A. Aiyegoro and A. I. Okoh, "Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC," *BMC Complementary and Alternative Medicine*, vol. 10, pp. 21–29, 2010.

[23] P. Rapisarda, F. Fanella, and E. Maccarone, "Reliability of analytical methods for determining anthocyanins in blood orange juices," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 6, pp. 2249–2252, 2000.

[24] T. K. Higuchi and A. Connors, "Phase-solubility techniques," *Advances in Analytical Chemistry and Instrumentation*, vol. 4, pp. 117–212, 1965.

[25] F. Sansone, P. Picerno, T. Mencherini et al., "Flavonoid microparticles by spray-drying: influence of enhancers of the dissolution rate on properties and stability," *Journal of Food Engineering*, vol. 103, no. 2, pp. 188–196, 2011.

[26] G. Cao, H. M. Alessio, and R. G. Cutler, "Oxygen-radical absorbance capacity assay for antioxidants," *Free Radical Biology and Medicine*, vol. 14, no. 3, pp. 303–311, 1993.

[27] C. Puglia, V. Cardile, A. M. Panico et al., "Evaluation of monooleine aqueous dispersions as tools for topical administration of curcumin: characterization, in vitro and ex-vivo studies," *Journal of Pharmaceutical Sciences*, vol. 102, no. 7, pp. 2349–2361, 2013.

[28] C. Puglia, M. R. Lauro, A. Offerta et al., "Nanostructured lipid carriers (NLC) as vehicles for topical administration of sesamol: in vitro percutaneous absorption study and evaluation of antioxidant activity," *Planta Medica*, vol. 83, no. 5, pp. 398–404, 2016.

[29] S. Derbré, J. Gatto, A. Pelleray, L. Coulon, D. Séraphin, and P. Richomme, "Automating a 96-well microtiter plate assay for identification of AGEs inhibitors or inducers: application to the screening of a small natural compounds library," *Anaytical and Bioanalytical Chemistry*, vol. 398, no. 4, pp. 1747–1758, 2010.

[30] D. Edelstein and M. Brownlee, "Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine," *Diabetes*, vol. 41, no. 1, pp. 26–29, 1992.

[31] L. Crascì, P. Vicini, M. Incerti, V. Cardile, S. Avondo, and A. Panico, "2-Benzisothiazolylimino-5-benzylidene-4-

thiazolidinones as protective agents against cartilage destruction," *Bioorganic and Medicinal Chemisty*, vol. 23, no. 7, pp. 1551–1556, 2015.

[32] M. D. L. M. Bilbao, C. Andrés-Lacueva, O. Jáuregui, and R. M. Lamuela-Raventos, "Determination of flavonoids in a citrus fruit extract by LC–DAD and LC–MS," *Food Chemistry*, vol. 101, no. 4, pp. 1742–1747, 2007.

[33] S. Hillebrand, M. Schwarz, and P. Winterhalter, "Characterization of anthocyanins and pyranoanthocyanins from blood orange [*Citrus sinensis* (L.) Osbeck] juice," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 24, pp. 7331–7338, 2004.

[34] S. R. Georgetti, R. Casagrande, C. R. F. Souza, W. P. Oliveira, and M. J. V. Fonseca, "Spray drying of the soybean extract: Effects on chemical properties and antioxidant activity," *LWT-Food Science and Technology*, vol. 41, no. 8, pp. 1521–1527, 2008.

[35] R. R. Araujo, C. C. C. Teixeira, and L. A. P. Freitas, "The preparation of ternary solid dispersions of an herbal drug via spray drying of liquid feed," *Drying Technology*, vol. 28, no. 3, pp. 412–421, 2010.

[36] C. R. F. Souza, I. A. Schiavetto, F. C. F. Thomazini, and W. P. Oliveira, "Processing of *Rosmarinus officinalis* linne extract on spray and spouted bed dryers," *Brazilian Journal of Chemical Engineering*, vol. 25, no. 1, pp. 59–69, 2008.

[37] R. L. Carrier, L. A. Miller, and I. Ahmed, "The utility of cyclodextrins for enhancing oral bioavailability," *Journal of Controlled Release*, vol. 123, no. 2, pp. 78–99, 2007.

[38] N. Phisut, "Spray drying technique of fruit juice powder: some factors influencing the properties of product," *International Food Research Journal*, vol. 19, no. 4, pp. 1297–1306, 2012.

[39] M. R. Lauro, F. De Simone, F. Sansone, P. Iannelli, and R. P. Aquino, "Preparations and release characteristics of naringin and naringenin gastro-resistant microparticles by spray-drying," *Journal of Drug Delivery Science and Tecnology*, vol. 17, no. 2, pp. 119–124, 2007.

[40] P. Picerno, F. Sansone, T. Mencherini et al., "Citrus bergamia juice: phytochemical and technological studies," *Natural Product Communinication*, vol. 6, no. 7, pp. 951–955, 2011.

[41] G. P. Panizzon, F. G. Bueno, T. Ueda-Nakamura, C. V. Nakamura, and B. P. Dias Filho, "Preparation of spray-dried soy isoflavone-loaded gelatin microspheres for enhancement of dissolution: formulation, characterization and *in vitro* evaluation," *Pharmaceutics*, vol. 6, no. 4, pp. 599–615, 2014.

[42] A. C. Templeton, S. R. Byrn, R. J. Haskell, and T. E. Prisinzano, *Discovering and Developing Molecules with Optimal Drug-Like Properties*, Springer, New York, NY, USA, 2015.

[43] B. B. Patel, J. K. Patel, S. Chakraborty, and D. Shukla, "Revealing facts behind spray dried solid dispersion technology used for solubility enhancement," *Saudi Pharmaceutical Journal*, vol. 23, no. 4, pp. 352–365, 2015.

[44] S. Jana, K. Trivedi, R. M. Tallapragada et al., "Characterization of physicochemical and thermal properties of chitosan and sodium alginate after biofield treatment," *Pharmaceutica Analytica Acta*, vol. 6, pp. 1–10, 2015.

[45] J. P. Soares, J. E. Santos, G. O. Chierice, and E. T. G. Cavalheiro, "Thermal behavior of alginic acid and its sodium salt," *Eclética Química*, vol. 29, no. 2, pp. 57–64, 2004.

[46] M. B. Nieto, "Structure and function of polysaccharide gum-based edible films and coatings," in *Edible films and Coatings for Food Applications*, K. C. Huber and M. E. Embuscado, Eds., Springer, New York, NY, USA, 2009.

[47] F. Sansone, A. Rossi, P. Del Gaudio, F. De Simone, R. P. Aquino, and M. R. Lauro, "Hesperidin gastroresistant microparticles by spray-drying: preparation, characterization, and dissolution profiles," *AAPS PharmSciTech*, vol. 10, no. 2, pp. 391–401, 2009.

[48] P. Laine, P. Kylli, M. Heinonen, and K. Jouppila, "Storage stability of microencapsulated cloudberry (*Rubus chamaemorus*) phenolics," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 23, pp. 11251–11261, 2008.

[49] M. Cisse, F. Vaillant, O. Acosta, C. Dhuique-Mayer, and M. Dornier, "Thermal degradation kinetics of anthocyanins from blood orange, blackberry, and roselle using Arrhenius, Eryng, and Ball models," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 14, pp. 6285–6291, 2009.

[50] A. Kirca and B. Cemeroglu, "Degradation kinetics of anthocyanins in blood orange juice and concentrate," *Food Chemistry*, vol. 81, pp. 583–587, 2003.

[51] C. Dhuique-Mayer, M. Tbatou, M. Carail, C. Caris-Veyrat, M. Dornier, and M. J. Amiot, "Thermal degradation of antioxidant micronutrients in citrus juice: kinetics and newly formed compounds," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 10, pp. 4209–4216, 2007.

[52] H. Chaaban, I. Ioannou, I. Chebil et al., "Effect of heat processing on thermal stability and antioxidant activity of six flavonoids," *Journal of Food Processing and Preservation*, vol. 41, no. 5, pp. 1–12, 2017.

[53] J. W. BaynesS. R. Thorpe, "Glycoxidation and lipoxidation in atherogenesis," *Free Radical Biology and Medicine*, vol. 28, no. 12, pp. 1708–1716, 2000.

[54] S. I. Yamagishi, N. Nakamura, M. Suematsu, K. Kaseda, and T. Matsui, "Advanced glycation end products: a molecular target for vascular complications in diabetes," *Molecular Medicine*, vol. 21, Supplement 1, pp. S32–S40, 2015.

[55] L. Crascì and A. Panico, "Protective effects of many citrus flavonoids on cartilage degradation process," *Journal of Biomaterilas and Nanobiotechnology*, vol. 4, pp. 279–283, 2013.

[56] L. Crascì, L. Basile, A. Panico et al.S. Guccione et al., "Correlating in vitro target-oriented screening and docking: inhibition of matrix metalloproteinases activities by flavonoids," *Planta Medica*, vol. 83, no. 11, pp. 901–911, 2017.

[57] H. I. Park, S. Lee, A. Ullah, Q. Cao, and Q. X. A. Sang, "Effects of detergents on catalytic activity of human endometase/matrilysin 2, a putative cancer biomarker," *Analytical Biochemistry*, vol. 396, no. 2, pp. 262–268, 2010.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 8315803, 7 pages
https://doi.org/10.1155/2017/8315803

Research Article

Effects of White Wine Consumption on Weight in Rats: Do Polyphenols Matter?

Ana Marija Milat,[1] Ivana Mudnić,[1] Ivica Grković,[2] Nikola Ključević,[2] Mia Grga,[1] Iva Jerčić,[1] Diana Jurić,[1] Danica Ivanković,[2] Benjamin Benzon,[2] and Mladen Boban[1]

[1]*Department of Pharmacology, University of Split School of Medicine, Split, Croatia*
[2]*Department of Anatomy, University of Split School of Medicine, Split, Croatia*

Correspondence should be addressed to Mladen Boban; mladen.boban@mefst.hr

Received 6 April 2017; Revised 10 August 2017; Accepted 25 September 2017; Published 31 October 2017

Academic Editor: Sara Arranz

Introduction. Effects of white wine and the role of wine polyphenols on weight gain in rats of different age were examined in the 4-week-voluntary-consumption trial. *Methods and Materials*. Biochemically characterized standard (low polyphenols, W) and macerated (high polyphenolic content, PW) white wines were compared. One- and three-month-old Sprague-Dawley male rats ($n = 78$) were used. Each age group was subdivided into water-only-drinking controls (C), W, and PW-drinking animals. Daily wine and total liquid consumption, food intake, and body weight were measured, and energy intake and feed efficiency index were calculated. *Results*. In both age categories, wine-drinking animals consumed less food and gained less weight in comparison to C (181 ± 2, 179 ± 6, and 201 ± 5 in younger animals and 32 ± 5, 28 ± 6, and 47 ± 4 grams in older animals, resp.), regardless of wine type. Total energy intake was the lowest in PW-drinking animals. *Conclusion*. Wine-drinking animals gained less weight in comparison to C, regardless of the wines' polyphenol content. Although our results are indicative of the major role of nonphenolic constituents of the wines (probably ethanol), the modifying role of wine phenolics on weight gain cannot be excluded as the group consuming PW had lower total energy intake than other groups.

1. Introduction

Despite large epidemiological evidence, it is still controversial whether alcohol intake represents a risk factor for weight gain and obesity.

Based on recent reviews of epidemiological studies regarding the effect of alcohol consumption on body weight, it seems that only heavy drinking is positively related with weight gain [1, 2]. Also, it appears that the type of alcoholic beverage is an important element in modifying the effect of alcohol consumption on weight gain with wine being regarded as an alcoholic beverage with more favourable effects [3]. Several animal studies, conducted under controlled experimental conditions, found that red wine intake did not cause an increase in body weight, compared to the water-only-drinking rats [4, 5]. Advantageous effects of wine were largely attributed to different biological actions of wine polyphenols. Among these, reduced appetite and/or nutrient absorption, promotion of energy expenditure, or prevention of energy storage have been proposed as potential mechanisms by which wine phenolics may contribute to the antiobesity effects of wine [6].

Because of much higher concentrations of wine polyphenols being found in red than in white wine, practically all studies examining effects of wine consumption on body weight have been conducted using red wine. In order to examine effects of white wine and the role of wine polyphenols on the weight gain in rats, we compared effects of white wines with low and high phenolic content in the 4-week-consumption trial.

Since it was observed that some differences in the impact of alcohol on body weight may be related to age [7], we

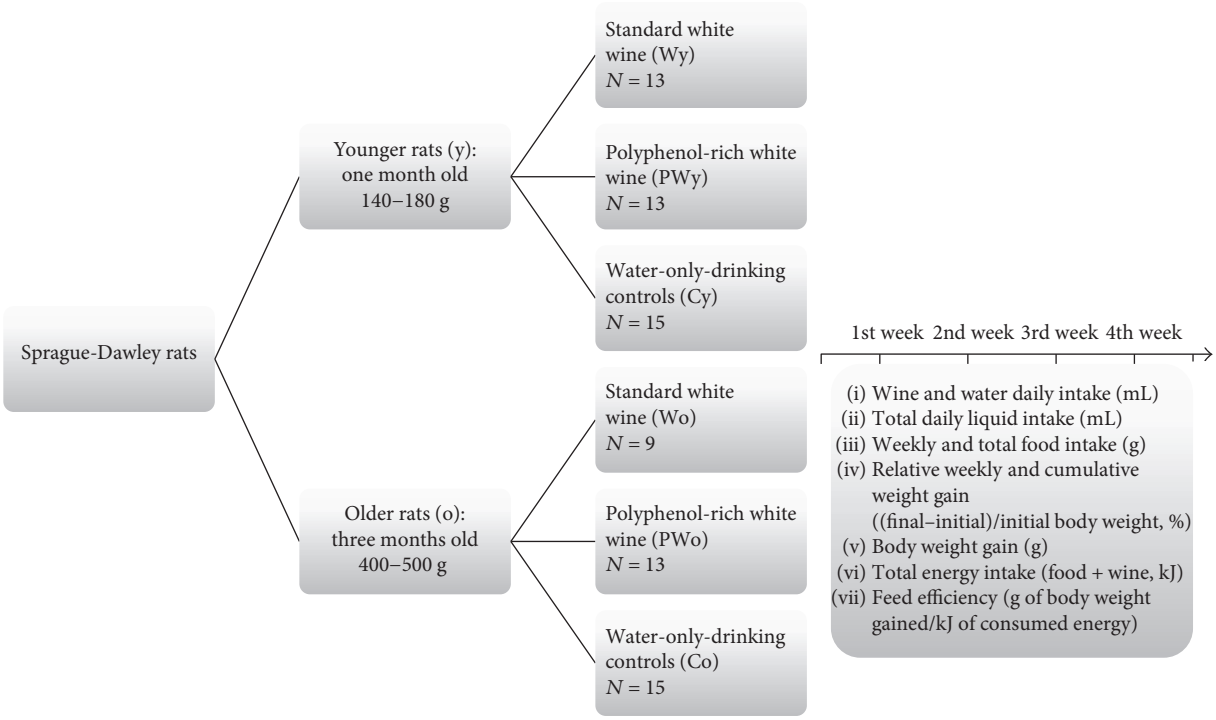


Figure 1: Methodological diagram of the sample, interventions, and main outcomes. W, standard white wine; PW, polyphenol-rich white wine; C, water-only-drinking control animals; y, younger animals; o, older animals.

studied two groups of animals, younger rats in the phase of fast growth and development and older animals that were close to reaching their body weight plateau.

2. Materials and Methods

2.1. Animals and Diets. With respect to the initial body mass and age, two groups of the Sprague-Dawley male rats were used: the younger group (fast-growing animals), one-month-old rats weighing 140–180 g, and the older group (slow-growing animals), approximately 3-month-old rats weighing 400–500 g. Both age groups were further divided into wine-drinking animals and water-only-drinking controls. The wine-drinking animals were offered a standard white wine or polyphenol-rich white wine ad libitum, 24 hours/day for 4 weeks with daily inclusion of tap water for 6 hours. Controls, water-only-drinking animals, were offered tap water without restrictions. Each age group of animals was finally divided into 3 subgroups: water-only-drinking controls (C), standard white wine (W), and polyphenol-rich white wine (PW)-drinking animals (Figure 1). The exclusion criterion for the wine-drinking animals was daily wine intake of less than 7 mL. The fresh beverages were supplied daily in small pet containers with a leak proof nozzle (Ferplast Small Pet Sippy Water Bottle, Castelgomberto, Italy). Fluid intake was recorded daily. The animals were fed a standard pellet diet (Mucedola S.R.L., Settimo Milanese, Milan, Italy) with caloric value of 16.54 kJ/g ad libitum. Body weight and food intake were measured weekly using Grundig KW 4060 Digital Scale (weight range 5 kg). The animals were kept in individual cages under standard temperature and light conditions (22–25°C, 12 h light/dark cycle). All procedures and experimental protocols were in accordance to European Convention on Animal Protection and Guidelines on Research Animal Use and were approved by the Ethics Committee of the School of Medicine University of Split and by the Ethics Committee of the Ministry of Agriculture of the Republic of Croatia.

2.2. Wines. The standard white wine used was Graševina, 2015, Krauthaker winery, Croatia. Polyphenol-rich white wine was obtained from the same grape variety within the same vineyard and harvest year by using the traditional Georgian wine production principles. It means that grape juice was first allowed spontaneous fermentation in contact with the hard parts of the grape. Following fermentation, without removing grape seeds and skins, the tanks were air-tight sealed at a constant temperature for 120 days. It resulted in production of white wine with orange or amber hue that was high in phenolic content, similar to red wines [8].

2.3. Biochemical Analysis of the Wines. The total phenolics and their flavonoid and nonflavonoid subgroup contents were measured spectrophotometrically. The total phenolic content of the samples was determined by the Folin-Ciocalteu method, and the results were expressed as milligrams of gallic acid equivalents (GAE) per liter. Nonflavonoid compounds were determined by the same method after flavonoid precipitation with formaldehyde, and the flavonoid content was calculated as the difference between total phenolic and nonflavonoid content. Absorbencies were monitored by UV-Vis spectrophotometer (Specord 200,

Table 1: Caloric value, ethanol, and phenolic content of the tested white wines.

	Ethanol content vol % (g/L)	Caloric value kJ/mL	Total phenolics mg GAE/L	Flavonoids mg GAE/L	Nonflavonoids mg GAE/L
Standard wine	13.0 (102.3)	3.04	305 ± 3*	3*	302 ± 2
Polyphenol-rich wine	13.3 (105)	3.12	2850 ± 35	2477	373 ± 3

Caloric value of tested wines is calculated as the product of ethanol concentration and caloric value of alcohol (29.7 kJ/g). Data on phenolic content are averages of at least three independent measurements and are shown as mean ± SD. Concentration of flavonoids is calculated as the difference between mean concentration of total phenolics and nonflavonoids and is expressed without SD; GAE: gallic acid equivalents; $^*P < 0.05$, Student's t-test.

Table 2: Concentrations of selected phenolic compounds in tested white wines.

	Gallic acid mg/L	(+)-catechin mg/L	(−)-epicatechin mg/L	Procyanidin B1 mg/L	Total resveratrol mg/L
Standard wine	1.45 ± 0.07	1.55 ± 0.07	1.25 ± 0.21	0.85 ± 0.07	0.30
Polyphenol-rich wine	34.75 ± 0.49	109.60 ± 0.28	67.65 ± 1.34	77.55 ± 0.49	2.30

Data are averages of two independent samples and are shown as mean ± SD values.

Analytik Jena Inc., Jena, Germany), equipped with a six-cell holder and a thermostatically controlled bath. A more detailed description of the above-mentioned methods has been previously published [9].

Individual polyphenols were identified and quantified by an Agilent HPLC system (type RRLC; Agilent Technologies, Santa Clara, CA) using a ZORBAX SB-C18 analytical column (15 × 2.1 mm, 1.8 μm). Phenolic compounds were identified by their retention times. Quantification was carried out by comparison with external standard calibration curves. Each sample was injected twice into the chromatographic system. A detailed description of the method has been published previously [10]. All analytical-grade chemicals and reagents were obtained from Sigma Aldrich (St. Louis, MO).

2.4. Statistical Analysis. The sample size was determined using the program G*Power 3.1 (G*Power, Dusseldorf, Germany). Results were expressed as means ± SEM. Data were compared by one-way ANOVA and post hoc Student t-test, using SPSS (version 24) software. The level of significance was set at $P < 0.05$.

3. Results

Results of the biochemical analysis of the wines including their caloric value, ethanol and phenolic content, and concentrations of selected individual phenolic compounds are presented in Tables 1 and 2.

Initial body weight among animal groups in both age categories was comparable at the beginning of the treatment. On average, younger animals in three experimental groups weighted 164 ± 4 g (standard white wine, W), 155 ± 3 g (polyphenol-rich white wine, PW), and 147 ± 3 g (water-only-drinking animals, C), whereas weights in corresponding groups in older animals were 453 ± 10 g, 439 ± 9 g, and 417 ± 6 g, respectively.

Expectedly, the rate and the extent of the weight gain in the younger animals were higher than those in the older animals. However, in both age categories, wine-drinking animals generally gained less weight both at weekly and cumulative basis in comparison to control, water-only-drinking animals (Figure 2). In the younger animals, mean body weight gain after 4 weeks of treatment was 181 ± 2 g, 179 ± 6 g, and 201 ± 5 g in W, PW, and C, respectively. In contrast to the water-only-drinking controls, there was no difference in weight gain between the wine-drinking animals ($P = 0.024$ for younger W versus C, $P = 0.020$ for younger PW versus C, and $P = 0.999$ for younger W versus PW, Figure 3(a)). In the older animals, at the same time point, total weight gain was 32 ± 5 g, 28 ± 6 g, and 47 ± 4 g for groups W, PW, and C, respectively. Again, there was no difference in weight gain between the animals drinking either wine ($P = 0.037$ for older W versus C, $P = 0.029$ for older PW versus C, and 0.989 for older W versus PW, Figure 3(b)).

All wine-drinking animals consumed less food at weekly and cumulative basis in comparison to controls, as follows: 154 ± 2 (W), 143 ± 4 (PW), and 176 ± 4 (C) g/week for younger animals and 163 ± 4 (W), 149 ± 4 (PW), and 178 ± 10 (C) g/week for older animals. Data on total food intake after 4 weeks of consumption trial are shown in Figure 4 for both younger and older animals. Following the pattern of weekly food intake, the wine-drinking animals did not significantly differ in their total food intake after 4 weeks regardless of the wine type ($P = 0.004$ for younger W versus C, $P < 0.001$ for younger PW versus C, and $P = 0.071$ for younger W versus PW; $P = 0.026$ for older W versus C, $P < 0.001$ for older PW versus C, and $P = 0.079$ for older W versus PW).

As to daily wine and total liquid intake, W, PW, and C groups ingested similar amounts regardless of the animals' age. Total energy intake (TEI) was calculated as the sum of energetic density of both food and wine, if applicable, in all animal groups after 4 weeks of follow-up. Total energy intake was somewhat lower in younger and older PW groups relative to all other experimental groups.

To assess the amount of body weight gained per kilojoule of consumed energy, we calculated feed efficiency (FE) as the ratio of these two parameters. In general, younger animals had higher FE than older animals as they gained more grams of body weight per kilojoule of consumed energy. However, feed efficiency did not differ between the animals of the same

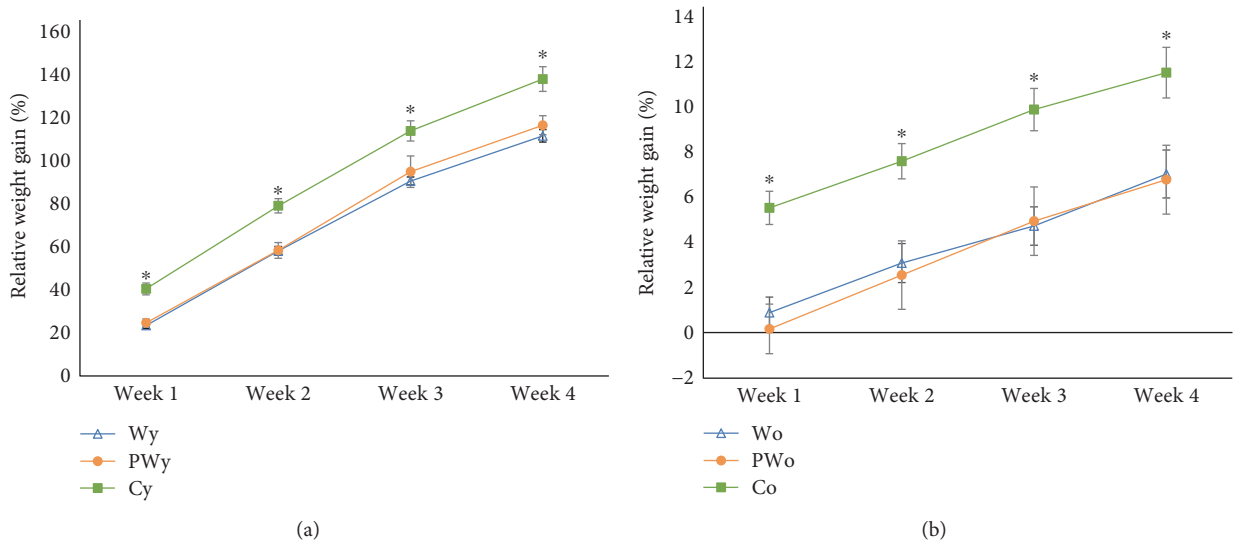


Figure 2: Relative body weight gain in younger (a) and older (b) rats drinking standard (W) or polyphenol-rich (PW) white wine and water-only-drinking controls (C) during 4 weeks. Relative weight gain is expressed as a percentage of initial weight. Final weight gain after 4 weeks is approximately 10 times higher in younger (y) animals than in older (o) ones, so ordinate scales are not matching. $^{*}P < 0.05$ for water-only-drinking group (C) versus wine-drinking groups (W and PW).

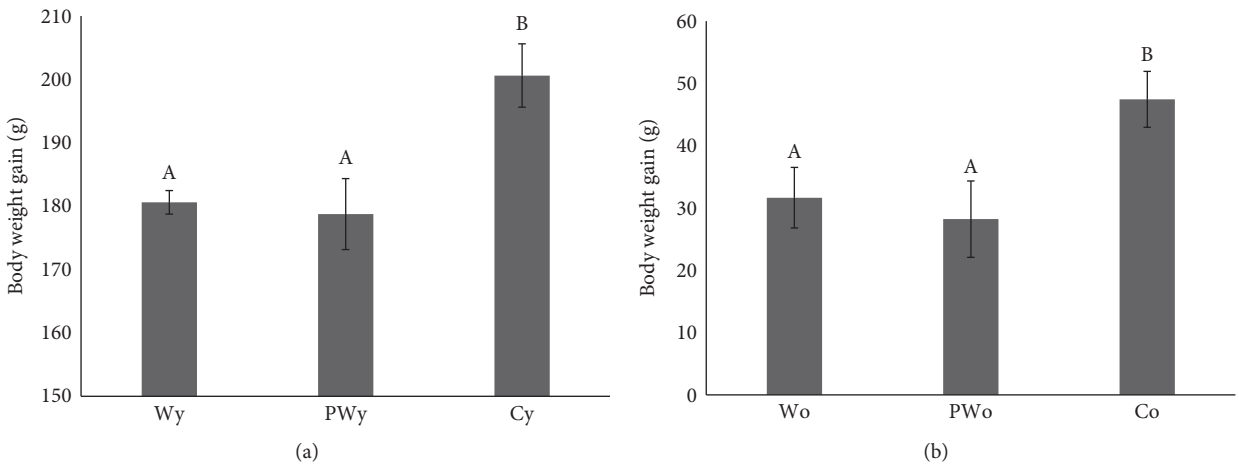


Figure 3: Body weight gain in younger (A) and older (B) rats drinking standard (W) or polyphenol-rich (PW) white wine and water only (C), after 4 weeks. Body weight gain is approximately 4 times higher in younger (y) animals in comparison to older (o) ones, so ordinate scales are not the same. Different letters indicate significant difference between groups ($P < 0.05$).

age regardless of the consumed beverage type. Summarized data on daily wine and total liquid intake, total energy intake, and feed efficiency are presented in Table 3.

4. Discussion

One of the key findings of our study is a significant association between white wine consumption and lower body weight gain in rats relative to water-only-drinking animals. This is largely in accordance with several other studies that reported similar results in rats and mice following ethanol and red wine consumption [4, 5, 11, 12]. Furthermore, for the first time, we showed that this holds true in both fast-growing younger animals and mature animals which were close to their body weight plateau. As a general rule, wine consumption was associated with decreased food intake in both age categories implying that additional calories provided by wine partially compensate for calories from other foodstuff (Figure 4).

Since no difference was observed between effects of standard and polyphenol-rich white wine on body weight gain and food intake, it appears that wine phenolics are of secondary importance relative to the other constituents of wine, ethanol being the most likely candidate. Namely, this would be in line with Monteiro et al.'s study showing that both red wine- and ethanol-consuming rats gained less

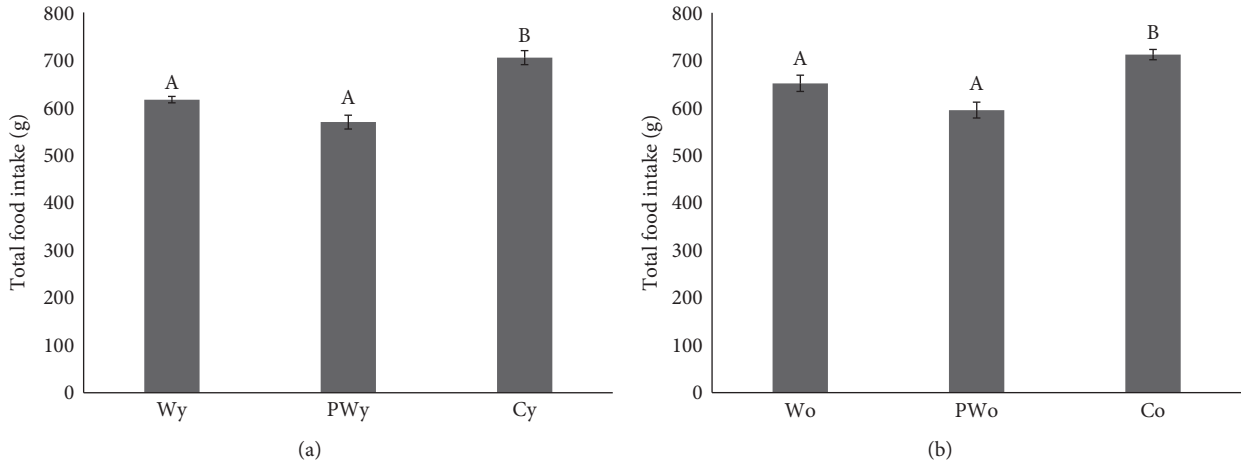


Figure 4: Total food intake in younger (a) and older (b) rats drinking standard (W) or polyphenol-rich (PW) white wine and water only (C), after 4 weeks. Different letters indicate significant difference between groups ($P < 0.05$).

Table 3: Average liquid intake, energy consumption, and feed efficiency among the animal groups.

	Daily wine intake mL/d	Total daily liquid intake (wine + water) mL/d	TE, total energy intake after 4 weeks kJ	Feed efficiency (BWG/TE) g/kJ
Wy	10 ± 1	30 ± 1	11088.4 ± 126.4	0.0163 ± 0.0003
PWy	11 ± 1	30 ± 1	10380.1 ± 205.0*	0.0172 ± 0.0004
Cy	n.a.	33 ± 1	11657.5 ± 244.8	0.0173 ± 0.0004
Wo	15 ± 2	29 ± 2	12097.6 ± 315.5	0.0026 ± 0.0004
PWo	11 ± 1	30 ± 1	10808.1 ± 282.4*	0.0025 ± 0.0005
Co	n.a.	32 ± 1	11776.7 ± 179.5	0.0040 ± 0.0004

n.a.: not applicable; BWG: body weight gained; C: control; W: standard white wine; PW: polyphenol-rich white wine; y: younger animals; o: older animals. Results are expressed as means ± SEM. *$P < 0.05$ for PWy and PWo versus groups within the same age category.

weight and ingested less food in comparison to controls, while there was no distinction between the effects of red wine, known to be rich in polyphenols, and ethanol [5].

Interestingly, the total energy intake, provided by calories from food and wine, was lower in polyphenol-rich-wine-drinking animals. This could be in part due to their tendency to consume less food in comparison to standard-wine-drinking animals. Indeed, polyphenols may be involved in appetite control over the central nervous system that monitors the food urge and feeling of satiety [6]. However, there was no difference in feed efficiency among animals of the same age category, regardless of the consumed beverage type. This is simply because animals which ate less gained less weight, so at the end of the experiment they gained similar weight per calorie of energy.

It is important to note that all animals consumed similar total amounts of liquid per day making our subgroups of animals substantially comparable. The seemingly paradoxical fact that younger animals, although weighing three times less than older animals, consume similar amounts of both liquid and food (Table 3 and Figure 4) is just due to their several times higher growth rate. In other words, proportionally higher consumption of food and liquid is necessary to support correspondingly higher metabolic turnover in younger, fast-growing animals. This is also indicated in their (several times) higher feed efficiency ratio (weight gain per calorie, shown in Table 3), relative to older animals. On the other side, steady liquid and food intake in older animals is required for maintaining their basic metabolic needs.

By eliminating differences between test wines, which could be due to differences in the grape variety, growing conditions, terrain, or harvesting time, we were able to determine the influence of wine-making technique and polyphenol content more objectively.

The results of the present study, however, did not determine the essential role of wine phenolics on the animals' weight gain. Although we did not examine the effects of ethanol directly in the present study, our results are indicative of the major role of ethanol, as reported by several different authors [5, 11, 12] who showed that ethanol in general (studied alone or in comparison to red wine) was not associated with body weight gain in the animals.

Generally, moderate drinkers (human or animals) often have significantly lower weight gain in comparison to non-drinkers. Although this may be due to confounding factors (e.g., physical activity), it may also reflect a true physiological effect of alcohol [13].

The energy derived from alcohol differs from other energy sources, since alcohol cannot be stored and is always first to be metabolized. It also has a very high potential

to affect metabolic pathways of other nutrients. Intake of alcohol can displace fat and carbohydrates from oxidative metabolism in the liver to a maximum level of 50% the resting value [14]. Indeed, energy stored this way can be more effectively utilized than calories derived from excessive carbohydrate intake [15].

Moreover, thermogenic response of alcohol is rather high and in healthy moderate alcohol consumers is between 15 and 25% of the energy content of the alcohol intake. As compared to fat (~13%) and carbohydrates (~8%), the alcohol energy can be considered as a less usable form of energy [16, 17]. Furthermore, alcohol stimulates sympathetic nervous system and decreases blood glucose by inhibition of liver gluconeogenesis [18]. Finally, when alcohol is consumed in conjunction with a meal, it lowers secretion of the protein leptin more than nonalcohol meals with the same energetic density [19].

Although not focused on underlying mechanisms of alcohol effects on body weight, our study provides additional experimental evidence on this matter. The model of consumption we used is comparable to the model of moderate voluntary wine consumption by Arola et al. where animals had free access to wine, without stressful treatments and non-physiologic consumption of wine (e.g., like in gastric gavage method) [20]. It is important to note that wine consumption by the animals observed in our study in terms of energy intake (approximately 8%) roughly corresponds to the contribution of alcoholic beverages to the total food stuff energy in the human light to moderate alcohol consumers [21].

Finally, the rationale of choosing wine as the alcoholic beverage lies in the fact that it represents an unavoidable constituent of Mediterranean diet. In contrast to red wine, to our best knowledge, the effects of white wine on weight gain in rats have not been studied.

Further studies are still needed to explore the relationship between wine consumption and body weight and to specify the impact of wine phenolics and ethanol. We hope that this simple yet straightforward study may be inspiring to deepen insight into this complex matter.

5. Conclusion

The rats drinking white wines gained less weight in comparison to water-only-drinking controls following 4-week-voluntary-consumption trial, regardless of the wine's phenolic content. Although our results are indicative of the major role of the nonphenolic constituents of white wine, the present study cannot exclude the modifying role of wine phenolics on energy metabolism and weight gain as the animals consuming polyphenol-rich white wine had lower total energy intake in comparison to all other experimental groups.

Disclosure

The results were partially presented as a poster at the Wine & Health meeting, Logrono, Spain, February 2017.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

The authors would like to thank Professor Alois Jungbauer and his associates from the University of Natural Resources and Applied Life Sciences, Department of Biotechnology (Vienna, Austria) for the assistance in the biochemical analysis of the wines. This study was supported by the Croatian Science Foundation (Project no. 8652).

References

[1] G. Traversy and J. P. Chaput, "Alcohol consumption and obesity: an update," *Current Obesity Reports*, vol. 4, no. 1, pp. 122–130, 2015.

[2] C. Sayon-Orea, M. A. Martinez-Gonzalez, and M. Bes-Rastrollo, "Alcohol consumption and body weight: a systematic review," *Nutrition Reviews*, vol. 69, no. 8, pp. 419–431, 2011.

[3] C. Sayon-Orea, M. Bes-Rastrollo, J. M. Nuñez-Cordoba, F. J. Basterra-Gortari, J. J. Beunza, and M. A. Martinez-Gonzalez, "Type of alcoholic beverage and incidence of overweight/obesity in a Mediterranean cohort: the SUN project," *Nutrition*, vol. 27, no. 7-8, pp. 802–808, 2011.

[4] M. Vadillo, A. Ardévol, J. Fernández-Larrea et al., "Moderate red-wine consumption partially prevents body weight gain in rats fed a hyperlipidic diet," *The Journal of Nutritional Biochemistry*, vol. 17, no. 2, pp. 139–142, 2006.

[5] R. Monteiro, R. Soares, S. Guerreiro, D. Pestana, C. Calhau, and I. Azevedo, "Red wine increases adipose tissue aromatase expression and regulates body weight and adipocyte size," *Nutrition*, vol. 25, no. 6, pp. 699–705, 2009.

[6] I. Fernandes, R. Pérez-Gregorio, S. Soares, N. Mateus, and V. de Freitas, "Wine flavonoids in health and disease prevention," *Molecules*, vol. 22, no. 2, 2017.

[7] L. Wang, I. M. Lee, J. E. Manson, J. E. Buring, and H. D. Sesso, "Alcohol consumption, weight gain, and risk of becoming overweight in middle-aged and older women," *Archives of Internal Medicine*, vol. 170, no. 5, pp. 453–461, 2010.

[8] N. S. Baghaturia, "Georgian winemaking. Theory and practice," private ed, 2010, Tbilisi Odishvili Z.G.

[9] V. Katalinić, M. Milos, D. Modun, I. Musić, and M. Boban, "Antioxidant effectiveness of selected wines in comparison with (+)-catechin," *Food Chemistry*, vol. 86, no. 4, pp. 593–600, 2004.

[10] A. Zoechling, E. Reiter, R. Eder, S. Wendelin, F. Liebner, and A. Jungbauer, "The flavonoid kaempferol is responsible for the majority of estrogenic activity in red wine," *American Journal of Enology and Viticulture*, vol. 60, no. 2, pp. 223–232, 2009.

[11] A. S. Aguiar, V. A. Da-Silva, and G. T. Boaventura, "Can calories from ethanol contribute to body weight preservation by malnourished rats?," *Brazilian Journal of Medical and Biological Research*, vol. 37, no. 6, pp. 841–846, 2004.

[12] R. R. Smith, J. Hong, A. E. Harvey, T. Lewis, D. Diaz, and N. P. Núñez, "Ethanol consumption does not promote weight gain in female mice," *Annals of Nutrition and Metabolism*, vol. 53, no. 3-4, pp. 252–259, 2008.

[13] W. L. Hellerstedt, R. W. Jeffery, and D. M. Murray, "The association between alcohol intake and adiposity in the general population," *American Journal of Epidemiology*, vol. 132, no. 4, pp. 594–611, 1990.

[14] Y. Schutz, "Role of substrate utilization and thermogenesis on body-weight control with particular reference to alcohol," *Proceedings of the Nutrition Society*, vol. 59, no. 4, pp. 511–517, 2000.

[15] P. M. Suter and A. Tremblay, "Is alcohol consumption a risk factor for weight gain and obesity?," *Critical Reviews in Clinical Laboratory Sciences*, vol. 42, no. 3, pp. 197–227, 2005.

[16] B. J. Sonko, A. M. Prentice, P. R. Murgatroyd, G. R. Goldberg, M. L. van de Ven, and W. A. Coward, "Effect of alcohol on postmeal fat storage," *The American Journal of Clinical Nutrition*, vol. 59, no. 3, pp. 619–625, 1994.

[17] P. R. Murgatroyd, M. L. van de Ven, G. R. Goldberg, and A. M. Prentice, "Alcohol and the regulation of energy balance: overnight effects on diet-induced thermogenesis and fuel storage," *British Journal of Nutrition*, vol. 75, no. 1, pp. 33–45, 1996.

[18] J. Delarue, P. Schneiter, S. Henry et al., "Effects of adrenergic blockade on hepatic glucose production during ethanol administration," *Clinical Physiology*, vol. 17, no. 5, pp. 509–521, 1997.

[19] A. Raben, L. Agerholm-Larsen, A. Flint, J. J. Holst, and A. Astrup, "Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake," *The American Journal of Clinical Nutrition*, vol. 77, no. 1, pp. 91–100, 2003.

[20] L. Arola, R. Roig, E. Cascón et al., "Model for voluntary wine and alcohol consumption in rats," *Physiology & Behavior*, vol. 62, no. 2, pp. 353–357, 1997.

[21] K. R. Westerterp, "Alcohol and body weight," in *Health Issues Related to Alcohol Consumption*, I. McDonald, Ed., pp. 103–123, Blackwell Press, Oxford, 1999.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 7467962, 10 pages
https://doi.org/10.1155/2017/7467962

Review Article

Dietary Polyphenols in the Prevention of Stroke

A. Tressera-Rimbau,[1,2] S. Arranz,[3] M. Eder,[4] and A. Vallverdú-Queralt[4]

[1]*Department of Nutrition, Food Science and Gastronomy, XaRTA, INSA-UB, School of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain*
[2]*CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain*
[3]*AZTI Tecnalia Food Research Division, Derio, Bizkaia, Spain*
[4]*INRA, UMR1083 Sciences pour l'œnologie, 2 Place Viala, 34060 Montpellier Cedex, France*

Correspondence should be addressed to A. Vallverdú-Queralt; avallverdu@ub.edu

Received 8 June 2017; Accepted 4 October 2017; Published 24 October 2017

Academic Editor: Manuela Curcio

Polyphenols have an important protective role against a number of diseases, such as atherosclerosis, brain dysfunction, stroke, cardiovascular diseases, and cancer. Cardiovascular diseases are the number one cause of death worldwide: more people die annually from cardiovascular diseases than from any other cause. The most important behavioural risk factors of heart disease and stroke are unhealthy diet, physical inactivity, tobacco use, and excess alcohol intake. The dietary consumption of polyphenols has shown to be inversely associated with morbidity and mortality by cardio- and cerebrovascular diseases. It is well-known that the protective effects of polyphenols *in vivo* depend on the grade how they are extracted from food and on their intestinal absorption, metabolism, and biological action with target tissues. The aim of this review was to summarise the relation between polyphenols of different plant sources and stroke in human intervention studies, animal models, and in vitro studies.

1. Introduction

Polyphenols are plant secondary metabolites and the most abundant dietary bioactive compounds. It is known that 100,000 to 200,000 secondary metabolites exist. Plant polyphenols include flavonoid and nonflavonoid compounds [1, 2]. Flavonoids are built from a basic structure made up of an oxygenated heterocycle and 2 phenolic rings. They are distinguished by the oxidation state of the heterocyclic pyran ring, forming several groups (e.g., flavonols, flavanols, and anthocyanins). Until now, over 4000 flavonoids have been described in plants [1], and this number is constantly expanding due to multiple ways by which primary substituents are replaced, yielding more complex structures. Moreover, flavan-3-ols are also found in oligomer and polymer forms, known as proanthocyanidins. The nonflavonoids include phenolic acids (benzoic and hydroxycinnamic acids) and stilbenes. Further nonflavonoids that may be found in nature are gallotannins, ellagitannins, stilbene oligomers, and lignans [2].

Polyphenols possess a wide range of beneficial effects against atherosclerosis, brain dysfunction, stroke, cardiovascular diseases (CVD), and cancer [1, 3]. The most important food sources are fruit and vegetables [3], red wine [4], black and green teas [5], coffee [6], extra virgin olive oil [7], and chocolate [8, 9]. Moreover, herbs, spices, and nuts are also potentially relevant polyphenol sources [10, 11]. The current dietary advice is that people should consume ≥5 portions of fruit and vegetables every day, each portion being at least 80 grams (U.S. Department of Health and Human Services, 2010), keep the body mass index from 18.5 to 24.9 kg/m^2, and perform physical exercise [12].

It is well-known that the protective effects of polyphenols *in vivo* depend on the grade how they are extracted from food and on their intestinal absorption, metabolism, and biological action with target tissues [13]. In the human digestive

system, polyphenols can be metabolized by intestine or hepatic cells or by intestinal microbiota. Advances in polyphenol bioavailability and metabolism, in the mechanisms of action, and in the evidence of health effects on animal models and humans have been reported [14]. There is an interindividual variability of polyphenol metabolites present in plasma and urine after the intake of different polyphenols which has been correlated with human gut microbiota composition and/or genetic polymorphisms, and it is potentially associated with specific health effects [15].

This review aims to summarise the current knowledge about the effects of dietary polyphenols from different food sources on stroke in human intervention studies, animal models, and *in vitro* studies.

2. Dietary Polyphenols and CVD

According to the reported information from the World Health Organization, CVD are the main cause of mortality worldwide. In 2012, CVD caused over 17.5 million deaths, which represented 31% of all deaths globally (7.4 and 6.7 million were caused by coronary heart disease and stroke, resp.). The main risk factors for CVD and stroke are lack of exercise, unhealthy diet, increased blood pressure, glucose and lipids, obesity, and extensive use of tobacco and alcohol. These risk factors should be controlled regularly and can be a sign of an increased risk for stroke, heart attack, and heart failure among others. Nonmodifiable risk factors for CVD are increasing age, gender, ethnicity, and family history. Thus, two-thirds of all strokes occur in people older than 65, mainly man and African-Caribbean.

Currently, the major risk factor for developing cardio- and cerebrovascular diseases is hypertension, and it is the major global health problem, affecting approximately 1 billion individuals and causing 7.6 million premature deaths, as well as 6% of all causes of disability worldwide [16]. Antihypertensives were the most distributed drugs (698 million prescriptions) in the US during 2013 [12].

The dietary consumption of polyphenols was shown to be inversely associated with cardio- and cerebrovascular diseases due to the anti-inflammatory and antiatherogenic properties of polyphenols, such as inhibition of peroxyl radical-induced DNA strand breakage, inhibition of platelet aggregation and of the expression of adhesion molecules to the endothelium, and protection of low-density lipoprotein (LDL) from oxidative damage [17, 18].

In certain inflammatory disease states, such as atherosclerosis, HDL particles may display atheroprotective functions in reverse cholesterol transport and antioxidant, anti-inflammatory, and antiapoptotic processes. Flavonoids show the potential to improve HDL function through their well-documented effects on cellular antioxidant status and inflammation [19]. Different clinical trials have revealed the atheroprotection of olive oil polyphenols through decreasing plasma LDL concentrations and LDL atherogenicity and improving HDL function [20, 21]. It has also been reported that polyphenols can reduce inflammation by blocking cytokines such as TNF-α, IL-1, IL-6, and IL-8 and C-reactive protein that play an important role as inflammatory markers in several diseases [18].

Several observational and short- or long-term intervention trials with food that possesses large amounts of polyphenols, such as virgin olive oil, cocoa, berries, and wine, have clearly shown that polyphenols have a positive effect on cardiovascular risk parameters. For example, BP levels are inversely correlated with the consumption of food rich in polyphenols. This may lead to the improvement in resistance artery function and decreases in peripheral artery vascular tone. However, most of these studies only take into account one sole source of polyphenols or one food item [22–27]. Noad et al. [28] demonstrated that a diet rich in polyphenols including six portions of fruits and vegetables, together with dark chocolate and a portion of berries each day, shows an improvement of microvascular function and decreases systolic BP. This is in accordance with several studies dealing with the vasodilator effects of polyphenols [29, 30]. Moreover, polyphenols can inhibit alpha-glucosidase and lipase activity [17].

Studies with wine and grape extracts have demonstrated improved endothelial function in conduit and resistance arteries [23, 26], possibly via nitric oxide- (NO-) dependent pathways [23]. In experimental studies, dietary polyphenols may help to stimulate NO endothelial secretion, which can decrease BP. Studies with tea and cocoa found an improvement in indirect measures of sympathetic nervous system activity patterns, possibly contributing to the lowering of peripheral vascular tone [27]. Moreover, hydroxytyrosol, oleuropein, and secoiridoids, found mainly in olives or virgin olive oils, may protect LDL particles from oxidation [12]. Further, cocoa flavanols have been assessed to play a role in maintaining endothelium-dependent vasodilation [12].

3. Dietary Polyphenols and Stroke

3.1. Stroke: Definition and Epidemiology. A stroke is a cerebrovascular disease related to atherosclerosis. It is caused by the interruption of the blood supply to the brain, usually because a vessel has either ruptured or been blocked by a blood clot. The result of this reduction in blood supply is a cut in the brain's oxygen causing the sudden death of some brain cells. Immediate symptoms are loss of speech, weakness, paralysis of one side of the body, dizziness, nausea, balance and coordination issues, and visual problems [31, 32].

Stroke is classified according to its aetiology as either haemorrhagic or ischaemic, being the last one as the most frequent (87% of cases). Haemorrhagic stroke is caused by a bleed into the brain tissue due to the rupture of blood vessels, aneurysms, or traumas. On the other hand, ischaemic stroke is caused by the occlusion of a cerebral artery, and it can be thrombotic or atherosclerotic and embolic or can be produced by microartery occlusion [31, 32].

Stroke is the second main cause of mortality worldwide and the main cause of adult disability, especially in middle- (12.8%) and high-income countries (8.7%). Ischaemic heart disease and stroke have been the world's biggest killers in the last 15 years. Only in 2015, they were together responsible of 15 million deaths around the world [33] and this burden is

predicted to increase due to the rapid rise of population older than 65 years [31].

3.2. In Vitro Assays Dealing with Stroke Risk. Different mechanisms of polyphenols on stroke prevention have been elucidated through in vitro studies. A study carried out by Gundimeda et al. [34] showed that trace amounts of green tea polyphenol (GTPP) and epigallocatechin-3-gallate (EGCG) could exhibit neurite outgrowth-inhibiting activity and the growth cone-collapsing activity of Nogo-66 (C-terminal region of Nogo-A). Resveratrol and polyphenols from green tea were able to reduce the reactive oxygen species (ROS) in mitochondria and cell swelling in endothelial cells [35]. Recent evidence also showed that resveratrol can be considered a signaling molecule in tissues and cells and thus act as a modulator for the expression of genes and proteins [36]. Thus, they may prevent, in part, brain edema and neural damage. One possible mechanism is that polyphenols may decrease endothelial cell swelling through Ca^{2+} reduction.

Mangiferin and morin, two natural antioxidants from mango peel and *Maclura pomifera,* (commonly known as the Osage orange), respectively, exhibited a wide spectrum of antioxidant and antiapoptotic activities in an in vitro model at micromolar concentrations [37]. These polyphenols are promising neuroprotectors for the diverse pathologies, such as cerebrovascular accident, epilepsy, stroke, brain trauma, and spinal cord damage.

Spigoni et al. [38] observed in endothelial cells that urolithins A and B and urolithin β-glucuronide had an influence on NO and NO synthase activation. Urolithin metabolism may be linked to peripheral cells. eNOS expression was activated by a mix of urolithins at $5\,\mu$M and urolithin β-glucuronide at $15\,\mu$M. It is known that decreased eNOS activation and reduction in NO bioavailability are two of the key factors for endothelial dysfunction. Moreover, the mix of urolithins at $5\,\mu$M following 24 h of incubation increased significantly nitrite/nitrate levels, also important in endothelial dysfunction.

Some reports show increased extracellular levels of glutamate in certain neurodegenerative diseases, such as stroke [39]. It is also very well-known that, in this brain disorder, increases in ROS levels occur, which have been associated with increased release and decreased uptake of glutamate [40]. Abib et al. [41] evaluated the effects of (−)-epicatechin-3-gallate (ECG), one of the three major green tea antioxidants, on C6 lineage cells. They observed that after 6 h, there was a beneficial cell response induced by ECG, indicating that ECG should be able to protect the brain against excitotoxicity induced by glutamate.

Another study reported that pycnogenol possesses scavenging activity against ROS and nitrogen species. In addition, effects on NO metabolism were proven in the macrophage cell line RAW 264.7 [42]. Cells were preincubated with pycnogenol, showing significant decreases in NO generation. This data sheds light on the biological activity of pycnogenol and possibly other polyphenols as beneficial agents in several human illnesses.

3.3. Animal Studies Determine the Molecular Mechanisms Involved in Stroke Prevention. Several epidemiological studies of dietary polyphenols on chronic disease prevention support a protective effect of these compounds against CVD [43–45]. Some observational studies have also shown an inverse association between the consumption of some classes of polyphenols and the overall mortality [44, 46]. Involved mechanisms have been elucidated from numerous studies conducted in animal models (mainly rodents) with nutritionally realistic levels of isolated flavonoids [47].

The health effect of polyphenols from tea, coffee, and cocoa has been extensively evaluated in experimental studies with animals, indicating beneficial effects on cardiovascular health and a reduced risk of stroke. While tea and cocoa exhibit beneficial effects on endothelial function, total and LDL cholesterol (tea only), and insulin sensitivity (cocoa only), moderate coffee consumption has been inversely associated with the risk of stroke. However, further long-term randomized clinical trials and prospective studies are necessary to provide clear mechanisms by which coffee, cocoa, and tea polyphenols offer cardiovascular health benefits. Antihypertensive, hypocholesterolemic, antioxidant, and anti-inflammatory activities have been proposed to these polyphenols, as well as improvement of vascular endothelial function and insulin sensitivity.

EGCG present in a limited number of plant-based foods and beverages, such as green tea, exhibited neuroprotective action in a cerebral ischaemia mouse model by controlling the inflammation cascade [48, 49], providing antioxidant and neuroprotective effects, possibly through the activation of the NF-E2-related factor 2/antioxidant responsive element signaling pathway [50]. A recent study described the first in vivo results supporting the potential of the coadministration of EGCG with a recombinant tissue plasminogen activator to extend its therapeutic window in treating acute brain ischaemia [51].

Quercetin, another flavonoid with similar antioxidant effects as those of green tea polyphenols, reduced levels of matrix metallopeptidase 9 (MMP-9) in cerebral ischaemia studies and attenuated blood-brain barrier disruption [52]. In a rat model of heat stroke, quercetin therapy (30 mg/kg) showed an improvement of heat stroke outcomes by attenuating excessive hyperthermia as well as myocardial injury. The protective effects of quercetin could be attributed to antilipid, peroxidative, antioxidant, and anti-inflammatory properties [53]. Antihypertensive effects in a rat model have also been attributed to quercetin that may induce a progressive and sustained reduction in BP, oxidative stress, or NO status [54]. Moreover, quercetin rutinoside (rutin) has been found to control neural damage in cerebral ischaemia [55].

Resveratrol has been extensively studied because of its ability to modulate certain parameters linked with increased cardiovascular risk [56]. Inhibition of lipid oxidation processes has been proposed as the main mechanism involved in neuroprotection. More recent results from a rat model of transient middle cerebral artery occlusion indicate that resveratrol significantly decreases apoptosis, mitochondrial lipid peroxidation, brain infarct volume, and edema [57, 58]. In

the endothelium, resveratrol can stimulate NOS activity, increasing the amount of NO in isolated rat aortas [59].

Polyphenols from grape powder, administered as a supplement in a diet, have been reported to protect the brain against ischaemic damage. The neuroprotective effects of the GP supplement may have a wide implication in the future for the prevention/protection against other neurodegenerative damages [60].

Antioxidative, anti-inflammatory, antilipidemic, and neuroprotective properties have been attributed to curcumin in experimental animal models [61]. In a porcine model with endothelial dysfunction, for example, curcumin can effectively block the detrimental effect of homocysteine on the vascular system [62].

Diets enriched in anthocyanins from blueberries provide neuroprotection after stroke induced in rats due to their antiatherogenic and anti-inflammatory properties [63, 64]. Consumption of pomegranate byproduct resulted in a 57% reduction in atherosclerotic lesion size in mice [65]. A red wine extract, rich in anthocyanin, reduces injury induced by cerebral ischaemia in rats and protects against ischaemia-induced excitotoxicity, energy failure, and oxidative stress [66].

Hydroxytyrosol and oleuropein, polyphenols from olive oil, are recognized as having an important role in the protection against CVD [67]. Olive oil supplementation in rabbits improved the outcome of atherosclerosis by improving the lipid profile and reducing platelet hyperactivity [68–70].

3.4. Dietary Polyphenols and Stroke: Results from Human Clinical and Epidemiological Studies. Epidemiologic studies are a useful tool to assess the association between dietary intake of polyphenols and human health. Although no cause-effect information can be obtained from these studies, they have been broadly used to evaluate the effects of dietary compounds on several diseases. The inverse association between high-polyphenol content food (e.g., fruits, vegetables, and their derivatives) and CVD risk has been reported elsewhere and seems indisputable. However, the question as to whether polyphenols are responsible for this protection is still under debate [71–73].

The consumption of alcoholic beverages with a high polyphenol content (i.e., wine and beer) and stroke have also been studied. The results of different observational studies suggest that the relative risk of ischaemic stroke is lowered by moderate alcohol consumption [74]. In fact, it is generally accepted that the association between alcohol intake and the risk of CVD and total mortality is demonstrated by a J-shaped curve. The conclusion derived from these graphs is that having one or two drinks per day, without binge drinking, protects against CVD, particularly coronary disease and ischaemic stroke [75, 76].

Since the formulation of the concept of the French paradox by epidemiologists in the 1980s, many efforts have been made to elucidate its causes. The low incidence of CVD in the French population despite a high intake of saturated fats has been on the table for decades. Debates have focused on wine consumption since it is the common element in all southern European countries [77]. Red wine contains around 200 mg of polyphenols per 100 g [78] mainly anthocyanins and flavan-3-ols and is one of the main contributors to total polyphenol intake in these countries [79–81].

Many experimental studies with resveratrol and stroke have been conducted in animals; however, to date, no clinical investigations have been performed in humans who have survived a stroke. It is plausible that resveratrol increases cerebral blood flow and enhances cerebrovascular perfusion in these patients since these effects have been observed in healthy subjects [82]. Other *in vivo* and animal studies have reported benefits of wine polyphenols on stroke protection [54].

Wine consumption has frequently been associated with CVD protection although beer has been also studied. Nevertheless, only three studies have provided prospective data concerning beer consumption and stroke [83–85], and only one study found a significant inverse association for ischaemic stroke on comparing subjects who drank 1–6 drinks per week with abstainers (61%; 95% CI: 16% to 81%) [86]. Thus, the data on beer and stroke currently available are inconclusive albeit promising.

The effect of alcohol on cerebrovascular events is due to its capacity to improve the lipid profile (increasing HDL cholesterol and decreasing LDL cholesterol) and reduce platelet aggregation and its anti-inflammatory effects. Additionally, nonalcoholic components of wine and beer such as polyphenols exert antiatherogenic and antithrombotic effects and regulate endothelial function through different mechanisms [22].

Other polyphenol-rich drinks such as coffee or tea have been associated with a lower risk of stroke. As with alcohol, high consumption of coffee is considered a risk factor for CVD, but moderate consumption may reduce the risk. A meta-analysis of 11 prospective studies pointed out that the risk of stroke was lower in subjects who consumed 6 or less cups of coffee per day compared with nonconsumers. The relative risks were similar for ischaemic and haemorrhagic stroke, and no differences were observed with gender at lower levels of coffee consumption (2 cups per day or less) [87]. More recent prospective studies confirmed these results for stroke mortality and incidence [88–90]. It should be pointed out that the many different ways of coffee preparation can affect the polyphenol content and may lead to biased results. Moreover, the mechanisms by which coffee may exert these beneficial effects are not clear [87, 91].

Similar to coffee, teas are very rich in polyphenols, mainly flavonoids. It is known that tea flavonoids improve endothelial function, BP, cholesterol levels, and blood glucose concentrations [91, 92]. The results of a meta-analysis of 14 prospective studies showed that the increment of 3 cups/day in green or black tea consumption decreased the relative risk by 13% (95% confidence interval (CI), 0.81–0.94). The association did not depend on gender but was slightly higher for green tea. Other results from two large prospective studies of green and black tea were in line with these findings [90, 92].

Other typically polyphenol-rich foods are chocolate and cocoa, nuts, and olive oil. Prospective studies on chocolate and stroke are still scarce, although a significant reduction

of 19% was observed in a meta-analysis of these studies when comparing the extreme of chocolate consumption [93].

Culinary herbs and spices are broadly used in meals to improve the flavour of food dishes and also as traditional medicines to prevent or treat different conditions. Scientific evidence about this is still scarce, but the results of the studies available are promising. Polyphenols may be found among all the active chemical components of herbs and spices. Due to the lack of water, the concentration of polyphenols in herbs and spices is higher than in fruits and vegetables. Several studies have focused on the effects of herbs, spices, and medicinal plants on glucose regulation and dyslipidemia in subjects who had metabolic syndrome and type 2 diabetes, both of which are major risk factors for stroke [94]. However, to our knowledge, no clinical trials or clinical studies have been performed to evaluate the direct relationship between spices/herbs and the risk of stroke.

Novel meta-analyses reported the effects of flavanol-containing products such as tea, cocoa, and apple products on body composition, blood pressure, and blood lipids. Bertoia et al. [95] indicated that foods rich in flavan-3-ols, flavonols, anthocyanins, and flavonoid polymers may help adults to control body weight. This type of studies may give a dietary pattern for obesity prevention and its possible consequences.

Several meta-analyses of randomized controlled trials have dealt with the effect of tea and different outcomes. The effects of black tea on blood cholesterol are contradictory. One meta-analysis found no effects of black tea on total cholesterol and serum concentrations of LDL and HDL cholesterol [96], while another one concluded that consumption of black tea lowered LDL cholesterol, especially in subjects at high cardiovascular risk [97]. Acute black tea and chocolate increased flow-mediated dilatation after acute and chronic intake and reduced systolic and diastolic blood pressure. Regarding green tea, results from randomized controlled trials point in the same direction. Its intake resulted in significant reductions in systolic blood pressure, total cholesterol, and LDL cholesterol [98–100].

Several randomized controlled trials have been conducted to assess the effects of cocoa and dark chocolate, which are rich sources of flavonoids, on serum lipids. Total cholesterol and LDL cholesterol decreased when consuming these foods. No effects were observed for HDL cholesterol and triglyceride concentration in plasma [101]. However, the observed changes depend on the intake dose and the health of the participants [102].

The PREDIMED (*Prevención con Dieta Mediterránea*) study was a prospective, randomized, multicentre, controlled trial aimed at determining the benefits of following a Mediterranean diet supplemented with either nuts or extra virgin olive oil on cardiovascular events (including myocardial infarction, stroke, and cardiovascular death). The control group followed a low-fat diet according to the guidelines of the American Heart Association. At the end of the study, the results revealed that both Mediterranean diet groups had a 30% lower incidence of the primary endpoint than the control group. Remarkably, incident stroke was reduced by nearly 50% in participants following the Mediterranean diet enriched with nuts (15 g walnuts, 7.5 g almonds, and 7.5 g hazelnuts) [103].

In an observational study within the PREDIMED trial, total polyphenol intake and polyphenol subclasses were calculated from the FFQ and the Phenol-Explorer database. Total polyphenols were associated with a 46% reduction in CVD risk on comparing the fifth versus the first quintiles. The intake of lignans, flavanols, and hydroxybenzoic acids was also inversely and significantly associated with cardiovascular events [104]. Besides this study, only a few more have directly evaluated the association between polyphenol intake and stroke, and only 2 found a significant inverse association: the Zutphen Elderly Study and the Finnish Mobile Clinic Health Examination Survey. In the first study, flavonol and flavone intakes, but not catechin intake [46], were associated with a lower incidence of stroke [105]. In the Finnish cohort, the polyphenolic groups inversely associated with stroke were flavonols, flavones, and flavanones [106]. However, similar studies did not find any association [107, 108].

4. Mechanisms

Polyphenols from fruits, vegetables, and beverages, such as wine, tea, cocoa, may exert protective effects on the cardiovascular system [109]. Increasing evidence suggests that dietary polyphenols prompt their health properties by the interaction with molecular signaling pathways [110] (Figure 1). The ability of polyphenols to target transcriptional networks that modulate gene expression favoring NO production, anti-inflammatory mediators, and energy expenses provides an attractive pharmacological approach to treat cardiovascular and metabolic diseases.

One of the most well-described mechanisms on neurovascular protection involves the ability of polyphenols to generate NO, a potent vasodilator generated by endothelial cells, which can also regulate cardiovascular-related gene expression. Consequently, polyphenols prevent the progress of endothelial dysfunction, decreasing the development of atherosclerotic plaque, vascular thrombosis, and occlusion. In early stages of atherosclerosis development, polyphenols contribute to reducing LDL oxidation, improving antioxidant status, and decreasing levels of inflammatory cytokines and adhesion molecules (Figure 1).

Oxidative stress is thought to be a key event in the pathogenesis of cerebral ischaemia. Overproduction of ROS during ischaemia may cause an imbalance between oxidative and antioxidative processes. ROS can damage lipids, proteins, and nucleic acids, inducing apoptosis or necrosis. It is hypothesized that plant polyphenols can provide protection against neurodegenerative changes associated with cerebral ischaemia [56]. Polyphenols can upregulate multiple redox enzymes, such as endothelial NO synthase, catalase, SOD1, and SOD2.

Most experimental and epidemiological studies suggest that dietary polyphenols activate antioxidant pathways and modulate immune response by inhibiting proinflammatory biomarkers.

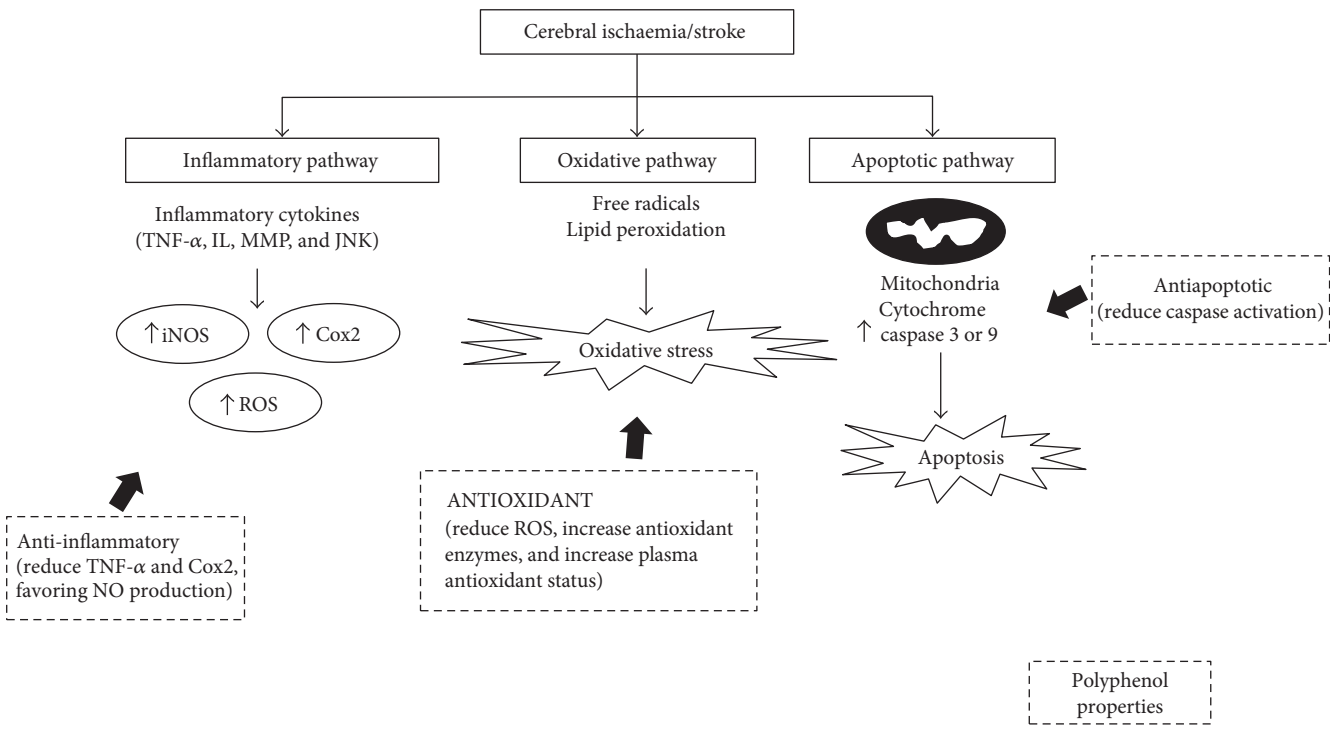


Figure 1: Molecular signaling pathways and molecular risk factors modulated by polyphenols in neurological disorders.

5. Conclusion

The results from experimental *in vitro* and *in vivo* studies and clinical evidence support the beneficial effects of some classes of polyphenols, mainly flavonoids, on multiple endpoints of cardiovascular risk. The most convincing mechanisms involve the reduction of BP by increasing NO production and improvement in endothelial function. However, the lack of information about polyphenolic food composition and metabolism does not allow conclusions about the effects and effectiveness in preventing CVD.

Moreover, future clinical trials evaluating the effect of dietary polyphenols should take into account different aspects to obtain conclusive results. It is important to include a proper control product regarding polyphenol content. It is also necessary to consider the specific target population included in the study to correctly design the nutritional intervention period duration (weeks, months), as well as the dose of the polyphenols investigated. To establish the effects of oral exposure to the most promising polyphenols, large-scale, long-term, well-controlled trials, preferably with clinical endpoints including cardiovascular morbidity/mortality, are required. Until now, there is no sufficient data to establish a dietary reference intake for each class of polyphenols. To ensure the safety of their consumption, the development of functional foods should only consider the same level of polyphenols as the best dietary source can deliver at dietary doses. Then, the bioactivity of this functional food should be tested with *in vivo* models. After proving the bioefficacy of the compounds, mechanism should be tested *in vitro*.

In the future, it is essential for the scientific community to strictly consider well-designed experiments (in terms of the nature and concentrations of metabolites used) in order to definitively identify the compounds biologically active in target tissues as well as the molecular mechanisms involved.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by ERAB: The European Foundation for Alcohol Research (EA 1324, EA 1514, EA 1515, EA1517, and CICYT(AGL2016-79113-R)) and the Instituto de Salud Carlos III (ISCIII) (CIBEROBN) from the Ministerio de Economía y Competitividad (MEC) (AEI/FEDER, UE) and Generalitat de Catalunya (GC) (2014 SGR 773).

References

[1] D. Del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges, and A. Crozier, *Dietary (Poly)-phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects against Chronic Diseases*, vol. 18, Antioxidants & Redox Signaling, 140 Huguenot Street, 3rd FloorNew Rochelle, NY 10801 USA, 2013.

[2] R. M. Lamuela-Raventós, A. Vallverdú-Queralt, O. Jáuregui, M. Martínez-Huélamo, and P. Quifer-Rada, "Improved characterization of polyphenols using liquid chromatography," *Polyphenols in Plants*, vol. 14, pp. 261–292, 2014.

[3] A. Crozier, I. B. Jaganath, and M. N. Clifford, "Dietary phenolics: chemistry, bioavailability and effects on health," *Natural Product Reports*, vol. 26, no. 8, pp. 1001–1043, 2009.

[4] A. Vallverdú-Queralt, N. Boix, E. Piqué et al., "Identification of phenolic compounds in red wine extract samples and zebrafish embryos by HPLC-ESI-LTQ-Orbitrap-MS," *Food Chemistry*, vol. 181, pp. 146–151, 2015.

[5] B. Frei and J. V. Higdon, "Antioxidant activity of tea polyphenols in vivo: evidence from animal studies," *The Journal of Nutrition*, vol. 133, no. 10, pp. 3275S–3284S, 2003.

[6] J. S. Bonita, M. Mandarano, D. Shuta, and J. Vinson, "Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies," *Pharmacological Research*, vol. 55, no. 3, pp. 187–198, 2007.

[7] N. Talhaoui, A. M. Gómez-Caravaca, L. León, R. De la Rosa, A. Fernández-Gutiérrez, and A. Segura-Carretero, "From olive fruits to olive oil: phenolic compound transfer in six different olive cultivars grown under the same agronomical conditions," *International Journal of Molecular Sciences*, vol. 17, no. 3, p. 337, 2016.

[8] F. Visioli, H. Bernaert, R. Corti et al., "Chocolate, lifestyle, and health," *Critical Reviews in Food Science and Nutrition*, vol. 49, no. 4, pp. 299–312, 2009.

[9] M. Rusconi and A. Conti, "*Theobroma cacao* L., the food of the gods: a scientific approach beyond myths and claims," *Pharmacological Research*, vol. 61, no. 1, pp. 5–13, 2010.

[10] A. Vallverdú-Queralt, J. Regueiro, M. Martínez-Huélamo, J. F. R. Alvarenga, L. N. Leal, and R. M. Lamuela-Raventos, "A comprehensive study on the phenolic profile of widely used culinary herbs and spices: rosemary, thyme, oregano, cinnamon, cumin and bay," *Food Chemistry*, vol. 154, pp. 299–307, 2014.

[11] J. Regueiro, C. Sánchez-González, A. Vallverdú-Queralt, J. Simal-Gándara, R. Lamuela-Raventós, and M. Izquierdo-Pulido, "Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap–Orbitrap mass spectrometry," *Food Chemistry*, vol. 152, pp. 340–348, 2014.

[12] R. M. Lamuela-Raventos and P. Quifer-Rada, "Effect of dietary polyphenols on cardiovascular risk," *Hear*, vol. 102, no. 17, pp. 1340-1341, 2016.

[13] M. Martínez-Huélamo, A. Vallverdú-Queralt, G. Di Lecce et al., "Bioavailability of tomato polyphenols is enhanced by processing and fat addition: evidence from a randomized feeding trial," *Molecular Nutrition & Food Research*, vol. 60, no. 7, pp. 1578–1589, 2016.

[14] F. Visioli, C. A. De La Lastra, C. Andres-Lacueva et al., "Polyphenols and human health: a prospectus," *Critical Reviews in Food Science and Nutrition*, vol. 51, no. 6, pp. 524–546, 2011.

[15] S. Bolca, T. Van de Wiele, and S. Possemiers, "Gut metabotypes govern health effects of dietary polyphenols," *Current Opinion in Biotechnology*, vol. 24, no. 2, pp. 220–225, 2013.

[16] A. Medina-Remon, R. Estruch, A. Tresserra-Rimbau, A. Vallverdú-Queralt, and R. M. Lamuela-Raventos, "The effect of polyphenol consumption on blood pressure," *Mini-Reviews in Medicinal Chemistry*, vol. 13, no. 8, pp. 1137–1149, 2013.

[17] A. C. de Camargo, M. A. B. Regitano-d'Arce, A. C. T. Biasoto, and F. Shahidi, "Low molecular weight phenolics of grape juice and winemaking byproducts: antioxidant activities and inhibition of oxidation of human low-density lipoprotein cholesterol and DNA strand breakage," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 50, pp. 12159–12171, 2014.

[18] C. Denny, J. G. Lazarini, M. Franchin et al., "Bioprospection of Petit Verdot grape pomace as a source of anti-inflammatory compounds," *Journal of Functional Foods*, vol. 8, pp. 292–300, 2014.

[19] C. L. Millar, Q. Duclos, and C. N. Blesso, "Effects of dietary flavonoids on reverse cholesterol transport, HDL metabolism, and HDL function," *Advances in Nutrition: An International Review Journal*, vol. 8, no. 2, pp. 226–239, 2017.

[20] Á. Hernáez, S. Fernández-Castillejo, M. Farràs et al., "Olive oil polyphenols enhance high-density lipoprotein function in humans: a randomized controlled trial," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 34, no. 9, pp. 2115–2119, 2014.

[21] A. Hernaez, A. T. Remaley, M. Farras et al., "Olive oil polyphenols decrease LDL concentrations and LDL atherogenicity in men in a randomized controlled trial," *Journal of Nutrition*, vol. 145, no. 8, pp. 1692–1697, 2015.

[22] G. Chiva-Blanch, E. Magraner, X. Condines et al., "Effects of alcohol and polyphenols from beer on atherosclerotic biomarkers in high cardiovascular risk men: a randomized feeding trial," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 25, no. 1, pp. 36–45, 2015.

[23] I. P. G. Botden, J. G. Langendonk, M. E. Meima et al., "Daily red wine consumption improves vascular function by a soluble guanylyl cyclase-dependent pathway," *American Journal of Hypertension*, vol. 24, no. 2, pp. 162–168, 2011.

[24] I. P. Botden, R. Draijer, B. E. Westerhof et al., "Red wine polyphenols do not lower peripheral or central blood pressure in high normal blood pressure and hypertension," *American Journal of Hypertension*, vol. 25, no. 6, pp. 718–723, 2012.

[25] D. W. Droste, C. Iliescu, M. Vaillant et al., "A daily glass of red wine associated with lifestyle changes independently improves blood lipids in patients with carotid arteriosclerosis: results from a randomized controlled trial," *Nutrition Journal*, vol. 12, no. 1, p. 147, 2013.

[26] G. Siasos, D. Tousoulis, E. Kokkou et al., "Favorable effects of concord grape juice on endothelial function and arterial stiffness in healthy smokers," *American Journal of Hypertension*, vol. 27, no. 1, pp. 38–45, 2013.

[27] A. Steptoe, E. L. Gibson, R. Vuononvirta et al., "The effects of tea on psychophysiological stress responsivity and post-stress recovery: a randomised double-blind trial," *Psychopharmacology*, vol. 190, no. 1, p. 91, 2007.

[28] R. L. Noad, C. Rooney, D. McCall et al., "Beneficial effect of a polyphenol-rich diet on cardiovascular risk: a randomised control trial," *Heart*, vol. 102, no. 17, pp. 1371–1379, 2016.

[29] A. Medina-Remón, A. Tresserra-Rimbau, A. Pons et al., "Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 25, no. 1, pp. 60–67, 2015.

[30] X. Guo, A. Tresserra-Rimbau, R. Estruch et al., "Effects of polyphenol, measured by a biomarker of total polyphenols in urine, on cardiovascular risk factors after a long-term follow-up in the PREDIMED study," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 2572606, 11 pages, 2016.

[31] H. Abbott and F. Sim, *Unit: Public Health Aspects of Stroke Workbook*, pp. 1–33, Lorraine Williams and Fiona Sim, London, 2010.

[32] R. Wittenauer and L. Smith, *Priority Medicines for Europe and the World "A Public Health Approach to Innovation"*.

Background Paper 6.6. Ischaemic and Haemorrhagic Stroke, WHO, Geneva, Switzerland, 2012.

[33] The World Health Organization, *The Top 10 Causes of Death*, 2017.

[34] U. Gundimeda, T. H. McNeill, B. A. Barseghian et al., "Polyphenols from green tea prevent antineuritogenic action of Nogo-A via 67-kDa laminin receptor and hydrogen peroxide," *Journal of Neurochemistry*, vol. 132, no. 1, pp. 70–84, 2015.

[35] K. S. Panickar, B. Qin, and R. A. Anderson, "Ischemia-induced endothelial cell swelling and mitochondrial dysfunction are attenuated by cinnamtannin D1, green tea extract, and resveratrol in vitro," *Nutritional Neuroscience*, vol. 18, no. 7, pp. 297–306, 2015.

[36] S. Doré, "Unique properties of polyphenol stilbenes in the brain: more than direct antioxidant actions; gene/protein regulatory activity," *Neurosignals*, vol. 14, no. 1-2, pp. 61–70, 2005.

[37] M. R. Campos-Esparza, M. V. Sánchez-Gómez, and C. Matute, "Molecular mechanisms of neuroprotection by two natural antioxidant polyphenols," *Cell Calcium*, vol. 45, no. 4, pp. 358–368, 2009.

[38] V. Spigoni, P. Mena, M. Cito et al., "Effects on nitric oxide production of urolithins, gut-derived ellagitannin metabolites, in human aortic endothelial cells," *Molecules*, vol. 21, no. 8, p. 1009, 2016.

[39] G. Campiani, C. Fattorusso, M. De Angelis et al., "Neuronal high-affinity sodium-dependent glutamate transporters (EAATs): targets for the development of novel therapeutics against neurodegenerative diseases," *Current Pharmaceutical Design*, vol. 9, no. 8, pp. 599–625, 2003.

[40] A. C. Bowling and M. F. Beal, "Bioenergetic and oxidative stress in neurodegenerative diseases," *Life Sciences*, vol. 56, no. 14, pp. 1151–1171, 1995.

[41] R. T. Abib, A. Quincozes-Santos, P. Nardin et al., "Epicatechin gallate increases glutamate uptake and S100B secretion in C6 cell lineage," *Molecular and Cellular Biochemistry*, vol. 310, no. 1, pp. 153–158, 2008.

[42] F. Virgili, H. Kobuchi, and L. Packer, "Procyanidins extracted from *Pinus maritima* (Pycnogenol®): scavengers of free radical species and modulators of nitrogen monoxide metabolism in activated murine RAW 264.7 macrophages," *Free Radical Biology & Medicine*, vol. 24, no. 7-8, pp. 1120–1129, 1998.

[43] S. Kuriyama, T. Shimazu, K. Ohmori et al., "Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study," *JAMA*, vol. 296, no. 10, pp. 1255–1265, 2006.

[44] P. J. Mink, C. G. Scrafford, L. M. Barraj et al., "Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women," *The American Journal of Clinical Nutrition*, vol. 85, no. 3, pp. 895–909, 2007.

[45] P. E. Szmitko and S. Verma, "Cardiology patient pages. Red wine and your heart," *Circulation*, vol. 111, no. 2, pp. e10–e11, 2005.

[46] I. C. Arts, P. C. Hollman, E. J. Feskens, H. B. Bueno de Mesquita, and D. Kromhout, "Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study," *The American Journal of Clinical Nutrition*, vol. 74, no. 2, pp. 227–232, 2001.

[47] G. D. Norata, P. Marchesi, S. Passamonti, A. Pirillo, F. Violi, and A. L. Catapano, "Anti-inflammatory and anti-atherogenic effects of cathechin, caffeic acid and trans-resveratrol in apolipoprotein E deficient mice," *Atherosclerosis*, vol. 191, no. 2, pp. 265–271, 2007.

[48] J.-W. Park, J.-S. Hong, K.-S. Lee, H.-Y. Kim, J.-J. Lee, and S.-R. Lee, "Green tea polyphenol (−)-epigallocatechin gallate reduces matrix metalloproteinase-9 activity following transient focal cerebral ischemia," *The Journal of Nutritional Biochemistry*, vol. 21, no. 11, pp. 1038–1044, 2010.

[49] M. Ashafaq, S. S. Raza, M. M. Khan et al., "Catechin hydrate ameliorates redox imbalance and limits inflammatory response in focal cerebral ischemia," *Neurochemical Research*, vol. 37, no. 8, pp. 1747–1760, 2012.

[50] J. Han, M. Wang, X. Jing, H. Shi, M. Ren, and H. Lou, "(−)-Epigallocatechin gallate protects against cerebral ischemia-induced oxidative stress via Nrf2/ARE signaling," *Neurochemical Research*, vol. 39, no. 7, pp. 1292–1299, 2014.

[51] Y.-P. You, "Epigallocatechin gallate extends the therapeutic window of recombinant tissue plasminogen activator treatment in ischemic rats," *Journal of Stroke and Cerebrovascular Diseases*, vol. 25, no. 4, pp. 990–997, 2016.

[52] J.-K. Lee, H.-J. Kwak, M.-S. Piao, J.-W. Jang, S.-H. Kim, and H.-S. Kim, "Quercetin reduces the elevated matrix metalloproteinases-9 level and improves functional outcome after cerebral focal ischemia in rats," *Acta Neurochirurgica*, vol. 153, no. 6, pp. 1321–1329, 2011.

[53] X. Lin, C.-H. Lin, T. Zhao et al., "Quercetin protects against heat stroke-induced myocardial injury in male rats: antioxidative and antiinflammatory mechanisms," *Chemico-Biological Interactions*, vol. 265, pp. 47–54, 2017.

[54] F. Perez-Vizcaino, J. Duarte, and R. Andriantsitohaina, "Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols," *Free Radical Research*, vol. 40, no. 10, pp. 1054–1065, 2006.

[55] M. M. Khan, A. Ahmad, T. Ishrat et al., "Rutin protects the neural damage induced by transient focal ischemia in rats," *Brain Research*, vol. 1292, pp. 123–135, 2009.

[56] A. Simonyi, Q. Wang, R. Miller et al., "Polyphenols in cerebral ischemia: novel targets for neuroprotection," *Molecular Neurobiology*, vol. 31, no. 1–3, pp. 135–147, 2005.

[57] S. Yousuf, F. Atif, M. Ahmad et al., "Resveratrol exerts its neuroprotective effect by modulating mitochondrial dysfunctions and associated cell death during cerebral ischemia," *Brain Research*, vol. 1250, pp. 242–253, 2009.

[58] J. A. Baur and D. A. Sinclair, "Therapeutic potential of resveratrol: the in vivo evidence," *Nature Reviews Drug Discovery*, vol. 5, no. 6, pp. 493–506, 2006.

[59] T. Wallerath, G. Deckert, T. Ternes et al., "Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase," *Circulation*, vol. 106, no. 13, pp. 1652–1658, 2002.

[60] Q. Wang, A. Simonyi, W. Li et al., "Dietary grape supplement ameliorates cerebral ischemia-induced neuronal death in gerbils," *Molecular Nutrition & Food Research*, vol. 49, no. 5, pp. 443–451, 2005.

[61] M. C. Ramirez-Tortosa, M. D. Mesa, M. C. Aguilera et al., "Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis," *Atherosclerosis*, vol. 147, no. 2, pp. 371–378, 1999.

[62] G. Ramaswami, H. Chai, Q. Yao, P. H. Lin, A. B. Lumsden, and C. Chen, "Curcumin blocks homocysteine-induced

endothelial dysfunction in porcine coronary arteries," *Journal of Vascular Surgery*, vol. 40, no. 6, pp. 1216–1222, 2004.

[63] D. Del Rio, G. Borges, and A. Crozier, "Berry flavonoids and phenolics: bioavailability and evidence of protective effects," *British Journal of Nutrition*, vol. 104, Supplement 3, pp. S67–S90, 2010.

[64] A. Basu, M. Rhone, and T. J. Lyons, "Berries: emerging impact on cardiovascular health," *Nutrition Reviews*, vol. 68, no. 3, pp. 168–177, 2010.

[65] X. Wu, J. Kang, C. Xie et al., "Dietary blueberries attenuate atherosclerosis in apolipoprotein E-deficient mice by upregulating antioxidant enzyme expression," *Journal of Nutrition*, vol. 140, no. 9, pp. 1628–1632, 2010.

[66] M.-F. Ritz, Y. Curin, A. Mendelowitsch, and R. Andriantsitohaina, "Acute treatment with red wine polyphenols protects from ischemia-induced excitotoxicity, energy failure and oxidative stress in rats," *Brain Research*, vol. 1239, pp. 226–234, 2008.

[67] M.-I. Covas, R. de la Torre, and M. Fito, "Virgin olive oil: a key food for cardiovascular risk protection," *British Journal of Nutrition*, vol. 113, Supplement 2, pp. S19–S28, 2015.

[68] J. P. De La Cruz, M. A. Villalobos, J. A. Carmona, M. Martin-Romero, J. M. Smith-Agreda, and F. S. de la Cuesta, "Antithrombotic potential of olive oil administration in rabbits with elevated cholesterol," *Thrombosis Research*, vol. 100, no. 4, pp. 305–315, 2000.

[69] M. Gonzalez-Santiago, E. Martin-Bautista, J. J. Carrero et al., "One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development," *Atherosclerosis*, vol. 188, no. 1, pp. 35–42, 2006.

[70] C. Manna, V. Migliardi, P. Golino et al., "Oleuropein prevents oxidative myocardial injury induced by ischemia and reperfusion," *The Journal of Nutritional Biochemistry*, vol. 15, no. 8, pp. 461–466, 2004.

[71] I. C. W. Arts and P. C. H. Hollman, "Polyphenols and disease risk in epidemiologic studies," *The American Journal of Clinical Nutrition*, vol. 81, Supplement 1, pp. 317S–325S, 2005.

[72] F. J. He, C. A. Nowson, and G. A. MacGregor, "Fruit and vegetable consumption and stroke: meta-analysis of cohort studies," *The Lancet*, vol. 367, no. 9507, pp. 320–326, 2006.

[73] E. Saita, K. Kondo, and Y. Momiyama, "Anti-inflammatory diet for atherosclerosis and coronary artery disease: antioxidant foods," *Clinical Medicine Insights: Cardiology*, vol. 8, Supplement 3, pp. 61–65, 2015.

[74] R. M. Pinder and M. Sandler, "Alcohol, wine and mental health: focus on dementia and stroke," *Journal of Psychopharmacology*, vol. 18, no. 4, pp. 449–456, 2004.

[75] G. de Gaetano, S. Costanzo, A. Di Castelnuovo et al., "Effects of moderate beer consumption on health and disease: a consensus document," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 26, no. 6, pp. 443–467, 2016.

[76] A. Di Castelnuovo, S. Costanzo, R. di Giuseppe, G. de Gaetano, and L. Iacoviello, "Alcohol consumption and cardiovascular risk: mechanisms of action and epidemiologic perspectives," *Future Cardiology*, vol. 5, no. 5, pp. 467–477, 2009.

[77] J. Ferrières, "The French paradox: lessons for other countries," *Heart*, vol. 90, no. 1, pp. 107–111, 2004.

[78] V. Neveu, J. Perez-Jiménez, F. Vos et al., "Phenol-Explorer: an online comprehensive database on polyphenol contents in foods," *Database*, vol. 2010, article bap024, 2010.

[79] A. Tresserra-Rimbau, A. Medina-Remón, J. Pérez-Jiménez et al., "Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 23, no. 10, pp. 953–959, 2013.

[80] J. Pérez-Jiménez, L. Fezeu, M. Touvier et al., "Dietary intake of 337 polyphenols in French adults," *The American Journal of Clinical Nutrition*, vol. 93, no. 6, pp. 1220–1228, 2011.

[81] J. Godos, S. Marventano, A. Mistretta, F. Galvano, and G. Grosso, "Dietary sources of polyphenols in the Mediterranean healthy eating, aging and lifestyle (MEAL) study cohort," *International Journal of Food Sciences and Nutrition*, vol. 68, no. 6, pp. 750–756, 2017.

[82] D. Bonnefont-Rousselot, "Resveratrol and cardiovascular diseases," *Nutrients*, vol. 8, no. 5, p. 250, 2016.

[83] T. Truelsen, M. Gronbaek, P. Schnohr, and G. Boysen, "Intake of beer, wine, and spirits and risk of stroke: the Copenhagen City Heart Study," *Stroke*, vol. 29, no. 12, pp. 2467–2472, 1998.

[84] L. Djoussé, J. J. Himali, A. Beiser, M. Kelly-Hayes, and P. A. Wolf, "Apolipoprotein E, alcohol consumption, and risk of ischemic stroke: the Framingham Heart Study revisited," *Journal of Stroke and Cerebrovascular Diseases*, vol. 18, no. 5, pp. 384–388, 2009.

[85] K. J. Mukamal, A. Ascherio, M. A. Mittleman et al., "Alcohol and risk for ischemic stroke in men: the role of drinking patterns and usual beverage," *Annals of Internal Medicine*, vol. 142, no. 1, pp. 11–19, 2005.

[86] K. J. Mukamal, H. Chung, N. S. Jenny et al., "Alcohol use and risk of ischemic stroke among older adults: the Cardiovascular Health Study," *Stroke*, vol. 36, no. 9, pp. 1830–1834, 2005.

[87] S. C. Larsson and N. Orsini, "Coffee consumption and risk of stroke: a dose-response meta-analysis of prospective studies," *American Journal of Epidemiology*, vol. 174, no. 9, pp. 993–1001, 2011.

[88] N. D. Freedman, Y. Park, C. C. Abnet, A. R. Hollenbeck, and R. Sinha, "Association of coffee drinking with total and cause-specific mortality," *The New England Journal of Medicine*, vol. 366, no. 20, pp. 1891–1904, 2012.

[89] A. Floegel, T. Pischon, M. M. Bergmann, B. Teucher, R. Kaaks, and H. Boeing, "Coffee consumption and risk of chronic disease in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany study," *The American Journal of Clinical Nutrition*, vol. 95, no. 4, pp. 901–908, 2012.

[90] Y. Kokubo, H. Iso, I. Saito et al., "The impact of green tea and coffee consumption on the reduced risk of stroke incidence in Japanese population: the Japan public health center-based study cohort," *Stroke*, vol. 44, no. 5, pp. 1369–1374, 2013.

[91] S. C. Larsson, "Coffee, tea, and cocoa and risk of stroke," *Stroke*, vol. 45, no. 1, pp. 309–314, 2014.

[92] S. C. Larsson, J. Virtamo, and A. Wolk, "Black tea consumption and risk of stroke in women and men," *Annals of Epidemiology*, vol. 23, no. 3, pp. 157–160, 2013.

[93] S. C. Larsson, J. Virtamo, and A. Wolk, "Chocolate consumption and risk of stroke: a prospective cohort of men and meta-analysis," *Neurology*, vol. 79, no. 12, pp. 1223–1229, 2012.

[94] K. S. Panickar, "Beneficial effects of herbs, spices and medicinal plants on the metabolic syndrome, brain and cognitive function," *Central Nervous System Agents in Medicinal Chemistry*, vol. 13, no. 1, pp. 13–29, 2013.

[95] M. L. Bertoia, E. B. Rimm, K. J. Mukamal, F. B. Hu, W. C. Willett, and 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*, vol. 352, p. i17, 2016.

[96] D. Wang, C. Chen, Y. Wang, J. Liu, and R. Lin, "Effect of black tea consumption on blood cholesterol: a meta-analysis of 15 randomized controlled trials," *PLoS One*, vol. 9, no. 9, article e107711, 2014.

[97] Y. Zhao, S. Asimi, K. Wu, J. Zheng, and D. Li, "Black tea consumption and serum cholesterol concentration: systematic review and meta-analysis of randomized controlled trials," *Clinical Nutrition*, vol. 34, no. 4, pp. 612–619, 2015.

[98] S. Khalesi, J. Sun, N. Buys, A. Jamshidi, E. Nikbakht-Nasrabadi, and H. Khosravi-Boroujeni, "Green tea catechins and blood pressure: a systematic review and meta-analysis of randomised controlled trials," *European Journal of Nutrition*, vol. 53, no. 6, pp. 1299–1311, 2014.

[99] A. Kim, A. Chiu, M. K. Barone et al., "Green tea catechins decrease total and low-density lipoprotein cholesterol: a systematic review and meta-analysis," *Journal of the American Dietetic Association*, vol. 111, no. 11, pp. 1720–1729, 2011.

[100] X.-X. Zheng, X. Y-L, S.-H. Li, X.-X. Liu, R. Hui, and X.-H. Huang, "Green tea intake lowers fasting serum total and LDL cholesterol in adults: a meta-analysis of 14 randomized controlled trials," *The American Journal of Clinical Nutrition*, vol. 94, no. 2, pp. 601–610, 2011.

[101] O. A. Tokede, J. M. Gaziano, and L. Djousse, "Effects of cocoa products/dark chocolate on serum lipids: a meta-analysis," *European Journal of Clinical Nutrition*, vol. 65, no. 8, pp. 879–886, 2011.

[102] L. Jia, X. Liu, Y. Y. Bai et al., "Short-term effect of cocoa product consumption on lipid profile: a meta-analysis of randomized controlled trials," *The American Journal of Clinical Nutrition*, vol. 92, no. 1, pp. 218–225, 2010.

[103] R. Estruch, E. Ros, J. Salas-Salvadó et al., "Primary prevention of cardiovascular disease with a Mediterranean diet," *New England Journal of Medicine*, vol. 368, no. 14, pp. 1279–1290, 2013.

[104] A. Tresserra-Rimbau, E. B. Rimm, A. Medina-Remón et al., "Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 24, no. 6, pp. 639–647, 2014.

[105] S. O. Keli, M. G. Hertog, E. J. Feskens, and D. Kromhout, "Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study," *Archives of Internal Medicine*, vol. 156, no. 6, pp. 637–642, 1996.

[106] P. Knekt, J. Kumpulainen, R. Järvinen et al., "Flavonoid intake and risk of chronic diseases," *The American Journal of Clinical Nutrition*, vol. 76, no. 3, pp. 560–568, 2002.

[107] T. Hirvonen, J. Virtamo, P. Korhonen, D. Albanes, and P. Pietinen, "Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers," *Stroke*, vol. 31, no. 10, pp. 2301–2306, 2000.

[108] L. Yochum, L. H. Kushi, K. Meyer, and A. R. Folsom, "Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women," *American Journal of Epidemiology*, vol. 149, no. 10, pp. 943–949, 1999.

[109] Y. Curin and R. Andriantsitohaina, "Polyphenols as potential therapeutical agents against cardiovascular diseases," *Pharmacological Reports*, vol. 57, pp. 97–107, 2005.

[110] J. Gonzalez-Gallego, M. V. Garcia-Mediavilla, S. Sanchez-Campos, and M. J. Tunon, "Fruit polyphenols, immunity and inflammation," *British Journal of Nutrition*, vol. 104, Supplement 3, pp. S15–S27, 2010.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 2962316, 13 pages
https://doi.org/10.1155/2017/2962316

Research Article

Neuroprotective and Cognitive-Enhancing Effects of Microencapsulation of Mulberry Fruit Extract in Animal Model of Menopausal Women with Metabolic Syndrome

Supannika Kawvised,[1,2] Jintanaporn Wattanathorn,[2,3] and Wipawee Thukham-mee[2,3]

[1]*Department of Physiology and Graduate School (Neuroscience Program), Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[2]*Integrative Complementary Alternative Medicine Research and Development Center, Khon Kaen University, Khon Kaen 40002, Thailand*
[3]*Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

Correspondence should be addressed to Jintanaporn Wattanathorn; jintanapornw@yahoo.com

Received 8 July 2017; Accepted 19 September 2017; Published 12 October 2017

Academic Editor: Anna Tresserra-Rimbau

Currently, the neuroprotectant and memory-enhancing agent for menopausal women with metabolic syndrome is required. Based on the advantages of polyphenolics on numerous changes observed in menopause with metabolic syndrome and the encapsulation method, we hypothesized that microencapsulated mulberry fruit extract (MME) could protect brain damage and improve memory impairment in an animal model of menopause with metabolic syndrome. To test this hypothesis, MME at doses of 10, 50, and 250 mg/kg was given to female Wistar rats which were induced experimental menopause with metabolic syndrome by bilateral ovariectomy (OVX) and fed with high-carbohydrate high-fat (HCHF) diet for 8 weeks. Spatial memory together with neuron density, oxidative stress status, acetylcholinesterase, and phosphorylation of Erk in the hippocampus was assessed at the end of the study. It was found that MME decreased memory impairment, oxidative stress status, and AChE activity but increased neuron density and Erk phosphorylation in the hippocampus. Therefore, the neuroprotective and memory-enhancing effects of MME might partly involve the enhanced cholinergic function and Erk phosphorylation but decreased oxidative stress status in hippocampus. Therefore, MME is the potential novel neuroprotectant and memory-enhancing agent for menopause with metabolic syndrome. However, further research especially clinical trial is still necessary.

1. Introduction

The number of menopausal women is continually increasing worldwide [1]. Recently, it has been demonstrated that the prevalence of chronic diseases including metabolic syndrome in menopausal women is increased [2, 3]. Moreover, both menopausal condition and metabolic syndrome can induce memory impairment and decrease hippocampal plasticity [4–7]. Based on the rising trends of both menopause and metabolic syndrome mentioned earlier, the memory impairment in menopausal women with metabolic syndrome is increasing its importance and is recognized as one of the important health problems. Unfortunately, less information concerning this issue is available. Moreover, the current therapeutic strategy is still not in satisfaction level. Therefore, this problem should be concerned and the successful strategies for combating this condition are required.

Recently, it has been demonstrated that the substances which are rich in polyphenolic compounds especially anthocyanins can improve both metabolic syndrome and cognitive impairment [8, 9]. Since polyphenolic compounds including anthocyanins have low stability toward environment condition during processing and storage, it is a hard task force to incorporate the mentioned substance into foods and health products. However, encapsulation has been reported to be an effective way to introduce such substances into the

mentioned products. Encapsulating agent can be served as a protector coat against adverse environmental condition resulting in the increased stability. In addition, encapsulated compounds are also easier to handle.

Ripen fruits of mulberry or *Morus alba* (Moraceae family) are rich in polyphenolic compounds especially anthocyanins. The mulberry fruit extract exerts the neuroprotective and memory-enhancing effects in an animal model of vascular dementia [10]. Since the neurodegeneration and memory impairment both in vascular dementia [10] and in a menopausal woman with metabolic syndrome [11] are associated with oxidative stress, the neuroprotective and cognitive-enhancing effects of mulberry extract have been focused. Based on the benefits of anthocyanins and mulberry extract together with the encapsulation advantage mentioned earlier, we hypothesized that the encapsulated mulberry extract could improve hippocampal damage and memory impairment in postmenopausal metabolic syndrome. To elucidate this issue, this study was carried out to determine the neuroprotective and cognitive-enhancing effects of encapsulated mulberry extract in an animal model of postmenopausal metabolic syndrome induced by high-carbohydrate high-fat diet.

2. Materials and Methods

2.1. Preparation of Mulberry Fruit Extract. Ripen mulberry fruits (*Morus alba* L.) were collected from the Queen Sirikit Department of Sericulture Center, Udon Thani Province. The fresh mulberry fruits were cleaned and dried with an oven (Memmert GmbH, USA) at 60°C for 72 hours. The dried mulberry was grounded to fine powder and extracted by maceration technique with 50% hydroalcoholic (1% *w*/*v*) for 24 h and then filtered with Whatman number 1 filter paper. All yielded extracts were dried with the oven (Memmert GmbH, USA) at 60°C for 24 hours and kept at 4°C until used.

2.2. Preparation of Microencapsulated Mulberry Extract. Maltodextrin dextrose equivalent 10 (DE10) was selected as encapsulation matrix. It was mixed with mulberry extract at the ratio of 9 : 1 (*w*/*w*). The mixture was dissolved in warm distilled water at 50°C and stirred for 30 minutes. The solution was frozen at −20°C for 18 hours in a freezer and subjected to drying in a freeze-dryer (Labconco freeze dryer, Labconco Corporation, Kansas City, MO, USA) for 48 hours (−86°C, 0.008 mbar). The dry sample was packaged and stored in a desiccator containing silica gel at 4°C.

2.3. Measurement of Total Phenolic Compound Contents. The total phenolic compound content of the encapsulated mulberry extract was determined by using the Folin-Ciocalteu colorimetric method in microplate reader (iMark™ Microplate Absorbance Reader) [12, 13]. A 20 μl of the extract was mixed with 158 μl of distilled water and 20 μl of 50% *v*/*v* Folin-Ciocalteu reagent (Sigma-Aldrich, USA) which was freshly prepared. The mixture was incubated for 8 minutes. Then, 30 μl of 20% Na_2CO_3 (Sigma-Aldrich, USA) was added and subjected to a 2-hour incubation period at room temperature in a dark room. The absorbance was measured at 765 nm. Result was expressed as mg gallic acid equivalent (GAE)/mg microencapsulated mulberry extract. Various concentrations of gallic acid (Sigma-Aldrich, USA) were used as a standard calibration curve.

2.4. Measurement of Flavonoid Content. The determination of flavonoid content was performed by aluminum chloride method [14]. This process was based on the formation of aluminum-flavonoid complexes. All assessments were performed in triplicate. In brief, 100 μl of the extract at various concentrations was mixed with 100 μl of 2% methanolic aluminum chloride (Sigma-Aldrich, USA) and subjected to the 30-minute incubation period at room temperature in dark room. Then, the absorbance at 415 nm was taken against the suitable blank. Various concentrations of quercetin (Sigma-Aldrich, USA) were used for the standard calibration curve preparation. Results were expressed as μg quercetin equivalent/mg microencapsulated mulberry extract.

2.5. Determination of Free Radical Scavenging Activities. The scavenging activity against free radicals of the encapsulated mulberry extract was assessed via 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays. For DPPH assay, an aliquot of 0.15 mM DPPH in methanol (180 μl) (Sigma-Aldrich, USA) was mixed with 20 μl of various concentrations of encapsulated mulberry extract and incubated for 30 minutes. The absorbance was measured against blank at 517 nm via microplate reader (iMark Microplate Absorbance Reader) [15, 16]. Ascorbic acid was used as positive control. Results were expressed in terms of EC_{50} (concentration in micrograms per milliliter required to inhibit DPPH radical formation by 50%).

The determination of antioxidant activity via FRAP assay is based on the ability of the tested substance to convert ferric tripyridyltriazine (Fe^{3+}-TPTZ) to ferrous tripyridyltriazine (Fe^{2+}-TPTZ). FRAP working solution containing 10 mM TPTZ (Sigma-Aldrich, USA), 20 mM ferric chloride solution ($FeCl_3$) (Sigma-Aldrich, USA), and 300 mM acetate buffer (Sigma-Aldrich, USA) at the volume of 190 μl was mixed with 10 μl of encapsulated mulberry extract. After a 10-minute incubation at 37°C, the solution was determined for the absorbance against blank at 593 nm [17]. Ascorbic was used as positive control, and results were expressed as EC_{50} value.

The 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was also used to determine the free radical-scavenging activity of the encapsulated mulberry extract [18]. In brief, various concentrations of the encapsulated mulberry extract (30 μl) were reacted with the stock solution containing 7 mM ABTS (Sigma-Aldrich, USA) and 2.45 mM potassium persulfate (Sigma-Aldrich, USA; freshly prepared). The absorbance was measured at 734 nm.

The antioxidant activity of each sample was expressed in terms of EC_{50} (concentration in micrograms per milliliter required to inhibit ABTS radical formation by 50%) calculated from the log-dose inhibition curve.

2.6. Measurement of Acetylcholinesterase Inhibitory (AChEI) Activity. AChE suppression activity of the encapsulated mulberry was determined by colorimetric method according to the method of Elmann et al. [19]. This method is based on the determination of a yellow color of 5,5′-dithiobis (2-nitrobenzoic acid) produced by the hydrolysis of acetylcholine by acetylcholinesterase (AChE). In brief, various concentrations of encapsulated mulberry extract at the volume of 25 μl each were added to the reaction mixture containing 50 μl of Tris-HCl (50 mM, pH 8.0) (Sigma-Aldrich, USA), 75 μl of 3 mM 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) (Sigma-Aldrich, USA), and 25 μl of 15 mM thiocholine iodide (ATCI) (Sigma-Aldrich, USA) and 25 μl of AChE (0.22 U/ml) (Sigma-Aldrich, USA). After mixing, the reaction mixture was incubated at room temperature for 5 minutes, and the absorbance at 412 nm was recorded with microplate reader (iMark Microplate Absorbance Reader). Percentage of inhibition was calculated by comparing the rate of enzymatic hydrolysis of ATCI for the samples to that of the blank (50% aqueous methanol in buffer). Donepezil (1–32 mM) (ARICEPT®, USA) was used as a reference standard. The AChE inhibition activity of each sample was expressed in terms of EC_{50}. Every sample was assessed in triplicate.

2.7. Finger Print Chromatogram Assessment. The phenolic profiles of mulberry extract and encapsulated mulberry extract consisting of cyanidin-3-glucoside (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), and quercetin-3-O-rutinoside (Sigma-Aldrich, USA) were determined by high-performance liquid chromatography (HPLC). Chromatography was performed by using a Waters® system equipped with a Waters 2998 photodiode array detector. Chromatographic separation was performed using Purospher® STAR, C-18 encapped (5 μm), LiChroCART® 250-4.6, and HPLC-Cartridge, Sorbet Lot number HX255346 (Merk, Germany). The mobile phase (HPLC grade) consisted of 100% methanol (solvent A) (Fisher Scientific, USA) and 2.5% acetic acid (solvent B) (Fisher Scientific, USA) in deionized (DI) water was used to induce gradient elution. The gradient elution was carried out at a flow rate of 1.0 ml/min with the following gradient: 0–17 min, 70% A; 18–20 min, 100% A; and 20.5–25 min, 10% A. The sample was filtered (0.45 μm, Millipore), and a direct injection of tested sample at the volume of 20 μl on the column was performed. The chromatograms were recorded at 280 nm using UV detector, and data analysis was performed using EmpowerTM3.

2.8. Experimental Protocol. Female Wistar rats (weighing 200–250 g, 10 weeks old) were obtained from National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. The rats were kept under standard laboratory conditions with food and water ad libitum and housed in standard metal cages (5 per cage). Temperature was controlled at 23 ± 2°C on 12 : 12 h light-dark cycle. All procedures and experimental protocols were approved by the Institutional Animal Ethics Committee of Khon Kaen University (Record number AEKKU 27/2017). After 1 week of acclimatization, the animals were divided into 7 groups as follows:

(a) Group I (normal diet (ND) + vehicle): all rats in this group received normal diet (4.5% fat, 42% carbohydrate, and 24%protein) and were treated with vehicle.

(b) Group II (HCHF + vehicle): all animals in this group received HCHF diet and treated with vehicle.

(c) Group III (OVX-HCHF diet + vehicle): all rats in this group were subjected to bilateral ovariectomy (OVX), received HCHF diet, and treated with vehicle.

(d) Group IV (OVX-HCHF diet + isoflavone): rats had been subjected to bilateral ovariectomy, fed with HCHF diet, and treated with isoflavone at a dose of 15 mg/kg BW.

(e) Group V-VII (OVX-HCHF diet + microencapsulated mulberry extract (MME)): all rats in these groups were subjected to OVX, received HCHF diet, and treated with MME at various doses ranging from 10, 50, and 250 mg/kg BW.

In this study, all OVX rats were anesthetized with thiopental sodium at a dose of 40 mg/kg BW prior to the induction of experimental menopause by bilateral ovariectomy. After 1 week of operation, OVX rats in group III–group VII were fed with high-carbohydrate high-fat diet (HCHF; 35.83% fat, 35.54% carbohydrate, and 28.63% protein) in order to induce metabolic syndrome. After 20 weeks of the feeding period, rats which showed percent change of body weight more than 25 percent, the homeostasis model assessment-estimated insulin resistance (HOMA-IR) index and plasma angiotensin-converting enzyme levels higher than the control group were selected for further study. Then, the recruited animals were randomly assigned for the interventions including vehicle, isoflavone, and MME at various doses ranging from 10, 50, and 250 mg/kg BW. All animals were assessed for spatial memory after the single dose of treatment and at the end of 8-week intervention period. In addition, neuron density, the oxidative stress status, AChE, and the expression of Erk signal transduction in the hippocampus were also investigated at the end of the study.

2.9. Morris Water Maze Test. The water maze is a metal circle pool at diameter of 147 cm and 60 cm in depth, and water (25 ± 1°C) was filled with white nontoxic milk powder to a depth of 40 cm. It was divided into 4 equal quadrants (Northeast, Southeast, Southwest, and Northwest) by 2 imaginary lines crossing the center of the pool. A removable platform was immersed in the center of one quadrant. Each animal was trained to memorize the location of the platform by forming the association information between its location and the location of platform by using external cues. After 4 training sessions, the animals were determined the time which required for finding the platform and climbed onto the platform or escape latency. The retention time was also determined by exposing the animals to the same condition except that the platform was

removed from the location previously immersed. In this case, the time which the animals spend swimming in the quadrant which previously contained the platform was regarded as retention time that was recorded.

2.10. Histological Procedure and Nissl Staining. The brains were perfused transcardially with fixative solution containing 4% paraformaldehyde (Sigma-Aldrich, USA) in 0.1 M phosphate buffer pH 7.4 overnight at 4°C. Then, they were infiltrated with 30% sucrose (Merck, Germany) solution for 48–72 h. Serial sections of tissues were cut frozen on cryostat (Thermo Scientific™ HM 525 Cryostat) at 10 μm thick. All sections were picked up on slides coated with 0.3% aqueous solution of gelatin containing 0.05% aluminum potassium sulfate (Sigma-Aldrich, USA). The triplicate coronal sections of the brains were stained with 0.25% cresyl violet (Sigma-Aldrich, USA), dehydrated through graded alcohols (70, 95, 100% 2x) (RCI LabScan, Thailand), placed in xylene (Merck, Germany), and mounted using DPX mountant (Merck, Germany). The evaluation of neuron density in the hippocampus was performed under Olympus light microscope model BH-2 (Japan) at 40x magnification. Counts were performed in three adjacent fields, and the mean number was calculated and expressed as the density of neurons per 255 μm^2.

2.11. Assessment of Oxidative Stress Status and Acetylcholine Activity in Hippocampus. At the end of the study, all animals were sacrificed. Anterior hippocampus was isolated, prepared as hippocampal homogenate with 50 volume of 0.1 M phosphate buffer saline. Then, the homogenate was used for the determination of the acetylcholinesterase (AChE) activity and oxidative status including malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). The protein concentration in brain homogenate was determined by using a Thermo Scientific NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and measured the optical density at the wavelength of 280 nm.

The determination of AChE in brain homogenate was evaluated according to the spectrophotometric method of Elmann et al. with a slight modification. [19]. The mixture of 20 μl of sample solution, 200 μl of 0.1 mM sodium phosphate buffer (pH 8.0) (Sigma-Aldrich, USA), and 10 μl of 0.2 M DTNB (5,5′-dithio-bis-(2-nitrobenzoic acid)) (Sigma-Aldrich, USA) were mixed and incubated at room temperature for 5 minutes. Then, 10 μl of 15 mM acetylcholine thiochloride (ACTI) (Sigma-Aldrich, USA) was added and incubated for 3 minutes and recorded the absorbance at 412 nm by using microplate reader (iMark Microplate Absorbance Reader). The activity of AChE was calculated according to the equation below and expressed as nmol/min·mg protein.

$$\text{AChE activity} = \frac{\Delta A}{1.36 \times 10^4} \times \frac{1}{20/230} C, \quad (1)$$

where ΔA = the difference of absorbance/minute and C = protein concentration of brain homogenate.

The level of MDA level, a lipid peroxidation product, was also assessed. Hippocampal homogenate was determined by thiobarbituric acid reaction according to the method of Ohkawa et al. [20, 21]. The reaction mixture containing 50 μl of sample solution, 50 μl of 8.1% sodium dodecyl sulfate (SDS) (Sigma-Aldrich, USA), 375 μl of 0.8% of thiobarbituric acid (TBA) (Sigma-Aldrich, USA), 375 μl of 20% acetic acid (Sigma-Aldrich, USA), and 150 μl of distilled water (DW) was boiled at 95°C in the water bath for 60 minutes. After boiling, it was cooled with tap water. Then, 250 μl of DW and 1250 μl of the solution containing n-butanol and pyridine (Merck, Germany) at the ratio of 15 : 1 were added, mixed together, and centrifuged at 4000 rpm for 10 minutes. The upper layer was separated and measured the absorbance at 532 nm. 1,1,3,3-tetramethoxy propane (0–15 μM) (Sigma-Aldrich, USA) was served as standard, and the level of MDA was expressed as ng/mg protein.

The determination of SOD activity was performed according to the method of Sun et al. [22]. In brief, the assay mixture containing 57 mM phosphate buffer solution (KH_2PO_4) (Sigma-Aldrich, USA), 0.1 mM EDTA (Sigma-Aldrich, USA), 10 mM cytochrome C (Sigma-Aldrich, USA) solution, and 50 μM of xanthine (Sigma-Aldrich, USA) solution at the volume of 200 μl was mixed with 20 μl of tissue sample. Then, 20 μl of xanthine oxidase (0.90 mU/ml) (Sigma-Aldrich, USA) solution was added and the absorbance at 415 nm was measured. SOD enzyme (Sigma-Aldrich, USA) activities at the concentrations of 0–25 units/ml were used as standard, and the results were expressed as units/mg protein.

Catalase activity was determined based on the ability of the enzyme to break down H_2O_2. In brief, 10 μl of sample was mixed with the reaction mixture containing 50 μl of 30 mM hydrogen peroxide (in 50 mM phosphate buffer, pH 7.0) (BDH Chemicals Ltd, UK), 25 μl of H_2SO_4 (Sigma-Aldrich, USA), and 150 μl of 5 mM $KMnO_4$ (Sigma-Aldrich, USA). After mixing thoroughly, the optical density was measured at 490 nm [23]. CAT enzyme (Sigma-Aldrich, USA) at the concentration range of 0–100 units/ml was used as standard, and the result was expressed as units/mg protein.

Glutathione peroxidase activity was also assessed. In brief, a mixture containing a 20 μl of sample solution and the reaction mixture consisting of 10 μl of 1 mM dithiothreitol (DTT) (Sigma-Aldrich, USA) in 6.67 mM potassium phosphate buffer (pH 7), 100 μl of 1 mM sodium azide (Sigma-Aldrich, USA) in 6.67 mM potassium phosphate buffer (pH 7), 10 μl of 50 mM glutathione (Sigma-Aldrich, USA) solution, and 100 μl of 30% hydrogen peroxide (BDH Chemicals Ltd, UK) were mixed thoroughly and incubated at room temperature for 5 minutes. Then, 10 μl of 10 mM DTNB (5,5-dithiobis-2-nitrobenzoic acid) (Sigma-Aldrich, USA) was added and the optical density at 412 nm was recorded at 25°C over a period of 5 min [24]. The standard calibration curve was prepared by using GSH-Px enzyme (Sigma-Aldrich, USA) at the concentration range of 0–5 units/ml. GSH-Px activity was expressed as units/mg protein.

2.12. Western Blotting Analysis. Anterior hippocampus of the brain was suspended and homogenized in mammalian protein extraction reagent (M-PER; Pierce Protein Biology

Table 1: The bioactive compounds and biological activities of microencapsulated mulberry fruit extract (MME).

Parameters	Mulberry fruit extract	Microencapsulated mulberry fruit extract	Standard reference
Total phenolic compounds (mg GAE/mg extract)	80.00 ± 0.98	103.89 ± 13.08	—
Flavonoids content (μg quercetin/mg extract)	8.89 ± 0.13	26.56 ± 1.26**	—
DPPH (EC_{50}, mg/ml)	2.56 ± 0.08	2.07 ± 0.79	0.03 ± 0.01, ascorbic acid
FRAP (EC_{50}, mg/ml)	280.04 ± 3.14	192.79 ± 15.94	21.04 ± 1.24, ascorbic acid
ABTS (EC_{50}, mg/ml)	4.08 ± 0.02	1.66 ± 0.08**	0.20 ± 0.002, Trolox
AChEI (EC_{50}, mg/ml)	0.07 ± 0.002	0.05 ± 0.003*	0.02 ± 0.001, donepezil

Data are presented as mean ± SEM. *p value <0.05 and **p value < 0.01, compared between MME and mulberry fruit extract.

Product, Rockford, IL, USA), with protease inhibitor cocktail (1 : 10) (Sigma-Aldrich, USA). Brain homogenate was subjected to a 12,000g centrifugation process for 10 minutes at 4°C. The supernatant was isolated and used for the determination of protein and Erk expression. Protein concentration was determined by using a Thermo Scientific NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). In addition, sixty micrograms of tissue lysates was adjusted to an appropriate concentration by using Tris-Glycine SDS-PAGE loading buffer (Bio-Rad, USA) and heated at 95°C for 10 minutes. Protein in tissue sample was isolated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by loading 20 μl of tissue sample on SDS-polyacrylamide gel. Then, the separated bands were transferred to nitrocellulose membrane, washed with 0.05% TBS-T, and incubated in blocking buffer (5% skim milk in 0.1% TBS-T) at room temperature for 1 hour. After the blocking process, the nitrocellulose membrane was incubated with anti-phospho-Erk1/2 (Thr202/Tyr204) (Cell Signaling Technology, USA; dilution 1 : 1000), anti-Erk1/2 (Cell Signaling Technology, USA; dilution 1 : 1000) antibodies at room temperature for 2 hours. The nitrocellulose membrane was rinsed with TBS-T (0.05%) again and incubated with anti-rabbit IgG, HRP-linked antibody (Cell Signaling Technology, USA; dilution 1 : 2000) at room temperature for 1 hour. The bands were visualized and quantitated by using the ECL detection systems (GE Healthcare) and LAS-4000 luminescent image analyzer (GE Healthcare). Band intensities were measured for statistical analysis using ImageQuant TL v.7.0 image analysis software (GE Healthcare). The expression was normalized using anti-total ERK1/2. Data were presented as a relative density to control normal diet group.

2.13. Statistical Analysis. All data are expressed as mean ± standard error of mean (SEM). Statistical significance was evaluated by using one-way analysis of variance (ANOVA), followed by Duncan's post hoc test. The statistical significance was regarded at p values < 0.05.

3. Results

3.1. Assessment of Bioactive Compounds and Biological Activities. Table 1 showed that the contents of polyphenolic compounds and flavonoids in mulberry fruit extract were 80 ± 0.98 mg of gallic acid equivalent (GAE)/mg sample extract and 8.89 ± 0.13 μg quercetin/mg sample extract whereas the contents of the substances just mentioned in microencapsulated mulberry fruit extract (MME) were 103.89 ± 13.08 mg GAE/mg sample extract and 26.56 ± 1.26 μg quercetin/mg sample extract. The flavonoid content in MME is significantly higher than that in mulberry fruit extract (p value < 0.01, compared between MME and mulberry fruit extract). Half maximal effective concentration (EC_{50}) of antioxidant evaluated via DPPH, FRAP, and ABTS assays of mulberry fruit extract was 2.56 ± 0.08, 280.04 ± 3.14, and 4.08 ± 0.02 mg/ml, respectively. EC_{50} of antioxidant activity of MME assessed by DPPH, FRAP, and ABTS were 2.07 ± 0.79, 192.79 ± 15.94, and 1.66 ± 0.08 mg/ml. Although MME shows the potent antioxidant activity than mulberry extract, the significant difference was observed only the EC_{50} assessed via ABTS assay (p value < 0.01, compared between MME and mulberry fruit extract). In addition, the EC_{50} of antioxidant effect of MME assessed via the mentioned methods was very much higher than the reference. EC_{50} of AChEI activity of a mulberry fruit extract was 0.07 ± 0.002 while that of MME was 0.05 ± 0.003 mg/ml. Interestingly, EC_{50} of AChEI of MME was significantly lower than that of mulberry extract (p value < 0.05, compared between MME and mulberry fruit extract). In addition, this value is close to the value of donepezil, a standard drug used for treating dementia.

3.2. The Finger Print of Microencapsulated Mulberry Fruit Extract. Figure 1 shows the fingerprint chromatogram of mulberry fruit extract and microencapsulated mulberry fruit extract (MME). The fingerprint chromatogram analysis obtained from this study revealed that mulberry fruit extract of 200 milligrams contained 253.04 ± 3.92 μg cyanidin-3-glucoside, 10.81 ± 0.29 μg gallic acid, and 265.84 ± 17.66 μg quercetin-3-O-rutinoside whereas MME of 200 milligrams contained of 293.62 ± 4.90 μg cyanidin-3-glucoside, 9.08 ± 0.09 μg gallic acid, and 243.51 ± 5.88 μg quercetin-3-O-rutinoside. However, no significant difference in gallic acid and quercetin-3-O-rutinoside contents between MME and mulberry fruit extract was observed (p value > 0.05). Interestingly, the cyanidin-3-glucoside content in MME is significantly higher than that in mulberry fruit extract (p value < 0.05, compared between MME and mulberry fruit extract).

3.3. Effect of MME on Spatial Memory. The effect of MME on escape latency was shown in Figure 2(a). After the single

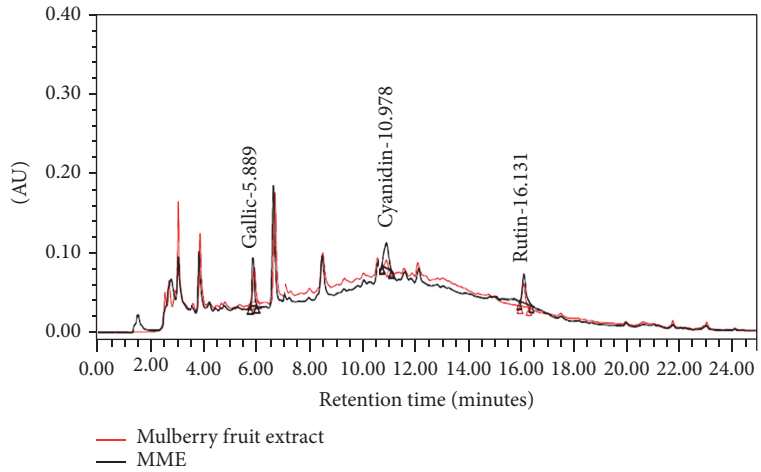


Figure 1: The fingerprint chromatogram of mulberry fruit extract and microencapsulated mulberry fruit extract (MME).

administration, no significant differences among groups were observed. When compared between rats which received HCHF diet and those which received normal diet, no significant change in escape latency was observed after 8 weeks of treatment. However, HCHF diet induced the increased escape latency in OVX rats (p value < 0.05, compared to control rats which received normal diet and vehicle). In addition, OVX rats which received HCHF diet and received isoflavone or received MME at doses of 10, 50, and 250 mg/kg significantly decreased escape latency (p value < 0.05 all, compared to OVX rats which received HCHF diet and vehicle).

Figure 2(b) shows the effect of MME on retention time. After the single administration, HCHF diet failed to produce the significant changes of retention time in both normal and OVX rats. OVX rats which received a high dose of MME significantly increased retention time after the single administration (p value < 0.01, compared to OVX rats which received HCHF and vehicle). After 8 weeks of intervention, it was found that HCHF failed to produce the significant change in retention time in normal rats which received vehicle. However, OVX rats which received HCHF diet significantly decreased retention time (p value < 0.01, compared to normal rats which received vehicle; p value < 0.001, compared to normal rats which received HCHF diet). Both OVX rats which fed with HCHF diet and received either isoflavone or MME (all doses used in this study) significantly enhanced retention time (p value < 0.01, 0.001, 0.001, and 0.001, respectively, compared to OVX which received HCHF diet and vehicle). Interestingly, MME at a dose of 250 mg/kg also enhanced retention time better than normal rats which received vehicle.

3.4. Effect of MME on Hippocampal Neuron. To further explore the possible underlying mechanism of the memory-enhancing effect of MME, the plasticity of the hippocampus was assessed via the assessment of the neuron density in CA1, CA2, CA3, and the dentate gyrus of the hippocampus (Figure 3). It was found that normal rats which received HCHF diet significantly decreased neuron density in CA1, CA2, CA3, and dentate gyrus (p value < 0.001, 0.001, 0.01, and 0.001, respectively, compared to normal rats which received normal diet and vehicle). OVX rats which received HCHF diet also showed the reduction of neuron density in all subregions mentioned earlier (p value < 0.001, 0.01, 0.01, and 0.001, respectively, compared to normal rats which received normal diet and vehicle). HCHF diet decreased the neuron density in CA2 of OVX rats higher than normal rats (p value < 0.05, compared between OVX rats which received HCHF diet and normal rats which received HCHF diet). Isoflavone could counteract the reduction of neuron density in CA1, CA2, CA3, and dentate gyrus in OVX rats which fed with HCHF diet (p value < 0.05, 0.01, 0.05, and 0.01, respectively, compared to OVX rats which received HCHF diet and vehicle). MME at all doses used in this study also enhanced neuron density in all subregions just mentioned in OVX rats which received HCHF diet (p value < 0.01, 0.05, and 0.001; p value < 0.001 all; p value < 0.01, 0.01, and 0.001; 0.01, 0.001, and 0.001, respectively, compared to OVX rats which received HCHF diet and vehicle). Interestingly, the increased neuron density in CA2 and CA3 induced by high dose of MME and the increased neuron density in CA2 induced by the low dose of MME were higher than the neuron density in the normal healthy rats which received normal diet and vehicle.

3.5. Effect of MME on Oxidative Stress Status and AChE in the Hippocampus. Table 2 shows the effect of MME on AChE activity and oxidative stress markers including MDA level and the activities of SOD, CAT, and GSH-Px. In this study, the effect of MME on AChE was assessed in order to indirectly indicate the effect on the available acetylcholine (ACh) and cholinergic function. The current data showed that HCHF diet failed to produce the significant change of AChE in normal rats but it significantly increased AChE activity in the hippocampus of OVX rats (p value < 0.001, compared to normal rats which received normal diet and vehicle; p value < 0.001, compared to normal rats which received HCHF diet and vehicle). OVX rats which received HCHF diet and isoflavone significantly decreased AChE activity in the hippocampus (p value < 0.01, compared to

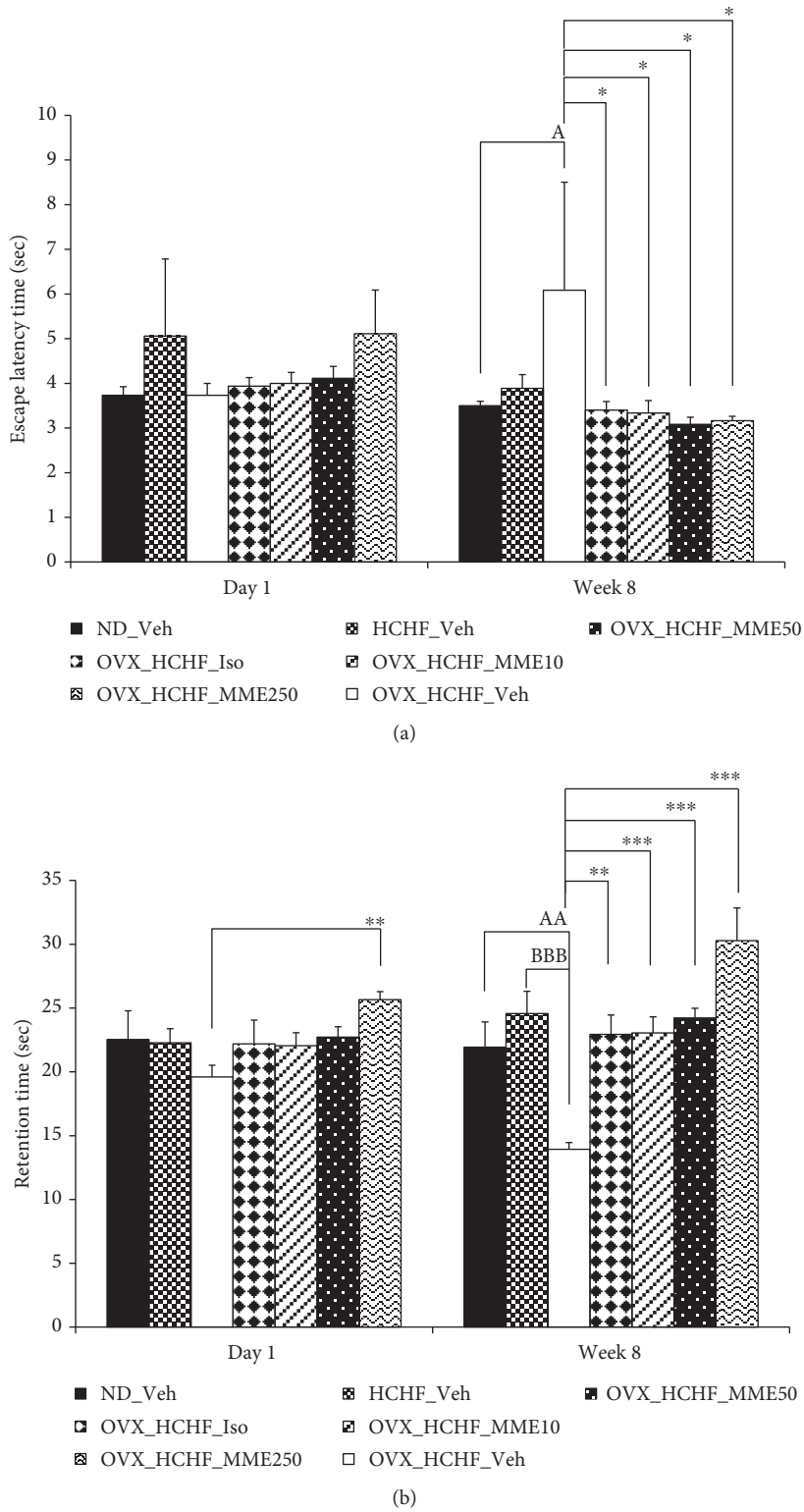
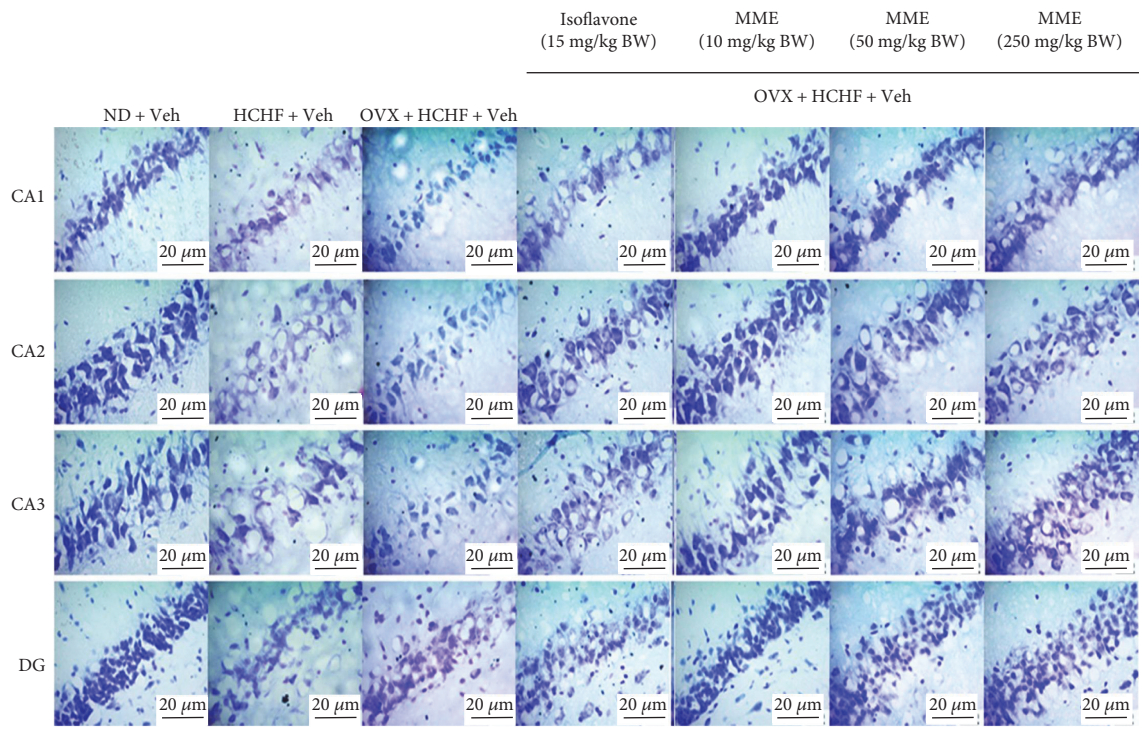
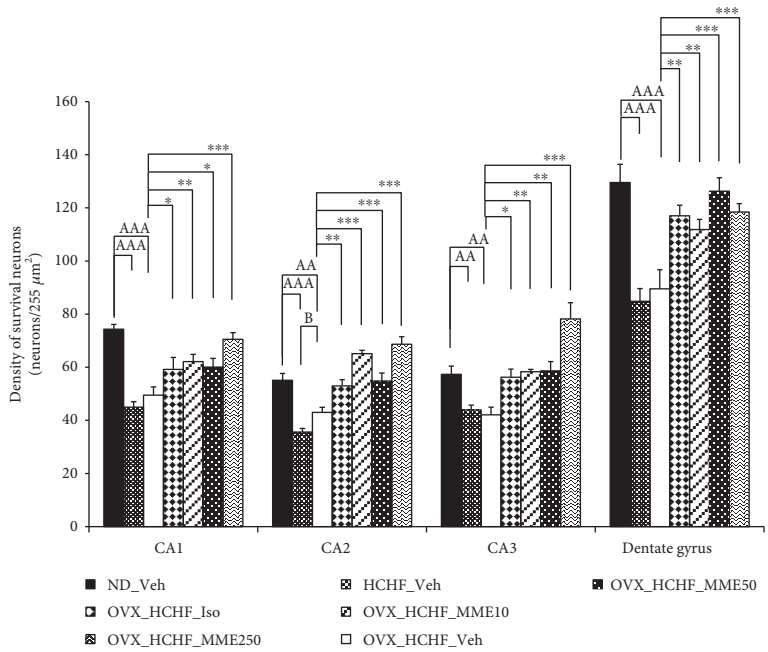


Figure 2: Effect of microencapsulated mulberry fruit extract (MME) on memory impairment via Morris Water Maze test ((a) escape latency time, (b) retention time). Data are presented as mean ± SEM ($n = 6$/group). ^{a}p value < 0.05 and ^{aa}p value < 0.01, compared to control rats which received normal diet and vehicle; ^{bbb}p value < 0.001, compared to normal rats which received HCHF diet and vehicle; and $^{*}p$ value < 0.05, $^{**}p$ value < 0.01, $^{***}p$ value < 0.001, compared to OVX rats which received HCHF and vehicle. ND: normal diet; HCHF: high-carbohydrate high-fat diet; OVX-HCHF: ovariectomized plus high-carbohydrate high-fat diet; Iso: the isoflavone at dose of 15 mg·kg^{-1} BW; MME10, 50, and 250: the microencapsulated mulberry fruit extract at dose of 10, 50, and 250 mg·kg^{-1} BW, respectively.



(a)



(b)

Figure 3: Effect of microencapsulated mulberry fruit extract on density of survival neurons in the various subregions of the hippocampus. (a) Light microscope of coronal sections in CA1, CA2, CA3, and dentate gyrus of the hippocampus were stained with cresyl violet at 40x magnification. (b) Density of survival neurons in CA1, CA2, CA3, and dentate gyrus of the hippocampus. Data are presented as mean ± SEM ($n = 6$/group). ^{aa}p value < 0.01 and ^{aaa}p value < 0.001, compared to control rats which received normal diet and vehicle; ^{b}p value < 0.05, compared to normal rats which received HCHF diet and vehicle; and $^{*}p$ value < 0.05, $^{**}p$ value < 0.01, $^{***}p$ value < 0.001, compared to OVX rats which received HCHF and vehicle. ND: normal diet; HCHF: high-carbohydrate high-fat diet; OVX-HCHF: ovariectomized plus high-carbohydrate high-fat diet; Iso: the isoflavone at dose of 15 mg·kg^{-1} BW; MME10, 50, and 250: the microencapsulated mulberry fruit extract at dose of 10, 50, and 250 mg·kg^{-1} BW, respectively.

Table 2: The effect of various doses of MME on acetylcholineseterase activity and oxidative stress markers in the hippocampus.

Treatment group	AChE activity (nmol/min·mg protein)	MDA (ng/mg protein)	SOD (units/mg protein)	CAT (units/mg protein)	GSH-Px (units/mg protein)
ND + vehicle	0.14 ± 0.01	4.38 ± 1.41	59.28 ± 6.40	780.07 ± 104.63	10.86 ± 1.04
HCHF + vehicle	0.17 ± 0.04	14.27 ± 2.36^{aaa}	47.39 ± 5.86^{a}	517.12 ± 53.99^{aa}	7.29 ± 0.96^{aa}
OVX + HCHF + vehicle	$0.43 \pm 0.12^{aaa,bbb}$	11.85 ± 0.85^{aa}	$14.28 \pm 0.82^{aaa,bbb}$	$201.80 \pm 12.04^{aaa,bbb}$	$3.64 \pm 0.52^{aaa,bbb}$
OVX + HCHF + isoflavone 15 mg/kg BW	$0.19 \pm 0.03^{**}$	$7.05 \pm 0.69^{*}$	$30.74 \pm 2.71^{*}$	$411.66 \pm 37.81^{*}$	4.72 ± 0.42
OVX + HCHF + MME 10 mg/kg BW	$0.20 \pm 0.03^{**}$	$6.51 \pm 0.98^{*}$	$38.15 \pm 2.53^{***}$	$446.31 \pm 51.80^{**}$	$6.94 \pm 0.69^{**}$
OVX + HCHF + MME 50 mg/kg BW	$0.20 \pm 0.03^{**}$	$4.93 \pm 0.61^{**}$	$30.07 \pm 0.56^{*}$	$427.58 \pm 15.64^{**}$	$6.06 \pm 0.36^{*}$
OVX + HCHF + MME 250 mg/kg BW	$0.26 \pm 0.02^{*}$	$5.06 \pm 0.69^{**}$	$31.40 \pm 1.64^{**}$	$464.92 \pm 38.16^{**}$	$7.27 \pm 0.43^{**}$

Data are presented as mean ± SEM ($n = 6$/group). ^{a}p value < 0.05, ^{aa}p value < 0.01, ^{aaa}p value < 0.001, compared to control rats which received normal diet and vehicle, ^{bbb}p value < 0.001, compared to normal rats which received HCHF diet and vehicle and $^{*}p$ value < 0.05, $^{**}p$ value < 0.01, $^{***}p$ value < 0.001, compared to OVX rats which received HCHF and vehicle.

OVX rats which received HCHF diet and vehicle). The reduction of AChE in this area was also observed in OVX rats which received MME at all doses used in this study (p value < 0.01, 0.01, and 0.05, respectively, compared to OVX rats which received HCHF diet and vehicle). The decreased activities of SOD, CAT, and GSH-Px (p value < 0.001 all, compared to normal rats which received normal diet and vehicle; p value < 0.001 all, compared to normal rats which received HCHF diet and vehicle) together with the increased MDA level (p value < 0.01, compared to normal rats which received normal diet) were also observed in OVX rats which received HCHF diet. Isoflavone treatment significantly increased SOD and CAT activities but decreased MDA level in OVX rats which received HCHF (p value < 0.05 all, compared to OVX rats which received HCHF diet and vehicle). MME at all doses used in this study also increased SOD (p value < 0.001, 0.05, and 0.01, respectively, compared to OVX rats which received HCHF diet and vehicle), CAT (p value < 0.01 all, compared to OVX rats which received HCHF diet and vehicle), and GSH-Px (p value < 0.01, 0.05, and 0.01, respectively, compared to OVX rats which received HCHF diet and vehicle) but decreased MDA level (p value < 0.05, 0.01, and 0.01, respectively, compared to OVX rats which received HCHF diet and vehicle) in hippocampus.

3.6. Effect of MME on the Phosphorylation of Erk in the Hippocampus. Figure 4 shows the effect of MME on the phosphorylation of Erk in the hippocampus. It was found that HCHF diet significantly decreased the phosphorylation of Erk in the hippocampus of both normal rats and OVX rats (p value < 0.001, compared to normal rats which received normal diet and vehicle). However, the magnitude of change in OVX rats was higher than that in normal rats (p value < 0.05, compared between normal rats which received HCHF diet and vehicle and OVX rats which received HCHF diet and vehicle). In addition, isoflavone and all doses of MME could counteract the reduction of Erk phosphorylation induced by HCHF diet in OVX rats (p value < 0.001, compared to OVX rats which received HCHF diet and vehicle).

4. Discussion

The present study has demonstrated that encapsulated mulberry fruit extract contains more polyphenolic compounds and flavonoid content. In addition, the biological activity including antioxidant and AChEI activities of the encapsulated mulberry fruit extract is also better than that of the mulberry fruit extract. In this study, both the mulberry fruit extract and encapsulated mulberry fruit extract were determined the contents of phenolic compounds and flavonoids 2 weeks after the preparation. Based on the antioxidant and AChEI of polyphenol [25, 26], the loss of some phenolic compounds and flavonoid contents during storage may possibly involve with the different contents of both substances mentioned earlier. The loss of phenolic compounds and flavonoid contents of mulberry fruit extract during storage due to environmental factors such as light and temperature [27, 28] may probably be more than the losses of the mentioned substances of encapsulated mulberry fruit giving rise to the higher contents of both substances in encapsulated mulberry fruit extract than that in mulberry fruit extract.

Our data also clearly demonstrates that the encapsulated mulberry fruit extract significantly improves spatial memory in an animal model of menopause with metabolic syndrome. Interestingly, the high dose of encapsulated mulberry fruit extract can significantly enhance retention time after the single administration while isoflavone fails to show the positive modulation effect on this parameter after the single-dose administration. However, at the end of the 8-week intervention period, isoflavone and all doses of encapsulated mulberry fruit extract used in this study show the positive modulation effect on spatial memory. Since the hippocampus is the brain area that plays an important role in spatial memory [29, 30], the changes of parameters contributing the important roles on learning and memory such as ACh [31]

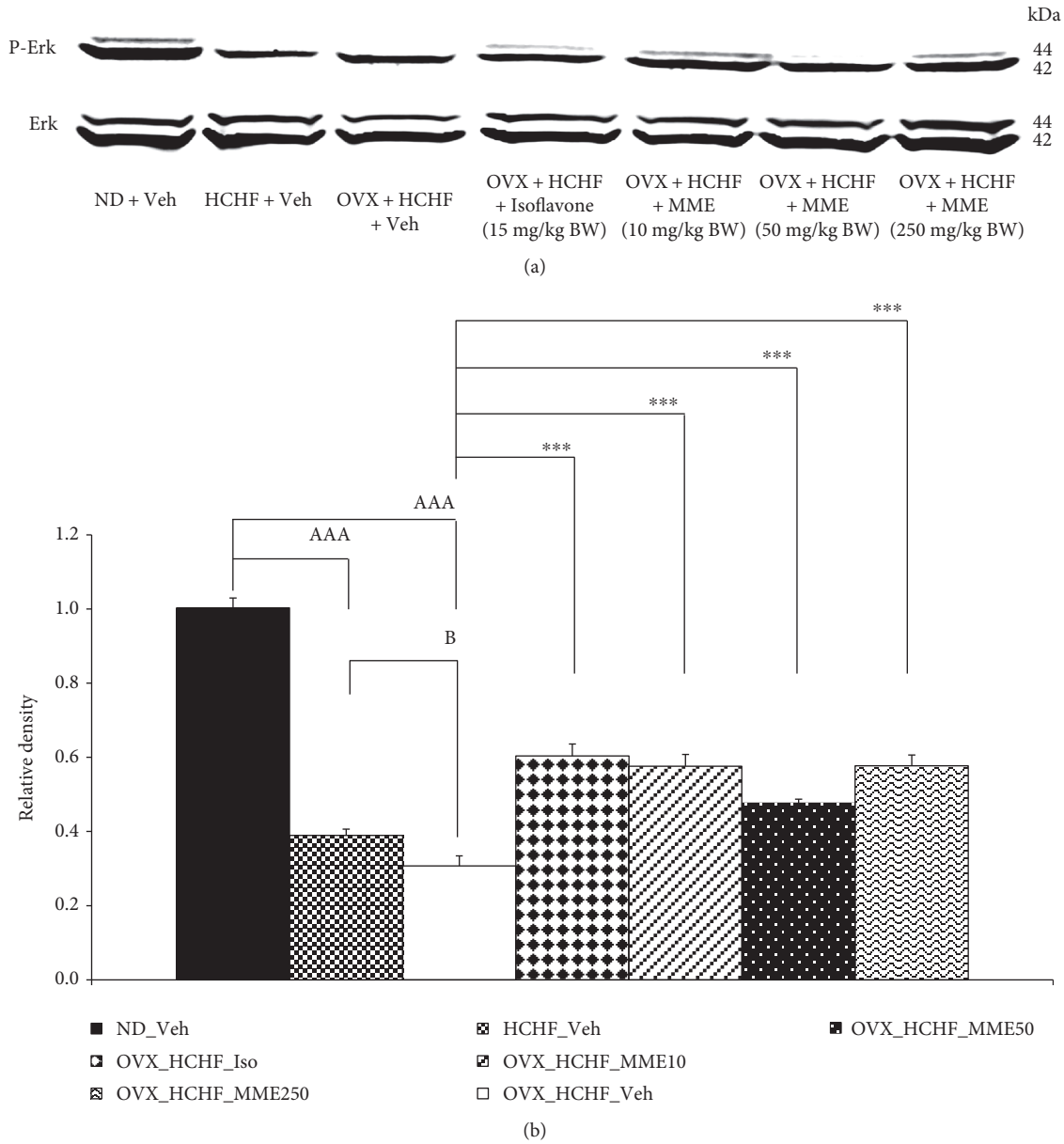


Figure 4: Effect of various doses of microencapsulated mulberry fruit extract on the relative density of total P-Erk/Erk in the hippocampus was detected by Western blotting (a) and quantitatively analysis (b). The levels of total P-Erk were normalized against the level of total Erk. Their relative phosphorylation levels (active form) were calculated against those of control normal diet plus vehicle rats. Data are presented as mean ± SEM ($n = 6$/group). ^{aaa}p value < 0.001, compared to control rats which received normal diet and vehicle, ^{b}p value < 0.05, compared to normal rats which received HCHF diet and vehicle, and $^{***}p$ value < 0.001, compared to OVX rats which received HCHF and vehicle. ND: normal diet; HCHF: high-carbohydrate high-fat diet; OVX-HCHF: ovariectomized plus high-carbohydrate high-fat diet; MME10, 50, and 250: the microencapsulated mulberry fruit extract at dose of 10, 50, and 250 mg·kg^{-1} BW, respectively.

and oxidative stress [32] were also investigated in this area. It has been found that in accompany with the improved spatial memory, the decreased AChE and MDA level together with the elevations of SOD, CAT, and GSH-Px were also present. Accumulative lines of evidence have shown that oxidative stress status in the hippocampus plays a pivotal role on the neuron survival in the hippocampus and memory performance [10, 29, 30]. Therefore, the improved learning and memory impairment observed in this study may occur partly via the improved function of cholinergic system via the increase of ACh and the decreased oxidative stress status in the hippocampus.

A pile of evidence has demonstrated that the brain plasticity which contributes a crucial role on learning and memory is associated with the neuron density in the hippocampus area [10, 33]. Therefore, the effects of encapsulated mulberry fruit extract on neuron densities in CA1, CA2, CA3, and dentate gyrus of the hippocampus were also explored. Interestingly, all doses of encapsulated mulberry fruit extract enhanced neuron density in all areas mentioned

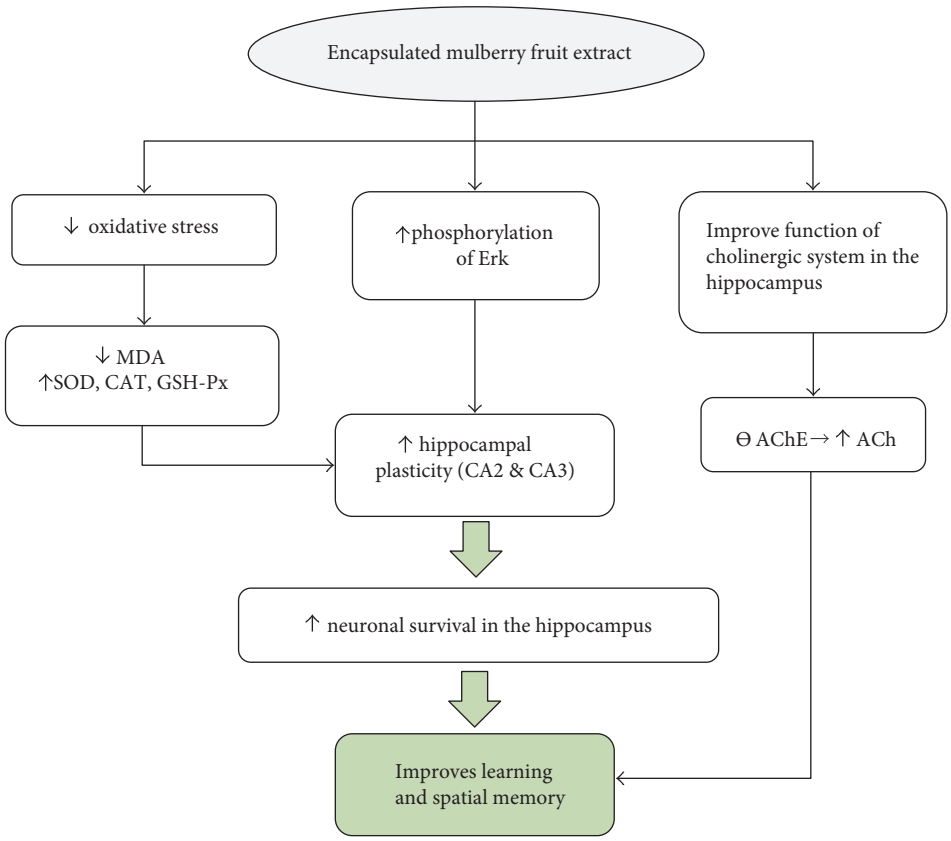


Figure 5: The possible underlying mechanism for the neuroprotective and cognitive-enhancing effect of encapsulated mulberry fruit extract on an animal model of menopause with metabolic syndrome.

earlier. It has been reported that the phosphorylation of extracellular signal-regulated kinase (ERK) also plays an important role on the neuroprotection and brain plasticity [34] so we also investigated the effect of encapsulated mulberry fruit extract on the phosphorylation of Erk. Our data have revealed that encapsulated mulberry fruit extract-treated rats also increased the phosphorylation of Erk. Taken all data together, we suggested that the increased brain plasticity which occurred via the increased phosphorylation of Erk might also play a role on the memory-enhancing effect of encapsulated mulberry fruit extract. Since, the increased neuron densities induced by MME at low and high doses of MME in CA2 and CA3 are higher than those in normal rats, we suggest that MME at the mentioned dose might also increase neurogenesis in the mentioned areas. However, further exploration is required to confirm this event.

No dose-dependent responses were observed in this study because the memory performance depended on many factors, so no simple linear relationship between the concentrations of the tested substance and the observed parameters was observed. In addition, the tested substance contained numerous ingredients and the effect of active ingredient can be masked by the others.

Based on the high contents of phenolic compounds such as cyanidin-3-glucoside, gallic acid, and quercetin-3-O-rutinoside in microencapsulated mulberry fruit extract together with the neuroprotection and memory-enhancing effects of the substances just mentioned [35–39], the neuroprotection and memory-enhancing effects of microencapsulated mulberry fruit extract observed in this study might be related to the contents of aforementioned phenolic compounds. However, the modulation effect induced by the interaction effects among various ingredients may also play the roles. Therefore, the precise understanding concerning this aspect required further study.

In summary, Figure 5 shows that MME exerts the memory-enhancing effect via multiple targets including the improved cholinergic function and the brain plasticity. MME can suppress AChE in the hippocampus which in turn improves cholinergic function in this area resulting in the improved memory performance. Moreover, MME also enhances plasticity of the hippocampus via both the decreased oxidative stress status and the increased Erk phosphorylation and finally giving rise to the improved memory. Since the low and high doses of MME can also increase neuron density in CA2 and CA3 than that in normal rats, MME at the doses mentioned earlier may probably increase neurogenesis in both subregions mentioned earlier. However, this point also requires further exploration.

5. Conclusion

Encapsulated mulberry fruit extract shows higher stability of phenolic compounds including flavonoids giving rise to the potent antioxidant and AChEI. The current preclinical data also clearly show that it improves hippocampal plasticity

and memory impairment in an animal model of experimental menopause with metabolic syndrome. Therefore, it may be served as the potential candidate for neuroprotectant and memory enhancer for menopausal women with metabolic syndrome. However, further researches concerning the possible active ingredients, the effect on neurogenesis, and clinical trial are still essential.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by Integrative Complementary Alternative Medicine Research and Development Center, Khon Kaen University, Khon Kaen, Thailand, Invitation Research Grant of Faculty of Medicine and Graduate School of Khon Kaen University (Grant no. IN59130), Thailand Research Fund via Research and Researchers for Industries (RRI) project, and The National Research Council of Thailand. The authors also would like to express their sincere thanks to Mr. Wiroje Kaewrueng and Queen Sirikit Department of Sericulture Center, Udon Thani Province, for providing mulberry fruit.

References

[1] E. Barrett-Connor, "Epidemiology and the menopause: a global overview," *International Journal of Fertility and Menopausal Studies*, vol. 38, Supplement 1, pp. 6–14, 1993.

[2] Y. Fu, Y. Yu, S. Wang et al., "Menopausal age and chronic diseases in elderly women: a cross-sectional study in Northeast China," *International Journal of Environmental Research and Public Health*, vol. 13, no. 10, p. 936, 2016.

[3] P. Chedraui, G. San Miguel, I. Vintimilla-Sigüenza et al., "The metabolic syndrome and its components in postmenopausal women," *Gynecological Endocrinology*, vol. 29, no. 6, pp. 563–568, 2013.

[4] S. Treviño, R. A. Vázquez-Roque, G. López-López et al., "Metabolic syndrome causes recognition impairments and reduced hippocampal neuronal plasticity in rats," *Journal of Chemical Neuroanatomy*, vol. 82, pp. 65–75, 2017.

[5] J. R. Sliwinski, A. K. Johnson, and G. R. Elkins, "Memory decline in peri- and post-menopausal women: the potential of mind-body medicine to improve cognitive performance," *Integrative Medicine Insights*, vol. 9, pp. 17–23, 2014.

[6] G. A. Greendale, R. G. Wight, M. H. Huang et al., "Menopause-associated symptoms and cognitive performance: results from the study of women's health across the nation," *American Journal of Epidemiology*, vol. 171, no. 11, pp. 1214–1224, 2010.

[7] E. G. Jacobs, B. K. Weiss, N. Makris et al., "Impact of sex and menopausal status on episodic memory circuitry in early midlife," *Journal of Neuroscience*, vol. 36, no. 39, pp. 10163–10173, 2016.

[8] J. Wei, G. Zhang, X. Zhang, J. Gao, J. Fan, and Z. Zhou, "Anthocyanin improving metabolic disorders in obese mice from *Aornia melanocarpa*," *Indian Journal of Pharmaceutical Education and Research*, vol. 50, no. 3, pp. 368–375, 2016.

[9] R. Krikorian, M. D. Shidler, T. A. Nash et al., "Blueberry supplementation improves memory in older adults," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 7, pp. 3996–4000, 2010.

[10] P. Kaewkaen, T. Tong-un, J. Wattanathorn, S. Muchimapura, W. Kaewrueng, and S. Wongcharoenwanakit, "Mulberry fruit extract protects against memory impairment and hippocampal damage in animal model of vascular dementia," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, Article ID 263520, 9 pages, 2012.

[11] M. Zacarías-Flores, M. A. Sánchez-Rodríguez, E. Correa-Muñoz, A. Arronte-Rosales, and V. M. Mendoza-Núñez, "Postmenopausal symptoms severity enhancement oxidative stress in women with metabolic syndrome," *Ginecologia y Obstetricia de México*, vol. 82, no. 12, pp. 796–806, 2014.

[12] P. G. Waterman and S. Mole, *Analysis of Phenolic Plant Metabolites*, Blackwell Scientific Oxford, United Kingdom, 1994.

[13] C. Quettier-Deleu, B. Gressier, J. Vasseur et al., "Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour," *Journal of Ethnopharmacology*, vol. 72, no. 1-2, pp. 35–42, 2000.

[14] A. Luximon-Ramma, T. Bahorun, M. A. Soobrattee, and O. I. Aruoma, "Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of *Cassia fistula*," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 18, pp. 5042–5047, 2002.

[15] M. A. Ebrahimzadeh, S. J. Hosseinimehr, A. Hamidinia, and M. Jafari, "Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves," *Pharmacology*, vol. 1, pp. 7–14, 2008.

[16] L. P. Leong and G. Shui, "An investigation of antioxidant capacity of fruits in Singapore markets," *Food Chemistry*, vol. 76, no. 1, pp. 69–75, 2002.

[17] I. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.

[18] N. J. Miller, C. Rice-Evans, M. J. Davies, V. Gopinathan, and A. Milner, "A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates," *Clinical Science*, vol. 84, no. 4, pp. 407–412, 1993.

[19] G. L. Elmann, K. D. Courtney, V. Andres Jr., and R. M. Featherstone, "A new and rapid colorimetric determination of acetylcholinesterase activity," *Biochemical Pharmacology*, vol. 7, no. 2, pp. 88–95, 1961.

[20] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Analytical Biochemistry*, vol. 95, no. 2, pp. 351–358, 1979.

[21] M. B. Harishekar and B. Kiran, "Studies on lipid peroxidation induced by lead, alcohol on liver and amelioration by vitamin E," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 4, no. 2, pp. 164–167, 2011.

[22] Y. Sun, L. W. Oberley, and Y. Li, "A simple method for clinical assay of superoxide dismutase," *Clinical Chemistry*, vol. 34, no. 3, pp. 497–500, 1988.

[23] L. Góth, "A simple method for determination of serum catalase activity and revision of reference range," *Clinica Chimica Acta*, vol. 196, no. 2-3, pp. 143–151, 1991.

[24] J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra, "Selenium: biochemical role as a component of glutathione peroxidase," *Science*, vol. 179, no. 4073, pp. 588–590, 1973.

[25] A. Scalbert, I. T. Johnson, and M. Saltmarsh, "Polyphenols: antioxidants and beyond," *American Journal of Clinical Nutrition*, vol. 81, 1 Supplement, pp. 215S–217S, 2005.

[26] L. B. Siero, A. P. Rauter, and M. L. M. Serralheiro, "Polyphenol as acetylcholinesterase inhibitors: structural specificity and impact on human diseases," *Nutrition and Aging*, vol. 1, no. 2, pp. 99–111, 2012.

[27] M. Shulman, M. Cohen, A. Soto-Gutierrez et al., "Enhancement of naringinin bioavailability by complexation with hydroxypropyl-β-cyclodextrin," *PLoS One*, vol. 6, no. 4, article e18033, 2011.

[28] A. M. Bakowska-Barczak and P. P. Kolodziejczyk, "Black currant polyphenols: their storage stability and microencapsulation," *Industrial Crops and Products*, vol. 34, no. 2, pp. 1301–1309, 2011.

[29] N. Sriraksa, J. Wattanathorn, S. Muchimapura, S. Tiamkao, K. Brown, and K. Chaisiwamongkol, "Cognitive-enhancing effect of quercetin in a rat model of parkinson's disease induced by 6-hydroxydopamine," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, Article ID 823206, 9 pages, 2012.

[30] P. Chonpathompikunlert, J. Wattanathorn, and S. Muchimapura, "Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease," *Food and Chemical Toxicology*, vol. 48, no. 3, pp. 798–802, 2010.

[31] M. E. Hasselmo, "The role of acetylcholine in learning and memory," *Current Opinion in Neurobiology*, vol. 16, no. 6, pp. 710–715, 2006.

[32] R. H. Silva, V. C. Abílio, A. L. Takatsu et al., "Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice," *Neuropharmacology*, vol. 46, no. 6, pp. 895–903, 2004.

[33] S. R. Burke and C. A. Barnes, "Neural plasticity in the ageing brain," *Nature Reviews Neuroscience*, vol. 7, no. 1, pp. 30–40, 2006.

[34] K. Jin, X. O. Mao, Y. Zhu, and D. A. Greenberg, "MEK and ERK protect hypoxic cortical neurons via phosphorylation of bad," *Journal of Neurochemistry*, vol. 80, no. 1, pp. 119–125, 2002.

[35] S. Nasri, M. Roghani, T. Baluchnejadmojarad, M. Balvardi, and T. Rabani, "Chronic cyanidin-3-glucoside administration improves short-term spatial recognition memory but not passive avoidance learning and memory in streptozotocin-diabetic rats," *Phytothery Research*, vol. 26, no. 8, pp. 1205–1210, 2012.

[36] J. Min, S. W. Yu, S. H. Baek et al., "Neuroprotective effect of cyanidin-3-O-glucoside anthocyanin in mice with focal cerebral ischemia," *Neuroscience Letters*, vol. 500, no. 3, pp. 157–161, 2011.

[37] L. G. Costa, J. M. Garrick, P. J. Roquè, and C. Pellacani, "Mechanisms of neuroprotection by quercetin: counteracting oxidative stress and more," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 2986796, 10 pages, 2016.

[38] S. K. Richetti, M. Blank, K. M. Capiotti et al., "Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish," *Behavioural Brain Resarch*, vol. 217, no. 1, pp. 10–15, 2011.

[39] S. Hajipour, A. Sarkaki, Y. Farbood, A. Eidi, P. Mortazavi, and Z. Valizadeh, "Effect of gallic acid on dementia type of Alzheimer disease in rats: electrophysiological and histological studies," *Basic and Clinical Neuroscience*, vol. 7, no. 2, pp. 97–106, 2016.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 6819281, 10 pages
https://doi.org/10.1155/2017/6819281

Research Article

Cardioprotective Effect of Resveratrol in a Postinfarction Heart Failure Model

Adam Riba,[1,2] Laszlo Deres,[1,2] Balazs Sumegi,[2,3,4] Kalman Toth,[1,2,4] Eszter Szabados,[1,2] and Robert Halmosi[1,2]

[1] *1st Department of Medicine, University of Pécs Medical school, Pécs, Hungary*
[2] *Szentagothai Research Centre, University of Pécs, Pécs, Hungary*
[3] *Department of Biochemistry and Medical Chemistry, University of Pécs Medical school, Pécs, Hungary*
[4] *MTA-PTE Nuclear-Mitochondrial Interactions Research Group, Pécs, Hungary*

Correspondence should be addressed to Eszter Szabados; szabados.eszter@pte.hu

Received 7 July 2017; Accepted 6 September 2017; Published 3 October 2017

Academic Editor: Anna V. Queralt

Despite great advances in therapies observed during the last decades, heart failure (HF) remained a major health problem in western countries. In order to further improve symptoms and survival in patients with heart failure, novel therapeutic strategies are needed. In some animal models of HF resveratrol (RES), it was able to prevent cardiac hypertrophy, contractile dysfunction, and remodeling. Several molecular mechanisms are thought to be involved in its protective effects, such as inhibition of prohypertrophic signaling molecules, improvement of myocardial Ca^{2+} handling, regulation of autophagy, and the reduction of oxidative stress and inflammation. In our present study, we wished to further examine the effects of RES on prosurvival (Akt-1, GSK-3β) and stress signaling (p38-MAPK, ERK 1/2, and MKP-1) pathways, on oxidative stress (iNOS, COX-2 activity, and ROS formation), and ultimately on left ventricular function, hypertrophy and fibrosis in a murine, and isoproterenol- (ISO-) induced postinfarction heart failure model. RES treatment improved left ventricle function, decreased interstitial fibrosis, cardiac hypertrophy, and the level of plasma BNP induced by ISO treatment. ISO also increased the activation of P38-MAPK, ERK1/$2^{Thr183\text{-}Tyr185}$, COX-2, iNOS, and ROS formation and decreased the phosphorylation of Akt-1, GSK-3β, and MKP-1, which were favorably influenced by RES. According to our results, regulation of these pathways may also contribute to the beneficial effects of RES in HF.

1. Introduction

Despite significant advances in therapy, heart failure (HF) is a constantly growing medical and social burden in western societies. Pharmacological inhibition of the RAAS and the adrenergic system resulted in substantial reduction in mortality [1]. However, further blocking of the neuroendocrine axis has failed to fulfill our hopes, so new ideas and approaches are needed in the treatment of heart failure. Oxidative stress is thought to play an important role in different cardiac pathological conditions such as I/R injury, fibrosis, cardiac hypertrophy, remodeling, and heart failure [2]. Excessive ROS may cause extensive oxidative damage to proteins, DNA, and lipids resulting in damaged cardiomyocyte functions including contractility, ion transport, and calcium cycling [3]. Moreover, ROS induce different pathological intracellular signaling pathways ultimately evoking apoptosis and necrosis. Regulation of intracellular ROS formation and modification of the stress responding signaling pathways may prevent or slow pathological processes in HF [4].

Resveratrol (3,5,4′-trihydroxystilbene) (RES) (Figure 1) is a natural phytoalexin found in a wide variety of plant species including grapes and nuts and present in varying concentration in red wines [5]. Numerous experimental studies have verified that RES interferes with several pathological processes in different cardiovascular diseases such as myocardial ischemia [6], myocarditis [7], cardiac hypertrophy [8], and heart failure [4]. Multiple mechanisms have been proposed to be responsible for the protective effects of RES in HF, including reducing oxidative stress and inflammation [9, 10],

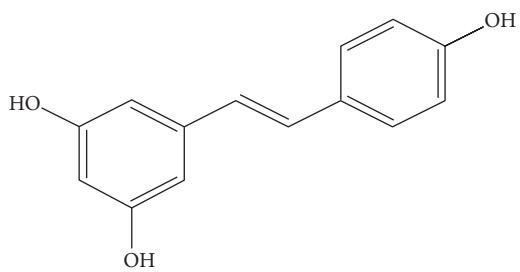


Figure 1: Chemical structure of resveratrol (RES).

inhibiting pathological hypertrophic signaling [11], improving Ca^{2+} handling [12], decreasing apoptosis, and modifying autophagy through different intracellular pathways [13].

The aim of the present study was to further examine RES in a postinfarction heart failure animal model, where isoproterenol, a strong sympathetic agent, was used to induce myocardial infarction, causing patchy, predominantly subendocardial necrosis and fibrosis [14]. We examined the effect of RES on left ventricular function, myocyte hypertrophy, collagen deposition, ROS production, and intracellular signaling pathways taking part in the process of cardiac remodeling and heart failure.

2. Methods

2.1. Animals. Male 20-week-old Wistar rats (410–480 g) were used for the experiments. The experiment was approved by the Animal Research Review Committee of the University of Pécs Medical School (Permit number: BA02/2000–2/2010), and the animals received care according to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication Number 85–23, revised 1996). The animals were housed under standardized conditions, 12 h dark-light cycle in solid-bottomed polypropylene cages, and received commercial rat chew ad libitum. Resveratrol of herbal origin (with >98% t-resveratrol content) was purchased from Argina Nutraceuticals (Fót, Hungary). Ethanol was used to prepare a stock solution of RES which was added to 30 ml of drinking water. We got a clean solution without any undissolved residue. Dosage of RES was set to 15 mg/kg/day. After finishing this first 30 ml of drinking water, the animals got clear water for the rest of the day [15].

2.2. Experimental Protocol. The rats were treated twice on two consecutive days with 80 mg/kg ISO (Sigma-Aldrich) or vehicle (physiological saline solution) subcutaneously to induce postinfarction remodeling as previously described. The animals were divided into four groups: control group (C) received clear water without ISO treatment; the second group of rats received two subcutaneous injections of isoproterenol at the dosage of 80 mg/kg (ISO); ISO + RES group (ISO + RES) received resveratrol with ISO treatment; and the resveratrol group (RES) received resveratrol without ISO treatment. RES inhibitor treatment was delayed 24 h to avoid suppression of infarct size. At the end of the 8-week-long period, body weights were measured, animals were sacrificed, and the hearts were removed. Atria and great vessels were trimmed from the ventricles; the weight of the ventricles was measured and normalized to body mass. Afterward, ventricles were fixed in 10% formalin for histology or freeze clamped for Western blot analysis [16].

2.3. Noninvasive Evaluation of Cardiac Structure and Function. Transthoracic echocardiography was performed under inhalation anesthesia at the beginning of the experiment and on the day of sacrifice. The rats were lightly anesthetized with a mixture of 1.5% isoflurane and 98.5% oxygen. The chest of the animals was shaved and acoustic coupling gel was applied. The animals were imaged in the left lateral position, and a warming pad was used to maintain normothermia. Cardiac diameter and functions were measured from short and long axis views at the midpapillary level using a VEVO 770 high-resolution ultrasound imaging system (VisualSonics, Toronto, Canada), which is equipped with a 25-MHz transducer. The investigators were blinded to the treatment protocol. Left ventricular (LV), ejection fraction (EF), LV end-diastolic volume, LV end-systolic volume, and the thickness of the septum and posterior wall were determined. EF (percentage) was calculated by $100 \times [(\text{LVEDV}-\text{LVESV})/\text{LVEDV}]$ [16, 17].

2.4. Histology. For histologic examination, ventricles were fixed in formalin and sliced and embedded in paraffin. Sections (5 μm thick) were cut serially from the base to apex. LV sections were stained with Masson's trichrome to detect interstitial fibrosis and quantified by the NIH ImageJ image processing program via its color deconvolution plug-in [17].

2.5. Nitrotyrosine Immunohistochemical Staining. We performed immunohistochemical staining for nitrotyrosine, a nitro-oxidative stress marker, using a previously described method. Extensively stained areas were also quantified using the NIH ImageJ image processing program via its color deconvolution plug-in [18].

2.6. Determination of Plasma B-Type Natriuretic Peptide Level. Blood samples were collected into Vacutainer tubes containing EDTA and aprotinin (0.6 IU/ml) and centrifuged at 1600g for 15 minutes at 4°C to obtain plasma, which was collected and kept at −70°C. Plasma B-type natriuretic peptide-45 levels (BNP-45) were determined by enzyme immunoassay method (BNP-45, Rat EIA Kit, Phoenix Pharmaceuticals Inc., CA, USA) [17].

2.7. Western Blot Analysis. Fifty milligrams of heart samples were homogenized in ice-cold Tris buffer (50 mmol/l, pH 8.0) containing 50 mM sodium vanadate and protease inhibitor cocktail (Sigma-Aldrich Co., Budapest, Hungary) and harvested in 2x concentrated SDS-polyacrylamide gel electrophoresis sample buffer. To ensure the same protein concentration in each well, protein levels were measured with Nanodrop. GAPDH was used only as a representative loading control. Proteins were separated on 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. After blocking (2 h with 3% nonfat milk in

Table 1: Effects of RES and ISO on the gravimetric parameters.

Group	Control	RES	ISO	ISO + RES
Weight (g)	595.86 ± 15.15	596.00 ± 21.30	544.50 ± 11.63	593.86 ± 18.41
Ventricular weight (g)	1.33 ± 0.01	1.31 ± 0.01	1.53 ± 0.02#	1.35 ± 0.01*
Tibia length (mm)	51.43 ± 0.72	51.57 ± 0.72	50.86 ± 0.55	49.86 ± 0.35
Ventricular weight/body weight (mg/g)	2.25 ± 0.06	2.21 ± 0.08	2.81 ± 0.06#	2.29 ± 0.09*
Ventricular weight/tibia length (mg/mm)	26.03 ± 0.47	25.33 ± 0.33	29.96 ± 0.28#	27.02 ± 0.13*

Eight weeks after ISO-induced myocardial infarction, body weight, mass of ventricles, and tibia length were measured. Ventricular weight/body weight (mg/g) and ventricular weight/tibia length (mg/mm) ratios were calculated. The results are expressed as mean ± SEM. #$P < 0.05$ versus control. *$P < 0.05$ versus ISO.

Tris-buffered saline), membranes were probed overnight at 4°C with antibodies recognizing the following antigens: phosphospecific mitogen-activated protein (MAP) kinase phosphatase-1 (*MKP-1*) Ser359 (1 : 1000), phosphospecific Akt-1/protein kinase B-α Ser473 (1 : 1000), phosphospecific glycogen synthase kinase (GSK)-3β Ser9 (1 : 1000), and phosphospecific p38 mitogen-activated protein kinase (p38-MAPK) Thr180-Gly-Tyr182 (1 : 1000), ERK1/2$^{\text{Thr183-Tyr185}}$, COX-2 (1 : 1000), and iNOS (1 : 1000). Membranes were washed six times for 5 min in Tris-buffered saline (pH 7.5) containing 0.2% Tween (TBST) before the addition of goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1 : 3000 dilution; Bio-Rad, Budapest, Hungary). Membranes were washed six times for 5 min in TBST, and the antibody-antigen complexes were visualized by means of enhanced chemiluminescence. The results of Western blots were quantified using the NIH ImageJ program.

2.8. Statistical Analysis. Statistical analysis was performed by analysis of variance, and all of the data were expressed as the mean ± SEM. The homogeneity of the groups was tested by *F*-test (Levene's test). There were no significant differences among the groups. Comparisons among groups were made by one-way ANOVA followed by Bonferroni correction or Tukey HSD's post hoc tests in SPSS for Windows, version 21.0. All data are expressed as mean ± SEM. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Resveratrol Treatment Improved the Gravimetric Parameters in ISO-Induced Heart Failure Model. Body weights did not differ significantly among the four groups at the beginning or the end of the experiment. Gravimetric measurements were performed and significantly elevated ventricular weight (WV, g) as well as ventricular weight normalized to body weight (WV/BW, mg/g) (C versus ISO, $P < 0.05$) and to tibia length (TL) (WV/TL, mg/mm) (C versus ISO, $P < 0.05$) were detected. Resveratrol treatment prevented the unfavorable changes in gravimetric parameters indicating hypertrophy in the ISO + RES group (ISO + RES versus ISO, $P < 0.05$) (Table 1).

3.2. Resveratrol Decreased the Heart Failure-Induced Elevation of Plasma BNP Level. ISO administration led to a significant increase in BNP level in the ISO group 8 weeks after myocardial infarction in our study (C versus ISO, $P < 0.05$). However, resveratrol significantly attenuated this response (ISO + RES versus ISO, $P < 0.05$) suggesting that resveratrol decreases the severity of postinfarction heart failure. There was no significant difference between the C and the RES groups (Figure 2).

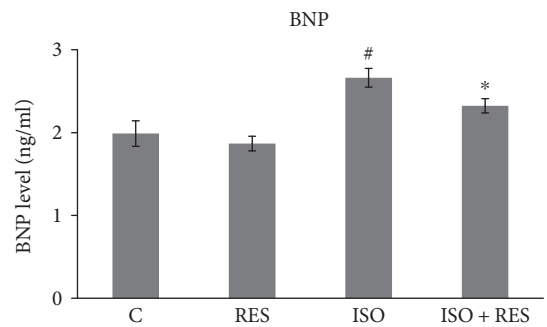


Figure 2: RES inhibited the heart failure-induced elevation of plasma BNP level. Plasma BNP level was determined using an ELISA method as described in Methods. C: control animals; RES: animals treated with resveratrol for 8 weeks; ISO: animals 8 weeks after ISO administration; ISO + RES: animals treated with resveratrol 8 weeks after ISO administration. Values are mean ± SEM, *$P < 0.05$ versus ISO, and #$P < 0.05$ versus C.

3.3. Resveratrol Improved Left Ventricular Function and Moderated Left Ventricular Hypertrophy in ISO-Treated Rats. The echocardiographic parameters of the animals did not differ significantly from each other at the beginning of the study. Heart rate did not differ significantly during anesthesia among the groups. LVESV and LVIDs were significantly higher in the ISO group (ISO versus C and ISO versus RES $P < 0.05$). The thickness of the septum and posterior wall and calculated LV mass were also higher in the ISO group (indicating the presence of ventricular hypertrophy) compared to the control group (ISO versus C, $P < 0.05$). Resveratrol treatment significantly reduced these unfavorable alterations. Systolic left ventricular function (EF %) was significantly lower in the ISO group (ISO versus C, $P < 0.05$), and this deterioration was significantly improved by resveratrol administration (ISO versus ISO + RES, $P < 0.05$) (Table 2, Figure 3).

Table 2: Resveratrol improved left ventricular function in ISO-treated rats and reduced left ventricular hypertrophy.

	Baseline	C	RES	ISO	ISO + RES
EF (%)	75.62 ± 0.87	71.70 ± 1.61	72.47 ± 1.69	56.96 ± 1.43#	67.49 ± 1.14*
Septum (mm)	1.63 ± 0.05	1.65 ± 0.10	1.61 ± 0.03	1.82 ± 0.03#	1.70 ± 0.02*
PW (mm)	1.57 ± 0.03	1.59 ± 0.07	1.59 ± 0.03	1.81 ± 0.06#	1.60 ± 0.02*
LVIDd (mm)	8.19 ± 0.11	8.44 ± 0.22	8.43 ± 0.17	7.88 ± 0.12	8.41 ± 0.23
LVIDs (mm)	4.42 ± 0.08	4.85 ± 0.09	4.69 ± 0.19	5.70 ± 0.2#	4.90 ± 0.12*
LVEDV (μl)	364.23 ± 10.38	393.36 ± 19.32	386.40 ± 16.82	365.54 ± 6.64	401.59 ± 18.63
LVESV (μl)	88.83 ± 4.40	109.9 ± 4.53	106.14 ± 7.26	157.71 ± 7.29#	130.27 ± 6.69*
LV mass (mg)	994.1 ± 21.8	1035.31 ± 59.79	1038.38 ± 44.44	1239.14 ± 76.5#	1041.85 ± 35.50*

C: control group; RES: resveratrol group; ISO: isoproterenol-treated group; ISO + RES: ISO + resveratrol group. EF: ejection fraction; LVESV: left ventricular end-systolic volume; LVEDV: left ventricular end-diastolic volume; LVIDd: diastolic left ventricular inner diameter; LVIDs: systolic left-ventricular inner diameter. The results are expressed as mean ± SEM. #$P < 0.05$ versus control. *$P < 0.05$ versus ISO.

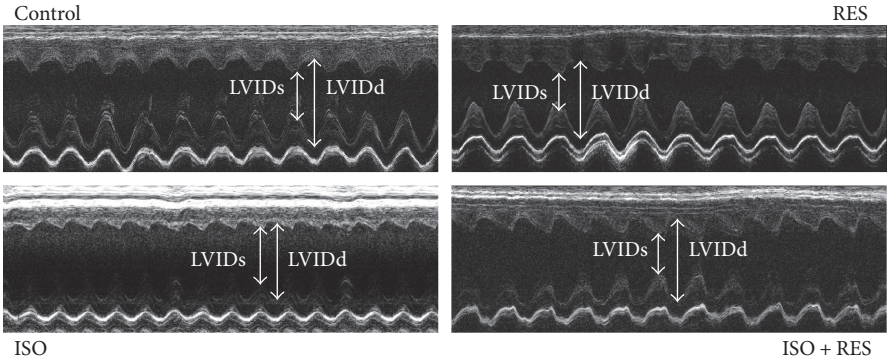


Figure 3: Representative echocardiographic M-mode images of left ventricles of animals from control, RES, ISO, and ISO + RES groups.

3.4. Resveratrol Decreased Interstitial Collagen Deposition in the Myocardium. Marked scar tissue formation was revealed by histological analysis after ISO stress in failing rat hearts compared to the control group ($P < 0.05$). Resveratrol treatment significantly decreased the extent of interstitial fibrosis ($P < 0.05$). Resveratrol alone did not cause any significant alterations in physiological conditions related to myocardial hypertrophy or interstitial collagen deposition (Figure 4(a)).

3.5. Effects of Resveratrol on the Oxidative Stress Marker Nitrotyrosine. The presence of oxidative stress was confirmed in our rodent heart failure model by the measurement of NT, which is a product of tyrosine nitration. Almost no immunostaining for NT was observed in myocardial sections of the control group. In contrast, in animals with heart failure (ISO), immunostaining was significantly more extensive ($P < 0.05$, C versus ISO), but this increase was attenuated by RES treatment ($P < 0.05$, ISO versus ISO + RES; Figure 4(b)).

3.6. Resveratrol Favorably Influenced the Phosphorylation of Akt-1^{Ser473} and GSK-3β^{Ser9} in Failing Myocardium. The level of Akt-1^{Ser473} was significantly higher in the RES and ISO + RES groups compared to the control and ISO groups ($P < 0.05$), respectively. Although the level of Akt-1^{Ser473} was significantly elevated in ISO-treated animals (ISO versus C $P < 0.05$), the highest activities were observed in the ISO + RES group (ISO versus ISO + RES $P < 0.05$). The level of GSK-3β^{Ser9} was also slightly elevated in the RES group compared to the control group and significantly decreased in ISO animals compared to control animals ($P < 0.05$, C versus ISO). The elevation in the ISO + RES group was significant compared to the ISO group too ($P < 0.05$, ISO + RES versus ISO). GAPDH was used as a loading control (Figure 5).

3.7. Resveratrol Attenuated the Phosphorylation of p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$ and ERK1/2$^{Thr183\text{-}Tyr185}$ and Increased the Amount of MKP-1 in ISO-Stressed Hearts. The level of phosphorylation of p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$ and ERK1/2$^{Thr183\text{-}Tyr185}$ was significantly elevated in the ISO group compared to the control group (C versus ISO $P < 0.05$). There was a significant reduction in the phosphorylation level of p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$ in the ISO + RES group versus the ISO group ($P < 0.05$, ISO versus ISO + RES). The activation of ERK1/2$^{Thr183\text{-}Tyr185}$ was also significantly reduced in the ISO + RES group compared to the ISO group ($P < 0.05$, ISO versus ISO + RES). Consequently, the steady state amount of MKP-1 was significantly elevated in the ISO + RES group compared to the ISO group. Interestingly,

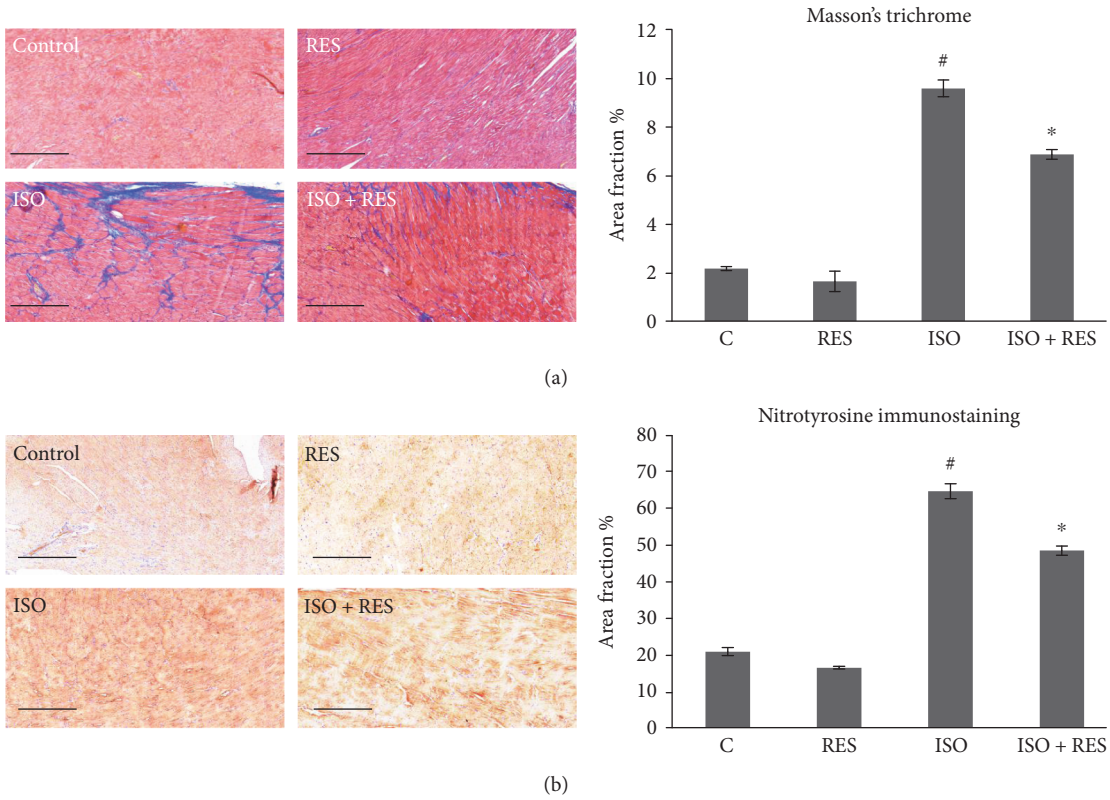


Figure 4: RES treatment moderated ISO-induced interstitial collagen deposition and protein nitrosylation in ISO-induced heart failure. (a) Representative sections stained with Masson's trichrome, scale bar: 500 μm, and magnification is10-fold. Control: age-matched control rats. RES: age-matched animals treated with resveratrol for 8 weeks. ISO: age-matched animals 8 weeks after ISO administration. ISO + RES: age-matched animals treated with resveratrol, 8 weeks after ISO administration. Values are mean ± SEM, $P < 0.05$ (ISO versus control group), and $P < 0.05$ (ISO + RES versus ISO group). (b) Representative immunohistochemical stainings for nitrotyrosine (NT, brown staining, scale bar: 500 μm, and 10x magnification) in the myocardium of the following: control: age-matched control rats; RES: age-matched animals treated with resveratrol for 8 weeks; ISO: age-matched animals 8 weeks after ISO administration; and ISO + RES: age-matched animals treated with resveratrol, 8 weeks after ISO administration. Values are mean ± SEM, $^{\#}P < 0.05$ (ISO versus control group), and $^{*}P < 0.05$ (ISO + RES versus ISO group).

there was a strong and significant elevation in the RES group compared to the control group ($P < 0.05$, C versus RES). GAPDH was used as a loading control (Figure 6).

3.8. Resveratrol Decreased the Expression of COX-2 and iNOS. The expression of COX-2 and iNOS was significantly elevated in ISO compared to the control ($P < 0.05$, C versus ISO) and significantly decreased in ISO + RES compared to ISO ($P < 0.05$, ISO versus ISO + RES). Although the activation level of COX-2 was markedly decreased in RES compared to the control, the only significant difference was between ISO + RES and ISO. GAPDH was used as a loading control (Figure 7).

4. Discussion

Chakraborty et al. examined the protective effect of resveratrol and atorvastatin and their combination on isoproterenol-induced cardiac hypertrophy in rats. They found that RES pretreatment was able to decrease infarct size and myocardial necroenzyme level and restored myocardial endogenous antioxidant level [19]. Moreover, our working group has previously demonstrated the beneficial effects of an alcohol-free red wine extract in an isoproterenol-induced heart failure model [8].

This time, we aimed to test the cardioprotective effect of RES on oxidative stress and different signaling pathways in an advanced stage of heart failure. Subcutaneous administration of ISO produces patchy myocardial necrosis with subsequent hypertrophy, fibrosis, and remodeling leading to heart failure similar to that observed in patients after myocardial infarction [20]. Plasma BNP levels (Figure 2), left ventricular wall thickness, and dimensions were increased and the systolic left ventricular function was significantly decreased in ISO-treated animals. RES was capable of preserving LV function and moderated the severity of heart failure (Table 2). Excessive collagen deposition is the main histological characteristic of ventricular remodeling. The damaged myocardium is replaced by scar tissue stabilizing the ventricular wall but leading to systolic and diastolic dysfunction and arrhythmias. In our study, RES prevented the marked fibrosis induced by ISO treatment (Figure 4(a)).

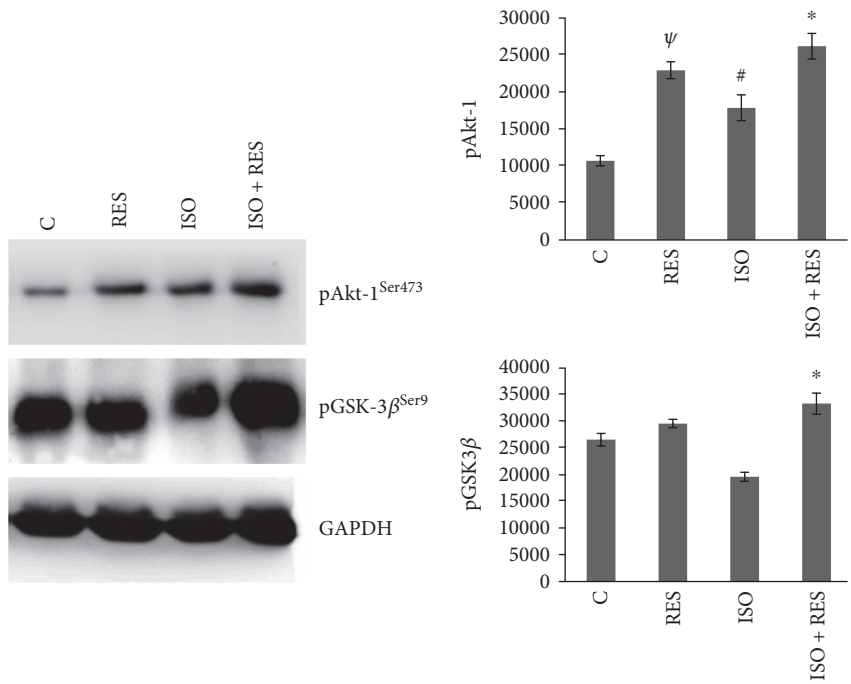


Figure 5: Effect of resveratrol treatment on Akt-1^{Ser473} and GSK-3β^{Ser9}. Representative Western blot analysis of Akt-1^{Ser473}, GSK-3β^{Ser9}, and p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$ and phosphorylation and densitometric evaluation are shown. GAPDH was used as a loading control. Representative blots and bar diagrams of three independent experiments are presented. C: control animals; RES: animals treated with resveratrol for 8 weeks; ISO: animals 8 weeks after ISO administration; ISO + RES: animals treated with resveratrol, 8 weeks after ISO administration. Values are mean ± SEM, $^{\#}P < 0.05$ versus control, $^{*}P < 0.05$ versus ISO, and $^{\psi}P < 0.05$ (C versus RES).

There is a large amount of evidence that ROS release plays a pivotal role in the development of heart failure [21]. We observed increased immunohistochemical staining against nitrotyrosine (NT)—a marker of oxidative stress—on ISO-treated animal heart samples, which was significantly decreased by RES treatment (Figure 4(b)) indicating that RES was able to reduce myocardial ROS production. This antioxidant property of RES was investigated many times in different heart failure models including hypertensive [22], pressure overload [23, 24], myocarditis [7], or chemotherapy induced [25, 26] and genetic models of HF [27].

Excessive ROS formation induces different intracellular signaling pathways regulating cardiac remodeling, myocyte survival, apoptosis, and necrosis [28]. Here, we investigated the effect of RES on ERK 1/2, p38-MAPK, Akt-1, and GSK-3β pathways which plays a critical role in cardiac hypertrophy and ventricular dilatation [29], myocyte survival, apoptosis, autophagy, and necrosis [29–31]. Akt activation inhibits cardiomyocyte apoptosis and improves surviving of cardiomyocytes in the ischemic heart [32]. Akt exerts its protective effect through phosphorylation of the Bcl-2 family and GSK-3β [33]. Akt-1 is a key molecule in the signaling of physiological hypertrophy, and it has a pivotal role in the prevention of pathological cardiac growth [29]. Interestingly, in our investigation, phosphorylation of Akt-1 and GSK-3β was elevated in all treated groups compared to the control group and the highest level of phosphorylation was in the ISO + RES group (Figure 5), indicating that RES presumably further augments the stress ISO-induced activity of endogenous prosurvival signaling pathways. Xi et al. examined the effect of RES against reperfusion injury on an isolated rat heart, and they also found that the cardioprotective effect of RES involved the increased phosphorylation of GSK-3β [11].

There are conflicting data in the literature regarding the role of MAP kinases in the regulation of cell survival [29, 30, 34–37]. A wide variety of extra and intracellular stress signals can induce sequential phosphorylation and activation of MAPK kinases via phosphorylation on both threonine and tyrosine residues [31]. The effect of RES on the activation of MAPK cascades was previously investigated on cardiomyocytes. Becatti et al. found that RES protected cardiomyocytes from oxidative injury by SIRT1 overexpression by reducing p38-MAPK activity [38]. Lv and Zhou demonstrated that RES attenuated cell death and apoptosis induced by oxidative stress by upregulating autophagy via inhibiting the p38 MAPK pathway [39]. In an animal study by Gao et al., they found that RES ameliorated diabetes-induced cardiac dysfunction through the AT1R-ERK/p38 MAPK signaling pathway [40]. We investigated the phosphorylation (activation) of p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$ and ERK1/2$^{Thr183\text{-}Tyr185}$ which was elevated in ISO-treated groups but RES significantly decreased their activation (Figure 6). These changes were probably due to the increased production of MAPK

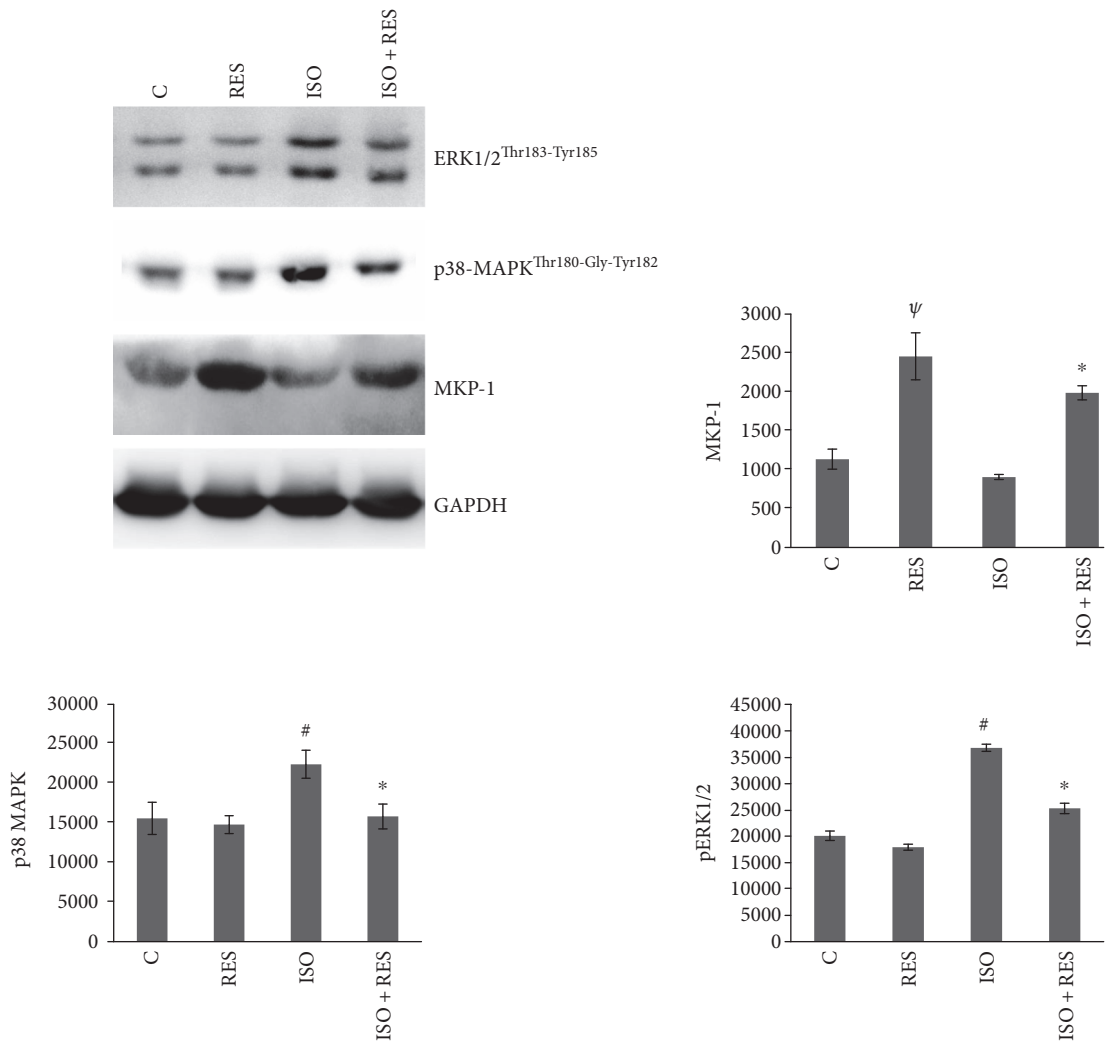


Figure 6: Effect of resveratrol treatment on the phosphorylation of p38-MAPK$^{\text{Thr180-Gly-Tyr182}}$, ERK1/2$^{\text{Thr183-Tyr185}}$, and on the amount of MKP-1. Representative Western blot analysis of p38-MAPK$^{\text{Thr180-Gly-Tyr182}}$, ERK1/2 phosphorylation, and MKP-1 level; a densitometric evaluation is shown. GAPDH was used as a loading control. Representative blots and bar diagrams of three independent experiments are presented. C: control animals; RES: animals treated with resveratrol for 8 weeks; ISO: animals 8 weeks after ISO administration; ISO + RES: animals treated with resveratrol, 8 weeks after ISO administration. Values are mean ± SEM, $^{\#}P < 0.05$ (control versus ISO), $^{\psi}P < 0.01$ (C versus RES), and $^{*}P < 0.05$ (ISO versus ISO + RES).

phosphatase-1 (MKP-1) which is the major regulator of MAPKs [31, 37, 41]. In accordance with this, the amount of MKP-1 was increased in the RES-treated groups compared to untreated animals (Figure 6).

Whereas COX-1 plays a housekeeping role, COX-2 plays a major part in inflammation, atherosclerosis, and tumor formation [42–44]. Previous studies showed that COX-2 is upregulated by p38-MAPK and ERK1/2 [45]. Moreover, prolonged activation of COX-2 produces cardiac cell death, leading to gradual loss of myocardial function, and eventually heart failure. The effect of RES on COX-2 activity was not investigated previously neither on cardiomyocytes nor on a heart failure animal model. We found in our postinfarction animal model that RES was able to reduce the activation of COX-2 induced by ISO treatment (Figure 7).

Previous in vivo animal and human studies demonstrated the elevated expression of iNOS in heart failure [46]. Overexpression of iNOS in the myocardium of mice resulted in peroxynitrite generation [47–50], which was also detected in our model and it has also been demonstrated that elevated nitrotyrosine formation results in an increase in iNOS levels, creating a vicious circle of harmful effects [51]. Such evidence indicates that iNOS is also a factor, in addition to COX-2, in the development of postinfarction heart failure [52]. It has been shown that iNOS, as well as COX-2 expression level, is strongly correlated with the phosphorylation level of the MAPKs (p38-MAPK and ERK1/2$^{\text{Thr183-Tyr185}}$) [53–55]. Also, it has been reported that flavonoids like quercetin, galangin, and apigenin can downregulate iNOS expression by modulating enzyme activity related to signal transduction [56, 57]. In our study, the

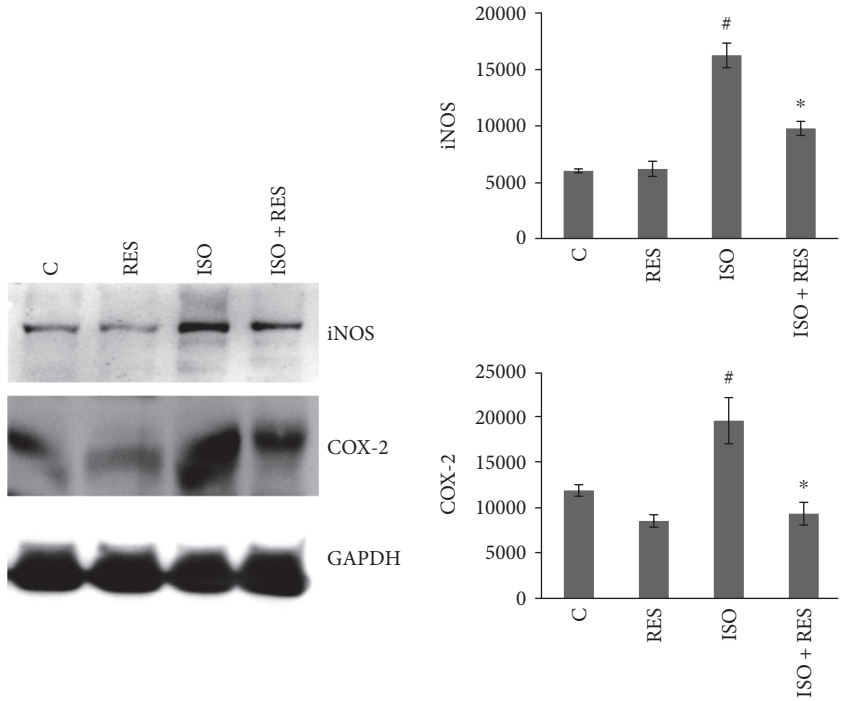


Figure 7: Effect of resveratrol treatment on COX-2 and iNOS. Representative Western blot analysis of COX-2 and iNOS activation and densitometric evaluation is shown. GAPDH was used as a loading control. Representative blots and bar diagrams of three independent experiments are presented. C: control animals; RES: animals treated with resveratrol for 8 weeks; ISO: animals 8 weeks after ISO administration; ISO + RES: animals treated with resveratrol, 8 weeks after ISO administration. Values are mean ± SEM, $^{\#}P < 0.05$ (control versus ISO), and $^{*}P < 0.05$ (ISO versus ISO + RES).

ISO-enhanced expression of iNOS was reduced by RES treatment (Figure 7) indicating suppressed free radical formation.

5. Conclusion

The protective effects of the nutritional agent RES against the development of postinfarction heart failure were investigated in a murine model. RES improved the left ventricular function and decreased myocardial hypertrophy, fibrosis, and the severity of heart failure. Moreover, RES decreased the oxidative stress and favorably modified the activity of several intracellular signaling pathways including Akt-1, GSK-3β, p38-MAPK, ERK1/2, MKP-1, COX-2, and iNOS. According to our results, regulation of these pathways may also contribute to the beneficial effects of RES in HF.

Abbreviations

RES: Resveratrol (3,5,4′-trihydroxystilbene)
ISO: Isoproterenol
LV: Left ventricular
VW: Ventricular weight
BW: Body weight
TL: Tibia length
EF: Ejection fraction
LVEDV: LV end-diastolic volume
LVESV: LV end-systolic volume
LVIDd: Diastolic left ventricular inner diameter
LVIDs: Systolic left-ventricular inner diameter
BNP: B-type natriuretic peptide
NT: Nitrotyrosine.

Conflicts of Interest

All authors have approved the manuscript for submission, and we have no conflicts of interest to disclose.

Acknowledgments

The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary. This study was supported by the Hungarian National Research Foundations Grant nos. (OTKA K104220, PTE ÁOK-KA-2015-05, GINOP-2.3.2-15-2016-00048, and GINOP 2.3.2-15-2016-00049) and MTA-PTE Nuclear-Mitochondrial Interactions Research Group, University of Pécs.

References

[1] A. L. Bui, T. B. Horwich, and G. C. Fonarow, "Epidemiology and risk profile of heart failure," *Nature Reviews Cardiology*, vol. 8, no. 1, pp. 30–41, 2011.

[2] K. Sugamura and J. F. Keaney Jr., "Reactive oxygen species in cardiovascular disease," *Free Radical Biology and Medicine*, vol. 51, no. 5, pp. 978–992, 2011.

[3] J. L. Park and B. R. Lucchesi, "Mechanisms of myocardial reperfusion injury," *The Annals of Thoracic Surgery*, vol. 68, no. 5, pp. 1905–1912, 1999.

[4] M. M. Sung and J. R. Dyck, "Therapeutic potential of resveratrol in heart failure," *Annals of the New York Academy of Sciences*, vol. 1348, no. 1, pp. 32–45, 2015.

[5] P. Raj, X. L. Louis, S. J. Thandapilly, A. Movahed, S. Zieroth, and T. Netticadan, "Potential of resveratrol in the treatment of heart failure," *Life Sciences*, vol. 95, no. 2, pp. 63–71, 2014.

[6] B. Burstein, A. Maguy, R. Clement et al., "Effects of resveratrol (*trans*-3,5,4′-trihydroxystilbene) treatment on cardiac remodeling following myocardial infarction," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 323, no. 3, pp. 916–923, 2007.

[7] Y. Yoshida, T. Shioi, and T. Izumi, "Resveratrol ameliorates experimental autoimmune myocarditis," *Circulation Journal*, vol. 71, no. 3, pp. 397–404, 2007.

[8] A. Palfi, E. Bartha, L. Copf et al., "Alcohol-free red wine inhibits isoproterenol-induced cardiac remodeling in rats by the regulation of Akt1 and protein kinase C α/β II," *The Journal of Nutritional Biochemistry*, vol. 20, no. 6, pp. 418–425, 2009.

[9] A. Csiszar, "Anti-inflammatory effects of resveratrol: possible role in prevention of age-related cardiovascular disease," *Annals of the New York Academy of Sciences*, vol. 1215, pp. 117–122, 2011.

[10] S. Das and D. K. Das, "Anti-inflammatory responses of resveratrol," *Inflammation & Allergy Drug Targets*, vol. 6, no. 3, pp. 168–173, 2007.

[11] J. Xi, H. Wang, R. A. Mueller, E. A. Norfleet, and Z. Xu, "Mechanism for resveratrol-induced cardioprotection against reperfusion injury involves glycogen synthase kinase 3β and mitochondrial permeability transition pore," *European Journal of Pharmacology*, vol. 604, no. 1–3, pp. 111–116, 2009.

[12] Q. Dong, Z. Wu, X. Li et al., "Resveratrol ameliorates cardiac dysfunction induced by pressure overload in rats via structural protection and modulation of Ca^{2+} cycling proteins," *Journal of Translational Medicine*, vol. 12, 2014.

[13] H. Kanamori, G. Takemura, K. Goto et al., "Resveratrol reverses remodeling in hearts with large, old myocardial infarctions through enhanced autophagy-activating AMP kinase pathway," *The American Journal of Pathology*, vol. 182, no. 3, pp. 701–713, 2013.

[14] A. Palfi, A. Toth, K. Hanto et al., "PARP inhibition prevents postinfarction myocardial remodeling and heart failure via the protein kinase C/glycogen synthase kinase-3β pathway," *Journal of Molecular and Cellular Cardiology*, vol. 41, no. 1, pp. 149–159, 2006.

[15] K. Robinson, C. Mock, and D. Liang, "Pre-formulation studies of resveratrol," *Drug Development and Industrial Pharmacy*, vol. 41, no. 9, pp. 1464–1469, 2015.

[16] A. Riba, L. Deres, K. Eros et al., "Doxycycline protects against ROS-induced mitochondrial fragmentation and ISO-induced heart failure," *PLoS One*, vol. 12, 2017.

[17] L. Deres, E. Bartha, A. Palfi et al., "PARP-inhibitor treatment prevents hypertension induced cardiac remodeling by favorable modulation of heat shock proteins, Akt-1/GSK-3β and several PKC isoforms," *PLoS One*, vol. 9, no. 7, article e102148, 2014.

[18] L. Liaudet, F. G. Soriano, É. Szabó et al., "Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose)polymerase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 18, pp. 10203–10208, 2000.

[19] S. Chakraborty, M. Pujani, and S. E. Haque, "Combinational effect of resveratrol and atorvastatin on isoproterenol-induced cardiac hypertrophy in rats," *Journal of Pharmacy and Bioallied Sciences*, vol. 7, pp. 233–238, 2015.

[20] J. R. Teerlink, J. M. Pfeffer, and M. A. Pfeffer, "Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats," *Circulation Research*, vol. 75, no. 1, pp. 105–113, 1994.

[21] D. O. Okonko and A. M. Shah, "Heart failure: mitochondrial dysfunction and oxidative stress in CHF," *Nature Reviews Cardiology*, vol. 12, pp. 6–8, 2014.

[22] S. J. Thandapilly, P. Wojciechowski, J. Behbahani et al., "Resveratrol prevents the development of pathological cardiac hypertrophy and contractile dysfunction in the SHR without lowering blood pressure," *American Journal of Hypertension*, vol. 23, no. 2, pp. 192–196, 2010.

[23] P. K. Gupta, D. J. DiPette, and S. C. Supowit, "Protective effect of resveratrol against pressure overload-induced heart failure," *Food Science & Nutrition*, vol. 2, no. 3, pp. 218–229, 2014.

[24] P. Wojciechowski, D. Juric, X. L. Louis et al., "Resveratrol arrests and regresses the development of pressure overload- but not volume overload-induced cardiac hypertrophy in rats," *The Journal of Nutrition*, vol. 140, no. 5, pp. 962–968, 2010.

[25] V. W. Dolinsky, K. J. Rogan, M. M. Sung et al., "Both aerobic exercise and resveratrol supplementation attenuate doxorubicin-induced cardiac injury in mice," *American Journal of Physiology - Endocrinology and Metabolism*, vol. 305, no. 2, pp. E243–E253, 2013.

[26] E. D. Danz, J. Skramsted, N. Henry, J. A. Bennett, and R. S. Keller, "Resveratrol prevents doxorubicin cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway," *Free Radical Biology and Medicine*, vol. 46, no. 12, pp. 1589–1597, 2009.

[27] M. Tanno, A. Kuno, T. Yano et al., "Induction of manganese superoxide dismutase by nuclear translocation and activation of SIRT1 promotes cell survival in chronic heart failure," *The Journal of Biological Chemistry*, vol. 285, no. 11, pp. 8375–8382, 2010.

[28] E. S. Park, D. H. Kang, J. C. Kang et al., "Cardioprotective effect of KR-33889, a novel PARP inhibitor, against oxidative stress-induced apoptosis in H9c2 cells and isolated rat hearts," *Archives of Pharmacal Research*, vol. 40, no. 5, pp. 640–654, 2017.

[29] J. Heineke and J. D. Molkentin, "Regulation of cardiac hypertrophy by intracellular signalling pathways," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 8, pp. 589–600, 2006.

[30] E. Bognar, Z. Sarszegi, A. Szabo et al., "Antioxidant and anti-inflammatory effects in RAW264.7 macrophages of malvidin, a major red wine polyphenol," *PLoS One*, vol. 8, no. 6, article e65355, 2013.

[31] K. Kondoh and E. Nishida, "Regulation of MAP kinases by MAP kinase phosphatases," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1773, no. 8, pp. 1227–1237, 2007.

[32] T. Matsui, J. Tao, F. del Monte et al., "Akt activation preserves cardiac function and prevents injury after transient cardiac

ischemia in vivo," *Circulation*, vol. 104, no. 3, pp. 330–335, 2001.

[33] S. Miyamoto, A. N. Murphy, and J. H. Brown, "Akt mediated mitochondrial protection in the heart: metabolic and survival pathways to the rescue," *Journal of Bioenergetics and Biomembranes*, vol. 41, no. 2, pp. 169–180, 2009.

[34] Z. Xia, M. Dickens, J. Raingeaud, R. J. Davis, and M. E. Greenberg, "Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis," *Science*, vol. 270, no. 5240, pp. 1326–1331, 1995.

[35] A. Palfi, A. Toth, G. Kulcsar et al., "The role of Akt and mitogen-activated protein kinase systems in the protective effect of poly(ADP-ribose) polymerase inhibition in Langendorff perfused and in isoproterenol-damaged rat hearts," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 315, no. 1, pp. 273–282, 2005.

[36] B. Veres, B. Radnai, F. Gallyas et al., "Regulation of kinase cascades and transcription factors by a poly(-ADP-ribose) polymerase-1 inhibitor, 4-hydroxyquinazoline, in lipopolysaccharide-induced inflammation in mice," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 310, no. 1, pp. 247–255, 2004.

[37] B. Racz, K. Hanto, A. Tapodi et al., "Regulation of MKP-1 expression and MAPK activation by PARP-1 in oxidative stress: a new mechanism for the cytoplasmic effect of PARP-1 activation," *Free Radical Biology and Medicine*, vol. 49, no. 12, pp. 1978–1988, 2010.

[38] M. Becatti, N. Taddei, C. Cecchi, N. Nassi, P. A. Nassi, and C. Fiorillo, "SIRT1 modulates MAPK pathways in ischemic-reperfused cardiomyocytes," *Cellular and Molecular Life Sciences*, vol. 69, no. 13, pp. 2245–2260, 2012.

[39] X. C. Lv and H. Y. Zhou, "Resveratrol protects H9c2 embryonic rat heart derived cells from oxidative stress by inducing autophagy: role of p38 mitogen-activated protein kinase," *Canadian Journal of Physiology and Pharmacology*, vol. 90, no. 5, pp. 655–662, 2012.

[40] Y. Gao, L. Kang, C. Li et al., "Resveratrol ameliorates diabetes-induced cardiac dysfunction through AT1R-ERK/p38 MAPK signaling pathway," *Cardiovascular Toxicology*, vol. 16, no. 2, pp. 130–137, 2016.

[41] C. S. Andrews, S. Matsuyama, B. C. Lee, and J. D. Li, "Resveratrol suppresses NTHi-induced inflammation via up-regulation of the negative regulator MyD88 short," *Scientific Reports*, vol. 6, article 34445, 2016.

[42] F. Cipollone and M. L. Fazia, "COX-2 and atherosclerosis," *Journal of Cardiovascular Pharmacology*, vol. 47, Supplement 1, pp. S26–S36, 2006.

[43] M. F. Linton and S. Fazio, "Cyclooxygenase-2 and inflammation in atherosclerosis," *Current Opinion in Pharmacology*, vol. 4, no. 2, pp. 116–123, 2004.

[44] M. E. Burleigh, V. R. Babaev, P. G. Yancey et al., "Cyclooxygenase-2 promotes early atherosclerotic lesion formation in ApoE-deficient and C57BL/6 mice," *Journal of Molecular and Cellular Cardiology*, vol. 39, no. 3, pp. 443–452, 2005.

[45] K. Bujold, D. Rhainds, C. Jossart, M. Febbraio, S. Marleau, and H. Ong, "CD36-mediated cholesterol efflux is associated with PPARγ activation via a MAPK-dependent COX-2 pathway in macrophages," *Cardiovascular Research*, vol. 83, no. 3, pp. 457–464, 2009.

[46] N. G. Vejlstrup, A. Bouloumie, S. Boesgaard et al., "Inducible nitric oxide synthase (iNOS) in the human heart: expression and localization in congestive heart failure," *Journal of Molecular and Cellular Cardiology*, vol. 30, no. 6, pp. 1215–1223, 1998.

[47] R. C. Starling, "Inducible nitric oxide synthase in severe human heart failure: impact of mechanical unloading," *Journal of the American College of Cardiology*, vol. 45, pp. 1425–1427, 2005, United States.

[48] Y. Xia and J. L. Zweier, "Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 13, pp. 6954–6958, 1997.

[49] A. Kumar, S. H. Chen, M. B. Kadiiska et al., "Inducible nitric oxide synthase is key to peroxynitrite-mediated, LPS-induced protein radical formation in murine microglial BV2 cells," *Free Radical Biology and Medicine*, vol. 73, pp. 51–59, 2014.

[50] J. M. Cotton, M. T. Kearney, and A. M. Shah, "Nitric oxide and myocardial function in heart failure: friend or foe?," *Heart*, vol. 88, pp. 564–566, 2002.

[51] C. L. Cooke and S. T. Davidge, "Peroxynitrite increases iNOS through NF-κB and decreases prostacyclin synthase in endothelial cells," *American Journal of Physiology - Cell Physiology*, vol. 282, no. 2, pp. C395–C402, 2002.

[52] B. D. Lamon, R. K. Upmacis, R. S. Deeb, H. Koyuncu, and D. P. Hajjar, "Inducible nitric oxide synthase gene deletion exaggerates MAPK-mediated cyclooxygenase-2 induction by inflammatory stimuli," *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 299, no. 3, pp. H613–H623, 2010.

[53] Q. Xia, Q. Hu, H. Wang et al., "Induction of COX-2-PGE2 synthesis by activation of the MAPK/ERK pathway contributes to neuronal death triggered by TDP-43-depleted microglia," *Cell Death and Disease*, vol. 6, no. 3, 2015.

[54] H. S. Chae, O. H. Kang, Y. S. Lee et al., "Inhibition of LPS-induced iNOS, COX-2 and inflammatory mediator expression by paeonol through the MAPKs inactivation in RAW 264.7 cells," *The American Journal of Chinese Medicine*, vol. 37, no. 1, pp. 181–194, 2009.

[55] C. J. Liou, W. B. Len, S. J. Wu, C. F. Lin, X. L. Wu, and W. C. Huang, "Casticin inhibits COX-2 and iNOS expression via suppression of NF-κB and MAPK signaling in lipopolysaccharide-stimulated mouse macrophages," *Journal of Ethnopharmacology*, vol. 158, Part A, pp. 310–316, 2014.

[56] E. A. Mazzio, D. Bauer, P. Mendonca, T. Equar, and K. F. Soliman, "Natural product HTP screening for evidence of attenuated cytokine-induced neutrophil chemo attractants (CINCs) and NO2 − in LPS/IFNγ activated glioma cells," *Journal of Neuroimmunology*, vol. 302, pp. 10–19, 2017.

[57] H. P. Kim, K. H. Son, H. W. Chang, and S. S. Kang, "Anti-inflammatory plant flavonoids and cellular action mechanisms," *Journal of Pharmacological Sciences*, vol. 96, no. 3, pp. 229–245, 2004.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 4749131, 9 pages
https://doi.org/10.1155/2017/4749131

Review Article

Beer Polyphenols and Menopause: Effects and Mechanisms—A Review of Current Knowledge

Berner Andrée Sandoval-Ramírez,[1] Rosa M. Lamuela-Raventós,[1,2] Ramon Estruch,[2,3] Gemma Sasot,[1,2] Monica Doménech,[2,3] and Anna Tresserra-Rimbau[1,2]

[1]*Department of Nutrition, Food Science and Gastronomy, XaRTA, INSA, School of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain*
[2]*CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain*
[3]*Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain*

Correspondence should be addressed to Anna Tresserra-Rimbau; annatresserra@ub.edu

Received 4 April 2017; Revised 26 June 2017; Accepted 10 July 2017; Published 17 August 2017

Academic Editor: Giuseppe Cirillo

Beer is one of the most frequently consumed fermented beverages in the world, and it has been part of the human diet for thousands of years. Scientific evidence obtained from the development of new techniques of food analysis over the last two decades suggests that polyphenol intake derived from moderate beer consumption may play a positive role in different health outcomes including osteoporosis and cardiovascular risk and the relief of vasomotor symptoms, which are commonly experienced during menopause and are an important reason why women seek medical care during this period; here, we review the current knowledge regarding moderate beer consumption and its possible effects on menopausal symptoms. The effect of polyphenol intake on vasomotor symptoms in menopause may be driven by the direct interaction of the phenolic compounds present in beer, such as 8-prenylnaringenin, 6-prenylnaringenin, and isoxanthohumol, with intracellular estrogen receptors that leads to the modulation of gene expression, increase in sex hormone plasma concentrations, and thus modulation of physiological hormone imbalance in menopausal women. Since traditional hormone replacement therapies increase health risks, alternative, safer treatment options are needed to alleviate menopausal symptoms in women. The present work aims to review the current data on this subject.

1. Introduction

Beer is one of the most frequently consumed alcoholic beverages in the world. Beer consumption ranks first in Europe, slightly above wine consumption, according to the World Health Organization [1] and third amongst alcoholic beverage preferences in North America [2]. Archaeological findings show that Chinese villagers brewed fermented alcoholic drinks as far back as 7000 BC on a small individual scale, with a production process and methods similar to those of ancient Egypt and Mesopotamia [3]. Throughout human history, products, ingredients, procedures, and techniques have evolved due to technological advances and the implementation of industrialized processes [4] further enhancing the long history of beer as a part of the human diet.

During the last two decades, scientific evidence has suggested that moderate consumption of alcoholic beverages has positive outcomes on different aspects of cardiovascular risk, as evidenced by Nogueira et al. who correlated regular daily intake of 330 ml of beer with positive changes in insulin sensitivity and lipid profiles [5]. Fermented beverages have also shown positive associations with different cardiovascular disease endpoints such as coronary heart disease, peripheral arterial disease, chronic heart failure, and stroke in which regular moderate consumption of alcohol reduced the prevalence of adverse events [6], and fermented beverages have shown anti-inflammatory properties [7]; these findings may explain the benefits of regular and moderate alcohol intake on cardiovascular disease risk [8–11]. In the last decade, the development of new techniques for food analysis has allowed

Table 1: Flavonoids contained in different types of beer.

Molecule	Mean content (mg/100 ml)			
	Alcohol-free	Ale	Dark	Regular
Chalcones				
Xanthohumol	0.0003	0.0100	0.0300	0.0014
Flavanols				
Catechin	0.1000	0.3300	0.0200	0.1100
Epicatechin	0.0056	0.0500	0.0100	0.0600
Procyanidin dimer B3				0.1600
Procyanidin trimer C2				0.0300
Prodelphinidin trimer C-GC-C				0.0200
Prodelphinidin trimer GC-C-C				0.0100
Prodelphinidin trimer GC-GC-C				0.0400
Prodelphinin dimer B3				0.1800
Flavanones				
6-Geranylnaringenin		0.0011	0.0027	0.0004
6-Prenylaringenin	0.0007	0.0200	0.0200	0.0026
8-Prenylaringenin	0.0003	0.0044	0.0092	0.0010
Isoxanthohumol	0.0100	0.2100	0.1200	0.0400
Naringin				0.0008
Flavones				
Apigenin				0.0042
Flavonols				
3,7-Dimethylquercetin				0.0003
Myricetin				0.0007
Quercetin				0.0067
Quercetin 3-*O*-arabinoside				0.0006
Quercetin 3-*O*-rutinoside				0.0900
Isoflavonoids				
Biochanin A		0.0005		0.0015
Daidzein		0.0005		
Genistein		0.0010		

Data from the Phenol-Explorer database [12].

the quantification of phenolic profiles [12], which, in turn, has led to new studies suggesting that regular polyphenol consumption might provide health benefits for menopausal and postmenopausal women, reducing vasomotor symptoms [13, 14] and osteoporosis [15].

Hop (*Humulus lupulus* L) is the ingredient used for beer making and is rich in phenolic compounds. Mass spectrometry analysis show that it contains around 14.4% of phenolic acids, flavonoids, proanthocyanidins, prenylated chalcones, and catechins [16]. Furthermore, malt provides 70%–80% of the total polyphenolic compounds found in beer [17]. It has been shown through high-performance liquid chromatography and posterior ultrasound separation that fermentation, boiling, and the amount of hop used to manufacture beer significantly influence the final polyphenol concentrations [18].

Menopause is induced by the permanent cessation of menstruation due to the end of ovarian follicular activity. This affects the physiology of women [19] and leads to a diminished production of estradiol which is correlated with the night sweats and hot flushes experienced by many menopausal women [20]. According to the Menopause Epidemiology Study, in which 4402 women were surveyed, these symptoms are one of the main reasons for women to seek medical care and over-the-counter treatments that provide some relief and improve the quality of life [21]. For the present work, we review the current knowledge found through online scientific libraries, PubMed and Scopus, regarding moderate beer consumption, polyphenol intake from beer, and their possible benefits for menopausal women.

2. Polyphenolic Compounds in Beer

Beer contains amino acids, carbohydrates, vitamins, minerals, and polyphenols. As mentioned above, beer contains a diversity of polyphenols mainly derived from hops and malt [16, 22]. Moreover, during the beer fermentation process, a resin produced by hops that contains monoacylphloroglucinols is converted into bitter acids such as humulones and isohumulones. These molecules act as bioactive antioxidants and provide additional beneficial effects [23].

Tables 1–3 show the polyphenols found in different types of beer. Malt contains many free and total (bound) polyphenolic compounds; according to composition analysis using a liquid chromatography-antioxidant technique before and after fermentation, the concentrations of polyphenolic compounds may be increased by up to threefold after the fermentation process [24]. The main polyphenolic compounds present in beer are sinapic, ferulic, and caffeic acids. Vanillic acids are present in bound and unbound forms while 4-hydroxyphenylacetic and p-coumaric acids are present as free forms [17]. The main phenolic acids found in beer are shown in Figure 1.

3. Polyphenol Metabolites in Plasma

Analysis of polyphenol concentrations in plasma reveals that after ingestion, beer goes through the gastrointestinal tract. An estimated amount of between 5–10% of beer is absorbed in the small intestine, with the remaining 90–95% continuing on to the colon where it is further fermented by the gut microbiota [25], increasing the amount of polyphenols such as 4-hydroxyphenylacetic and vanillic acids absorbed [26–28]. After being absorbed, polyphenols undergo hepatic conjugation reactions with S-adenosyl methionine, sulfates, glucuronates, or a combination of them [29]. After 30 minutes, the plasma levels of nonconjugated hydroxyphenylacetic acid significantly increase. Vanillic, caffeic, and ferulic acid levels raise equally as conjugated and nonconjugated forms, with a slight prevalence of sulfate over glucuronate isoforms [30]. Composition analysis carried out in human urine samples after ingestion of wine, tea, beer, or coffee has shown that polyphenol compounds and metabolites such as resveratrol [31], 4-*O*-methylgallic acid, isoferulic acid [32], and isoxanthohumol [33] are excreted through renal filtration. Table 4 provides detailed information about the plasma levels of polyphenol metabolites after the ingestion of beer.

Table 2: Phenolic acids contained in different types of beer.

Molecule	Mean content (mg/100 ml)			
	Alcohol-free	Ale	Dark	Regular
Hydroxybenzoic acids				
2,6-Dihydroxybenzoic acid				0.0900
2-Hydroxybenzoic acid	0.0011			0.2000
3,5-Dihydroxybenzoic acid				0.0300
3-Hydroxybenzoic acid				0.0300
4-Hydroxybenzoic acid	0.0073	0.1100	0.0700	0.9600
Gallic acid		0.1100	0.0300	0.0700
Gallic 3-O-gallate				0.2600
Gentisic acid				0.0300
Protocatechuic acid	0.2700	0.0600	0.0400	0.0500
Syringic acid		0.1100		0.0200
Vanillic acid	0.0300	0.2900	0.1700	0.0700
Hydroxycinnamic acids				
4-Caffeoylquinic acid				0.0100
5-Caffeoylquinic acid				0.0800
Caffeic acid	0.0100	0.0075	0.0300	0.0300
Ferulic acid	0.1200	0.3300	0.0900	0.2600
m-Coumaric acid				0.0200
o-Coumaric acid				0.1500
p-Coumaric acid	0.4000	0.1200	0.0500	0.1000
Sinapic acid	0.0073	0.0700	0.0300	0.0200
Hydroxyphenylacetic acids				
4-Hydroxyphenylacetic acid				0.0300
Homovanillic acid				0.0500

Data from the Phenol-Explorer database [12].

4. Menopause: Physiology, Symptoms, and Current Treatment

Menopause is defined as the permanent cessation of menstruation as a direct result of the end of ovarian follicular activity [35]. Follicular development is a cyclical process that occurs on average every 28 days during reproductive life. However, with age, these cycles become irregular and then stop completely. This cessation causes abnormal fluctuations of sex hormones, such as the follicle-stimulating hormone (FSH), anti-Müllerian hormone, estrogen, and insulin-like growth factors-I (ILGF-I), which eventually lead to physiological and morphological changes in many organs and systems in women [36].

These physiological changes induce different symptoms and signs which are characteristic of menopausal women, such as irregular bleeding, night sweats, hot flashes, tachycardia, breast pain, lack of energy, dyspareunia, joint soreness, atrophic vaginitis, interrupted sleeping patterns, anxiety, mood swings, dry skin, and loss of libido [37, 38]. Moreover, menopause may also predispose women to a series of risks, such as an increased risk of atherosclerosis [39–43], osteopenia, and osteoporosis [44, 45] (Figure 2).

Hot flashes are one of the most frequent symptoms presented by women undergoing menopause. They have a profound impact on the quality of life and increase health costs [46]. Vasomotor symptoms represent one of the main reasons why menopausal women seek medical care and treatments in the hope of relieving their discomfort [47]. Hot flashes are the result of the brain's response to diminished and fluctuating sex hormone concentrations that occur in menopause [48, 49]. Mechanisms of temperature homeostasis on the hypothalamus and peripheral vasculature are influenced by different hormones such as ovarian hormones, norepinephrine, and serotonin. Kronenberg described the links between vasomotor symptoms and different thermal, hormonal, and autonomic parameters, demonstrating the relevance of hormones in the deregulation of core body temperature that leads to hot flashes in menopause [50].

Current menopausal treatment includes estrogen hormone replacement therapy (HRT); selective estrogen

Table 3: Other phenolic compounds contained in beer.

Molecule	Mean content (mg/100 ml)			
	Alcohol-free	Ale	Dark	Regular
2,3-Dihydroxy-1-guaiacylpropanone	0.0025			0.0034
3-Methylcatechol			0.0029	0.0001
4-Ethylcatechol			0.0010	0.0006
4-Hydroxycoumarin				0.1100
4-Methylcatechol			0.0022	
4-Vinylguaiacol		0.0100	0.0300	0.1500
4-Vinylphenol			0.0300	0.0045
Catechol			0.0100	0.0011
Esculin				0.0200
Pyrogallol			0.0300	0.0047
Tyrosol	0.2700			0.3200
Umbelliferone				0.0017
Vanillin	0.0048			0.0200

Data from the Phenol-Explorer database [12].

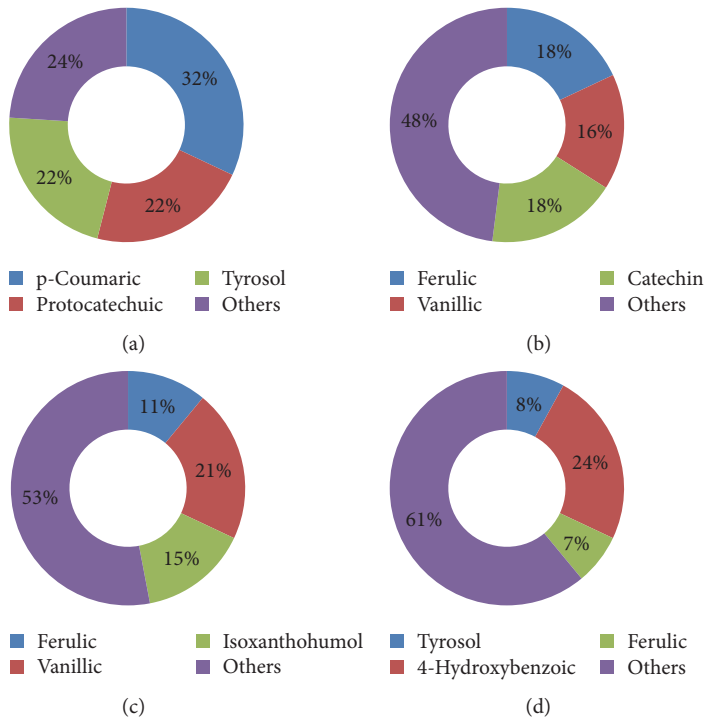


Figure 1: Main polyphenol content in different types of beer presented in percentages: (a) alcohol-free beer; (b) ale beer; (c) dark beer; (d) regular beer.

receptor modulators, such as tamoxifen and raloxifene [51]; and other medications such as selective serotonin reuptake inhibitors that alleviate vasomotor symptoms [52]. However, in different studies carried out in human patients, it has been suggested that HRT has no benefit in preventing cardiovascular disease and may even lead to an increased risk of arterial and venous thrombotic events [53], ovarian cancer [54], nonalcoholic steatohepatitis [55], and other diseases. These reports have encouraged scientists to find alternative and safer treatment options for menopausal symptoms.

5. Moderate Beer Intake and Health

Although it is well known that ethanol is a carcinogenic substance for humans [56], several studies have shown that regular and moderate intake of fermented beverages, such as wine and beer, may be associated with different positive

Table 4: Mean plasmatic levels of polyphenolic metabolites after beer intake.

Polyphenolic metabolite	Dose per day	Mean concentration (plasma)	T-Max (h)	Ref.
Ferulic acid	500 ml	0.11 μmol/l	0.5	[34]
4-Hydroxyphenylacetic acid	500 ml	1.4 μmol/l	0.5	[30]
Vanillic acid	500 ml	0.11 μmol/l	0.5	[30]
p-Coumaric acid	500 ml	0.05–0.07 μmol/l	0.5	[30]
Caffeic acid	500 ml	0.05–0.07 μmol/l	0.5	[30]

T-Max: time when maximal concentration is achieved.

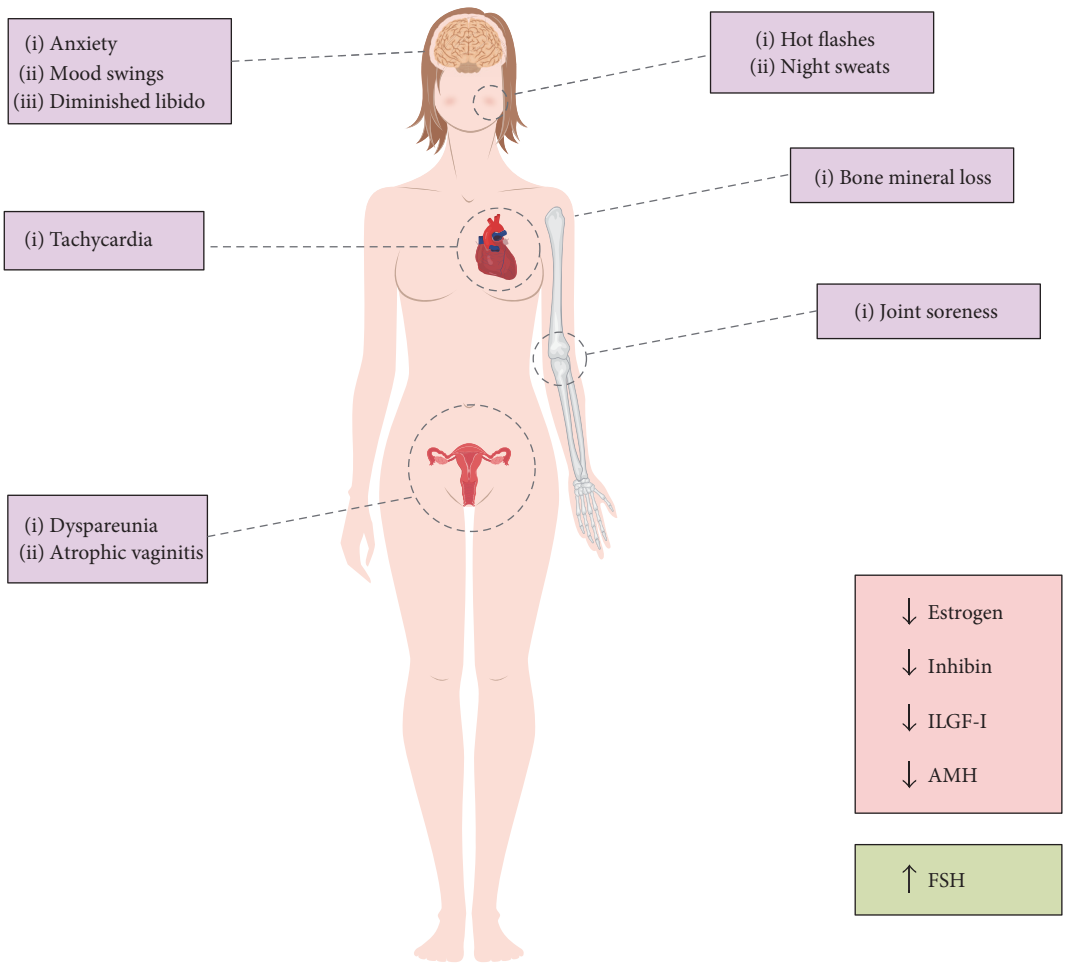


Figure 2: Sexual hormone status and common clinical manifestations in menopause. Insulin-like growth factor I (ILGF-I); anti-Müllerian hormone (AMH); follicle-stimulating hormone (FSH).

health effects, such as the reduction in the risk of cardiovascular disease as evidenced by the J-shaped relation found in wine [57] and beer [58] intake on cardiovascular risk, the reduction in atheroma plaque formation [59], prevention on different cancer types [23, 60, 61], and the reduction in bone mineral loss that leads to osteoporosis and osteopenia [15, 62]. The lack of evidence attributing the same effects to spirit intake suggests that polyphenolic compounds might play an important role in the beneficial effects of moderate alcoholic beverage intake on several health outcomes [63–66].

6. Beer and Menopause

Several intervention studies have evaluated the effects of beer and menopause. An 8-week, randomized, double-blind, cross-over trial showed that consuming 8-prenylnaringenin (8-PN), a characteristic polyphenol from hops and beer, resulted in a significant reduction in menopause symptoms [14] and discomforts [67]. Vasomotor symptoms are believed to be caused by a slight increase in body temperature in conjunction with a smaller thermo-neutral zone [68]. These processes are controlled by a region of the anterior

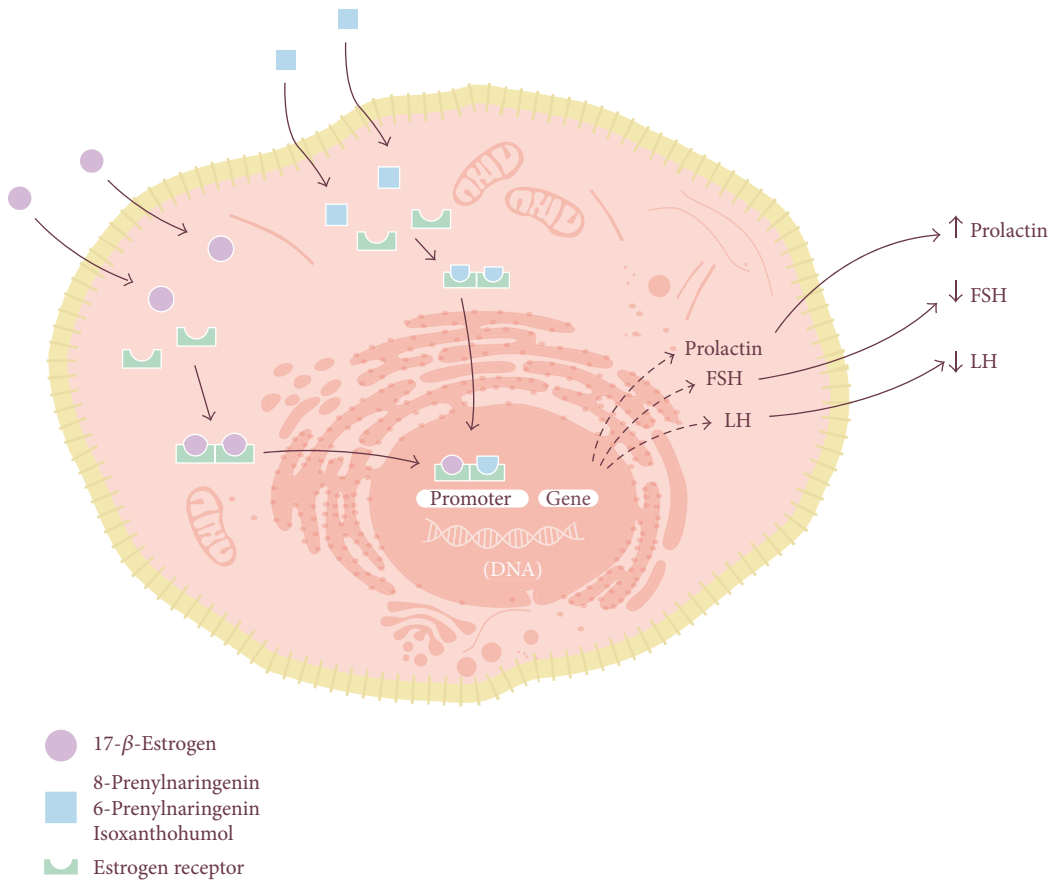


Figure 3: Mechanism of phytoestrogens. Like estrogen, phytoestrogen molecules travel through plasma and diffuse into the target cells, where they bind to cytoplasmatic estrogen receptors (ER). The new molecule-ER complex then dimerizes and is translocated into the nucleus, where it binds to specific promoters that decrease the expression of genes translating hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and promote the translation of prolactin. The use of estrogen receptors by 17-b-estrogen and molecules such as 8-prenylaringenin, 6-prenylaringenin, and isoxanthohumol explains why these molecules can be used to decrease the intensity of many classical symptoms of menopause.

hypothalamus called the thermoregulatory nucleus. This area responds to sex hormones as shown by experimental models with ovariectomized rats. These rats presented significant differences in body temperature compared to a unovariectomized control group, and the differences reversed when the rats were treated with estrogens or clonidine, an alpha-adrenoceptor used for vasomotor symptom treatment, suggesting that temperature irregularities in menopause may be due to changes in the sex hormone regulatory system [69]. In the same animal model, low doses of approximately 400 μg/kg/day of 8-prenylnaringenin were also able to alleviate menopausal vasomotor symptoms [70].

The effect of 8-prenylnaringenin may be explained by its strong affinity for both alpha and beta estrogen receptors (ER). The binding of 8-PN and the consequent activation of ERs lead to the stimulation of alkaline phosphatase activity and upregulate the activity of progesterone receptors and presenelin-2 [14], both of which are estrogen-stimulated genes (Figure 3). In addition, low doses of 8-prenylnaringenin increase the libido of menopausal women [71].

The absorption of hop phenolic acid and the pharmacokinetics and possible health benefits of hops have been studied in women [72]; however, at present, no clinical trial has assessed the effects of moderate beer consumption on menopausal women.

7. Summary

Menopause is a physiological condition that causes significant discomfort in many women around the world with the presentation of a myriad of symptoms related to an imbalance in sex hormone levels. Hot flashes and night sweats are two of the most common clinical findings in menopausal women that lead them to seek medical care. Since traditional hormone replacement therapies increase health risks, alternative, safer treatment options are needed. Hop and beer polyphenols seem to be an alternative to alleviate the menopausal symptoms presented by women.

There is evidence that regular and moderate intake of the polyphenols commonly found in hop and beer may help to relieve many common symptoms presented by women undergoing menopause. Said benefits can also be obtained by menopausal women from regular alcohol-free beer consumption, since ingredients used and most processes are

shared between alcohol-free and regular beer. Alcohol-free beer could provide women with all the same possible benefits, without the risk of gastrointestinal pathologies and cancer that frequent alcohol consumption represents to health. Nonetheless, randomized intervention clinical trials are needed to confirm their efficacy.

Disclosure

No foundation or institution was involved in the writing of the manuscript or the decision to submit the manuscript for publication.

Conflicts of Interest

Anna Tresserra-Rimbau, Rosa M. Lamuela-Raventós, and Ramon Estruch have received funding from The European Foundation for Alcohol Research (ERAB). Rosa M. Lamuela-Raventós and Ramon Estruch report serving on the board of and receiving lecture fees from Research Foundation on Wine and Nutrition (FIVIN) and Cerveceros de España. Rosa M. Lamuela-Raventós has received lecture fees and travel support from PepsiCo, and Ramon Estruch reports serving on the boards of the Mediterranean Diet Foundation, receiving lecture fees from Sanofi-Aventis, and receiving grant support through his institution from Novartis.

Acknowledgments

This work was supported by the European Foundation for Alcohol Research (ERAB) (EA 1324, EA 1514, EA 1515, and EA 1517), the CICYT (AGL2016-79113-R), and the Instituto de Salud Carlos III (ISCIII) (CIBEROBN) from the Ministerio de Economía, Industria y Competitividad (MEIC) (AEI/FEDER, UE) and Generalitat de Catalunya (GC) (2014 SGR 773).

References

[1] World Health Organization, *Alcohol in the European Union. Consumption, Harm and Policy Approaches*, World Health Organization, Regional office for Europe, Copenhaguen, Denmark, 2012.

[2] M. Adjemian and R. Volpe, *Alcohol consumption and food-at-home dietary quality in the United States*, Journal of Wine Economics. 2012 Annual Meeting, August 12-14, Agricultural and Applied Economics Association, Seattle, Washington, WA, USA, 2012.

[3] W. Jiajing, L. Li, B. Terry, Y. Linjie, L. Yuanqing, and X. Fulai, "Revealing a 5,000-y-old beer recipe in China," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 23, pp. 6444–6448, 2016.

[4] R. W. Unger, *Beer in the Middle Ages and the Renaissance Philadelphia*, University of Pennsylvania Press, Philadelphia, PA, USA, 2007.

[5] L. C. Nogueira, R. F. do Rio, P. C. Lollo, and I. M. Ferreira, "Moderate alcoholic beer consumption: the effects on the lipid profile and insulin sensitivity of adult men," *Journal of Food Science*, vol. 82, no. 7, pp. 1720–1725, 2017.

[6] C. Matsumoto, M. D. Miedema, P. Ofman, J. M. Gaziano, and H. D. Sesso, "An expanding knowledge of the mechanisms and effects of alcohol consumption on cardiovascular disease," *Journal of Cardiopulmonary Rehabilitation and Prevention*, vol. 34, no. 3, pp. 159–171, 2014.

[7] S. Arranz, G. Chiva-Blanch, P. Valderas-Martínez, A. Medina-Remón, R. M. Lamuela-Raventós, and R. Estruch, "Wine, beer, alcohol and polyphenols on cardiovascular disease," *Nutrients*, vol. 4, no. 7, pp. 759–781, 2012.

[8] A. Hernandez-Hernandez, A. Gea, M. Ruiz-Canela et al., "Mediterranean alcohol-drinking pattern and the incidence of cardiovascular disease and cardiovascular mortality: the SUN project," *Nutrients*, vol. 7, no. 11, pp. 9116–9126, 2015.

[9] G. Chiva-Blanch, E. Magraner, X. Condines et al., "Effects of alcohol and polyphenols from beer on atherosclerotic biomarkers in high cardiovascular risk men: a randomized feeding trial," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 25, no. 1, pp. 36–45, 2015.

[10] A. Di Castelnuovo, S. Costanzo, R. di Giuseppe, G. de Gaetano, and L. Iacoviello, "Alcohol consumption and cardiovascular risk: mechanisms of action and epidemiologic perspectives," *Future Cardiology*, vol. 5, no. 5, pp. 467–477, 2009.

[11] T. Brügger-Andersen, V. Pönitz, S. Snapinn, and K. Dickstein, "Moderate alcohol consumption is associated with reduced long-term cardiovascular risk in patients following a complicated acute myocardial infarction," *International Journal of Cardiology*, vol. 133, no. 2, pp. 229–232, 2009.

[12] J. A. Rothwell, J. Pérez-Jiménez, V. Neveu et al., "Phenol-explorer 3.0: a major update of the phenol-explorer database to incorporate data on the effects of food processing on polyphenol content," *Database: The Journal of Biological Databases and Curation*, vol. 2013, 2013.

[13] K. Annekathrin, Z. Oliver, and K. Georg, "Hop extracts and hop substances in treatment of menopausal complaints," *Planta Medica*, vol. 79, no. 7, pp. 576–579, 2013.

[14] A. Fatemeh, M. Hamid, and R. Nasibeh, "Hops for menopausal vasomotor symptoms: mechanisms of action," *Journal of Menopausal Medicine*, vol. 22, no. 2, pp. 62–64, 2016.

[15] J. Pedrera-Zamorano, J. Lavado-Garcia, R. Roncero-Martin, J. Calderon-Garcia, T. Rodriguez-Dominguez, and M. Canal-Macias, "Effect of beer drinking on ultrasound bone mass in women," *Nutrition*, vol. 25, no. 10, pp. 1057–1063, 2009.

[16] A. W. Taylor, E. Barofsky, J. A. Kennedy, and M. L. Deinzer, "Hop (Humulus lupulus L.) proanthocyanidins characterized by mass spectrometry, acid catalysis, and gel permeation chromatography," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 14, pp. 4101–4010, 2013.

[17] P. Quifer-Rada, A. Vallverdú-Queralt, M. Martínez-Huélamo et al., "A comprehensive characterisation of beer polyphenols by high resolution mass spectrometry (LC–ESI-LTQ-Orbitrap-MS)," *Food Chemistry*, vol. 169, pp. 336–343, 2015.

[18] D. Intelmann, G. Haseleu, and T. Hofmann, "LC-MS/MS quantitation of hop-derived bitter compounds in beer using the ECHO technique," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 4, pp. 1172–1182, 2009.

[19] J. E. Hall, "Neuroendocrine physiology of the early and late menopause," *Endocrinology and Metabolism Clinics of North America*, vol. 33, no. 4, pp. 637–659, 2004.

[20] A. Ziv-Gal and J. A. Flaws, "Factors that may influence the experience of hot flushes," *Journal of Women's Health*, vol. 19, no. 10, pp. 1905–1914, 2010.

[21] F. Kronenberg, "Menopausal hot flashes: a review of physiology and biosociocultural perspective on methods of assessment," *The Journal of Nutrition*, vol. 140, no. 7, pp. 1380S–1385S, 2010.

[22] D. De Keukeleire, L. De Cooman, H. Rong, A. Heyerick, J. Kalita, and S. R. Milligan, "Functional properties of hop polyphenols," *Basic Life Sciences*, vol. 66, pp. 739–760, 1999.

[23] C. Gerhauser, "Beer constituents as potential cancer chemopreventive agents," *European Journal of Cancer*, vol. 41, no. 13, pp. 1941–1954, 2005.

[24] C. Leitao, E. Marchioni, M. Bergaentzlé et al., "Effects of processing steps on the phenolic content and antioxidant activity of beer," *Journal of Agricoultural and Food Chemistry*, vol. 59, no. 4, pp. 1249–1255, 2011.

[25] M. N. Clifford, "Diet-derived phenols in plasma and tissues and their implications for health," *Planta Medica*, vol. 70, no. 12, pp. 1103–1114, 2004.

[26] C. Cueva, I. Gil-Sánchez, B. Ayuda-Durán et al., "An integrated view of the effects of wine polyphenols and their relevant metabolites on gut and host health," *Molecules*, vol. 22, no. 1, 2017.

[27] M. Monagas, M. Urpi-Sarda, F. Sánchez-Patán et al., "Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites," *Food & Function*, vol. 1, no. 3, pp. 233–253, 2010.

[28] T. Requena, M. Monagas, M. Pozo-Bayón et al., "Perspectives of the potential implications of wine polyphenols on human oral and gut microbiota," *Trends in Food Science & Technology*, vol. 21, no. 7, pp. 332–344, 2010.

[29] A. Scalbert and G. Williamson, "Dietary intake and bioavailability of polyphenols," *Journal of Nutrition*, vol. 130, no. 8S, pp. 2073S–2085S, 2000.

[30] M. Nardini, F. Natella, C. Scaccini, and A. Chiselli, "Phenolic acids from beer are absorbed and extensively metabolized in humans," *The Journal of Nutritional Biochemistry*, vol. 17, no. 1, pp. 14–22, 2006.

[31] R. Zamora-Ros, M. Urpi-Sarda, R. M. Lamuela-Raventós et al., "Diagnostic performance of urinary resveratrol metabolites as a biomarker of moderate wine consumption," *Clinical Chemistry*, vol. 52, no. 7, pp. 1373–1380, 2006.

[32] J. M. Hodgson, S. Y. Chan, I. B. Puddey et al., "Phenolic acid metabolites as biomarkers for tea- and coffee-derived polyphenol exposure in human subjects," *The British Journal of Nutrition*, vol. 91, no. 2, pp. 301–306, 2004.

[33] P. Quifer-Rada, M. Martínez-Huelamo, G. Chiva-Blanch, O. Jauregui, R. Estruch, and R. M. Lamuela-Raventós, "Urinary isoxanthohumol is a specific and accurate biomarker of beer consumption," *The Journal of Nutrition*, vol. 144, no. 4, pp. 484–488, 2014.

[34] R. A. Canccetta, K. D. Croft, L. J. Beilin, and I. B. Puddey, "Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivo lipoprotein oxidizability," *American Journal of Clinical Nutrition*, vol. 71, no. 1, pp. 67–74, 2000.

[35] J. S. Berek, *Berek and Novak's Gynecology*, Lippincott Williams and Wilkins, Philadelphia, PA, USA, 15th edition, 2012.

[36] M. R. Sowers, A. D. Eyvazzadeh, D. McConnell et al., "Anti-Mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 9, pp. 3478–3483, 2008.

[37] P. Llaneza, M. P. García-Portilla, D. Llaneza-Suárez, B. Armott, and F. R. Pérez-López, "Depressive disorders and the menopause transition," *Maturitas*, vol. 71, no. 2, pp. 120–130, 2012.

[38] B. Hoffman, J. Schorge, J. Schaffer, L. Halvorson, K. Bradshaw, and F. Cunningham, *Williams Gynecology*, McGraw-Hill Medical, New York, NY, USA, 2nd edition, 2012.

[39] H. Souza and G. Tezini, "Autonomic cardiovascular damage during post-menopause: the role of physical training," *Aging and Disease*, vol. 4, no. 6, pp. 320–328, 2013.

[40] V. Malinauskiene and A. Tamosiunas, "Menopause and myocardial infarction risk among employed women in relation to work and family psychosocial factors in Lithuania," *Maturitas*, vol. 66, no. 1, pp. 94–98, 2010.

[41] I. A. Ebong, K. E. Watson, D. C. Goff et al., "Age at menopause and incident heart failure: the multi-ethnic study of atherosclerosis," *Menopause*, vol. 21, no. 6, pp. 585–591, 2014.

[42] E. Barrett-Connor, "Menopause, atherosclerosis, and coronary artery disease," *Current Opinion in Pharmacology*, vol. 13, no. 2, pp. 186–191, 2013.

[43] M. Wellons, P. Ouyang, P. J. Schreiner, D. M. Herrington, and D. Vaidya, "Early menopause predicts future coronary heart disease and stroke: the multi-ethnic study of atherosclerosis," *Menopause*, vol. 19, no. 10, pp. 1081–1087, 2012.

[44] D. McLemon, J. Powell, R. Jugdaohsingh, and H. Macdonald, "Do lifestyle choices explain the effect of alcohol on bone mineral density in women around menopause," *The American Journal of Clinical Nutrition*, vol. 95, no. 5, pp. 1261–1269, 2012.

[45] L. Baig, F. A. Mansuri, and S. A. Karim, "Association of menopause with osteopenia and osteoporosis: results from population based study done in Karachi," *Journal of the College of Physicians and Surgeons–Pakistan*, vol. 19, no. 4, pp. 240–244, 2009.

[46] W. H. Utian, "Psychosocial and socioeconomic burden of vasomotor symptoms in menopause: a comprehensive review," *Health and Quality of Life Outcomes*, vol. 3, no. 1, p. 47, 2005.

[47] R. E. Williams, L. Kalilani, D. B. DiBenedetti, X. Zhou, S. E. Fehnel, and R. V. Clark, "Healthcare seeking and treatment for menopausal symptoms in the United States," *Maturitas*, vol. 58, no. 4, pp. 348–358, 2007.

[48] R. R. Friedman, "Pathophysiology and treatment of menopausal hot flashes," *Seminars in Reproductive Medicine Journal*, vol. 23, no. 2, pp. 117–125, 2005.

[49] D. C. Deecher, "Physiology of thermoregulatory dysfunction and current approaches to the treatment of vasomotor symptoms," *Expert Opinion on Investigational Drugs*, vol. 14, no. 4, pp. 434–448, 2005.

[50] F. Kronenberg, "Hot flashes: epidemiology and physiology," *Annals of the New York Academy of Sciences*, vol. 592, pp. 52–86, 1990.

[51] S. R. Davis, I. Dinatale, L. Rivera-Woll, and S. Davison, "Postmenopausal hormone therapy: from monkey glands to transdermal patches," *Journal of Endocrinology*, vol. 185, no. 2, pp. 207–222, 2005.

[52] M. S. Krause and S. T. Nakajima, "Hormonal and nonhormonal treatment of vasomotor symptoms," *Obstetrics and Gynecology Clinics of North America*, vol. 42, no. 1, pp. 163–179, 2015.

[53] H. M. Boardman, L. Hartley, A. Eisinga et al., "Hormone therapy for preventing cardiovascular disease in post-menopausal women," *The Cochrane Database of Systematic Reviews*, vol. 10, no. 3, 2015.

[54] C. La Vecchia, "Ovarian cancer: epidemiology and risk factors," *European Journal of Cancer Prevention*, vol. 26, no. 1, pp. 55–62, 2017.

[55] J. D. Yang, M. F. Abdelmalek, C. D. Guy et al., "Patient sex, reproductive status, and synthetic hormone use associate with histologic severity of nonalcoholic steatohepatitis," *Clinical Gastroenterology and Hepatology*, vol. 15, no. 1, pp. 127–131, 2017.

[56] World Health Organization and International Agency for Research on Cancer. IARC, "Monographs on the evaluation of carcinogenic risks to humans: alcohol consumption and ethyl carbamate," in *Monographs on the Evaluation of Carcinogenic Risks to Humans*, World Health Organization, International Agency for Research on Cancer, Lyon, France, 2010, Report No.: 96.

[57] R. Estruch, M. Urpi-Sarda, G. Chiva, E. S. Romero, M. I. Covas, and J. Salas-Salvadó, *Cerveza, Dieta Mediterránea y Enfermedad Cardiovascular. Dossier de Prensa*, Madrid, Spain, Centro de Información Cerveza y Salud, Cerveza y Salud, 2011.

[58] S. Costanzo, A. Di Castelnuovo, M. B. Donati, L. Iacoviello, and W. de Gaetano, "Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: a meta-analysis," *European Journal of Epidemiology*, vol. 26, no. 11, pp. 833–850, 2011.

[59] L. Brown, P. Kroon, D. Das et al., "The biological responses to resveratrol and other polyphenols from alcoholic beverages," *Alcoholism, Clinical and Experimental Research*, vol. 33, no. 9, pp. 1513–1526, 2009.

[60] M. N. Gronbaek, T. I. Sorensen, D. Johansen et al., "Beer, wine, spirits and mortality," *Läkartidningen*, vol. 98, no. 21, pp. 2585–2588, 2001.

[61] S. Renaud, D. Lanzmann-Petithory, R. Gueguen, and P. Conard, "Alcohol and mortality from all causes," *Biological Research*, vol. 37, pp. 183–187, 2004.

[62] K. L. Tucker, R. Jugdaohsing, J. J. Powell et al., "Effects of beer, wine, and liquor intakes on bone mineral density in older men and women," *American Journal of Clinical Nutrition*, vol. 89, no. 4, pp. 1188–1196, 2009.

[63] P. Gresele, C. Cerletti, G. Guglielmini, P. Pignatelli, G. de Gaetano, and F. Violi, "Effects of resveratrol and other wine polyphenols on vascular function: an update," *Journal of Nutritional Biochemistry*, vol. 22, no. 3, pp. 202–211, 2011.

[64] N. Martinez, M. Urpi-Sarda, M. A. Martinez-Gonzalez, C. Andres-Lacueva, and M. T. Mitjavila, "Dealcoholised beers reduce atherosclerosis and expression of adhesion molecules in apoE-deficient mice," *British Journal of Nutrition*, vol. 105, no. 5, pp. 721–730, 2011.

[65] A. Piazzon, M. Forte, and M. Nardini, "Characterization of phenolics content and antioxidant activity of different beer types," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 19, pp. 10677–10683, 2010.

[66] R. Estruch, E. Sacanella, E. Badia et al., "Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial. Effects of wine on inflammatory markers," *Atherosclerosis*, vol. 175, no. 1, pp. 117–123, 2004.

[67] R. Erkkola, S. Vervarcke, S. Vansteelandt, P. Rompotti, D. DeKeukeleire, and A. Heyerick, "A randomized, double-blind, placebo-controlled, cross-over pilot study on the use of a standardized hop extract to alleviate menopausal discomforts," *Phytomedicine*, vol. 17, no. 6, pp. 389–396, 2010.

[68] R. R. Friedman, "Hot flashes: behavioral treatments, mechanisms, and relation," *American Journal of Medicine*, vol. 118, no. 12Bf, pp. 124–130, 2005.

[69] H. H. Berendsen, A. H. Weekers, and H. J. Kloosterboer, "Effect of tibolone and raloxifene on the tail temperature of oestrogen-deficient rats," *European Journal of Pharmacology*, vol. 419, no. 1, pp. 47–54, 2001.

[70] J. Bowe, X. F. Li, J. Kinsey-Jones et al., "The hop phytoestrogen, 8-prenylnaringenin, reverses the ovariectomy-induced rise in skin temperature in an animal model of menopausal hot flushes," *Journal of Endocrinology*, vol. 191, no. 2, pp. 399–405, 2006.

[71] A. Keiler, O. Zierau, and G. Kretzschmar, "Hop extracts and hop substances in treatment of menopausal complaints," *Planta Medica*, vol. 79, no. 7, pp. 576–579, 2013.

[72] R. B. Van Breemen, Y. Yang, S. Banuvar et al., "Pharmacokinetics of prenylated hop phenols in women following oral administration of a standardized extract of hops," *Molecular Nutrition & Food Research*, vol. 58, no. 10, pp. 1962–1969, 2014.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 5812401, 18 pages
https://doi.org/10.1155/2017/5812401

Review Article

Effects of Polyphenol Intake on Metabolic Syndrome: Current Evidences from Human Trials

Gemma Chiva-Blanch[1,2] and Lina Badimon[1,2]

[1]*Cardiovascular Science Institute (ICCC)-Biomedical Research Institute Sant Pau (IIB-Sant Pau), Hospital de la Santa Creu I Sant Pau, Barcelona, Spain*
[2]*CiberCV, Institute Carlos III, Barcelona, Spain*

Correspondence should be addressed to Gemma Chiva-Blanch; gchiva@santpau.cat

Received 17 April 2017; Revised 3 July 2017; Accepted 13 July 2017; Published 15 August 2017

Academic Editor: Ryuichi Morishita

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors which severely increases the risk of type II diabetes and cardiovascular disease. Several epidemiological studies have observed a negative association between polyphenol intake and MetS rates. Nevertheless, there are relatively small numbers of interventional studies evidencing this association. This review is focused on human interventional trials with polyphenols as polyphenol-rich foods and dietary patterns rich in polyphenols in patients with MetS. Current evidence suggests that polyphenol intake has the potential to alleviate MetS components by decreasing body weight, blood pressure, and blood glucose and by improving lipid metabolism. Therefore, high intake of polyphenol-rich foods such as nuts, fruits, vegetables, seasoning with aromatic plants, spices, and virgin olive oil may be the cornerstone of a healthy diet preventing the development and progression of MetS, although there is no polyphenol or polyphenol-rich food able to influence all MetS features. However, inconsistent results have been found in different trials, and more long-term randomized trials are warranted to develop public health strategies to decrease MetS rates.

1. Introduction

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors which severely increases the risk of type II diabetes [1] and cardiovascular disease (CVD) [2, 3]. CVD and diabetes are major causes of disability, whose prevalence is increasing worldwide [4]. Therefore, strategies to decrease the onset and progression of MetS and their associated pathologies are of extreme interest.

In terms of the relationship between nutrition and MetS, undernutrition and overnutrition from an energy balance focus are the most studied topics, because a triggering factor of MetS is obesity. Nevertheless, intense research from the last decades has shown that not only energy balance but macro- and micronutrient composition of the diet may influence MetS onset and progression. On those grounds, diet is considered a major environmental insult contributing to the increase in metabolic disease incidence, especially in younger individuals, and current evidence highlights that the overall quality diets such as the Mediterranean diet, the Nordic diet, and Dietary Approaches to Stop Hypertension (DASH) diets protect against MetS or are even able to improve the MetS phenotype [5–7]. A diet is a complex mixture of food and compounds with multiple interactions and varying bioavailability, which hampers the identification and isolation of the effect of one single component in a total meal or diet. Although individual dietary components have to be considered in the context of a whole dietary pattern to evaluate their effects on MetS, phytochemicals such as polyphenols and their metabolites have been shown to modulate MetS through different mechanisms.

Polyphenols are biomolecules found in products from plant origin and have been shown to exert antioxidant and anti-inflammatory effects both *in vitro* and *in vivo* [8]. However, controlled trials in MetS subjects with single phenolic compounds or specific food/beverage/extract do not provide strong evidence for the promising protective effects of polyphenols on cardiovascular diseases as reported in numerous animal and cell studies. This can be attributable to the peculiar

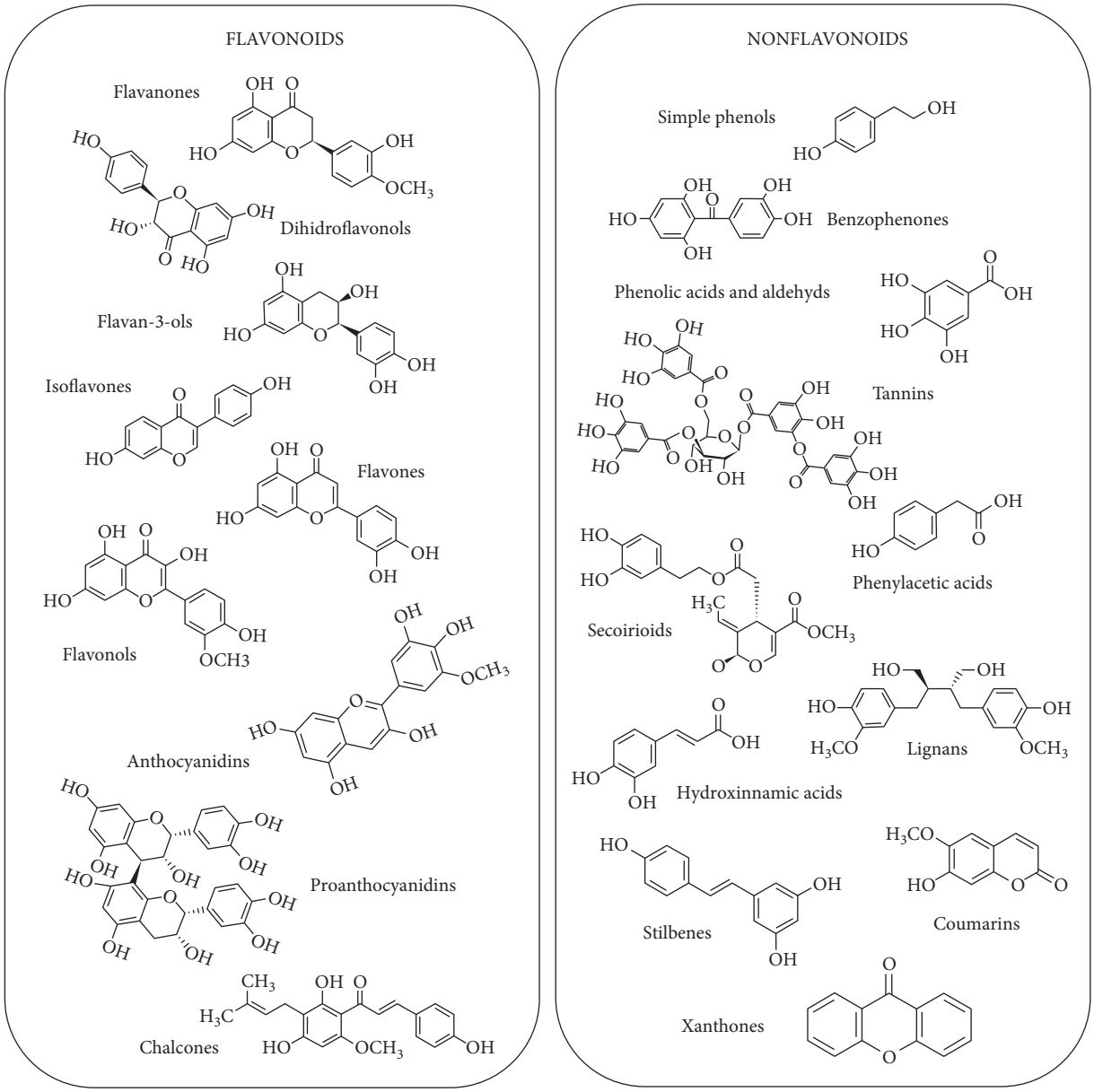


Figure 1: Representative chemical structures of major groups of polyphenols. Adapted from Phenol-Explorer (http://phenol-explorer.eu/) and Andres-Lacueva et al. [132].

characteristics of MetS patients, the use of overdoses in experimental models compared to human trials, and the low bioavailability of polyphenols in the small intestine (5 to 10%).

Therefore, the aim of this review is to update the knowledge on the effects of polyphenol intake in MetS patients, derived from interventional human studies with polyphenols as polyphenol-rich foods and dietary patterns rich in polyphenols.

2. Phenolic Compounds: Occurrence, Intake, and Bioavailability

Polyphenols (phenolic compounds) are the most widely distributed secondary metabolites from plants in dietary sources. Although they are not considered essential micronutrients, a huge body of literature evinces their beneficial effects on human health, especially in diets associated with high consumption of fruits and vegetables.

Polyphenols share a phenol carbon ring but have different structures, and more than 500 different molecules have been found in foods [9]. According to their chemical structure, they can be divided into two major groups: flavonoids and nonflavonoids, with subsequent subgroups as depicted in Figure 1. Table 1 displays the main sources of polyphenols in the diet.

Worldwide consumption of polyphenols differs between countries. For instance, mean polyphenol intake of an adult subject is about 283–1000 mg of total polyphenols/day in

Table 1: Major food sources of dietary polyphenols.

Food group	Polyphenols
Wine	Phenolic acids, stilbenes, flavonols, dihydroflavonols, anthocyanins, flavanol monomers (catechins), and flavanol polymers (proanthocyanidins)
Beer	Prenylated flavonoids, phenolic acids, simple phenols, flavanols, hydroxycoumarins, flavonols, and flavones
Coffee	Phenolic acids
Tea	Catechins, phenolic acids, flavonols, and proanthocyanidins
Cocoa	Flavanols (catechins and proanthocyanidins), phenolic acids, flavonols, some stilbenes, simple phenols, and isocoumarins
Vegetables	Flavonols (kaempferol and quercetin derivatives) and hydroxycinnamic acids (cabbages) Hydroxycinnamic acids, flavonols, and flavanones (tomatoes) Flavonoids, phenolic acids, and capsaicinoids (pepper) Hydroxycinnamic acids and anthocyanins (eggplant) Hydroxycinnamic acids, flavones, and flavonols (leaf vegetables) Flavonols such as quercetin (onions) Phenolic acids (roots)
Fruits	Anthocyanins, ellagitannins, and proanthocyanidins (berries) Flavanone glycosides, polymethoxylated flavones, and traces of flavonols and hydroxycinnamic acids (citrus) Chlorogenic acids, anthocyanins, flavonols, catechins, and proanthocyanidins (pommes and drupes)
Nuts	Catechins, proanthocyanidins, ellagitannins, and ellagic acid
Pulses	Proanthocyanidins, flavonols, flavanones, and hydroxycinnamic acids
Soy	Isoflavonoids
Virgin or extra virgin olive oil	Tyrosols
Sesame oil	Lignans and phenolic acids
Aromatic plants	Phenolic acids, flavones, phenolic diterpenes, and flavanones
Spices	Phenylpropenes, phenolic acids, flavones, and flavonols

Data extracted from Phenol-Explorer (http://phenol-explorer.eu/).

France [10, 11], 500–1100 mg/day in Spain [12], about 700 mg/d in Italy [13, 14], 890 mg/day in Finland [15], 534 mg/day in Brazil [16], and around 1500 mg/day in Japan [17], while total flavonoid intake is about 190 mg/day in the UK and Ireland [18], about 240–350 mg/day in the US [19, 20], around 450 mg/day in Australia [21], between 50 and 500 mg/day in China [22], and around 320 mg/day in Korea [23]. In addition, there is a great variability in polyphenol intake within countries depending on the type of diet consumed. Because polyphenols are from plant origin, vegetarians and vegans should have higher intakes of polyphenols than people who follow westernized diets. However, in the US and Canada, coffee consumption is more determinant in the amount of total polyphenol intake than the dietary pattern itself [24].

Besides differences between countries and dietary patterns, differences in polyphenol intake can also be attributed to the analytical method used to quantify polyphenols in foods. To determine polyphenol content in foods, there are two main approaches: by measuring total polyphenol content with the Folin-Ciocalteu colorimetric assay (by quantifying the total reducing capacity of a sample) and by measuring single polyphenols or metabolites by chromatographic techniques [25]. The Folin-Ciocalteu method gives higher values of polyphenols than chromatographic techniques because several other reducing agents contained in foods such as vitamin C or some nitrogen-containing compounds [26] interfere in the quantification. Although it is an estimation of total polyphenols, it should strictly be considered a measurement of the *in vitro* antioxidant capacity of foods. On the other hand, chromatographic techniques offer higher sensitivity and sensibility than the colorimetric methods.

To determine polyphenol intake in humans, there are also two different approaches: by determining biomarkers of intake (namely, total polyphenols or single polyphenols or their metabolites in biological samples with chromatographic techniques or again with the Folin-Ciocalteu method) or by estimating its intake.

The estimation of the intake of polyphenols is performed by administering food frequency questionnaires or food recalls to the study subjects and translating this information to single or total polyphenols by using databases to calculate polyphenol intake from consumed food. The major public databases used for polyphenol content of foods and beverages are the United States Department of Agriculture (USDA) databases for flavonoids, proanthocyanidins, and isoflavones and the Phenol-Explorer database. USDA databases only present values for flavonoids, but Phenol-Explorer is a more complete database because it gathers information on all classes and types of polyphenols, including their metabolites [27]. Nevertheless, this approach is limited due to self-reporting bias and because of the seasonal and geographical variability in the polyphenolic composition of foods, the ripeness of the food at time of harvest and its storage before consumption, and the limited data on the polyphenolic composition of food databases. Moreover,

Table 2: Metabolic syndrome definition.

Cardiovascular risk factor	Measurement	Definition		
		Region	Men	Women
Central obesity	Waist circumference	Asia	≥90 cm	≥80 cm
		Europe, Africa, and Middle-East	≥94 cm	≥80 cm
		America	≥102 cm	≥88 cm
Insulin resistance	Fasting blood glucose	≥100 mg/dL (5.55 mmol/L)		
Hypertension	Blood pressure	≥130/85 mmHg		
Dyslipidemia*	Fasting blood triglycerides	≥150 mg/dL		
	Fasting blood HDL cholesterol	≤50 and ≤40 mg/dL for women and men, respectively		

According to the International Diabetes Federation (IDF), metabolic syndrome is defined as central obesity plus 2 or more cardiovascular risk factors [133], and according to the American Heart Association (AHA), metabolic syndrome is defined by having 3 or more cardiovascular risk factors [134]. *High triglyceride levels and low HDL cholesterol levels are considered independent risk factors for the definition of metabolic syndrome. HDL denotes high-density lipoprotein.

polyphenols are not homogeneously distributed within foods but are usually concentrated in the outer layers. Therefore, peeling or processing foods may substantially decrease its polyphenol content [28]. In addition, cooking foods provokes either losses or increases in some polyphenol content of foods [29].

The use of the Folin-Ciocalteu method carries the same limitations described above, and thus, chromatographic analyses provide the most reliable results, although only few polyphenols and their metabolites have been quantified in fluids or tissues. In addition, given the low bioavailability of polyphenols (5–10%) [30] and that maximal plasma concentrations are reached within the first two hours after ingestion and fall to baseline levels within 8 to 12 hours [31], 24 h urine provides a more accurate measure of the total polyphenol absorption, metabolism, and excretion over a 24 h period, even for polyphenols with short half-lives [32].

3. The Metabolic Syndrome

MetS is a cluster of several interrelated and well-documented cardiovascular risk factors (hyperglycemia, hypertension, dyslipidemia, insulin resistance, and central adiposity) that may provably result from the increasing prevalence of obesity and seems to be triggered by insulin resistance [33]. Many international organizations and expert groups, such as the World Health Organization (WHO), the European Group for the Study of Insulin Resistance (EGIR), the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII), the American Association of Clinical Endocrinology (AACE), the International Diabetes Federation (IDF), and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), have attempted to incorporate all the different parameters used to define MetS [34, 35]. However, the consensus definition of the IDF [34] seems the most suitable for practical use in clinical medicine, taking into account the incorporation of different thresholds for different ethnicities as shown in Table 2, recognizing that the risk associated with a particular waist measurement will differ in different populations.

As depicted in Figure 2, MetS is a pathological condition defined by a chronic, systemic, and low-grade inflammation [36] and oxidative status and characterized by the cluster of three or more independent CV risk factors, namely, abdominal obesity, hyperglycemia/insulin resistance, hypertriglyceridemia, low high-density lipoprotein (HDL) and/or high low-density lipoprotein (LDL) cholesterol, and high blood pressure [37], in which each factor contributes to the development and progression of each other. Overall, this leads to excessive tissue damage, endothelial dysfunction, thrombosis, insulin resistance, and high blood pressure. Accumulation of these cardiometabolic risk factors has been associated with increased CVD [38], diabetes, some forms of cancer, arthritis, neurodegenerative diseases [39], and all-cause mortality [40]. The prevalence of both obesity and type 2 diabetes has increased dramatically in recent decades worldwide, and both conditions represent substantial risk factors for the development of atherosclerotic disease and the resulting increased incidence of myocardial infarction and stroke [41].

Worldwide prevalence of MetS ranges between 10 and 84% depending on the ethnicity, age, gender, and race of the population [3, 42, 43]. As an average, one-quarter of the world's population has MetS [44]. Therefore, MetS has been highlighted as a major socioeconomic problem throughout the world.

4. Effects of Polyphenol Intake on the Metabolic Syndrome

Considering that a pro-oxidant status and low-grade chronic inflammation are hallmarks of MetS and that its severity seems to depend on the prevalent number of components of MetS, polyphenols appear as good dietary candidates to prevent MetS progression given their well-described antioxidant and anti-inflammatory actions [45]. Furthermore, polyphenols have been shown to improve insulin resistance [46], to decrease blood pressure [47] and body weight [48], and to improve the lipid profile [49]. Nevertheless, dietary strategies may be less effective for patients with a cluster of risk factors of MetS as a whole than for those patients with one or two risk factors. Along this line, the effects of polyphenol intake on healthy volunteers or low-moderate CV risk patients may differ from those on patients with MetS because of

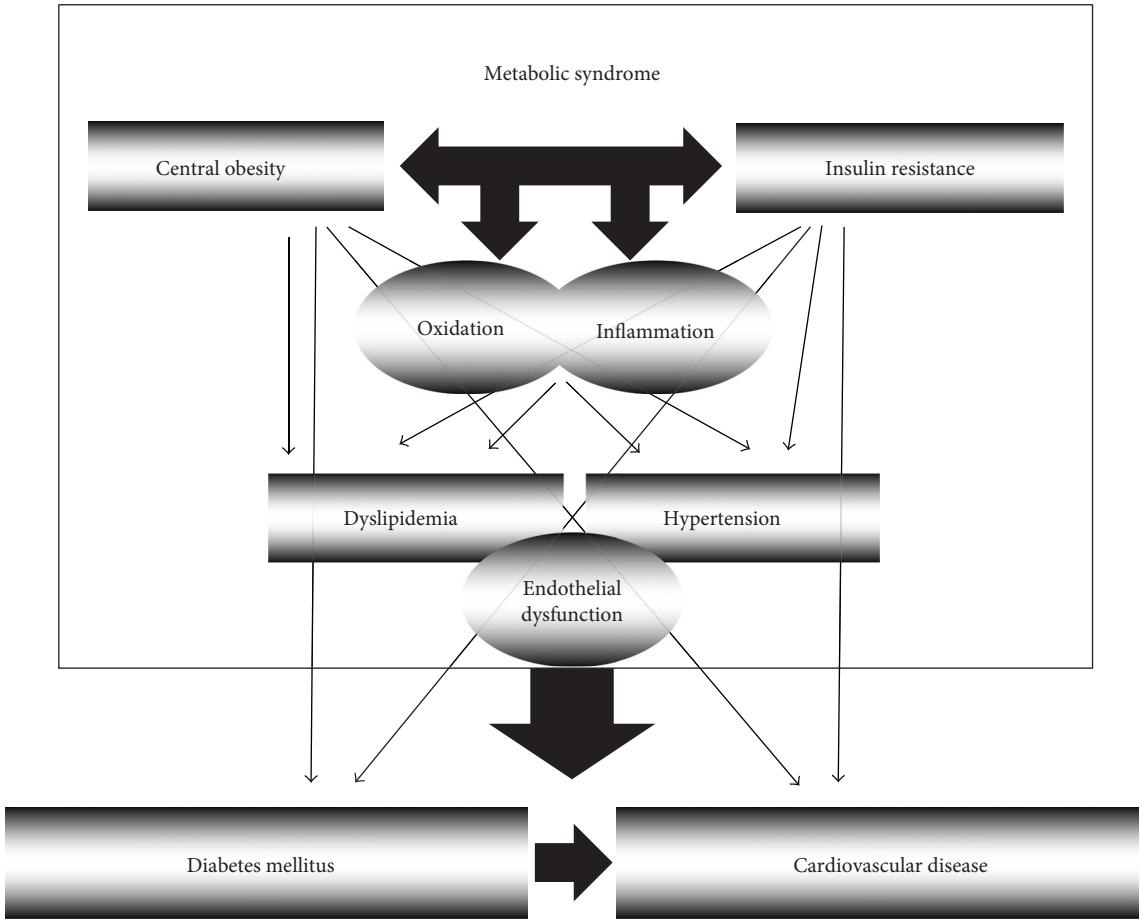


Figure 2: Pathological processes involved in the metabolic syndrome and their potential interactions.

their pathological characteristics. Thus, the results reported in humans are still inconsistent, and the metabolic benefits of polyphenols may strongly depend on the population studied. For instance, urolithins are the microbial metabolites of ellagitannin. Ellagitannin-metabolizing phenotypes (urolithin metabotypes A, B, and 0) differ among individuals depending on their body mass index. In fact, urolithin A in MetS patients only correlated inversely with glucose after intake of 30 g of nuts, while in healthy and overweight patients, urolithin A positively correlated with apolipoprotein A-I and HDL cholesterol after the intake of nuts [50]. Along the same line, a systematic review on the effects of grape polyphenols on MetS components [51] shows differential effects of grape polyphenols on MetS components according to the number of components in each patient. Moreover, in healthy volunteers, extra virgin olive oil consumption improved glycemia, insulin sensitivity, and the inflammatory phenotype, but these effects were observed in a significantly lesser extent in MetS patients [52]. A recent systematic review also postulates that polyphenols are effective in reducing some MetS features, but there is no single food, extract, or polyphenol able to act on all MetS features [53], suggesting that the whole dietary pattern and not food or extract supplements may contribute in controlling MetS progression. Considering their low bioavailability and metabolism, the protective functions of polyphenols may become effective only through frequent and sustained intake at a long term, in the context of a healthy and diversified diet. Given the complex nature of MetS, we aimed to review the modulation of MetS features by polyphenol intake with a special focus on MetS patients, defined according to the IDF, beyond their anti-inflammatory and antioxidant effects.

4.1. Epidemiological Evidence

4.1.1. Association between Polyphenol Intake and Central Obesity. In the past decades, polyphenols have attracted media interest because of their potential effects on lipid and energy metabolism and on reducing body weight. Several *in vitro* and animal studies have proposed different mechanisms by which polyphenols could play a potential role in reducing obesity, but few cohort studies have evaluated the association between polyphenol intake and body weight. In addition, interventional trials are very limited too.

In a Chinese cohort, regular tea consumers showed less percentage of body fat and waist-to-hip ratio compared to subjects who did not consume tea on a regular basis [54]. A longitudinal analysis from the Netherlands Cohort Study has shown that increased intake of flavones, flavonols, and catechins is associated with a lower increased body mass index (BMI) associated with age in women but not in men

[55]. Chocolate intake in a cohort of subjects under statin treatment without known cardiovascular disease or diabetes was associated with lower BMI, despite that higher chocolate intake was associated with higher calorie and saturated fat intake [56]. However, as recently reviewed, resveratrol intake does not mediate body weight loss in overweight/obese patients, showing a great difference in the response to resveratrol intake between animal and human studies [57].

Despite the potential effects of polyphenols on reducing body weight, a recent systematic review points out that the weight loss induced by polyphenols is not clinically relevant in overweight and obese individuals [58]. In addition, many of the interventional trials have a duration of less than 3 months, and therefore, long-term randomized interventional trials are needed to properly elucidate the role of polyphenols in weight loss and obesity prevention.

4.1.2. Association between Polyphenol Intake and Insulin Resistance. Polyphenol intake has been related to decreased risk of insulin resistance and type 2 diabetes. In a Mediterranean cohort at high cardiovascular risk, total polyphenol, total flavonoid (flavanones and dihydroflavonols), and stilbene intake was associated with decreased risk of type 2 diabetes [12]. In the Nurses' Health Studies (NHS) I and II, urinary excretion of flavanones (naringenin and hesperetin) and flavonols (quercetin and isorhamnetin), as well as caffeic acid, was associated with about 39%–48% decreased risk of type 2 diabetes at a middle term (5 years) but not at a long term (up to 11 years) [59]. In this study, only hesperetin was associated with decreased long-term risk of type 2 diabetes. However, in another substudy of women from the NHS I and II and men from the Health Professionals Follow-up Study, higher intakes of anthocyanins, but not of total flavonoids or other flavonoid subclasses, were significantly associated with lower risk of type 2 diabetes [60]. Oppositely, the European Prospective Investigation into Cancer and Nutrition- (EPIC-) InterAct case-cohort study observed that flavan-3-ol monomers and proanthocyanidin dimers and trimers, but not proanthocyanidins with greater polymerization degrees, were associated with decreased risk of type 2 diabetes [61]. Nevertheless, the Iowa Women's Health Study found no association between flavonoid consumption and the risk of type 2 diabetes [62], in accordance with the Women's Health Study [63], in which dietary intake of total flavonols and flavones, quercetin, kaempferol, myricetin, apigenin, and luteolin was not associated with decreased risk of type 2 diabetes. However, apple and tea consumption was associated with lower risk of type 2 diabetes. In this line, green tea (but not black tea) intake of more than 3 cups/day has been inversely associated with type 2 diabetes risk in a Japanese cohort [64]. On the other hand, the relationship between coffee intake and type 2 diabetes is a matter of controversy [65].

4.1.3. Association between Polyphenol Intake and Dyslipidemia. A meta-analysis has shown that green tea but not black tea [66] consumption decreases total and LDL cholesterol [67] with no effects on HDL cholesterol, although some studies observed that green tea consumption increased HDL cholesterol levels [68]. In the TOSCA.IT study with type 2 diabetic patients, high polyphenol intake is associated with slightly lower levels of LDL and triglycerides and higher levels of HDL cholesterol [14]. In the Moli-sani cohort, higher intakes of polyphenols were associated with lower total and LDL cholesterol, lower triglycerides, and higher levels of HDL cholesterol [69]. In the PREDIMED cohort, increased polyphenol intake at 5 years measured by total urinary polyphenol excretion was inversely associated with triglyceride levels but not with total, LDL, or HDL cholesterol levels [70]. In a subset of the NHANES cohort, urinary enterolignan concentrations (enterolactone and enterodiol) were associated with increased levels of HDL cholesterol and decreased levels of triglycerides, but no association was found for total or HDL cholesterol [71]. In a subset cohort of the ATHENA study, total polyphenol and anthocyanin intakes were not associated with an improved lipid profile, but individuals with the serum paraoxonase/arylesterase 1 single-nucleotide polymorphisms rs854549 and rs854552 showed a positive association between HDL cholesterol levels and total polyphenol and anthocyanin intakes [72].

4.1.4. Association between Polyphenol Intake and Hypertension. Several observational studies have revealed a positive correlation between greater intake of fruits and vegetables and a decreased prevalence of hypertension (reviewed in [73]). In a Mediterranean population at high cardiovascular risk, total polyphenol intake measured by urinary polyphenol excretion was associated with lower blood pressure levels and lower prevalence of hypertension [74]. However, in a Brazilian cohort, an inverse relationship was found for hypertension and intake of lignans, stilbenes, tyrosols, alkylphenols, and other polyphenols, but not for total polyphenol, flavonoid, or phenolic acid intake [75]. A meta-analysis of the effects of hibiscus tea showed that supplementation with this sour tea decreased blood pressure levels [76], and another meta-analysis has shown that green tea consumption decreases systolic blood pressure, although the size of the effects appears to be relatively small (about 2 mmHg) [67] in accordance with another meta-analysis [77]. High intake of blueberries and strawberries (rich in anthocyanins) has been associated with decreased risk of hypertension in subsets of the cohorts of women from the NHS I and II and men from the Health Professionals Follow-up Study (HPFS) [78]. Furthermore, in a cross-sectional study on women aged 18–75 years old, higher anthocyanin intake, but not intake of other flavonoids, was associated with lower systolic blood pressure and mean arterial pressure, whereas anthocyanin and flavone intake was associated with lower pulse wave velocity [79]. However, a recent meta-analysis pinpoints that blueberry supplementation (and consequently anthocyanin supplementation) does not decrease blood pressure [80]. Therefore polyphenols, and specifically flavonoids, show potential antihypertensive effects, which may differ in relation to the disease status (healthy versus pre-hypertensive versus hypertensive individuals).

4.1.5. Association between Polyphenol Intake and the Metabolic Syndrome. Several epidemiological studies have

observed a negative association between polyphenol intake and MetS rates. In a cross-sectional study comprising more than 8800 subjects, BMI, waist circumference, blood pressure, and triglycerides were significantly lower among individuals in the higher quartiles of polyphenol intake, assessed by food frequency questionnaires. In addition, the odds ratio for MetS was about 0.75 for subjects at the highest quartile of polyphenol intake [81].

Coffee consumption has been inversely associated with the prevalence of MetS in Korean women [82] and Japanese [83] and Danish men and women [84], although these associations may vary depending on the body mass index [85].

Tea drinking, especially 240 ml or more of tea daily, was inversely associated with incidence of MetS in elderly Taiwanese males [86]. A cross-sectional study of US adults showed that intake of hot (brewed) tea, but not of iced tea, was inversely associated with obesity and biomarkers of MetS and CVDs [87]. On the other hand, a cross-sectional study of a Japanese cohort observed no such association [83].

In a Spanish cohort, moderate red wine consumption was negatively associated with MetS prevalence, accompanied by reduced waist circumference, blood pressure, and fasting glucose and higher HDL levels [88]. On the other hand, in another Spanish cohort, no relationship was found between wine consumption and MetS prevalence, but beer consumption was associated with higher risk for MS [89].

Overall, epidemiologic data is very useful from a hypothesis-generating perspective, but usually, nutritional data relies on the transformation of food frequency questionnaires to food or single polyphenol intake, and large epidemiological trials do not often use reliable biomarkers of intake. Therefore, caution should be taken when interpreting epidemiological data.

4.2. Evidences from Interventional Studies. As previously stated, MetS is triggered by a maintained pro-oxidant status and low-grade chronic inflammation. Therefore, antioxidant and anti-inflammatory compounds may appear protective for the MetS onset and progression, but despite the well-known antioxidant effects of polyphenols *in vitro* in numerous clinical studies, antioxidant and anti-inflammatory effects were not significant after polyphenol supplementation in patients with MetS (reviewed in [53]).

4.2.1. Antioxidant Activity of Polyphenols. In MetS women, 480 mL/day of cranberry juice for 8 weeks increased plasma antioxidant capacity and decreased oxidized LDL and malondialdehyde [90], and 50 g freeze-dried blueberries for eight weeks decreased serum oxidized LDL and malondialdehyde and increased hydroxynonenal concentrations in both men and women with MetS [91]. However, in women with MetS, 22 g freeze-dried blueberry powder for eight weeks showed no effect on superoxide dismutase levels [92], but 300 mL/day of pomegranate juice for 6 weeks decreased the levels of thiobarbituric acid reactive substances (TBARS) in erythrocytes [93]. MetS patients receiving 10 mL/day of extra virgin olive oil showed increased total radical-trapping antioxidant parameter (TRAP)/uric acid ratio, but no effects were observed on the levels of hydroperoxide, advanced oxidation protein product (AOPP) or AOPP/TRAP, and TRAP/AOPP indexes [94].

In MetS patients, a Mediterranean diet for five years supplemented with extra virgin olive oil or nuts increased plasma levels and activity of superoxide dismutase and catalase, increased levels of nitrates, and decreased xanthine oxidase activity [95], and a Mediterranean-style diet for 3 months decreased lipid and protein oxidation and increased plasma, erythrocyte, and platelet antioxidant enzymes [96].

The antioxidant potential of polyphenols is defined by their chemical structure, the number and position of hydroxyl groups, conjugation groups, degree of glycosylation, and the presence of donor electrons in the ring structure, considering that the aromatic group is able to endure the disappearance of electrons. However, whether the effects of polyphenols are relevant in oxidative stress remains to be elucidated. Despite their low bioavailability and rapid metabolism and elimination, the antioxidant effects of polyphenols may be of potential clinical relevance when considered in the context of a diet rich in fruits and vegetables. However, being polyphenol-rich foods and also antioxidant vitamin-rich foods, the antioxidant activity observed in these trials may be a sum of the effects of antioxidant vitamins and polyphenols, because polyphenols represent <1-2% of the plasma antioxidants, which include proteins, ascorbate, tocopherol, carotenoids, bilirubin, uric acid, and several other compounds [97]. Therefore, polyphenols may exert their protective effects far beyond their antioxidant activity.

4.2.2. Anti-Inflammatory Activity of Polyphenols. Besides the antioxidant activity of polyphenols, they exert effects on enzymes, cell signaling pathways, and gene expression related to inflammation that may better explain their beneficial effects on endothelial function, metabolic disturbances, and vascular inflammation. Berry supplementation in the form of juice, powder, or extract has been shown to decrease serum levels of interleukin- (IL-) 12, monocyte expression of monocyte-to-macrophage differentiation-associated (MMD) and C-C motif chemokine receptor 2 (CCR2), and the overall inflammation score [98] and to improve endothelial function measured by the reactive hyperemia index [99] in MetS patients. However, in those patients, berry supplementation had no effect on C-reactive protein (CRP) [91, 92], intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), adiponectin [91], IL-6 [90], CRP, tumor necrosis factor- (TNF-) α, or monocyte chemoprotein- (MCP-) 1 [100]. On the other hand, men with MetS show decreased ICAM-1 levels and increased flow-mediated dilation after 46 g/day of freeze-dried grape polyphenol powder for 30 days [101], and green tea supplementation for eight weeks through infusion or powder did not significantly modify serum levels of adiponectin, CRP, IL-6, IL-1β, VCAM-1, ICAM-1, leptin, or leptin : adiponectin ratio [102] in both men and women with MetS. In those patients, a Mediterranean-style diet for 3 months decreased CRP levels [96] and serum concentrations of IL-6, IL-7, and IL-18 and increased the endothelial function score after 2 years [103]. These controversial results may partly be explained by differences in polyphenol doses in each trial and also suggest that

MetS should be addressed through a multifactorial approach considering the whole dietary pattern and also increased physical activity, known to be a good anti-inflammatory strategy [104].

4.2.3. Acute Interventions with Polyphenol-Rich Foods in Patients with Metabolic Syndrome. As pinpointed in a previous section, given the low bioavailability of polyphenols and that maximal plasma concentrations are reached within the first two hours after ingestion and fall to baseline levels within 8 to 12 hours, the protective effects of polyphenols may be reached through a long-term regular, daily basis consumption. However, minimizing postprandial metabolic stress may help in the control of the chronic progression of MetS. Therefore, polyphenols may be more active when consumed in the principal meals, which provokes greater postprandial metabolic stress. While several studies reported beneficial postprandial effects of polyphenol intake on healthy subjects or on subjects with one or two cardiovascular risk factors, very few acute interventional trials have been performed in MetS patients. Diets rich in polyphenols have been shown to decrease postprandial triglyceride total area under the curve in plasma and large VLDLs, as well as to decrease 24 h urinary 8-isoprostane in these patients [105]. Acute intake of extra virgin olive oil improved glycemia and insulin sensitivity in healthy subjects but not in MetS patients. In addition, the authors observed that acute extra virgin olive oil consumption switched peripheral blood mononuclear cells to a less deleterious inflammatory phenotype, but weaker effects were observed in patients with MetS [52]. Taking into account these differences and given the fact that MetS could affect polyphenol bioavailability as shown in rats [106], more acute interventional trials in MetS patients are warranted in order to elucidate the capability of polyphenols to minimize postprandial metabolic stress in MetS patients.

4.2.4. Long-Term Interventions with Polyphenol-Rich Foods in Patients with Metabolic Syndrome. On the other hand, clinical trials are currently the best approach to demonstrate the effects of foods or food compounds such as polyphenols on human health. Nevertheless, it has to be taken into account that the fact that a food or a single polyphenol modulates some or several biomarkers related to MetS may not imply an improvement of the MetS progression itself. In addition, while several randomized trials have evaluated the effects of polyphenols on patients with abdominal obesity and or some of the MetS components, few randomized trials have evaluated the effects of polyphenols on MetS patients. Therefore, more and large randomized trials are needed to definitively elucidate the role of polyphenols on MetS. In this subsection and in Table 3, the main clinical trials on polyphenols and MetS are summarized.

(1) Fruit Polyphenols. Berries are very rich in anthocyanins, being pelargonidin, cyanidin, delphinidin, petunidin, peonidin, and malvidin the most predominant. Randomized trials analyzing the effect of berry consumption (as fresh fruit, juice, or extracts) are performed at a relatively short term, and therefore, their long-term effects still remain unknown. Blueberry consumption has been shown to decrease blood pressure [92] and oxidative stress [91] and improve endothelial function [99] and insulin sensitivity [100] in MetS patients. On the other hand, inconsistent results have been found for other berries in the improvement of MetS. In MetS patients, neither bilberry [98] nor cranberry supplementation [90] showed differences in body weight, glucose, or lipid metabolism, compared to a control group. However, compelling evidence shows that overall berry intake improves the oxidative and inflammatory profile, as reviewed [107].

In middle-aged women with metabolic syndrome, 300 mL of pomegranate juice (rich in flavonoids) daily for 6 weeks decreased erythrocyte but not plasma TBARS levels. Nevertheless, they observed no effects on blood pressure [93].

(2) Olive Oil Polyphenols. It is worth pointing out that olive oil polyphenols are contained in extra virgin olive oil, whereas olive oil is almost deprived of polyphenols because of the refining process [108]. Polyphenols from extra virgin olive oil, mainly (hydroxy) tyrosol and oleuropein, elicit antioxidant and anti-inflammatory effects, improve the lipid profile and the endothelial function, and may exert some antithrombotic effects [109]. Although the beneficial effects of olive oil polyphenols on the general population are not under debate, to our knowledge, only a clinical trial has been performed to evaluate the effects of extra virgin olive oil as a single food in MetS patients. These patients showed decreased waist perimeter after consuming 10 mL of extra virgin olive oil a day for 3 months, but no changes in the lipid profile, glucose, insulin resistance, or blood pressure were observed [94].

(3) Tea Polyphenols. Tea is a beverage especially rich in catechins. The beneficial effects of tea (higher for green than for black tea intake) on MetS have generally been observed in most human studies at least at three to four cups or more per day. One of the protective main effects of tea consumption in MetS is by reducing body weight [110], by decreasing digestion and absorption of some macronutrients, by altering the gut microbiota, by inhibiting anabolism, and by stimulating catabolism in liver, muscle, and adipose tissues [48]. However, current evidence suggests that this effect may only be observed at a short term and not at a long term [111].

(4) Grape and Red Wine Polyphenols. Grape and wine (red wine in a higher degree than white wine) are very rich in phenolic acids, stilbenes, flavonols, dihydroflavonols, anthocyanins, catechins, and proanthocyanidins. Consumption of dried grape polyphenol powder for a month resulted in decreased systolic blood pressure [112] and increased flow-mediated dilation in men with MetS, but no effects were observed in insulin resistance, lipid profile, or body weight [101]. Intake of red wine or dealcoholized red wine for 30 days significantly decreased SBP, DBP, glucose, triglycerides, total cholesterol, and C-reactive protein and increased HDL levels in MetS patients [113]. In parallel and as will be further discussed, red wine polyphenols (in both interventions)

Table 3: Summary of interventional trials with polyphenol-rich foods on metabolic syndrome.

Ref.	Type of study	Number of patients	Patients' characteristics	Age (years)	Intervention	Dose	Duration	Measured outcomes	Results
[92]	Placebo-controlled	48	Postmenopausal women with pre- and stage 1 hypertension	55–65	Blueberries	22 g/day powder	8 weeks	Blood pressure, arterial stiffness, CRP, nitric oxide, and superoxide dismutase	Decreased blood pressure and arterial stiffness and increased nitric oxide after blueberry intervention: no effects on CRP
[91]	Placebo-controlled	48	MetS	47–53	Blueberries	50 g/day powder	8 weeks	Blood pressure, lipid profile, HOMA index, oxidation, and inflammation parameters	Decreased blood pressure, no changes in body weight, HOMA index or lipid profile. Decreased oxLDL, MDA, and HNE. No changes in inflammatory biomarkers
[99]	Placebo-controlled	44	MetS	53–61	Blueberries	45 g/day powder	6 weeks	Blood pressure, endothelial function, and insulin sensitivity	Improved endothelial function. No changes in blood pressure or insulin sensitivity
[100]	Placebo-controlled	32	Obese, nondiabetic, and insulin-resistant	46–57	Blueberries	45 g/day powder	6 weeks	Insulin sensitivity, inflammatory biomarkers, and adiposity	Improved insulin sensitivity but no changes in adiposity or inflammatory biomarkers
[98]	Placebo-controlled	27	MetS	43–59	Bilberries	400 g fresh	8 weeks	Body weight, blood pressure, glucose, lipid profile, and inflammatory parameters	Decreased CRP, IL-6, IL-12, and LPS concentrations and decreased expression of MMD and CCR2 in monocytes. No changes in body weight, blood pressure, glucose, or lipid metabolism
[90]	Placebo-controlled	31	MetS women	46–60	Cranberries	480 mL/day juice	8 weeks	Blood pressure, glucose and lipid profile, markers of inflammation, and oxidation	Increased plasma antioxidant capacity and decreased oxLDL and MDA. No changes in blood pressure, glucose and lipid profiles, CRP, and IL-6
[93]	Placebo-controlled	23	MetS women	40–60	Pomegranate	300 mL/day juice	6 weeks	Lipid peroxidation and phospholipid fatty acid composition of plasma and erythrocytes, blood pressure, and lipid profile	Decreased plasma arachidonic acid and increased saturated fatty acids. Decreased TBARS and arachidonic acid and increased monounsaturated fatty acids in erythrocytes. No changes in blood pressure or lipid profile
[94]	Placebo-controlled	102	MetS	43–60	Extra virgin olive oil	10 mL/day	90 days	Blood pressure, BMI, HOMA index, lipid profile, CRP, and oxidative parameters	Decreased waist perimeter and increased TRAP but no changes in blood pressure, lipid profile, HOMA index, BMI, or CRP

Table 3: Continued.

Ref.	Type of study	Number of patients	Patients' characteristics	Age (years)	Intervention	Dose	Duration	Measured outcomes	Results
[112]	Placebo-controlled	65	Normal and overweight	18–50	Green tea extract	9 capsules/day containing >0.06 g EGCG and 0.03–0.05 g caffeine per capsule	12 weeks	Body weight, fat mass index, resting energy expenditure	No differences in body weight, fat mass index, or resting energy expenditure
[113]	Placebo-controlled	27	MetS	25–80	Grape seed extract	300 or 150 mg/day	4 weeks	Serum lipids, blood glucose, and blood pressure	Decreased blood pressure. No changes in glucose or lipid profile. No differences between doses
[101]	Randomized crossover	24	MetS men	30–70	Grape seed powder or placebo	46 g/day (267 mg polyphenols)	30 days	Blood pressure, endothelial function, lipid profile, glucose, and body weight	Decreased systolic blood pressure and ICAM-1 and increased FMD. No differences in diastolic blood pressure, nitric oxide, body weight, glucose, or lipids
[114]	Randomized crossover	10	MetS		Red wine and dealcoholized red wine	272 mL/day	30 days	Body weight, blood pressure, glucose and insulin, lipid profile, CRP, and LPS	Decreased systolic and diastolic blood pressure, glucose, triglycerides, total cholesterol, CRP, and LPS and increased serum levels of HDL cholesterol. No changes in body weight
[95]	Randomized parallel	75	MetS	55–80	Mediterranean diet supplements with extra virgin olive oil or nuts and control diet	—	5 years	Catalase, SOD, myeloperoxidase, and XOX activities and protein levels; protein carbonyl derivatives; and nitrotyrosine, nitrite, and nitrate levels	Increased plasma activity and protein levels of SOD and catalase, increased plasma nitrate levels, and decreased XOX activity in the Mediterranean diets compared to the control diet
[96]	Case-control	36	MetS and healthy individuals	42–64	Mediterranean diet	—	3 months	Insulin resistance and oxidative and inflammatory status	Decreased plasma, erythrocyte, and platelet antioxidant enzymes and a rise in lipid and protein oxidation, plasma CRP, and fibrinogen in MetS patients
[103]	Randomized parallel	180	MetS	37–50	Mediterranean diet and prudent diet	—	2 years	Endothelial function score, lipid profile and glucose, insulin sensitivity, circulating levels of CRP, and IL-6, IL-7, and IL-18	Decreased body weight and insulin resistance, decreased concentrations of CRP, IL-6, IL-7, and IL-18, and improved endothelial function score in the Mediterranean diet group

Table 3: Continued.

Ref.	Type of study	Number of patients	Patients' characteristics	Age (years)	Intervention	Dose	Duration	Measured outcomes	Results
[102]	Randomized controlled	35	MetS	40–44	Green tea	Green tea (4 cups/day), green tea extract (2 capsules and 4 cups water/day)	8 weeks	Body weight, lipid profile, blood pressure, and inflammatory biomarkers	Decreased total and LDL cholesterol after green tea extracts. No changes in body weight, blood pressure or in serum levels of adiponectin, CRP, IL-6, IL-1β, VCAM-1, ICAM-1, leptin, or leptin : adiponectin ratio
[105]	Randomized parallel	86	Overweight/obese individuals with a large waist circumference and any other component of the metabolic syndrome	44–64	Isoenergetic diets with high and low polyphenol content	—	8 weeks	Fasting and postmeal TRLs and 8-isoprostane concentrations	Reduced fasting triglyceride concentrations and large VLDL, reduced postprandial triglyceride total area under the curve in plasma and large VLDLs, and decreased urinary 8-isoprostane after high polyphenol intake
[52]	Case-control	24	Healthy and MetS	27–38	Extra virgin olive oil	50 mL/single dose	Acute	Glycemia, insulin sensitivity, lipid profile, and gene and miRNA expression of peripheral blood mononuclear cells	Improved glycemia and insulin sensitivity in healthy subjects but not in MetS patients. No changes in lipid profile in either population

Ref.: reference number; MetS: metabolic syndrome; HOMA: homeostasis model assessment of β-cell function and insulin resistance; BMI: body mass index; CRP: C-reactive protein; LPS: lipopolysaccharide; SOD: superoxide dismutase; XOX: xanthine oxidase; IL: interleukin; TRL: triglyceride-rich lipoprotein; oxLDL: oxidized low-density lipoprotein; VLDL: very low-density lipoprotein; MDA: malondialdehyde; HNE: hydroxynonenal; MMD: monocyte-to-macrophage differentiation-associated; CCR2: C-C motif chemokine receptor 2; TBARS: thiobarbituric acid reactive substances; TRAP: total peroxyl radical-trapping antioxidant potential; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular adhesion molecule 1; FMD: flow-mediated dilation.

increased the number of fecal bifidobacteria and *Lactobacillus* (intestinal barrier protectors) and butyrate-producing bacteria and decreased lipopolysaccharide- (LPS-) producing bacteria, which was translated to lower LPS systemic levels, suggesting that red wine polyphenols are capable of switching microbiota phenotype to a more protective one in patients with MetS [113]. However, a recent meta-analysis concludes that grape polyphenols do not have a significant effect on glycemia, blood pressure, or the lipid profile in MetS patients, although limited evidence suggests a positive effect on insulin sensitivity [51], in contraposition to another meta-analysis that concluded that grape polyphenols are able to decrease systolic but not diastolic blood pressure in MetS patients [114].

(5) Mediterranean Diet. The Mediterranean diet is characterized by the high consumption of polyphenol-rich foods such as fruits, vegetables, nuts, olive oil, aromatic plants, spices, whole-grain cereals, and red wine. Several cross-sectional and prospective studies have suggested that the Mediterranean diet has protective effects on different components of MetS [115] and its associated low-grade inflammation and pro-oxidative status. Intervention with a Mediterranean diet for five years supplemented with extra virgin olive oil or nuts increased plasma antioxidant capacity and decreased xanthine oxidase activity in MetS patients [95]. This is in concordance with another study with MetS patients, which reported that a Mediterranean-style diet for 3 months improved the antioxidant enzyme activities in plasma, erythrocytes, and platelets [96]. Moreover, MetS patients under a Mediterranean diet for 2 years showed reduced prevalence of MetS by decreasing body weight, reducing serum concentrations of inflammatory markers, decreasing insulin resistance, and improving endothelial function [103]. Concurrently, Mediterranean diet intervention for one year supplemented with extra virgin olive oil or nuts was associated with decreased incidence [116] and increased reversion of MetS [117], even at five years of intervention [118], suggesting that the Mediterranean diet may be a promising tool for both MetS prevention and management. In the same cohort, one-year intervention with a Mediterranean diet supplemented with extra virgin olive oil or nuts has been shown to decrease blood pressure, and these changes were associated with a significant increase in total polyphenols in urine and plasma nitric oxide [119]. Overall, a Mediterranean diet for five years supplemented with extra virgin olive oil or nuts, despite the increased fat intake, was not associated with increased visceral adiposity or body weight [120], suggesting that indeed, olive oil and nut polyphenols, among other compounds, may have an effect on body weight maintenance or loss.

4.3. Gut Microbiota and Metabolic Syndrome. The human colonic microbiota is a large and complex microbial community. In total, over 1000 bacterial species have been identified, of which many remain uncultured, with about 160 species being found in the gut of any individual, making an important contribution to human metabolism and health by contributing enzymes that are not encoded by the human genome [121]. Given the low bioavailability of polyphenols, the remaining polyphenols (90–95% of total polyphenol intake) may accumulate in the large intestinal lumen up to the millimolar range where they are subjected to the enzymatic activities of the gut microbial community prior to their reabsorption, modulating the composition of the microbiota and influencing the metabolic balance and the health status. In this line, red wine intake has been shown to increase global fecal microbial diversity in healthy volunteers [122], and different profiles of urinary polyphenol metabolites derived from microbial metabolism have been shown after one-month red wine intake between healthy individuals and obese/diabetic patients [123].

Once the polyphenols have been metabolized to their aglycones or the polymers have been converted to monomers, they are extensively degraded by components of the colonic microbiota via dehydroxylation, decarboxylation, and ring breakage ultimately generating simpler phenolic compounds, such as hydroxyphenyl-acetic acids and hydroxyphenylpropionic acids [124]. Given the high variability in the microbial composition between individuals, polyphenols may be differently metabolized, thus affecting the bioavailability of both the parental polyphenols and their metabolites and influencing their bioactivity. For instance and as previously mentioned, daidzein (a soy isoflavone) is metabolized by two different pathways depending on the gut microbiota of the subjects, producing O-desmethylangolensin by *Clostridium*, or on (S)-equol via dihydrodaidzein and tetrahydrodaidzein by *Streptococcus intermedius*, *B. ovatus*, *Ruminococcus productus*, *Eggerthella sp.* Julong732, *Adlercreutzia equolifaciens*, *Slakia isoflavoniconvertens*, and *Slakia equolifaciens* [125]. In addition, different responses to a mix of epigallocatechin-3-gallate and resveratrol have been observed between men and women. In men, this polyphenol mixture decreased the amount of fecal bacteroidetes and tended to decrease *Faecalibacterium prausnitzii*, but these changes were not observed in women [126], highlighting the complex nature of intestinal microbiota. Moreover, MetS features may also influence intestinal microbiota and polyphenol metabolism and therefore their bioactivity [127, 128]. A randomized study in MetS patients has shown that bioavailability of ellagitannins depends on the gut composition of microbiota [98]. As previously remarked, ellagitannin-metabolizing phenotypes (urolithin metabotypes A, B, and 0) differ among individuals depending on their body mass index, and urolithin A in MetS patients only correlated inversely with glucose after intake of 30 g of nuts, while in healthy and overweight patients, urolithin A positively correlated with apolipoprotein A-I and HDL cholesterol after the intake of nuts [50]. In MetS patients, red wine polyphenols in the form of red wine or dealcoholized red wine significantly increased the number of fecal protective species (bifidobacteria and *Lactobacillus*) and butyrate-producing bacteria (*Faecalibacterium prausnitzii* and *Roseburia*), consequently decreasing the amount of groups of nonprotective bacteria such as LPS producers (*Escherichia coli* and *Enterobacter cloacae*) and approaching the microbiome profile of MetS patients to the profile of healthy volunteers. Indeed, before the red wine intervention,

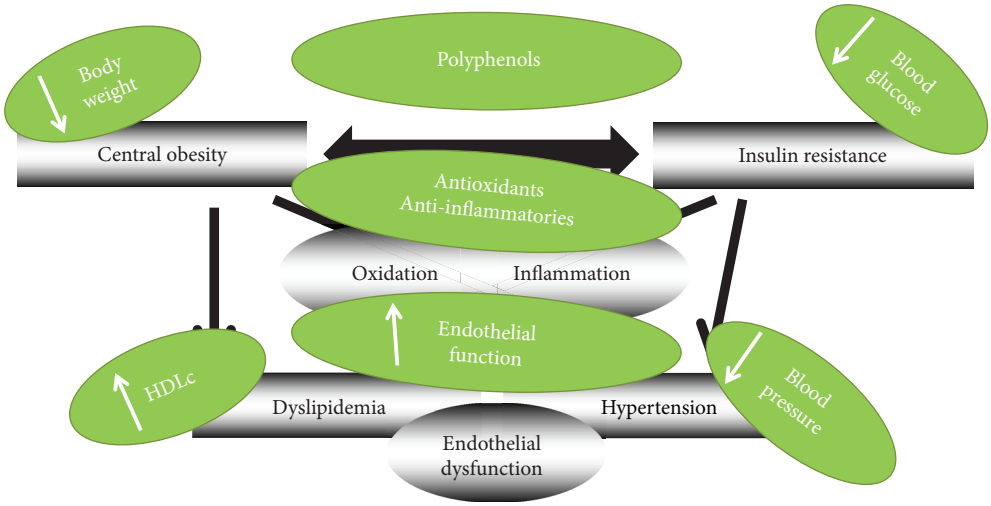


Figure 3: Main potential effects of polyphenols on metabolic syndrome components.

MetS patients had higher amounts of Proteobacteria and Firmicutes compared to healthy subjects, while after the red wine and dealcoholized red wine intake periods, no significant differences were observed between healthy individuals and MetS patients [113]. Overall, the (complex) interaction between nutrients and the gut microbiome is an emerging field of intensive research that will provide new and promising insights into the relationship between nutrition and health in the near future.

5. Conclusions

As summarized in Figure 3, compelling evidence suggests that polyphenols at maintained doses may delay or prevent MetS onset by decreasing body weight, blood pressure, and blood glucose and by improving lipid metabolism. Because of the complex polyphenol composition of polyphenol-rich foods, it is difficult to emphasize the bioactivity of a specific polyphenol. Moreover, it seems plausible that they have additive or synergistic effects. Therefore, high intake of polyphenol-rich foods such as nuts, fruits, vegetables, seasonings with aromatic plants, spices, and virgin olive oil may be the cornerstone of a healthy diet preventing the development and progression of MetS. However, human epidemiological and interventional studies have shown inconsistent results. There is a small number of interventional studies evidencing the benefits of polyphenol intake in the improvement of MetS phenotype in these patients, and more long-term randomized trials are warranted in order to evaluate the possible preventive effects of a higher consumption of polyphenols by a combination of their diverse dietary sources, as suggested by some epidemiological observations [12, 61, 129–131] and in order to conclude whether single polyphenols or polyphenol-rich foods are indeed related to a reduction in MetS-related symptoms or whether their action is merely affecting a biomarker.

In conclusion, current evidence suggests that polyphenol intake has the potential to alleviate MetS components. However, there is still a long way to run before establishing the role of polyphenols in MetS progression. Safe doses have to be determined, as the effects greatly vary among polyphenols and food sources, and no specific food or polyphenol is able to improve all components of MetS. Overall, a varied diet rich in polyphenol-rich foods may be beneficial in the onset and progression of MetS and in decreasing the associated risk of developing diabetes or cardiovascular disease.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Gemma Chiva-Blanch is a Juan de la Cierva-Incorporación Postdoctoral Fellow (IJCI-2015-26358) from the Spanish Ministry of Economy and Competitiveness (MINECO), Spain. This work was supported by grants from the Spanish Ministry of Economy and Competitiveness of Science (SAF2016-76819-R to Lina Badimon); the Institute of Health Carlos III (ISCIII) (TERCEL RD16/0011/0018 and CIBERCV CB16/11/0041 to Lina Badimon); the FEDER "Una Manera de Hacer Europa"; and the "CERCA Programme/Generalitat de Catalunya," Spain. The authors thank Fundació d'Investigació Cardiovascular (FIC)-Fundacion Jesús Serra, Barcelona, Spain, for their continuous support.

References

[1] A. J. G. Hanley, A. J. Karter, K. Williams et al., "Prediction of type 2 diabetes mellitus with alternative definitions of the metabolic syndrome: the Insulin Resistance Atherosclerosis Study," *Circulation*, vol. 112, no. 24, pp. 3713–3721, 2005.

[2] European Association for Cardiovascular Prevention & Rehabilitation, Z. Reiner, A. L. Catapano et al., "ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS)," *European Heart Journal*, vol. 32, no. 14, pp. 1769–1818, 2011.

[3] A. S. Gami, B. J. Witt, D. E. Howard et al., "Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies," *Journal of the American College of Cardiology*, vol. 49, no. 4, pp. 403–414, 2007.

[4] WHO, *Global Atlas on Cardiovascular Disease Prevention and Control*, WHO, 2015, http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf.

[5] Z. Hosseini, S. J. Whiting, and H. Vatanparast, "Current evidence on the association of the metabolic syndrome and dietary patterns in a global perspective," *Nutrition Research Reviews*, vol. 29, no. 2, pp. 152–162, 2016.

[6] H. Robberecht, T. De Bruyne, and N. Hermans, "Effect of various diets on biomarkers of the metabolic syndrome," *International Journal of Food Sciences and Nutrition*, vol. 68, pp. 627–641, 2017.

[7] N. Babio, M. Bulló, and J. Salas-Salvadó, "Mediterranean diet and metabolic syndrome: the evidence," *Public Health Nutrition*, vol. 12, no. 9A, pp. 1607–1617, 2009.

[8] G. Chiva-Blanch and F. Visioli, "Polyphenols and health: moving beyond antioxidants," *Journal of Berry Research*, vol. 2, pp. 63–71, 2012.

[9] Phenol-Explorer, "Database on polyphenol content in foods," April 2017, http://phenol-explorer.eu/.

[10] P. Brat, S. Georgé, A. Bellamy et al., "Daily polyphenol intake in France from fruit and vegetables," *The Journal of Nutrition*, vol. 136, no. 9, pp. 2368–2373, 2006.

[11] C. Julia, M. Touvier, C. Lassale et al., "Cluster analysis of polyphenol intake in a French middle-aged population (aged 35-64 years)," *Journal of Nutritional Science*, vol. 5, article e28, 2016.

[12] A. Tresserra-Rimbau, M. Guasch-Ferre, J. Salas-Salvado et al., "Intake of total polyphenols and some classes of polyphenols is inversely associated with diabetes in elderly people at high cardiovascular disease risk," *The Journal of Nutrition*, vol. 146, no. 4, pp. 767–777, 2016.

[13] J. Godos, S. Marventano, A. Mistretta, F. Galvano, and G. Grosso, "Dietary sources of polyphenols in the Mediterranean healthy eating, aging and lifestyle (MEAL) study cohort," *International Journal of Food Sciences and Nutrition*, vol. 68, pp. 750–756, 2017.

[14] M. Vitale, O. Vaccaro, M. Masulli et al., "Polyphenol intake and cardiovascular risk factors in a population with type 2 diabetes: the TOSCA.IT study," *Clinical Nutrition*, 2016.

[15] M.-L. Ovaskainen, R. Törrönen, J. M. Koponen et al., "Dietary intake and major food sources of polyphenols in Finnish adults," *The Journal of Nutrition*, vol. 138, no. 3, pp. 562–566, 2008.

[16] M. A. Nascimento-Souza, P. G. de Paiva, J. Pérez-Jiménez, S. D. Franceschini, and A. Q. Ribeiro, "Estimated dietary intake and major food sources of polyphenols in elderly of Viçosa, Brazil: a population-based study," *European Journal of Nutrition*, 2016, In press.

[17] C. Taguchi, Y. Fukushima, Y. Kishimoto et al., "Estimated dietary polyphenol intake and major food and beverage sources among elderly Japanese," *Nutrients*, vol. 7, no. 12, pp. 10269–10281, 2015.

[18] K. Beking and A. Vieira, "An assessment of dietary flavonoid intake in the UK and Ireland," *International Journal of Food Sciences and Nutrition*, vol. 62, no. 1, pp. 17–19, 2011.

[19] M. E. Goetz, S. E. Judd, M. M. Safford, T. J. Hartman, W. M. McClellan, and V. Vaccarino, "Dietary flavonoid intake and incident coronary heart disease: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study," *The American Journal of Clinical Nutrition*, vol. 104, no. 5, pp. 1236–1244, 2016.

[20] W. Bai, C. Wang, and C. Ren, "Intakes of total and individual flavonoids by US adults," *International Journal of Food Sciences and Nutrition*, vol. 65, no. 1, pp. 9–20, 2014.

[21] L. Johannot and S. M. Somerset, "Age-related variations in flavonoid intake and sources in the Australian population," *Public Health Nutrition*, vol. 9, no. 8, pp. 1045–1054, 2006.

[22] Z. Q. Zhang, L. P. He, Y. H. Liu, J. Liu, Y. X. Su, and Y. M. Chen, "Association between dietary intake of flavonoid and bone mineral density in middle aged and elderly Chinese women and men," *Osteoporosis International*, vol. 25, no. 10, pp. 2417–2425, 2014.

[23] S. Jun, S. Shin, and H. Joung, "Estimation of dietary flavonoid intake and major food sources of Korean adults," *The British Journal of Nutrition*, vol. 115, no. 03, pp. 480–489, 2016.

[24] N. Burkholder-Cooley, S. Rajaram, E. Haddad, G. E. Fraser, and K. Jaceldo-Siegl, "Comparison of polyphenol intakes according to distinct dietary patterns and food sources in the Adventist Health Study-2 cohort," *The British Journal of Nutrition*, vol. 115, no. 12, pp. 2162–2169, 2016.

[25] J. P. E. Spencer, M. M. Abd El Mohsen, A.-M. Minihane, and J. C. Mathers, "Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research," *The British Journal of Nutrition*, vol. 99, no. 1, pp. 12–22, 2008.

[26] M. Ikawa, T. D. Schaper, C. A. Dollard, and J. J. Sasner, "Utilization of Folin-Ciocalteu phenol reagent for the detection of certain nitrogen compounds," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 7, pp. 1811–1815, 2003.

[27] P. Pinto and C. N. Santos, "Worldwide (poly)phenol intake: assessment methods and identified gaps," *European Journal of Nutrition*, vol. 56, no. 4, pp. 1393–1408, 2017.

[28] J. A. Rothwell, A. Medina-Remón, J. Pérez-Jiménez et al., "Effects of food processing on polyphenol contents: a systematic analysis using Phenol-Explorer data," *Molecular Nutrition & Food Research*, vol. 59, no. 1, pp. 160–170, 2015.

[29] G. Hithamani and K. Srinivasan, "Bioaccessibility of polyphenols from wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), green gram (*Vigna radiata*), and chickpea (*Cicer arietinum*) as influenced by domestic food processing," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 46, pp. 11170–11179, 2014.

[30] A. Klinder, Q. Shen, S. Heppel, J. A. Lovegrove, I. Rowland, and K. M. Tuohy, "Impact of increasing fruit and vegetables and flavonoid intake on the human gut microbiota," *Food & Function*, vol. 7, no. 4, pp. 1788–1796, 2016.

[31] M. D'Archivio, C. Filesi, R. Di Benedetto, R. Gargiulo, C. Giovannini, and R. Masella, "Polyphenols, dietary sources and bioavailability," *Annali dell'Istituto Superiore di Sanità*, vol. 43, no. 4, pp. 348–361, 2007.

[32] J. Pérez-Jiménez, J. Hubert, L. Hooper et al., "Urinary metabolites as biomarkers of polyphenol intake in humans: a systematic review," *The American Journal of Clinical Nutrition*, vol. 92, no. 4, pp. 801–809, 2010.

[33] R. H. Eckel, S. M. Grundy, and P. Z. Zimmet, "The metabolic syndrome," *The Lancet*, vol. 365, no. 9468, pp. 1415–1428, 2005.

[34] K. G. M. Alberti, P. Zimmet, and J. Shaw, "IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition," *Lancet*, vol. 366, no. 9491, pp. 1059–1062, 2005.

[35] E. Kassi, P. Pervanidou, G. Kaltsas, G. Chrousos, M. Carnethon, and S. Heymsfield, "Metabolic syndrome: definitions and controversies," *BMC Medicine*, vol. 9, no. 1, p. 48, 2011.

[36] E. Fuentes, F. Fuentes, G. Vilahur, L. Badimon, and I. Palomo, "Mechanisms of chronic state of inflammation as mediators that link obese adipose tissue and metabolic syndrome," *Mediators of Inflammation*, vol. 2013, Article ID 136584, 11 pages, 2013.

[37] R. Weiss, A. A. Bremer, and R. H. Lustig, "What is metabolic syndrome, and why are children getting it?" *Annals of the New York Academy of Sciences*, vol. 1281, no. 1, pp. 123–140, 2013.

[38] L. S. Sperling, J. I. Mechanick, I. J. Neeland et al., "The cardiometabolic health alliance: working toward a new care model for the metabolic syndrome," *Journal of the American College of Cardiology*, vol. 66, no. 9, pp. 1050–1067, 2015.

[39] F. Samad and W. Ruf, "Inflammation, obesity, and thrombosis," *Blood*, vol. 122, no. 20, pp. 3415–3422, 2013.

[40] WHO, *Global Status Report on Noncommunicable Diseases 2014*, 2014, http://www.who.int/cardiovascular_diseases/publications/atlas_cvd/en/.

[41] S. M. Grundy, R. Pasternak, P. Greenland, S. Smith, and V. Fuster, "Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology," *Circulation*, vol. 100, no. 13, pp. 1481–1492, 1999.

[42] M. Aguilar, T. Bhuket, S. Torres, B. Liu, and R. J. Wong, "Prevalence of the metabolic syndrome in the United States, 2003-2012," *Journal of the American Medical Association*, vol. 313, no. 19, p. 1973, 2015.

[43] D. Gu, K. Reynolds, X. Wu et al., "Prevalence of the metabolic syndrome and overweight among adults in China," *Lancet (London, England)*, vol. 365, no. 9468, pp. 1398–1405, 1996.

[44] S. O'Neill and L. O'Driscoll, "Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies," *Obesity Reviews*, vol. 16, no. 1, pp. 1–12, 2015.

[45] S. Upadhyay and M. Dixit, "Role of polyphenols and other phytochemicals on molecular signaling," *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 504253, 15 pages, 2015.

[46] S. Gothai, P. Ganesan, S.-Y. Park, S. Fakurazi, D.-K. Choi, and P. Arulselvan, "Natural phyto-bioactive compounds for the treatment of type 2 diabetes: inflammation as a target," *Nutrients*, vol. 8, no. 8, p. 461, 2016.

[47] J. L. de Brito Alves, V. P. de Sousa, M. P. Cavalcanti Neto et al., "New insights on the use of dietary polyphenols or probiotics for the management of arterial hypertension," *Frontiers in Physiology*, vol. 7, p. 448, 2016.

[48] C. S. Yang, J. Zhang, L. Zhang, J. Huang, and Y. Wang, "Mechanisms of body weight reduction and metabolic syndrome alleviation by tea," *Molecular Nutrition & Food Research*, vol. 60, no. 1, pp. 160–174, 2016.

[49] A. Murillo and M. Fernandez, "The relevance of dietary polyphenols in cardiovascular protection," *Current Pharmaceutical Design*, vol. 23, no. 999, pp. 1–1, 2017.

[50] M. V. Selma, A. González-Sarrías, J. Salas-Salvadó et al., "The gut microbiota metabolism of pomegranate or walnut ellagitannins yields two urolithin-metabotypes that correlate with cardiometabolic risk biomarkers: comparison between normoweight, overweight-obesity and metabolic syndrome," *Clinical Nutrition*, 2017, In press.

[51] J. Woerdeman, E. van Poelgeest, J. C. F. Ket, E. C. Eringa, E. H. Serné, and Y. M. Smulders, "Do grape polyphenols improve metabolic syndrome components? A systematic review," *European Journal of Clinical Nutrition*, 2017, In press.

[52] S. D'Amore, M. Vacca, M. Cariello et al., "Genes and miRNA expression signatures in peripheral blood mononuclear cells in healthy subjects and patients with metabolic syndrome after acute intake of extra virgin olive oil," *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, vol. 1861, no. 11, pp. 1671–1680, 2016.

[53] M. J. Amiot, C. Riva, and A. Vinet, "Effects of dietary polyphenols on metabolic syndrome features in humans: a systematic review," *Obesity Reviews*, vol. 17, no. 7, pp. 573–586, 2016.

[54] C.-H. Wu, F.-H. Lu, C.-S. Chang, T.-C. Chang, R.-H. Wang, and C.-J. Chang, "Relationship among habitual tea consumption, percent body fat, and body fat distribution," *Obesity Research*, vol. 11, no. 9, pp. 1088–1095, 2003.

[55] L. A. E. Hughes, I. C. W. Arts, T. Ambergen 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," *The American Journal of Clinical Nutrition*, vol. 88, no. 5, pp. 1341–1352, 2008.

[56] B. A. Golomb, S. Koperski, and H. L. White, "Association between more frequent chocolate consumption and lower body mass index," *Archives of Internal Medicine*, vol. 172, no. 6, p. 519, 2012.

[57] A. Fernández-Quintela, C. Carpéné, M. Fernández et al., "Anti-obesity effects of resveratrol: comparison between animal models and humans," *Journal of Physiology and Biochemistry*, 2016, In press.

[58] G. Farhat, S. Drummond, and E. A. S. Al-Dujaili, "Polyphenols and their role in obesity management: a systematic review of randomized clinical trials," *Phytotherapy Research: PTR*, vol. 31, no. 7, pp. 1005–1018, 2017.

[59] Q. Sun, N. M. Wedick, S. S. Tworoger et al., "Urinary excretion of select dietary polyphenol metabolites is associated with a lower risk of type 2 diabetes in proximate but not remote follow-up in a prospective investigation in 2 cohorts of US women," *The Journal of Nutrition*, vol. 145, no. 6, pp. 1280–1288, 2015.

[60] N. M. Wedick, A. Pan, A. Cassidy et al., "Dietary flavonoid intakes and risk of type 2 diabetes in US men and women," *The American Journal of Clinical Nutrition*, vol. 95, no. 4, pp. 925–933, 2012.

[61] R. Zamora-Ros, N. G. Forouhi, S. J. Sharp et al., "Dietary intakes of individual flavanols and flavonols are inversely associated with incident type 2 diabetes in European populations," *The Journal of Nutrition*, vol. 144, no. 3, pp. 335–343, 2014.

[62] J. A. Nettleton, L. J. Harnack, C. G. Scrafford, P. J. Mink, L. M. Barraj, and D. R. Jacobs, "Dietary flavonoids and flavonoid-rich foods are not associated with risk of type 2 diabetes in postmenopausal women," *The Journal of Nutrition*, vol. 136, no. 12, pp. 3039–3045, 2006.

[63] Y. Song, J. E. Manson, J. E. Buring, H. D. Sesso, and S. Liu, "Associations of dietary flavonoids with risk of type 2

diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis," *Journal of the American College of Nutrition*, vol. 24, no. 5, pp. 376–384, 2005.

[64] H. Iso, C. Date, K. Wakai, M. Fukui, A. Tamakoshi, and JACC Study Group, "The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults," *Annals of Internal Medicine*, vol. 144, no. 8, pp. 554–562, 2006.

[65] A. H. Lee, L. B. Tan, N. Hiramatsu et al., "Plasma concentrations of coffee polyphenols and plasma biomarkers of diabetes risk in healthy Japanese women," *Nutrition Diabetes*, vol. 6, no. 6, p. e212, 2016.

[66] D. Wang, C. Chen, Y. Wang, J. Liu, and R. Lin, "Effect of black tea consumption on blood cholesterol: a meta-analysis of 15 randomized controlled trials," *PLoS One*, vol. 9, no. 9, article e107711, 2014.

[67] I. Onakpoya, E. Spencer, C. Heneghan, and M. Thompson, "The effect of green tea on blood pressure and lipid profile: a systematic review and meta-analysis of randomized clinical trials," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 24, no. 8, pp. 823–836, 2014.

[68] K. Imai and K. Nakachi, "Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases," *BMJ*, vol. 310, no. 6981, pp. 693–696, 1995.

[69] G. Pounis, M. Bonaccio, A. Di Castelnuovo et al., "Polyphenol intake is associated with low-grade inflammation, using a novel data analysis from the Moli-sani study," *Thrombosis and Haemostasis*, vol. 115, no. 2, pp. 344–352, 2016.

[70] X. Guo, A. Tresserra-Rimbau, R. Estruch et al., "Effects of polyphenol, measured by a biomarker of total polyphenols in urine, on cardiovascular risk factors after a long-term follow-up in the PREDIMED Study," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 2572606, 11 pages, 2016.

[71] J. L. Penalvo and P. Lopez-Romero, "Urinary enterolignan concentrations are positively associated with serum HDL cholesterol and negatively associated with serum triglycerides in U.S. adults," *The Journal of Nutrition*, vol. 142, no. 4, pp. 751–756, 2012.

[72] F. Rizzi, C. Conti, E. Dogliotti et al., "Interaction between polyphenols intake and PON1 gene variants on markers of cardiovascular disease: a nutrigenetic observational study," *Journal of Translational Medicine*, vol. 14, no. 1, p. 186, 2016.

[73] S. Davinelli and G. Scapagnini, "Polyphenols: a promising nutritional approach to prevent or reduce the progression of prehypertension," *High Blood Pressure & Cardiovascular Prevention*, vol. 23, no. 3, pp. 197–202, 2016.

[74] A. Medina-Remón, R. Zamora-Ros, M. Rotchés-Ribalta et al., "Total polyphenol excretion and blood pressure in subjects at high cardiovascular risk," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 21, no. 5, pp. 323–331, 2011.

[75] A. M. Miranda, J. Steluti, R. M. Fisberg, and D. M. Marchioni, "Association between polyphenol intake and hypertension in adults and older adults: a population-based study in Brazil," *PLoS One*, D. Delmas, Ed., vol. 11, no. 10, article e0165791, 2016.

[76] C. Serban, A. Sahebkar, S. Ursoniu, F. Andrica, and M. Banach, "Effect of sour tea (Hibiscus sabdariffa L.) on arterial hypertension," *Journal of Hypertension*, vol. 33, no. 6, pp. 1119–1127, 2015.

[77] X. Peng, R. Zhou, B. Wang et al., "Effect of green tea consumption on blood pressure: a meta-analysis of 13 randomized controlled trials," *Scientific Reports*, vol. 4, no. 1, p. 6251, 2014.

[78] A. Cassidy, É. J. O'Reilly, C. Kay et al., "Habitual intake of flavonoid subclasses and incident hypertension in adults," *The American Journal of Clinical Nutrition*, vol. 93, no. 2, pp. 338–347, 2011.

[79] A. Jennings, A. A. Welch, S. J. Fairweather-Tait et al., "Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women," *The American Journal of Clinical Nutrition*, vol. 96, no. 4, pp. 781–788, 2012.

[80] Y. Zhu, J. Sun, W. Lu et al., "Effects of blueberry supplementation on blood pressure: a systematic review and meta-analysis of randomized clinical trials," *Journal of Human Hypertension*, vol. 31, no. 3, pp. 165–171, 2017.

[81] G. Grosso, U. Stepaniak, A. Micek, D. Stefler, M. Bobak, and A. Pająk, "Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study," *European Journal of Nutrition*, vol. 56, no. 4, pp. 1409–1420, 2017.

[82] K. Kim, K. Kim, and S. M. Park, "Association between the prevalence of metabolic syndrome and the level of coffee consumption among Korean women," *PLoS One*, vol. 11, no. 12, article e0167007, 2016.

[83] H. Takami, M. Nakamoto, H. Uemura et al., "Inverse correlation between coffee consumption and prevalence of metabolic syndrome: baseline survey of the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study in Tokushima, Japan," *Journal of Epidemiology*, vol. 23, no. 1, pp. 12–20, 2013.

[84] A. T. Nordestgaard, M. Thomsen, and B. G. Nordestgaard, "Coffee intake and risk of obesity, metabolic syndrome and type 2 diabetes: a Mendelian randomization study," *International Journal of Epidemiology*, vol. 44, no. 2, pp. 551–565, 2015.

[85] E. Suliga, D. Kozieł, E. Cieśla, D. Rębak, and S. Głuszek, "Coffee consumption and the occurrence and intensity of metabolic syndrome: a cross-sectional study," *International Journal of Food Sciences and Nutrition*, vol. 68, no. 4, pp. 507–513, 2017.

[86] C.-S. Chang, Y.-F. Chang, P.-Y. Liu, C.-Y. Chen, Y.-S. Tsai, and C.-H. Wu, "Smoking, habitual tea drinking and metabolic syndrome in elderly men living in rural community: the Tianliao Old People (TOP) Study 02," *PLoS One*, vol. 7, no. 6, article e38874, 2012.

[87] J. A. Vernarelli and J. D. Lambert, "Tea consumption is inversely associated with weight status and other markers for metabolic syndrome in US adults," *European Journal of Nutrition*, vol. 52, no. 3, pp. 1039–1048, 2013.

[88] A. Tresserra-Rimbau, A. Medina-Remón, R. M. Lamuela-Raventós et al., "Moderate red wine consumption is associated with a lower prevalence of the metabolic syndrome in the PREDIMED population," *The British Journal of Nutrition*, vol. 113, no. S2, pp. S121–S130, 2015.

[89] M. T. Barrio-Lopez, M. Bes-Rastrollo, C. Sayon-Orea et al., "Different types of alcoholic beverages and incidence of metabolic syndrome and its components in a Mediterranean cohort," *Clinical Nutrition*, vol. 32, no. 5, pp. 797–804, 2013.

[90] A. Basu, N. M. Betts, J. Ortiz, B. Simmons, M. Wu, and T. J. Lyons, "Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with

metabolic syndrome," *Nutrition Research*, vol. 31, no. 3, pp. 190–196, 2011.

[91] A. Basu, M. Du, M. J. Leyva et al., "Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome," *The Journal of Nutrition*, vol. 140, no. 9, pp. 1582–1587, 2010.

[92] S. A. Johnson, A. Figueroa, N. Navaei et al., "Daily blueberry consumption improves blood pressure and arterial stiffness in postmenopausal women with pre- and stage 1-hypertension: a randomized, double-blind, placebo-controlled clinical trial," *Journal of the Academy of Nutrition and Dietetics*, vol. 115, no. 3, pp. 369–377, 2015.

[93] M. I. Kojadinovic, A. C. Arsic, J. D. Debeljak-Martacic et al., "Consumption of pomegranate juice decreases blood lipid peroxidation and levels of arachidonic acid in women with metabolic syndrome," *Journal of the Science of Food and Agriculture*, vol. 97, no. 6, pp. 1798–1804, 2017.

[94] D. Venturini, A. N. C. Simão, M. R. Urbano, and I. Dichi, "Effects of extra virgin olive oil and fish oil on lipid profile and oxidative stress in patients with metabolic syndrome," *Nutrition*, vol. 31, no. 6, pp. 834–840, 2015.

[95] A. Sureda, M. Bibiloni, M. Martorell et al., "Mediterranean diets supplemented with virgin olive oil and nuts enhance plasmatic antioxidant capabilities and decrease xanthine oxidase activity in people with metabolic syndrome: the PREDIMED study," *Molecular Nutrition & Food Research*, vol. 60, no. 12, pp. 2654–2664, 2016.

[96] L. Bekkouche, M. Bouchenak, W. Malaisse, and D. Yahia, "The Mediterranean diet adoption improves metabolic, oxidative, and inflammatory abnormalities in Algerian metabolic syndrome patients," *Hormone and Metabolic Research*, vol. 46, no. 04, pp. 274–282, 2014.

[97] P. C. H. Hollman, A. Cassidy, B. Comte et al., "The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established," *The Journal of Nutrition*, vol. 141, no. 5, pp. 989S–1009S, 2011.

[98] M. Kolehmainen, O. Mykkänen, P. V. Kirjavainen et al., "Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome," *Molecular Nutrition & Food Research*, vol. 56, no. 10, pp. 1501–10, 2012.

[99] A. Stull, K. Cash, C. Champagne et al., "Blueberries improve endothelial function, but not blood pressure, in adults with metabolic syndrome: a randomized, double-blind, placebo-controlled clinical trial," *Nutrients*, vol. 7, no. 6, pp. 4107–4123, 2015.

[100] A. J. Stull, K. C. Cash, W. D. Johnson, C. M. Champagne, and W. T. Cefalu, "Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women," *The Journal of Nutrition*, vol. 140, no. 10, pp. 1764–1768, 2010.

[101] J. Barona, J. C. Aristizabal, C. N. Blesso, J. S. Volek, and M. L. Fernandez, "Grape polyphenols reduce blood pressure and increase flow-mediated vasodilation in men with metabolic syndrome," *The Journal of Nutrition*, vol. 142, no. 9, pp. 1626–1632, 2012.

[102] A. Basu, M. Du, K. Sanchez et al., "Green tea minimally affects biomarkers of inflammation in obese subjects with metabolic syndrome," *Nutrition*, vol. 27, no. 2, pp. 206–213, 2011.

[103] K. Esposito, R. Marfella, M. Ciotola et al., "Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome," *Journal of the American Medical Association*, vol. 292, no. 12, p. 1440, 2004.

[104] N. M. Berry, K. Davison, A. M. Coates, J. D. Buckley, and P. R. C. Howe, "Impact of cocoa flavanol consumption on blood pressure responsiveness to exercise," *The British Journal of Nutrition*, vol. 103, no. 10, pp. 1480–1484, 2010.

[105] G. Annuzzi, L. Bozzetto, G. Costabile et al., "Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial," *The American Journal of Clinical Nutrition*, vol. 99, no. 3, pp. 463–471, 2014.

[106] M. Margalef, Z. Pons, L. Iglesias-Carres, F. I. Bravo, B. Muguerza, and A. Arola-Arnal, "Flavanol plasma bioavailability is affected by metabolic syndrome in rats," *Food Chemistry*, vol. 231, pp. 287–294, 2017.

[107] S. Vendrame, C. Del Bo', S. Ciappellano, P. Riso, and D. Klimis-Zacas, "Berry fruit consumption and metabolic syndrome," *Antioxidants*, vol. 5, no. 4, p. 34, 2016.

[108] F. Visioli, "Nutritional support in the pharmacological treatment of metabolic syndrome," *European Journal of Pharmacology*, vol. 668, pp. S43–S49, 2011.

[109] J. López-Miranda, F. Pérez-Jiménez, E. Ros et al., "Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 20, no. 4, pp. 284–294, 2010.

[110] R. Hursel, W. Viechtbauer, and M. S. Westerterp-Plantenga, "The effects of green tea on weight loss and weight maintenance: a meta-analysis," *International Journal of Obesity*, vol. 33, no. 9, pp. 956–961, 2009.

[111] P. L. Janssens, R. Hursel, and M. S. Westerterp-Plantenga, "Long-term green tea extract supplementation does not affect fat absorption, resting energy expenditure, and body composition in adults," *The Journal of Nutrition*, vol. 145, no. 5, pp. 864–870, 2015.

[112] B. Sivaprakasapillai, I. Edirisinghe, J. Randolph, F. Steinberg, and T. Kappagoda, "Effect of grape seed extract on blood pressure in subjects with the metabolic syndrome," *Metabolism*, vol. 58, no. 12, pp. 1743–1746, 2009.

[113] I. Moreno-Indias, L. Sánchez-Alcoholado, P. Pérez-Martínez et al., "Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients," *Food & Function*, vol. 7, no. 4, pp. 1775–1787, 2016.

[114] S.-H. Li, P. Zhao, H.-B. Tian, L.-H. Chen, and L.-Q. Cui, "Effect of grape polyphenols on blood pressure: a meta-analysis of randomized controlled trials," *PLoS One*, vol. 10, no. 9, article e0137665, 2015.

[115] C.-M. Kastorini, H. J. Milionis, K. Esposito, D. Giugliano, J. A. Goudevenos, and D. B. Panagiotakos, "The effect of Mediterranean diet on metabolic syndrome and its components," *Journal of the American College of Cardiology*, vol. 57, no. 11, pp. 1299–1313, 2011.

[116] J. Mayneris-Perxachs, A. Sala-Vila, M. Chisaguano et al., "Effects of 1-year intervention with a Mediterranean diet on plasma fatty acid composition and metabolic syndrome in a population at high cardiovascular risk," *PLoS One*, vol. 9, no. 3, article e85202, 2014.

[117] J. Salas-Salvadó, J. Fernández-Ballart, E. Ros et al., "Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status," *Archives of Internal Medicine*, vol. 168, no. 22, p. 2449, 2008.

[118] N. Babio, E. Toledo, R. Estruch et al., "Mediterranean diets and metabolic syndrome status in the PREDIMED

randomized trial," *Canadian Medical Association Journal*, vol. 186, no. 17, pp. E649–E657, 2014.

[119] A. Medina-Remón, A. Tresserra-Rimbau, A. Pons et al., "Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 25, no. 1, pp. 60–67, 2015.

[120] R. Estruch, M. A. Martínez-González, D. Corella et al., "Effect of a high-fat Mediterranean diet on bodyweight and waist circumference: a prespecified secondary outcomes analysis of the PREDIMED randomised controlled trial," *The Lancet Diabetes and Endocrinology*, vol. 4, no. 8, pp. 666–676, 2016.

[121] I. Rowland, G. Gibson, A. Heinken et al., "Gut microbiota functions: metabolism of nutrients and other food components," *European Journal of Nutrition*, 2017, In press.

[122] E. Barroso, I. Muñoz-González, E. Jiménez et al., "Phylogenetic profile of gut microbiota in healthy adults after moderate intake of red wine," *Molecular Nutrition & Food Research*, vol. 61, no. 3, article 1600620, 2017.

[123] R. Vázquez-Fresno, R. Llorach, A. Perera et al., "Clinical phenotype clustering in cardiovascular risk patients for the identification of responsive metabotypes after red wine polyphenol intake," *The Journal of Nutritional Biochemistry*, vol. 28, pp. 14–20, 2016.

[124] A. Rechner, "Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products," *Free Radical Biology and Medicine*, vol. 36, no. 2, pp. 212–225, 2004.

[125] I. Rowland, M. Faughnan, L. Hoey, and K. Wähälä, "Bioavailability of phyto-oestrogens," *British Journal of Haematology*, vol. 89, pp. S45–S58, 2003.

[126] J. Most, J. Penders, M. Lucchesi, G. H. Goossens, and E. E. Blaak, "Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women," *European Journal of Clinical Nutrition*, 2017, In press.

[127] X. Mora-Cubillos, S. Tulipani, M. Garcia-Aloy, M. Bulló, F. J. Tinahones, and C. Andres-Lacueva, "Plasma metabolomic biomarkers of mixed nuts exposure inversely correlate with severity of metabolic syndrome," *Molecular Nutrition & Food Research*, vol. 59, no. 12, pp. 2480–2490, 2015.

[128] J. R. Marchesi, D. H. Adams, F. Fava et al., "The gut microbiota and host health: a new clinical frontier," *Gut*, vol. 65, no. 2, pp. 330–339, 2016.

[129] R. Zamora-Ros, M. Rabassa, A. Cherubini et al., "High concentrations of a urinary biomarker of polyphenol intake are associated with decreased mortality in older adults," *The Journal of Nutrition*, vol. 143, no. 9, pp. 1445–1450, 2013.

[130] R. Zamora-Ros, N. G. Forouhi, S. J. Sharp et al., "The association between dietary flavonoid and lignan intakes and incident type 2 diabetes in European populations: the EPIC-InterAct study," *Diabetes Care*, vol. 36, no. 12, pp. 3961–3970, 2013.

[131] A. Tresserra-Rimbau, E. B. Rimm, A. Medina-Remón et al., "Polyphenol intake and mortality risk: a re-analysis of the PREDIMED trial," *BMC Medicine*, vol. 12, no. 1, p. 77, 2014.

[132] C. Andres-Lacueva, A. Medina-Remon, R. Llorach et al., "Phenolic compounds. Chemistry and occurrence in fruits and vegetables," in *Fruit and vegetable phytochemicals: chemistry, nutritional value and stability*, pp. 53–88, Blackwell Publishing, 2010.

[133] K. G. M. M. Alberti, P. Zimmet, and J. Shaw, "Metabolic syndrome—a new world-wide definition. A Consensus Statement from the InternationalDiabetes Federation," *Diabetic Medicine*, vol. 23, pp. 469–480, 2006.

[134] S. M. Grundy, J. I. Cleeman, S. R. Daniels et al., "Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement," *Circulation*, vol. 112, pp. 2735–2752, 2005.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 6723931, 16 pages
https://doi.org/10.1155/2017/6723931

Review Article

Dietary Polyphenols, Mediterranean Diet, Prediabetes, and Type 2 Diabetes: A Narrative Review of the Evidence

Marta Guasch-Ferré,[1] Jordi Merino,[2] Qi Sun,[1,3] Montse Fitó,[4,5] and Jordi Salas-Salvadó[5,6]

[1]*Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA*
[2]*Diabetes Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA*
[3]*Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA*
[4]*Cardiovascular Risk and Nutrition (Regicor Study Group), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain*
[5]*CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain*
[6]*Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Faculty of Medicine and Health Sciences, IISPV, Rovira I Virgili University, Reus, Spain*

Correspondence should be addressed to Marta Guasch-Ferré; mguasch@hsph.harvard.edu

Received 28 March 2017; Revised 19 June 2017; Accepted 3 July 2017; Published 13 August 2017

Academic Editor: Giuseppe Cirillo

Dietary polyphenols come mainly from plant-based foods including fruits, vegetables, whole grains, coffee, tea, and nuts. Polyphenols may influence glycemia and type 2 diabetes (T2D) through different mechanisms, such as promoting the uptake of glucose in tissues, and therefore improving insulin sensitivity. This review aims to summarize the evidence from clinical trials and observational prospective studies linking dietary polyphenols to prediabetes and T2D, with a focus on polyphenol-rich foods characteristic of the Mediterranean diet. We aimed to describe the metabolic biomarkers related to polyphenol intake and genotype-polyphenol interactions modulating the effects on T2D. Intakes of polyphenols, especially flavan-3-ols, and their food sources have demonstrated beneficial effects on insulin resistance and other cardiometabolic risk factors. Several prospective studies have shown inverse associations between polyphenol intake and T2D. The Mediterranean diet and its key components, olive oil, nuts, and red wine, have been inversely associated with insulin resistance and T2D. To some extent, these associations may be attributed to the high amount of polyphenols and bioactive compounds in typical foods conforming this traditional dietary pattern. Few studies have suggested that genetic predisposition can modulate the relationship between polyphenols and T2D risk. In conclusion, the intake of polyphenols may be beneficial for both insulin resistance and T2D risk.

1. Introduction

In the past few decades, type 2 diabetes (T2D), in parallel with the obesity epidemic, has become a public health challenge for many countries [1]. Increasing evidence has demonstrated that the combinations of several unhealthy lifestyle factors, including a sedentary lifestyle, unhealthy diets, overweight/obesity, smoking, and excessive alcohol intake, were responsible for 90% of T2D cases [2]. For this reason, strategies focused on lifestyle and the promotion of a healthy diet to prevent T2D have been identified as a cornerstone of researchers and policymakers.

Recently, growing interest has emerged on the beneficial effects of plant-based diets for the prevention of chronic diseases including obesity, diabetes, and cardiovascular disease [3, 4]. Such diets are based on foods derived from plants, including fruits, vegetables, whole grain cereals, legumes, and nuts, with limited animal products. As an example, the Mediterranean diet, which has been associated with many health benefits [5], is characterized by a high intake of fruits,

Table 1: Main food sources of polyphenols.

Polyphenol	Compound	Main food sources, excluding seasoning	Main food sources, only seasoning
Total polyphenols		Coffee, oranges, apples, grapes, olives and olive oil, red wine, cocoa powder, dark chocolate, tea, black elderberry, nuts, whole grains, legumes	Cloves, dried peppermint, star anise
Flavonoids	Flavones	Virgin olive oil, oranges, whole grain wheat-flour bread, refined-grain wheat-flour bread, whole grain wheat four, black olives	Celery seed, dried peppermint, dried, common verbena
	Flavonols	Spinach, beans, onions, shallot	Capers, saffron, dried oregano
	Flavanols	Red wine, apples, peaches, cocoa powder, nuts, dark chocolate	
	Flavanones	Grapefruit/pomelo juice, oranges, orange juice, grapefruit juice	Dried peppermint, dried oregano, fresh rosemary
	Isoflavones	Soy flour, soy paste, roasted soy bean, beans	Soy sauce
	Anthocyanins	Cherries, red wine, olives, hazelnuts, almonds, black elderberry, black chokeberry, blueberries	
Phenolic acids	Benzoic acid	Olives, virgin olive oil, red wine, walnuts, pomegranate juice, red raspberry, American cranberry	Chestnut, cloves, star anise
	Cinnamic acid	Coffee, maize oil, potatoes	Dried peppermint. Common verbena, dried rosemary
Stilbenes	Resveratrol	Grapes, red wine, nuts	
Lignans		Virgin olive oil, whole grain rye flour, bread from whole grain rye flour, flaxseed	Sesame seed oil, black sesame oil, flaxseed

vegetables, legumes, nuts, and olive oil; a moderate consumption of dairy products and wine; and low intake of red and processed meat, butter, cream, and sugar drinks. One of the dietary constituents common in plant-based diets are polyphenols, which are especially abundant not only in fruits, vegetables, whole grains, and legumes but also in cocoa, tea, coffee, and red wine [6].

Polyphenols are a large and heterogeneous group of phytochemicals containing phenol rings and are divided into flavonoids, phenolic acids, stilbenes, and lignans [7] (Table 1). Flavonoids are classified into flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins [8]. Among others, fruits like apples, grapes, pears, and berries typically contain high amounts of polyphenols (200–300 mg per 100 g) [9]. The most common phenolic acids are caffeic acid and ferulic acid, which is a major phenolic compound in coffee and cereals, respectively [10]. The best-studied stilbene is resveratrol in grapes, grape products, and red wine [10]. Other main dietary sources of polyphenols include vegetables, chocolate, tea, whole grains, dry legumes, nuts, and olive oil [10]. Polyphenols are the most abundant antioxidants in the diet, and their intake has been associated with a reduced incidence of T2D in humans [11–13]. Polyphenols have anti-inflammatory effects and may influence glycemia through different mechanisms, including the inhibition of glucose absorption in the gut and the improvement of insulin resistance [9].

This review aims to summarize the relevant evidence linking dietary polyphenols to prediabetes and T2D with a focus on polyphenol-rich food characteristics of the Mediterranean diet (MedDiet). In addition, the present work aims to describe genotype-polyphenol interactions and metabolic biomarkers to understand the relationship between polyphenols and T2D.

2. Dietary Polyphenols, Insulin Sensitivity, and Resistance

Abundant evidence generated in human studies collectively suggests that the intake of polyphenols and their major food sources may exert beneficial effects on improving insulin resistance and related diabetes risk factors, such as inflammation and oxidative stress (as reviewed by Scalbert et al. [9]). Given the amount of such evidence, this narrative review does not seek to provide a comprehensive summary of all study findings, but rather focuses on well-conducted clinical trials and observational prospective studies. In this regard, only randomized and controlled trials have been considered, and regarding the prospective cohort studies, only those with appropriate control of potentially confounding variables have been included. We have performed a narrative review in MEDLINE up to June 2017. Our search terms combined the exposures (polyphenols, flavonoids, phenolic acids, stilbenes, lignans; and also, MedDiet, extra-virgin olive oil, nuts, and red wine) with several outcomes (insulin resistance, insulin sensitivity, oxidation, inflammation, and T2D). We also conducted a specific search for well-powered human clinical studies with the potential for clinical translational findings. We reviewed the evidence of polyphenols and T2D from a nutritional genomic perspective, particularly from metabolomics and metagenomics. We acknowledge other omics studies using transcriptomics, proteomics, and

epigenomics may also have an important role on understanding polyphenol mechanisms of action, but given the clinical translational nature of this review, we have not included these studies.

Among all polyphenols, the beneficial effects of flavanols (flavan-3-ols) and their primary food sources, including cocoa, chocolate, and red wine, have been most widely examined in clinical trials. In a meta-analysis of 24 trials among 1106 individuals that examined the effects of cocoa intake for 2–18 weeks, Shrime et al. showed significant effects of cocoa intake on improving insulin sensitivity [14]. Homeostatic model assessment of insulin resistance (HOMA-IR) decreased by 0.94 points (95% CI = 0.59, 1.29; $P < 0.001$) with the consumption of flavonoid-rich cocoa. This meta-analysis included healthy participants and patients with hypertension, diabetes, and overweight. Total flavonoid intake ranged from 16.6 mg/d to 1080 mg/d and control group included low-flavonoid cocoa, white chocolate, skim milk, and placebo capsules. In nine studies, at least one author was employed by a chocolate company [14]. In another meta-analysis of 42 trials comprised of 1297 individuals which was conducted in 2012, Hooper et al. showed that acute to short-term (≤18 weeks) intake of cocoa, chocolate, and flavan-3-ols significantly decreased insulin resistance [15]. Healthy participants and participants with elevated blood pressure, serum cholesterol, and diabetes as well as other health conditions were included. Interventions were cocoa drinks, dark or milk chocolate, cocoa supplements, solid chocolate plus cocoa drinks, and a whole diet (all foods provided) including cocoa powder and chocolate. These were compared with low flavan-3-ol versions of the same foods, drinks, or supplements [15].

Data on the effects of long-term chronic consumption of flavan-3-ols on cardiometabolic health are scarce. In a randomized, placebo-controlled trial in 93 patients with T2D, the intake of 7 grams per day of flavonoid-enriched chocolate (containing 850 mg flavan-3-ols and 100 mg isoflavones) for 1 year significantly improved insulin resistance. In this long-term trial, effects on hemoglobin A1c (HbA1c) and glucose were not observed [16]. However, the authors acknowledged that the dropout rate was high and limited to postmenopausal women receiving diabetes therapy.

Another food source of flavan-3-ols, green tea, has also been extensively examined in short-term clinical trials evaluating its beneficial effects on cardiometabolic health. For example, catechins from green tea exerted beneficial effects on lowering fasting glucose, although effects on fasting insulin, HbA1c, and HOMA-IR were not significant in a meta-analysis summarizing results of 22 randomized clinical trials with 1584 participants and ranging interventions from 3 to 24 weeks [17]. Green tea catechin dosage ranged from 240 mg/d to 1207 mg/d and was compared with water or placebo.

Clinical trials that aim to assess the effects of anthocyanins are relatively sparse. In a double-blinded 6-week clinical trial among 32 obese nondiabetic individuals, Stull et al. found that intake of a smoothie with added blueberry powder (equivalent to 668 mg/day anthocyanins) as compared to a smoothie of equal nutritional value without added blueberry bioactivities significantly improved insulin sensitivity measured by a hyperinsulinemic-euglycemic clamp [18]. Beneficial effects of anthocyanins (either purified anthocyanin (320 mg/d) derived from bilberry and black currant or placebo) on improving insulin resistance were also observed in a 12-week trial conducted in 74 patients with nonalcoholic fatty liver disease [19]. Of note, the descriptions of the control foods and extracts used as in the abovementioned clinical trials were poorly described and no indication on its nature is given.

The consumption of beverages with added freeze-dried strawberries rich in anthocyanins compared to placebo for 12 weeks did not exert effects on inflammatory markers or glucose measurements in 60 individuals with abdominal adiposity and hypercholesterolemia, although a significant reduction of total and low-density lipoprotein (LDL) cholesterol was observed in this trial [20].

Isoflavones are a class of flavonoids with estrogenic effects that are found in soya beans and are highly consumed in East Asian populations. Probably because of these estrogenic properties, most of the clinical trials evaluating the effects of isoflavone supplementation focused on postmenopausal or perimenopausal women. In a meta-analysis of 24 trials comprised of 1518 men and women, intervention groups included isoflavone extracts as supplements and whole soy foods into the diet and compared the isolated soy protein that contained isoflavones with milk or animal protein. The soy protein content ranged from 0 to 40 g soy protein/d, and the isoflavone content ranged from 36 to 132 mg isoflavones/d. Results from this meta-analysis have shown that soy intake did not exert significant effects on measures of glucose metabolism, including fasting glucose and insulin, HOMA-IR, HbA1c, and 2-hour glucose or insulin levels [21]. Restricting the analysis to postmenopausal women generated similar results.

Interestingly, in another meta-analysis that only considered clinical trials conducted among non-Asian perimenopausal or postmenopausal women, soy isoflavones or genistein supplements for 3 months to 2 years significantly reduced serum insulin and HOMA-IR, but had no effects on fasting blood glucose [22].

Lignans are a group of polyphenols that also possess estrogenic properties. A few trials have been conducted to elucidate the effects of flaxseed supplements that contain high amount of lignans on insulin resistance and other cardiometabolic intermediate outcomes. In a small crossover trial among 9 obese subjects with glucose intolerance, flaxseed intake (40 g/day) compared to 40 g wheat bran for 12 weeks significantly improved insulin resistance [23]. In another small randomized trial comparing flaxseed intake (40 g/day) with hormone replacement therapy among 25 postmenopausal women, flaxseed intake significantly reduced glucose and insulin levels, although these benefits were comparable to those of hormone replacement therapy [24]. However, sample sizes of these studies were small and replication of the results in other populations is needed to make strong conclusions. In a crossover, randomized trial conducted among ~70 patients with T2D, Pan et al. showed that supplementation of 360 mg/day of lignans derived from

flaxseed for 12 weeks significantly reduced HbA1c [25] and C-reactive protein (CRP) [26] in comparison with the placebo group. Interestingly, such effects were primarily observed in women, whilst no effects on CRP levels were found in men [26]. In the largest trial testing the efficacy of flaxseed supplements so far, flaxseed lignan supplements (543 mg/day) (as compared to placebo) did not exert significant effects on lowering fasting blood glucose concentrations or inflammatory markers in 100 Canadian healthy men and women followed for 6 months [27]. Moreover, in a recent trial among 99 prediabetic patients, flaxseed supplementation did not improve insulin sensitivity [28]. This randomized, clinical trial compared two groups receiving 40 g and 20 g per day of flaxseed powder daily for 12 weeks and the third group was the control group (no intervention) [28].

Evidence regarding other polyphenols or their food sources is sporadic. In a crossover trial conducted in overweight men and women, supplementation of 150 mg/day resveratrol compared to placebo capsules for 4 weeks had no effects on insulin sensitivity or inflammatory markers [29]. Supplements of chlorogenic acid (1 g/d, a polyphenol highly present in coffee, significantly reduced glucose and insulin concentrations 15 minutes following an oral glucose tolerance test (OGTT), although no overall improvement on OGTT measurements was observed in a small crossover trial (three interventions: 12 g decaffeinated coffee, 1 g chlorogenic acid, 500 mg trigonelline, and placebo (1 g mannitol)) including 15 overweight men [30]. Of note, trials that examined the effects of coffee consumption per se did not generate consistent findings regarding whether insulin resistance could be improved, although all these trials were small in sample size (ranging from 10 to 47 participants) [31–33]. Of note, because the portions of coffee are larger in North European and American populations and therefore the intake of polyphenols from this food greater, some differences may exist among populations.

In summary, intake of polyphenols, especially flavan-3-ols and their food sources, have demonstrated overall beneficial effects on decreasing insulin resistance, chronic systematic inflammation, oxidative stress, and improving other cardiometabolic risk factors. However, some publication bias may be present as several clinical trials have been sponsored by the food industry, particularly for cocoa, chocolate, and tea, and this may explain the greater amount of evidence on this food groups. In addition, some clinical trials on this topic were of short duration which may dilute the effect of polyphenol intake on a long term. Although surrogate endpoints measured in most clinical trials can help to monitor changes during follow-up and are relevant for the prediction of T2D, more research is warranted to elucidate the metabolic effects of polyphenols in larger trials with longer intervention term and risk of T2D. In this regard, the ongoing Cocoa Supplement and Multivitamin Outcomes Study (COSMOS) [34], which aims to evaluate the efficacy of a concentrated cocoa extract using a five-year randomized trial among 18,000 healthy men and women, may provide conclusive evidence on the health benefits of cocoa (rich in polyphenols) on cardiometabolic hard outcomes. Finally, novel metabolomic techniques will help to determine more objective plasma concentrations of polyphenols and had a better picture of availability and potential beneficial effects on insulin resistance and T2D.

3. Dietary Polyphenols and Risk of Type 2 Diabetes

Table 2 summarizes the evidence on prospective studies evaluating polyphenol intake and risk of T2D. Several studies have evaluated the associations between the intake of total flavonoids or specific flavonoids and the risk of T2D. Four studies including data from 6 cohorts on healthy participants have evaluated the associations between dietary total flavonoids and risk of T2D [35]. Results from a meta-analysis including 18,146 incident T2D cases and 284,806 participants showed that the relative risk of T2D for the highest intake of total flavonoids compared with the lowest was 0.91 (95% CI: 0.87, 0.96). A dose-response analysis of these results revealed that 500 mg/d increase in total flavonoid intake was associated with a significant 5% reduction of T2D incidence. These beneficial effects were especially observed in younger individuals and in those studies with larger follow-up [35]. Two new studies not included in this meta-analysis were published later [36, 37]. In the European Prospective Investigation into Cancer and Nutrition-InterAct (EPIC-InterAct) study, an inverse association between total flavonoid intake and T2D risk was reported in a case-cohort study including 12,403 incident cases of diabetes and 16,154 healthy participants [36]. In contrast, total flavonoid intake was nonsignificantly associated with the risk of T2D in a report from the Framingham Offspring Study, which included 2915 participants followed for 11.9 years [37].

Several studies have evaluated the association between the consumption of different subtypes of flavonoids and T2D risk with some controversial results. The Finnish Mobile Clinic Health Examination Survey (FMCHES) [38], the Womens' Health Study [39], both including healthy participants, and the α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study, which included male smokers [40], examined the associations between the intake of total or selected flavonol or flavone molecules (quercetin, kaempferol, myricetin, hesperetin, and naringenin) and T2D risk. In the FMCHES, higher intake of quercetin and myricetin showed a trend towards a reduction in the risk of T2D, but not for the rest of the compounds [38]. No significant associations were observed between total or selected types of flavonol and flavone intake and T2D in the Women's Health Study [39] and the ATBC Study cohorts [40].

Findings from large-scale prospective studies have evaluated the associations between flavonoids and T2D risk. After pooling the results of three large cohorts (Nurses' Health Study (NHS), NHSII and Health Professionals Follow-up Study (HPFS); 3,645,585 person-years of follow-up; 12,611 incident cases of diabetes), higher intakes of anthocyanins and anthocyanin-rich fruits were associated with a decreased risk of T2D after adjusting for multiple potential confounders, whereas no associations were shown for total flavonols, flavones, flavanones, and flavan-3-ols [11]. A case-

Table 2: Prospective studies evaluating polyphenol intake on the risk of type 2 diabetes.

First author, publication year, study name, location	Sex	Follow-up (years)	Age at baseline (years) (mean)	Number of cases/ participants	Exposure assess ment and case ascertainment	Types of polyphenols analyzed	Relative risk (95% CI) (highest versus lowest category)	Adjustment for covariates
Knekt 2002, The FMCHES, Finland	Both	18	39.3 ± 15.8	526/9878	FFQ > 100 items/ identified from Social Insurance Institution Finland	Quartiles of dietary intake of major flavonoid subclasses (total flavonoid intake 24.2 mg/d)	Q4 versus Q1 0.98 (0.78, 1.24)	Sex and age
Song 2005, WHS, United States	F	8.8	≥45 (53)	1614/ 38,018	131-item semiquantitative validated FFQ/self-report and confirmed with supplementary questionnaire about symptoms, American Diabetes Criteria	mg/d quintiles of dietary intake of total or individual flavonols and flavones and flavonoid-rich foods	Q5 versus Q1 (median intake mg/d: 47.2 versus 8.85) of total flavonoids 0.92 (0.78, 1.09)	Age, BMI, energy, total fat, smoking, exercise, alcohol use, history of hypertension, high cholesterol, family history of diabetes, fiber intake, glycemic load, magnesium
Nettleton 2006, Iowa Women's Health Study, United States	F	18	55–69 (61)	3395/ 35,816	Validated 127-item FFQ/self-reported were determined by the following question: "Were you diagnosed for the first time by a doctor as having sugar diabetes?"	mg/d quintiles of flavonoid and flavonoid sources	Q5 versus Q1 (median intake mg/d: 680.4 versus 90.4) of total flavonoids 0.97 (0.86, 1.10)	Age, energy, education level, BMI, waist:hip ratio, activity level, smoking status, multivitamin use, and hormone therapy
Kataja-Tuomola 2011, ATBC, Finland	M	10.2	50–69 (57.5)	660/ 25,505	Validated FFQ 275 food items/self-reported or medical diagnosis and Social Insurance Institution Finland	Quintiles of flavonols and flavones	Nonsignificant associations for kaempferol, luteolin, myricetin, quercetin	Age, supplementation, BMI, cigarettes smoked daily, smoking years, blood pressure, total cholesterol, high-density lipoprotein cholesterol, leisure-time physical activity, and daily intake of alcohol and energy

Table 2: Continued.

First author, publication year, study name, location	Sex	Follow-up (years)	Age at baseline (years) (mean)	Number of cases/ participants	Exposure assessment and case ascertainment	Types of polyphenols analyzed	Relative risk (95% CI) (highest versus lowest category)	Adjustment for covariates
Wedick 2012, NHS, NHSII, HPFS, United States	F (NHS)	24	30–55 (50)	6878/ 70,359	131-item semiquantitative validated FFQ/ self-report and confirmed with supplementary questionnaire about symptoms, the National Diabetes Group criteria	mg/d quintiles of dietary intake of major flavonoid subclasses	Q5 versus Q1 (median intake mg/d: 718.1 versus 105.2) of total flavonoids 0.85 (0.79, 0.92)	Age, BMI, smoking status, alcohol intake, multivitamin use, physical activity, family history of diabetes, postmenopausal status and hormone use, ethnicity, total energy, intakes of red meat, fish, whole grains, coffee, high-calorie sodas, and trans fat
	F (NHSII)	16	25–42 (36)	3084/ 89,201	Same as above	mg/d quintiles of dietary intake of major flavonoid subclasses	Q5 versus Q1 (median intake mg/d: 770.3 versus 112.1) of total flavonoids 0.99 (0.89, 1.11)	Same as above plus oral contraceptive use
	M (HPFS)	20	40–75 (53)	2649/ 41,334	Same as above	mg/d quintiles of dietary intake of major flavonoid subclasses	Q5 versus Q1 (median intake mg/d: 624.3 versus 112.5) of total flavonoids 0.92 (0.81, 0.94)	Same as above except postmenopausal status and hormone use and oral contraceptive use
Zamora-Ros 2013, Epic-InterAct, 8 European countries	Both	3.99 million person-years of follow-up	52.4 (9.1)	12,403/ 16,154	Country-specific FFQ/ self-report and linkage to primary and secondary care registers, hospital and mortality data	mg/d quintiles of dietary flavonoids, types of flavonoids and lignans intake	Q5 versus Q1 (median intake mg/d: 817.5 versus 126.8) of total flavonoids 0.90 (0.77, 1.04)	Age, sex, and total energy intake, educational level, physical activity, smoking status, BMI, alcohol intake, intakes of red meat, processed meat, sugar-sweetened soft drinks, and coffee, intakes of fiber, vitamin C, and magnesium

Table 2: Continued.

First author, publication year, study name, location	Sex	Follow-up (years)	Age at baseline (years) (mean)	Number of cases/ participants	Exposure assess ment and case ascertainment	Types of polyphenols analyzed	Relative risk (95% CI) (highest versus lowest category)	Adjustment for covariates
Jacques 2013, Framingham Offspring Cohort, United States	Both	11.9	54.2 (53.8, 54.5)	308/2915	Validated FFQ/fasting glucose concentrations and/or a medical and medication use history obtained by a physician at each study examination	6 flavonoid classes and total flavonoids	HR per 2.5-fold difference in flavonoid intake (cumulative mean flavonoid intake) 0.89 (0.75, 1.05)	Sex, age, cardiovascular disease, current smoker (y/n), BMI, and cumulative mean energy intake, vegetable and fruit intake
Zamora-Ros 2014, EPIC-InterAct, United States	Both	3.99 million person-years of follow-up	52.4 (9.1)	12,403/ 16,154	Country-specific FFQ/ self-report and linkage to primary and secondary care registers, hospital and mortality data	mg/d quintiles of dietary flavanol and flavonol intake	Q5 versus Q1 of sum of flavanols and flavonols (median intake in mg/d: 713.6 versus 97.6). Inverse associations between all flavan-3-ol monomers, proanthocyanidin dimers and trimers (Q5 versus Q1 0.81 (0.71, 0.92) and 0.91 (0.80, 1.04), resp.)	Age, sex, and total energy intake, educational level, physical activity, smoking status, BMI, alcohol intake, intakes of red meat, processed meat, sugar-sweetened soft drinks, and coffee, intakes of fiber, vitamin C, and magnesium
Tresserra-Rimbau 2016, PREDIMED, Spain	Both	5.51	55–80	314/ 3430	Validated 137-item FFQ/ fasting plasma glucose ≥7 mmol/L or 2 h plasma glucose ≥11.1 mmol/L after a 75 g oral glucose load, confirmed by a second test using the same criteria, the American Diabetes Association criteria	Total polyphenols, flavonoids, stilbenes, lignans	T3 versus T1 (mean intake 1002 versus 600) of total polyphenols 0.72 (0.52, 0.99)	Age, sex, recruitment center, intervention group. Smoking, BMI, physical activity, dyslipidemia, hypertension, education level, total energy intake, alcohol intake, adherence to the Mediterranean diet, and fasting glucose

Table 2: Continued.

First author, publication year, study name, location	Sex	Follow-up (years)	Age at baseline (years) (mean)	Number of cases/ participants	Exposure assess ment and case ascertainment	Types of polyphenols analyzed	Relative risk (95% CI) (highest versus lowest category)	Adjustment for covariates
Ding 2016, NHS, NHS2, HPFS, United States	F (NHS)	8	30–55 (50)	3671/ 63,115	131-item semiquantitative validated FFQ/self-report and confirmed with supplementary questionnaire about symptoms, the National Diabetes Group criteria	mg/d quintiles of isoflavone consumption	Q5 versus Q1 (median intake mg/d: 2.78 versus 0.17) of isoflavones 0.97 (0.88, 1.07)	Age, race, family history of T2D, baseline disease status, BMI, physical activity, overall dietary pattern (alternate Healthy Eating Index score, in quintiles), total energy intake and smoking status and menopausal status, postmenopausal hormone use
	F (NHSII)	8	25–42 (36)	3920/ 79,061	Same as above	mg/d quintiles of isoflavone consumption	Q5 versus Q1 (median intake mg/d: 5.73 versus 0.17) of isoflavones 0.85 (0.76, 0.95)	Same as above
	M (HPFS)	8	40–75 (53)	742/ 21,281	Same as above	mg/d quintiles of isoflavone consumption	Q5 versus Q1 (median intake mg/d: 5.09 versus 0.31) of isoflavones 0.80 (0.62, 1.02)	Same as above except postmenopausal status and hormone use

ATBC, α-Tocopherol, β-Carotene Cancer Prevention Study; WHS, Women's Health Study; FFQ, food frequency questionnaire; NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; PREDIMED, Prevención con Dieta Mediterránea; EPIC, The European Prospective Investigation into Cancer and Nutrition; FMCHES, Finnish Mobile Clinic Health Examination Survey.

cohort study within the frame of the EPIC-InterAct cohort, which included healthy middle-aged participants from eight countries, showed that when comparing extreme quintiles of consumption of flavonoid subclasses, total flavonols and flavanols, including flavan-3-ol monomers, were inversely related to the diabetes risk, whereas no associations were reported for the intake of lignans [36]. It is important to highlight that these associations were observed within the context of a European-wide population with a large heterogeneity in the intake of these compounds. In a following report from the same study [41], significant inverse trends for the intakes of proanthocyanidin dimers and trimers and incident T2D were observed, but not for proanthocyanidins with a greater polymerization degree. Among the flavonol subclasses, only myricetin was associated with a lower incidence of T2D [41]. Finally, in the Framingham Offspring Cohort, each 2.5-fold increase in flavan-3-ol intake was marginally associated with an 11% lower incidence of T2D, but no other associations between other flavonoid classes (flavonols, flavones, flavanones, anthocyanins, and polymeric flavonoids) and risk of T2D were reported [37].

In relation to the intake of isoflavones, findings from the NHS, NHSII, and HPFS have shown inverse associations for total isoflavones and major individual isoflavones, especially those present in soy and soy products, including daidzein and genistein, with the risk of developing diabetes [42]. However, no significant association between total isoflavone intake and T2D has been reported in the EPIC-InterAct Study [36].

In summary, results from prospective cohort studies have suggested inverse associations between the intake of total flavonoids and specific flavonoid subclasses and the risk of T2D although some controversial results exist.

4. Characteristic Polyphenols of the Mediterranean Diet, Prediabetes, and Type 2 Diabetes

As described before, dietary polyphenols come mainly from plant-based foods [8]. Olive oil, nuts, red wine, legumes, fruits, and vegetables, key components of the MedDiet, are all polyphenol-rich foods [5]. The much-appreciated MedDiet is well-known for its antioxidant and anti-inflammatory effects [43, 44]. The cardioprotective effect of this dietary pattern has been attributed, in part, to the high amount of antioxidant components such as the phenolic compounds [43]. Moreover, the MedDiet has been shown to be beneficial for glycemic control and T2D [45, 46]; however, few studies have evaluated the effects of specific polyphenols from food products characteristic of this diet and insulin resistance or T2D. In this section, we aim to provide further information on characteristic food of the MedDiet that contain large amounts of polyphenols.

4.1. Extra-Virgin Olive Oil. Extra-virgin olive oil is probably one of the components that most differentiates the MedDiet from other dietary patterns. Phenolic components such as oleuropein and hydroxytyrosol, flavonoids, specially flavones, and lignans are abundant in extra-virgin olive oil [47]. Extra-virgin olive oil is the best quality olive oil, is rich in taste and color, and also contains high amount of bioactive compounds compared to other types of olive oil (such as common olive oil, which is refined, has less flavor, color, and aroma, and contains fewer amounts of antioxidants and vitamin E). The phenolic composition of olive oil ranges from 50 to 800 mg/L depending on several factors like the variety, cultivation techniques, degree of ripeness, and climate, among others [48].

Evidence from clinical trials on the effects of olive oil polyphenols and biomarkers of T2D is scarce. Only two randomized trials have been published so far evaluating the effect of olive leaf polyphenols on markers of insulin sensitivity [49, 50]. Main polyphenols in olive leaves (oleuropein and hydroxytyrosol) are similar than those in fruit and fruit oil; however, its concentration is greater. In the first intervention study, including 70 adults with T2D, those participants consuming olive leaf extract (500 mg) exhibited significantly lower HbA1c and fasting plasma insulin levels; however, postprandial plasma insulin levels did not differ significantly by treatment group [50]. The second study demonstrated that participants who received capsules of olive leaf extract for 12 weeks (51.1 mg oleuropein and 9.7 mg hydroxytyrosol per day) compared to placebo improved pancreatic β-cell function by 28% and also significantly improved insulin sensitivity compared to the placebo [49]. Other clinical trials have evaluated the effect of extra-virgin olive oil rich in polyphenols on glycemic biomarkers [51, 52]. Daily consumption of polyphenol-rich extra-virgin olive oil for 8 weeks significantly reduced fasting plasma glucose and HbA1c, as well as other circulating inflammatory adipokines, in overweight patients with T2D [52]. In contrast, no significant effects on fasting glucose after supplementation with 20 mL per day of polyphenol-rich olive oil during 6 weeks compared to 20 mL dose of low phenolic oil were observed in healthy participants [51]. The PREDIMED trial has provided conclusive evidence of the beneficial effects of a MedDiet supplemented with extra-virgin olive oil on glucose metabolism and T2D [45, 46]. In this regard, a MedDiet supplemented with extra-virgin olive oil reduced the risk of T2D by 40% (HR: 0.60; 95% CI: 0.43, 0.85) after a median of 4.1 years of follow-up among participants at high cardiovascular risk compared to the control group [46]. Previous reports from the PREDIMED Study also showed that a MedDiet supplemented with extra-virgin olive oil reduced fasting plasma glucose improved insulin resistance and inflammatory biomarkers [45, 53]. Another randomized crossover trial in healthy volunteers, the EUROLIVE study, which tested the effect of the daily 3-week administration of 25 mL of 3 olive oils with low, medium, and high phenolic content, demonstrated beneficial effects of phenolic compounds on oxidative damage on lipids and HDL cholesterol [54]. However, given that the volunteers were healthy, no effects were observed on glucose levels; nevertheless, the objective of the study was the lipid and lipoperoxidation. Also in a postprandial state study with healthy volunteers, after 50 mL of virgin olive oil load, the expression of candidate genes related to insulin sensitivity was observed in peripheral mononuclear cells [55].

4.2. Nuts. Nuts, which are well-known for their unique nutritional composition (rich in unsaturated fatty acids, fiber, antioxidant vitamins, minerals, and other bioactive compounds), are also consumed in high amounts in the MedDiet. Several seeds and nuts are among the richest sources of polyphenols: chestnuts and walnuts are rich in ellagitannins; while hazelnuts, pecan, and almonds are rich in proanthocyanidins; and flaxseed is rich in lignans [8]. Although the effects of nut consumption on glycemic markers and T2D have been well studied over the past decades [56, 57], specific studies on polyphenols from nuts are lacking. However, we have some indirect evidence that polyphenols from tree nuts may have beneficial effects on glucose metabolism. Some evidence has suggested that ellagic acid, which is found in a considerable amount in several nuts (especially walnuts), could be beneficial for diabetes control [58]. In addition, urolithin A glucuronide in plasma, a metabolite of dietary ellagic acid derivatives which has shown to be a discriminative biomarker of nut exposure, was inversely correlated with insulin resistance measured by HOMA-IR. However, further clinical trials are needed to confirm these associations [59].

4.3. Red Wine. One of the main characteristics of the traditional MedDiet is the moderate intake of wine consumed with meals. Wine, particularly red wine, is rich in phenolic compounds including flavonoids (anthocyanins, tannins, and catechin), stilbenes like resveratrols, tyrosols, and hydroxytyrosols. Evidence suggests that red wine consumption exerts some benefits on cardiovascular health [60]; however, whether these effects are due to ethanol or to nonalcoholic components of red wine is still unclear. Along these lines, a randomized clinical trial including sixty-seven men at high cardiovascular risk was designed to compare the effects of moderate intake during 4 weeks of red wine, a high polyphenolic alcoholic beverage (the equivalent amount of dealcoholized wine), a high polyphenolic nonalcoholic beverage, and gin (without polyphenols) on glucose metabolism and lipid profile [61]. Findings of that study suggested that red wine rich in polyphenols with or without alcohol, but not gin, improved glucose metabolism, as measured by HOMA-IR [61]. Previous clinical trials have demonstrated improvements in insulin sensitivity with the consumption of red wine [62] and red grape juice [63], but others reported no significant effects on these parameters [64, 65]. In addition, the positive effects on glucose metabolism of supplementation with grape derived polyphenol and resveratrol, one of the main polyphenols in red wine, have been observed in intervention studies [66, 67]. Finally, a 2-year randomized clinical trial in patients with T2D suggests that initiating moderate red wine intake, among well-controlled diabetics as part of a healthy diet, is apparently safe and modestly decreases cardiometabolic risk [68]. Moreover, genetic interactions may play a role in glucose metabolism, and red wine's effects also involve nonalcoholic constituents such as polyphenols. In this trial, slow ethanol metabolizers (alcohol dehydrogenase alleles [ADH1B ∗ 1] carriers) significantly benefited from the effect of red wine on glycemic control (fasting plasma glucose, HOMA-IR, and hemoglobin A1c) compared with fast ethanol metabolizers (persons homozygous for ADH1B ∗ 2) [68].

4.4. Other. Fruits, vegetables, whole grains, legumes, and coffee are also primary sources of polyphenols [8] and are beneficial for glycemic control and prevention of T2D [4, 69]. Other beverages and foods that are rich in polyphenols include green tea, soy, chocolate, and cocoa and could also play a role in the prevention of T2D [70].

In summary, the MedDiet and its key components, extra virgin olive oil, nuts, and red wine, are inversely associated with insulin resistance and T2D. To some extent, these effects may be attributed to its high amount of polyphenols and other bioactive compounds.

5. New Frontiers in Nutrition Research: Genotype-Polyphenol Interaction on Type 2 Diabetes

Because of their chemical structures, dietary polyphenols exert multiple activities by interacting with several molecular pathways particularly relevant for glucose homeostasis. In Figure 1, we summarize relevant mechanisms linking dietary polyphenols and T2D risk. These include slowing carbohydrate digestion and glucose absorption, stimulation of insulin secretion, modulation of glucose release, and activation of insulin receptors and glucose uptake in the insulin-sensitive tissues [71]. A growing body of evidence from in vitro and animal studies has shown that polyphenols can activate and/or silence transcription factors, and consequently influence gene expression, and regulate different signaling pathways in the muscle, the liver, pancreatic β-cells, the hypothalamus, and adipose tissue, thereby contributing to glucose homoeostasis [72, 73].

Although promising data aimed at understanding how different polyphenol classes can modulate genetic regulation and expression have been published, few studies have investigated whether genetic predisposition modifies the relationship among polyphenols, intermediate phenotypes of insulin resistance, and T2D risk [74–79]. An initial and very preliminary track of evidence for genotype-polyphenol interaction is emerging from studies of coffee, the consumption of which is highly spread in Mediterranean regions. In a prospective population-based cohort study including 4077 normal glycemic individuals over a 4-year follow-up, habitual coffee intake outweighed the hazard of unfavorable genetic predisposition on 3 well-known T2D-increasing risk genetic loci, including *IGF2BP2*, *CDKAL1*, and *KCNJ11* [74]. Along these lines, an independent study including 1180 nondiabetic young to middle-aged participants with stage 1 hypertension, baseline coffee consumption was longitudinally associated with the risk of impaired glucose tolerance only in carriers of CYP1A2 ∗ 1F allele. Among participants homozygous for the ∗ 1A allele, which is responsible for fast caffeine metabolism, the favorable action of polyphenols or other bioactive agents balanced the genetic and metabolic risk for T2D [75]. Finally, in a prospective epidemiological study from the EPIC-InterAct cohort, including 8086 incident T2D cases in 11,035 participants over

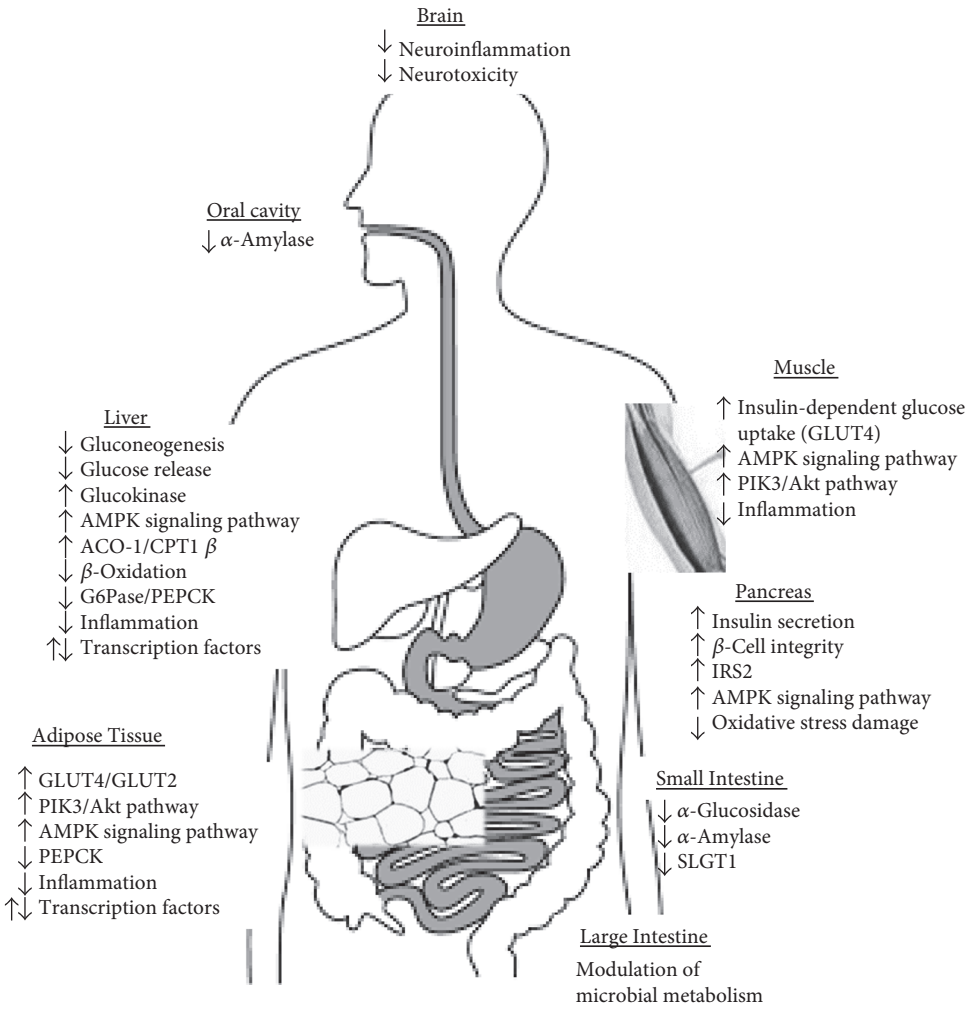


Figure 1: Relevant mechanisms linking dietary polyphenols and T2D risk. Polyphenols can exert a beneficial effect on type 2 diabetes by a number of mechanisms including (a) slowing carbohydrate digestion and glucose absorption by interacting with oral cavity and intestinal α-amylase and intestinal α-glucosidase and sodium-dependent glucose transporter (SLGT1); (b) stimulating insulin secretion in the pancreas via increasing $5'$ adenosine monophosphate-activated protein kinase (AMPK) pathway, and insulin receptor substrate (ISRS) and decreasing β-cell oxidative damage which preserves β-cell integrity; (c) modulating liver glucose release due to increase in acyl-CoA oxidase 1 (ACO-1) and carnitine palmitoyl transferase 1-β (CPT1-β) and diminishing glucose 6 phosphatase (G6Pase) and phosphoenolpyruvate carboxylase (PEPCK); and (d) activation of glucose uptake receptors in the insulin-sensitive tissue. Additionally, the modulation of microbial metabolism can synergically benefit glucose homeostasis.

12.5 years, habitual coffee consumption was associated with a 7% T2D risk reduction among carriers of the diabetes increasing risk allele at transcription factor 7-like 2 locus (TCF7L2) [76]. In addition, an interaction between an incretin-specific genetic risk score, designed to capture the genetic predisposition to defects in postprandial insulin secretion and coffee consumption on T2D risk was observed (i.e., each additional cup of coffee was associated with 5% lower T2D risk in individuals carrying high number of risk alleles). In line with previous results, adherence to the MedDiet has been reported to be able to reduce the adverse effect of the TCF7L2 polymorphism on fasting glucose and blood lipids and, importantly, on stroke incidence [77]. Unfortunately, with this type of study, we cannot differentiate the effect of polyphenols from other dietary compounds of the MedDiet.

Despite nutrigenomic evidence being still preliminary, and major challenges existing in analytical strategies and replication, the role of polyphenol-rich components on TCF7L2 locus is likely to be relevant. TCF7L2 is the strongest common genomic region associated with T2D (OR = 1.4) and encodes a transcription factor for proteins involved in the proper functioning of the Wnt signaling pathway, essential for insulin secretion and beta-cell proliferation [78, 79]. It would be rational, therefore, to envision that nutrients which tend to stimulate both insulin and incretin secretion will tend to partly compensate for any genetic defects in the incretin system and thus confer higher protection from T2D in individuals carrying the predisposing polymorphisms. Understanding gene-diet interactions in T2D holds promise to achieve the goal to develop personalized diet and lifestyle interventions based on genetic profile; nevertheless, further

research is warranted before implementing preliminary results to clinical practice is still required.

6. Biomarkers of Polyphenol Intake

Along with the development of sophisticated genomics techniques, recent advancements in analytic procedures have allowed the measurement of molecules that provide a close representation of what is encoded by the genome and modified by diet, gut microbiome, and other environmental exposures [80]. In this regard, metabolomics has greatly contributed in the identification of new biomarkers (of dietary exposure, nutritional status, and health/disease). In a prospective nested case-control study including 1107 diabetic women and 1107 control women participating in the NHS, metabolites from different classes of polyphenols were associated with T2D risk. For example, urinary excretion of enterolignans, especially enterolactone, was significantly associated with a lower T2D risk during up to 13 years of follow-up [12]. Comparing the extreme quartiles of urinary excretion of enterolactone, the relative risk of T2D was 0.62 (95% CI: 0.44, 0.88; P for trend = 0.003). In contrast, metabolites from flavanones (naringenin and hesperetin), flavonols (quercetin and isorhamnetin), and phenolic acids (caffeic acid) in spot urine samples were significantly associated with a lower T2D risk within a relatively short period of follow-up (≤4.6 years) after sample collection, but not during the entire follow-up [81]. Such a temporal pattern may be explained by the significant variability of these metabolites over time, especially when they are measured in spot urine samples [81]. In a separate analysis, Ding et al. found an inverse association between urinary excretion of daidzein and genistein, but not other isoflavone metabolites, and T2D risk in the NHS and NHSII [82]. An inverse association between isoflavone biomarkers and diabetes risk was also observed in two other prospective analyses conducted among minority populations. Among 431 Native American participating in the Strong Heart Family Study [83], Zhao et al. used an untargeted metabolomics approach to examine metabolite levels in plasma. From seven metabolites related to diabetes risk after adjustment by multiple testing, an isoflavone metabolite [(3S)-7-hydroxy-2′,3′,4′,5′,8-pentamethoxyisoflavan] was identified as being significantly associated with a lower risk [83]. Moreover, in 1391 Korean men and women, higher plasma concentrations of genistein were significantly associated with a lower T2D risk among women who were equol producers, which is a metabolite of isoflavone, but not in men or nonequol producers [84]. Although equol is biotransformed from daidzein by the metabolism caused by gut bacteria, some individuals cannot metabolize daidzein to equol. There is increasing evidence that the endocrine-related clinical efficacy of isoflavone may be modified by equol-producing status [84].

A recent prospective analysis conducted in the PREDIMED trial showed that over a 5-year follow-up, increased total polyphenol excretion was significantly associated with lower levels of triglycerides, glucose, and diastolic blood pressure among 573 participants at high cardiovascular risk [85]. In a small cross-sectional analysis conducted among 57 Japanese women, higher plasma levels of chlorogenic acid were significantly associated with lower levels of fasting blood glucose, HbA1c, and CRP [86]. In the nationwide representative National Health and Nutrition Examination Survey (NHANES), urinary excretion of enterolignans was inversely associated with CRP levels and metabolic syndrome components [87–89].

7. Metagenomics

Another layer of complexity in human nutritional studies comes from the intricate interplay between the gut microbiome and dietary intake. Obesity, insulin resistance, and T2D have been correlated with an altered gut microbiota composition. The largest metagenomics-based study to date, which includes 368 Chinese individuals, identified a moderate degree of gut bacterial dysbiosis with a decline in butyrate-producing bacteria and an increase in opportunistic bacteria among T2D patients as well as an enrichment of other microbial functions conferring sulphate reduction and oxidative stress resistance [90]. This is particularly relevant because only a limited number of bacterial species have been identified as being involved in the metabolism of polyphenols [91].

The 3-week consumption of a phenolic compounds enriched virgin olive oil, containing a mixture of olive oil and thyme phenolic compounds, decreases systemic oxidized LDL and increases bifidobacterium population and microbial metabolites of phenolic compound in faeces from hypercholesterolemic humans [92]. However, this was a small randomized controlled trial only including 12 participants. For instance, in vitro digestion of water-insoluble cocoa fractions to investigate the biotransformation of polyphenols demonstrated that bacterial fermentation of the insoluble material was associated with an increase of bifidobacteria and lactobacilli as well as butyrate production [93]. Flavanols were converted into phenolic acids by the microbiota resulting in an increasing concentration of 3-hydroxyphenylpropionic acid [93]. In a separate randomized clinical trial, these microbial changes were associated with significant reductions in plasma triacylglycerol and C-reactive proteins, suggesting the potential benefits associated with the dietary inclusion of flavanol-rich foods [94].

Undoubtedly, the interplay between gut microbiome and host and its modulation by nutrition will benefit from the integration of information from a biology-wide systems approach. Integration of gene sequence, metabolomics, and other "omics" sources will pave the way towards a better molecular understanding of the complex organisms. System-wide computational approaches will aid testing mechanistic hypothesis in silico on whole systems, such as effects of diet and modulations of metabolic diseases.

8. Conclusions

In the past decades, active strategies for the prevention of T2D focusing on healthy diets, and lifestyle in general, have become a priority for researchers and policymakers. Growing interest has particularly emerged on the beneficial effects of plant-based diets for the prevention of chronic diseases such as obesity and diabetes [4]. Polyphenols are highly

prevalent in plant-based diets, such as the MedDiet, and especially abundant in fruits, vegetables, legumes, cocoa, coffee, and red wine [7]. In addition, extra-virgin olive oil and nuts, both key components of the MedDiet, are also polyphenol-rich foods.

Given the abundant evidence from human studies regarding the intake of polyphenols, and their food sources, on related-diabetes risk factors, this narrative review has focused on well-conducted clinical trials and prospective cohort studies with special attention paid to extra-virgin olive oil, nut, and red wine consumption but also other polyphenol-rich foods. These key components of the MedDiet were inversely associated with insulin resistance and T2D risk in observational studies [46]. The intake of specific polyphenols, especially flavan-3-ols and their food sources, has demonstrated overall beneficial effects on improving insulin resistance, chronic systemic inflammation, oxidative stress, and other cardiometabolic risk factors in trials that tested effects of acute, moderate-term, or relatively long-term (up to a year) intake of flavan-3-ols [14–16]. Moreover, findings from prospective cohort studies have suggested inverse associations between the intake of total flavonoids and specific flavonoid subclasses and the risk of T2D [13, 35–37], although some controversial results still exist.

Given the chemical structures of dietary polyphenols, multiple bioactivities are displayed by interacting with molecular pathways particularly relevant for glucose homeostasis. A preliminary track of evidence for genotype-polyphenol interaction is emerging from coffee consumption and benefits on glycemia control [74]. In addition, adherence to the MedDiet, a polyphenol-rich dietary pattern, has been shown to reduce the adverse effect of the TCF7L2 polymorphism on fasting glucose [77]. The role of polyphenol-rich foods on TCF7L2 locus is therefore likely to be relevant.

The advancement in sophisticated omics methodologies has allowed the determination of molecules involved in the nutritional genomics (genetic/epigenetic, transcriptome/epigenomics, proteome, and metabolome), metagenomics, and other environmental exposures, but mainly as markers of compliance. Therefore, the integration of gene sequencing and omics techniques will lead towards a molecular understanding of the complex organisms. System-wide computational approaches will thus contribute to the study of the effects of diet on modulation of metabolic diseases by testing the hypothetical mechanisms in silico on whole systems.

In conclusion, the intake of polyphenols may be beneficial for both insulin resistance and T2D risk.

Abbreviations

CRP: C-reactive protein
HbA1c: Hemoglobin A1c
HOMA-IR: Homeostasis model assessment of insulin resistance
QUICK: Quantitative insulin sensitivity index
T2D: Type 2 diabetes.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] International Diabetes Federation, *IDF Diabetes Atlas*, International Diabetes Federation, Brussels, Belgium, 7th edition, 2015.

[2] L. Chen, D. J. Magliano, and P. Z. Zimmet, "The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives," *Nature Reviews Endocrinology*, vol. 8, pp. 228–236, 2012.

[3] U.S. Department of Agriculture and U.S. Department of Helath and Human Services, *Scientific Report of the 2015 Dietary Guidelines Advisory Committee*, US Departmentof Health and Human Services, Washington (DC), 2015.

[4] A. Satija, S. N. Bhupathiraju, E. B. Rimm et al., "Plant-based dietary patterns and incidence of type 2 diabetes in US men and women: results from three prospective cohort studies," *PLoS Medicine*, vol. 13, article e1002039, 2016.

[5] M. A. Martínez-González, J. Salas-Salvadó, R. Estruch, D. D. Corella, M. Fitó, and E. Ros, "Benefits of the Mediterranean diet: insights from the PREDIMED study," *Progress in Cardiovascular Diseases*, vol. 58, pp. 50–60, 2015.

[6] O. Rangel-Huerta, B. Pastor-Villaescusa, C. Aguilera, and A. Gil, "A systematic review of the efficacy of bioactive compounds in cardiovascular disease: phenolic compounds," *Nutrients*, vol. 7, pp. 5177–5216, 2015.

[7] K. B. Pandey and S. I. Rizvi, "Plant polyphenols as dietary antioxidants in human health and disease," *Oxidative Medicine and Cellular Longevity*, vol. 2, pp. 270–278, 2009.

[8] J. Pérez-Jiménez, V. Neveu, F. Vos, and A. Scalbert, "Identification of the 100 richest dietary sources of polyphenols: an application of the phenol-explorer database," *European Journal of Clinical Nutrition*, vol. 64, Supplement 3, pp. S112–S120, 2010.

[9] A. Scalbert, C. Manach, C. Morand, C. Rémésy, and L. Jiménez, "Dietary polyphenols and the prevention of diseases," *Critical Reviews in Food Science and Nutrition*, vol. 45, pp. 287–306, 2005.

[10] C. Manach, A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez, "Polyphenols: food sources and bioavailability," *The American Journal of Clinical Nutrition*, vol. 79, pp. 727–747, 2004.

[11] N. M. Wedick, A. Pan, A. Cassidy et al., "Dietary flavonoid intakes and risk of type 2 diabetes in US men and women," *The American Journal of Clinical Nutrition*, vol. 95, pp. 925–933, 2012.

[12] Q. Sun, N. M. Wedick, A. Pan et al., "Gut microbiota metabolites of dietary lignans and risk of type 2 diabetes: a prospective investigation in two cohorts of U.S. women," *Diabetes Care*, vol. 37, pp. 1287–1295, 2014.

[13] A. Tresserra-Rimbau, M. Guasch-Ferre, J. Salas-Salvado et al., "Intake of total polyphenols and some classes of polyphenols is inversely associated with diabetes in elderly people at high cardiovascular disease risk," *The Journal of Nutrition*, vol. 146, pp. 767–777, 2016.

[14] M. G. Shrime, S. R. Bauer, A. C. McDonald, N. H. Chowdhury, C. E. M. Coltart, and E. L. Ding, "Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies," *The Journal of Nutrition*, vol. 141, pp. 1982–1988, 2011.

[15] L. Hooper, C. Kay, A. Abdelhamid et al., "Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials," *The American Journal of Clinical Nutrition*, vol. 95, pp. 740–751, 2012.

[16] P. J. Curtis, M. Sampson, J. Potter, K. Dhatariya, P. A. Kroon, and A. Cassidy, "Chronic ingestion of flavan-3-ols and isoflavones improves insulin sensitivity and lipoprotein status and attenuates estimated 10-year CVD risk in medicated postmenopausal women with type 2 diabetes: a 1-year, double-blind, randomized, controlled trial," *Diabetes Care*, vol. 35, pp. 226–232, 2012.

[17] X.-X. Zheng, Y.-L. Xu, S.-H. Li, R. Hui, Y.-J. Wu, and X.-H. Huang, "Effects of green tea catechins with or without caffeine on glycemic control in adults: a meta-analysis of randomized controlled trials," *The American Journal of Clinical Nutrition*, vol. 97, pp. 750–762, 2013.

[18] A. J. Stull, K. C. Cash, W. D. Johnson, C. M. Champagne, and W. T. Cefalu, "Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women," *The Journal of Nutrition*, vol. 140, pp. 1764–1768, 2010.

[19] P.-W. Zhang, F.-X. Chen, D. Li, W.-H. Ling, and H.-H. Guo, "A CONSORT-compliant, randomized, double-blind, placebo-controlled pilot trial of purified anthocyanin in patients with nonalcoholic fatty liver disease," *Medicine (Baltimore)*, vol. 94, article e758, 2015.

[20] A. Basu, N. M. Betts, A. Nguyen, E. D. Newman, D. Fu, and T. J. Lyons, "Freeze-dried strawberries lower serum cholesterol and lipid peroxidation in adults with abdominal adiposity and elevated serum lipids," *The Journal of Nutrition*, vol. 144, pp. 830–837, 2014.

[21] Z.-M. Liu, Y.-M. Chen, and S. C. Ho, "Effects of soy intake on glycemic control: a meta-analysis of randomized controlled trials," *The American Journal of Clinical Nutrition*, vol. 93, pp. 1092–1101, 2011.

[22] E. Ricci, S. Cipriani, F. Chiaffarino, M. Malvezzi, and F. Parazzini, "Effects of soy isoflavones and genistein on glucose metabolism in perimenopausal and postmenopausal non-Asian women," *Menopause*, vol. 17, pp. 1080–1086, 2010.

[23] Y. Rhee and A. Brunt, "Flaxseed supplementation improved insulin resistance in obese glucose intolerant people: a randomized crossover design," *Nutrition Journal*, vol. 10, p. 44, 2011.

[24] A. Lemay, S. Dodin, N. Kadri, H. Jacques, and J.-C. Forest, "Flaxseed dietary supplement versus hormone replacement therapy in hypercholesterolemic menopausal women," *Obstetrics and Gynecology*, vol. 100, pp. 495–504, 2002.

[25] A. Pan, J. Sun, Y. Chen et al., "Effects of a flaxseed-derived lignan supplement in type 2 diabetic patients: a randomized, double-blind, cross-over trial," *PLoS One*, vol. 2, article e1148, 2007.

[26] A. Pan, W. Demark-Wahnefried, X. Ye et al., "Effects of a flaxseed-derived lignan supplement on C-reactive protein, IL-6 and retinol-binding protein 4 in type 2 diabetic patients," *The British Journal of Nutrition*, vol. 101, p. 1145, 2009.

[27] S. M. Cornish, P. D. Chilibeck, L. Paus-Jennsen et al., "A randomized controlled trial of the effects of flaxseed lignan complex on metabolic syndrome composite score and bone mineral in older adults," *Applied Physiology, Nutrition, and Metabolism*, vol. 34, pp. 89–98, 2009.

[28] A. Javidi, H. Mozaffari-Khosravi, A. Nadjarzadeh, A. Dehghani, and M. H. Eftekhari, "The effect of flaxseed powder on insulin resistance indices and blood pressure in prediabetic individuals: a randomized controlled clinical trial," *Journal of Research in Medical Sciences*, vol. 21, p. 70, 2016.

[29] S. M. Madevan der, J. Plat, and R. P. Mensink, "Resveratrol does not influence metabolic risk markers related to cardiovascular health in overweight and slightly obese subjects: a randomized, placebo-controlled crossover trial," *PLoS One*, vol. 10, article e0118393, 2015.

[30] A. E. Dijkvan, M. R. Olthof, J. C. Meeuse, E. Seebus, R. J. Heine, and R. M. Damvan, "Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance," *Diabetes Care*, vol. 32, pp. 1023–1025, 2009.

[31] V. Lecoultre, G. Carrel, L. Egli et al., "Coffee consumption attenuates short-term fructose-induced liver insulin resistance in healthy men," *The American Journal of Clinical Nutrition*, vol. 99, pp. 268–275, 2014.

[32] K. Kempf, C. Herder, I. Erlund et al., "Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial," *The American Journal of Clinical Nutrition*, vol. 91, pp. 950–957, 2010.

[33] N. M. Wedick, A. M. Brennan, Q. Sun, F. B. Hu, C. S. Mantzoros, and R. M. Damvan, "Effects of caffeinated and decaffeinated coffee on biological risk factors for type 2 diabetes: a randomized controlled trial," *Nutrition Journal*, vol. 10, p. 93, 2011.

[34] "COcoa Supplement and Multivitamin Outcomes Study (COSMOS)," https://www.clinicaltrials.gov/ct2/show/NCT02422745, 2016.

[35] Y.-J. Liu, J. Zhan, X.-L. Liu, Y. Wang, J. Ji, and Q.-Q. He, "Dietary flavonoids intake and risk of type 2 diabetes: a meta-analysis of prospective cohort studies," *Clinical Nutrition*, vol. 33, pp. 59–63, 2014.

[36] R. Zamora-Ros, N. G. Forouhi, S. J. Sharp et al., "The association between dietary flavonoid and lignan intakes and incident type 2 diabetes in European populations: the EPIC-InterAct study," *Diabetes Care*, vol. 36, pp. 3961–3970, 2013.

[37] P. F. Jacques, A. Cassidy, G. Rogers, J. J. Peterson, J. B. Meigs, and J. T. Dwyer, "Higher dietary flavonol intake is associated with lower incidence of type 2 diabetes," *The Journal of Nutrition*, vol. 143, pp. 1474–1480, 2013.

[38] P. Knekt, J. Kumpulainen, R. Järvinen et al., "Flavonoid intake and risk of chronic diseases," *The American Journal of Clinical Nutrition*, vol. 76, pp. 560–568, 2002.

[39] Y. Song, J. E. Manson, J. E. Buring, H. D. Sesso, and S. Liu, "Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis," *Journal of the American College of Nutrition*, vol. 24, pp. 376–384, 2005.

[40] M. K. Kataja-Tuomola, J. P. Kontto, S. Männistö, D. Albanes, and J. Virtamo, "Intake of antioxidants and risk of type 2 diabetes in a cohort of male smokers," *European Journal of Clinical Nutrition*, vol. 65, pp. 590–597, 2011.

[41] R. Zamora-Ros, N. G. Forouhi, S. J. Sharp et al., "Dietary intakes of individual flavanols and flavonols are inversely associated with incident type 2 diabetes in European populations," *The Journal of Nutrition*, vol. 144, pp. 335–343, 2014.

[42] M. Ding, A. Pan, J. E. Manson et al., "Consumption of soy foods and isoflavones and risk of type 2 diabetes: a pooled analysis of three US cohorts," *European Journal of Clinical Nutrition*, vol. 70, pp. 1381–1387, 2016.

[43] M. Bulló, R. Lamuela-Raventós, and J. Salas-Salvadó, "Mediterranean diet and oxidation: nuts and olive oil as important sources of fat and antioxidants," *Current Topics in Medicinal Chemistry*, vol. 11, pp. 1797–1810, 2011.

[44] A. Medina-Remón, R. Casas, A. Tressserra-Rimbau et al., "PREDIMED study investigators: polyphenol intake from a Mediterranean diet decreases inflammatory biomarkers related to atherosclerosis: a substudy of the PREDIMED trial," *British Journal of Clinical Pharmacology*, vol. 83, pp. 114–128, 2017.

[45] R. Estruch, M. A. Martínez-González, D. Corella et al., "Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial," *Annals of Internal Medicine*, vol. 145, pp. 1–11, 2006.

[46] J. Salas-Salvadó, M. Bulló, R. Estruch et al., "Prevention of diabetes with Mediterranean diets: a subgroup analysis of a randomized trial," *Annals of Internal Medicine*, vol. 160, pp. 1–10, 2014.

[47] E. Ros, "Olive oil and CVD: accruing evidence of a protective effect," *The British Journal of Nutrition*, vol. 108, pp. 1931–1933, 2012.

[48] S. Martín-Peláez, M. I. Covas, M. Fitó, A. Kušar, and I. Pravst, "Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims," *Molecular Nutrition & Food Research*, vol. 57, pp. 760–771, 2013.

[49] M. Bockde, J. G. B. Derraik, C. M. Brennan et al., "Olive (Olea europaea L.) leaf polyphenols improve insulin sensitivity in middle-aged overweight men: a randomized, placebo-controlled, crossover trial," *PLoS One*, vol. 8, article e57622, 2013.

[50] J. Wainstein, T. Ganz, M. Boaz et al., "Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats," *Journal of Medicinal Food*, vol. 15, pp. 605–610, 2012.

[51] S. Silva, M. R. Bronze, M. E. Figueira et al., "Impact of a 6-wk olive oil supplementation in healthy adults on urinary proteomic biomarkers of coronary artery disease, chronic kidney disease, and diabetes (types 1 and 2): a randomized, parallel, controlled, double-blind study," *The American Journal of Clinical Nutrition*, vol. 101, pp. 44–54, 2015.

[52] C. Santangelo, C. Filesi, R. Varì et al., "Consumption of extra-virgin olive oil rich in phenolic compounds improves metabolic control in patients with type 2 diabetes mellitus: a possible involvement of reduced levels of circulating visfatin," *Journal of Endocrinological Investigation*, vol. 39, pp. 1295–1301, 2016.

[53] A. Lasa, J. Miranda, M. Bulló et al., "Comparative effect of two Mediterranean diets versus a low-fat diet on glycaemic control in individuals with type 2 diabetes," *European Journal of Clinical Nutrition*, vol. 68, pp. 767–772, 2014.

[54] M.-I. Covas, K. Nyyssönen, H. E. Poulsen et al., "The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial," *Annals of Internal Medicine*, vol. 145, pp. 333–341, 2006.

[55] V. Konstantinidou, O. Khymenets, M.-I. Covas et al., "Time course of changes in the expression of insulin sensitivity-related genes after an acute load of virgin olive oil," *Omics*, vol. 13, pp. 431–438, 2009.

[56] P. Casas-Agustench, P. Lopez-Uriarte, M. Bullo, E. Ros, J. J. Cabre-Vila, and J. Salas-Salvado, "Effects of one serving of mixed nuts on serum lipids, insulin resistance and inflammatory markers in patients with the metabolic syndrome," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 21, pp. 126–135, 2011.

[57] C. Luo, Y. Zhang, Y. Ding et al., "Nut consumption and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a systematic review and meta-analysis," *The American Journal of Clinical Nutrition*, vol. 100, pp. 256–269, 2014.

[58] I. Kang, T. Buckner, N. F. Shay, L. Gu, and S. Chung, "Improvements in metabolic health with consumption of Ellagic acid and subsequent conversion into urolithins: evidence and mechanisms," *Advances in Nutrition: An International Review Journal*, vol. 7, pp. 961–972, 2016.

[59] X. Mora-Cubillos, S. Tulipani, M. Garcia-Aloy, M. Bulló, F. J. Tinahones, and C. Andres-Lacueva, "Plasma metabolomic biomarkers of mixed nuts exposure inversely correlate with severity of metabolic syndrome," *Molecular Nutrition & Food Research*, vol. 59, pp. 2480–2490, 2015.

[60] M. M. Marcadenti, M. Markoski, J. Garavaglia, A. Oliveira, and J. Olivaes, "Molecular properties of red wine compounds and cardiometabolic benefits," *Nutrition and Metabolic Insights*, vol. 9, p. 51, 2016.

[61] G. Chiva-Blanch, M. Urpi-Sarda, E. Ros et al., "Effects of red wine polyphenols and alcohol on glucose metabolism and the lipid profile: a randomized clinical trial," *Clinical Nutrition*, vol. 32, pp. 200–206, 2013.

[62] R. Napoli, D. Cozzolino, V. Guardasole et al., "Red wine consumption improves insulin resistance but not endothelial function in type 2 diabetic patients," *Metabolism*, vol. 54, pp. 306–313, 2005.

[63] P. Castilla, R. Echarri, A. Dávalos et al., "Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects," *The American Journal of Clinical Nutrition*, vol. 84, pp. 252–262, 2006.

[64] L. Cordain, C. L. Melby, A. E. Hamamoto et al., "Influence of moderate chronic wine consumption on insulin sensitivity and other correlates of syndrome X in moderately obese women," *Metabolism*, vol. 49, pp. 1473–1478, 2000.

[65] J. W. J. Beulens, R. M. Beersvan, R. P. Stolk, G. Schaafsma, and H. F. J. Hendriks, "The effect of moderate alcohol consumption on fat distribution and adipocytokines," *Obesity (Silver Spring)*, vol. 14, pp. 60–66, 2006.

[66] M. Hokayem, E. Blond, H. Vidal et al., "Grape polyphenols prevent fructose-induced oxidative stress and insulin resistance in first-degree relatives of type 2 diabetic patients," *Diabetes Care*, vol. 36, pp. 1454–1461, 2013.

[67] S. Timmers, E. Konings, L. Bilet et al., "Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans," *Cell Metabolism*, vol. 14, pp. 612–622, 2011.

[68] Y. Gepner, R. Golan, I. Harman-Boehm et al., "Effects of initiating moderate alcohol intake on cardiometabolic risk in adults with type 2 diabetes: a 2-year randomized, controlled trial," *Annals of Internal Medicine*, vol. 163, pp. 569–579, 2015.

[69] L. Mamluk, M. G. O'Doherty, P. Orfanos et al., "Fruit and vegetable intake and risk of incident of type 2 diabetes: results from the consortium on health and ageing network of cohorts in Europe and the United States (CHANCES)," *European Journal of Clinical Nutrition*, vol. 71, pp. 83–91, 2017.

[70] Y. Kim, J. B. Keogh, and P. M. Clifton, "Polyphenols and glycemic control," *Nutrients*, vol. 8, p. 17, 2016.

[71] K. Hanhineva, R. Törrönen, I. Bondia-Pons et al., "Impact of dietary polyphenols on carbohydrate metabolism," *International Journal of Molecular Sciences*, vol. 11, pp. 1365–1402, 2010.

[72] G. Berná, M. J. Oliveras-López, E. Jurado-Ruíz et al., "Nutrigenetics and nutrigenomics insights into diabetes etiopathogenesis," *Nutrients*, vol. 6, pp. 5338–5369, 2014.

[73] P. V. A. Babu, D. Liu, and E. R. Gilbert, "Recent advances in understanding the anti-diabetic actions of dietary flavonoids," *The Journal of Nutritional Biochemistry*, vol. 24, pp. 1777–1789, 2013.

[74] J. K. Lee, K. Kim, Y. Ahn, M. Yang, and J. E. Lee, "Habitual coffee intake, genetic polymorphisms, and type 2 diabetes," *European Journal of Endocrinology*, vol. 172, pp. 595–601, 2015.

[75] P. Palatini, E. Benetti, L. Mos et al., "Association of coffee consumption and CYP1A2 polymorphism with risk of impaired fasting glucose in hypertensive patients," *European Journal of Epidemiology*, vol. 30, pp. 209–217, 2015.

[76] InterAct Consortium, "Investigation of gene-diet interactions in the incretin system and risk of type 2 diabetes: the EPIC-InterAct study," *Diabetologia*, vol. 59, pp. 2613–2621, 2016.

[77] D. Corella, P. Carrasco, J. V. Sorli et al., "Mediterranean diet reduces the adverse effect of the TCF7L2-rs7903146 polymorphism on cardiovascular risk factors and stroke incidence: a randomized controlled trial in a high-cardiovascular-risk population," *Diabetes Care*, vol. 36, pp. 3803–3811, 2013.

[78] Grant SFA, G. Thorleifsson, I. Reynisdottir et al., "Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes," *Nature Genetics*, vol. 38, pp. 320–323, 2006.

[79] Y. Zhou, S.-Y. Park, J. Su et al., "TCF7L2 is a master regulator of insulin production and processing," *Human Molecular Genetics*, vol. 23, pp. 6419–6431, 2014.

[80] R. D. Beger, W. Dunn, M. A. Schmidt et al., "Metabolomics enables precision medicine: "a white paper, community perspective"," *Metabolomics*, vol. 12, p. 149, 2016.

[81] Q. Sun, N. M. Wedick, S. S. Tworoger et al., "Urinary excretion of select dietary polyphenol metabolites is associated with a lower risk of type 2 diabetes in proximate but not remote follow-up in a prospective investigation in 2 cohorts of US women," *The Journal of Nutrition*, vol. 145, pp. 1280–1288, 2015.

[82] M. Ding, A. A. Franke, B. A. Rosner et al., "Urinary isoflavonoids and risk of type 2 diabetes: a prospective investigation in US women," *The British Journal of Nutrition*, vol. 114, pp. 1694–1701, 2015.

[83] J. Zhao, Y. Zhu, N. Hyun et al., "Novel metabolic markers for the risk of diabetes development in American Indians," *Diabetes Care*, vol. 38, pp. 220–227, 2015.

[84] K.-P. Ko, C.-S. Kim, Y. Ahn et al., "Plasma isoflavone concentration is associated with decreased risk of type 2 diabetes in Korean women but not men: results from the Korean Genome and Epidemiology Study," *Diabetologia*, vol. 58, pp. 726–735, 2015.

[85] X. Guo, A. Tresserra-Rimbau, R. Estruch et al., "Effects of polyphenol, measured by a biomarker of total polyphenols in urine, on cardiovascular risk factors after a long-term follow-up in the PREDIMED study," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 2572606, 11 pages, 2016.

[86] A. H. Lee, L'. Tan, N. Hiramatsu et al., "Plasma concentrations of coffee polyphenols and plasma biomarkers of diabetes risk in healthy Japanese women," *Nutrition & Diabetes*, vol. 6, article e212, 2016.

[87] M. Eichholzer, A. Richard, H. L. Nicastro, E. A. Platz, J. Linseisen, and S. Rohrmann, "Urinary lignans and inflammatory markers in the US National Health and Nutrition Examination Survey (NHANES) 1999-2004 and 2005-2008," *Cancer Causes & Control*, vol. 25, pp. 395–403, 2014.

[88] T. Struja, A. Richard, J. Linseisen, M. Eichholzer, and S. Rohrmann, "The association between urinary phytoestrogen excretion and components of the metabolic syndrome in NHANES," *European Journal of Nutrition*, vol. 53, pp. 1371–1381, 2014.

[89] C. Xu, Q. Liu, Q. Zhang, A. Gu, and Z.-Y. Jiang, "Urinary enterolactone is associated with obesity and metabolic alteration in men in the US National Health and Nutrition Examination Survey 2001-10," *The British Journal of Nutrition*, vol. 113, pp. 683–690, 2015.

[90] J. Qin, Y. Li, Z. Cai et al., "A metagenome-wide association study of gut microbiota in type 2 diabetes," *Nature*, vol. 490, pp. 55–60, 2012.

[91] S. Moco, F.-P. J. Martin, and S. Rezzi, "Metabolomics view on gut microbiome modulation by polyphenol-rich foods," *Journal of Proteome Research*, vol. 11, pp. 4781–4790, 2012.

[92] S. Martín-Peláez, J. I. Mosele, N. Pizarro et al., "Effect of virgin olive oil and thyme phenolic compounds on blood lipid profile: implications of human gut microbiota," *European Journal of Nutrition*, vol. 56, pp. 119–131, 2017.

[93] V. Fogliano, M. L. Corollaro, P. Vitaglione et al., "In vitro bioaccessibility and gut biotransformation of polyphenols present in the water-insoluble cocoa fraction," *Molecular Nutrition & Food Research*, vol. 55, Supplement 1, pp. S44–S55, 2011.

[94] X. Tzounis, A. Rodriguez-Mateos, J. Vulevic, G. R. Gibson, C. Kwik-Uribe, and J. P. Spencer, "Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study," *The American Journal of Clinical Nutrition*, vol. 93, pp. 62–72, 2011.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 5982809, 11 pages
https://doi.org/10.1155/2017/5982809

Research Article

Dietary Polyphenol Intake, but Not the Dietary Total Antioxidant Capacity, Is Inversely Related to Cardiovascular Disease in Postmenopausal Polish Women: Results of WOBASZ and WOBASZ II Studies

Anna M. Witkowska,[1] Anna Waśkiewicz,[2] Małgorzata E. Zujko,[1] Danuta Szcześniewska,[2] Andrzej Pająk,[3] Urszula Stepaniak,[3] and Wojciech Drygas[2,4]

[1]*Department of Food Biotechnology, Medical University of Bialystok, Bialystok, Poland*
[2]*Department of Epidemiology, Cardiovascular Disease Prevention and Health Promotion, National Institute of Cardiology, Warsaw, Poland*
[3]*Department of Epidemiology and Population Studies, Institute of Public Health, Jagiellonian University Medical College, Krakow, Poland*
[4]*Department of Social and Preventive Medicine, Medical University of Lodz, Lodz, Poland*

Correspondence should be addressed to Anna M. Witkowska; witam@umb.edu.pl

Received 4 March 2017; Revised 4 May 2017; Accepted 22 May 2017; Published 20 June 2017

Academic Editor: Anna Tresserra

The aim of the study was to assess the relationship between the dietary polyphenol intake (DPI) and the dietary total antioxidant capacity (DTAC) and the prevalence of cardiovascular disease (CVD) in postmenopausal women. Participants were 916 postmenopausal women diagnosed with CVD and 1683 postmenopausal women without history of CVD, who took part in the population-based studies carried out in Poland: WOBASZ (2003–2005) and WOBASZ II (2013-2014). Nutritional data were collected using a single 24-hour dietary recall. DPI and DTAC in the CVD women were significantly lower and accounted for 1766.39 mg/d and 10.84 mmol/d, respectively, versus 1920.57 mg/d and 11.85 mmol/d in the women without CVD, but these differences disappeared after the standardization for energy input. Also, in the multiple-adjustment model, higher DPI, but not DTAC, was associated with the reduced odds ratio for the prevalence of CVD. Beverages, mainly coffee and tea, contributed in more than 40% to DPI and in more than a half to DTAC. In this study, higher dietary polyphenol intake, but not the dietary total antioxidant capacity, was inversely associated with CVD in postmenopausal women, which points to the health benefits of increased polyphenol intake from food sources for these women.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in postmenopausal women [1]. Unfavorable behavioral factors during the life as tobacco use, physical inactivity, unhealthy diet, and harmful use of alcohol are fundamental for CVD risk [2]. Deleterious lifestyle patterns translate to the occurrence of intermediate risk factors as raised blood glucose and lipid levels, increased blood pressure, and overweight/obesity [3]. CVD incidence increases with age, and particularly in women, which raises after menopause. Termination of the secretory ovarian function contributes to this morbidity [4]. Because life expectancy has improved and can be up to one-third after menopause and due to increased morbidity and mortality, postmenopausal women should be targeted with all sorts of activities that would reduce the risk of CVD.

At the ground of several chronic diseases that include CVD, diabetes, neurodegenerative diseases (Parkinson's disease and Alzheimer's disease), and cancer and aging is oxidative stress [5]. An excessive generation of extremely reactive

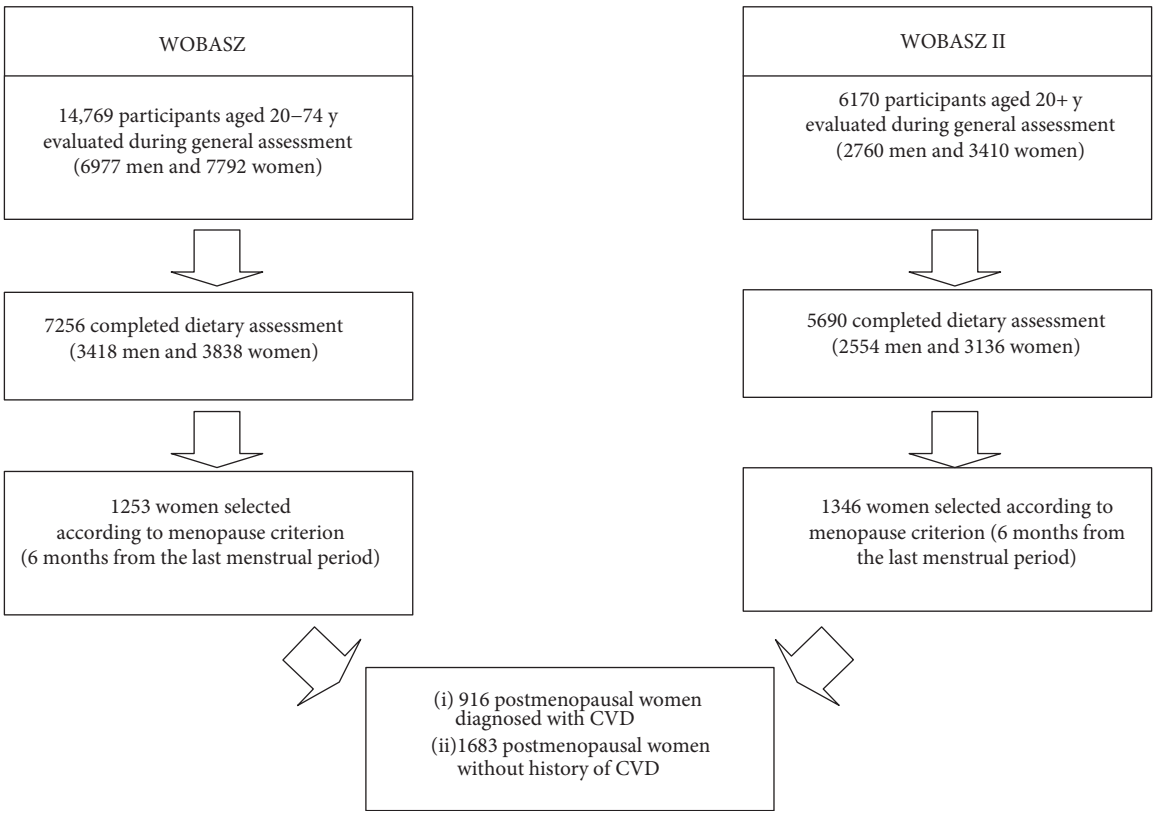


Figure 1: Flow chart of the study participants.

oxygen species (ROS) in the body leads to oxidative damage to cellular structures [6]. Some evidence suggests that mechanisms of antioxidant protection can deteriorate with age [7]. Furthermore, heart tissues of elderly people tend to be more vulnerable to oxidative stress, because of the disturbed metabolism in the mitochondria, which leads to oxidative injury [8]. Dietary antioxidants are one of the several lines of defense against cardiovascular disease, diabetes, and cancer. The most important are redox-active dietary constituents as vitamins C and E, carotenoids, and polyphenols [9].

Polyphenols, which are secondary plant metabolites, display potential health effects. The main classes of phenolic compounds include phenolic acids, flavonoids, stilbenes, and lignans [10]. Food products are generally sources of all classes of polyphenols, but they occur in different proportions. The most abundant dietary polyphenols, however, are phenolic acids and flavonoids [11].

The aim of the study was to assess the relationship between the dietary polyphenol intake (DPI) and the dietary total antioxidant capacity (DTAC) and the prevalence of CVD in postmenopausal women and to establish the main dietary sources of polyphenols and antioxidants in postmenopausal women with and without history of CVD.

2. Material and Methods

2.1. Participants. The study examined general, anthropometric, biochemical, and dietary data collected from 2599 postmenopausal women, participants of the two largest population-based cross-sectional studies carried out in Poland: Polish National Multicenter Health Examination Surveys—WOBASZ (2003–2005) and WOBASZ II (2013-2014), which were carried out by the National Institute of Cardiology, Warsaw, Poland, in collaboration with five Polish medical universities. The rationale, design, and methods of the WOBASZ and WOBASZ II studies were described in previous publications [12–14]. Women were selected for the current analysis on the basis of the occurrence of natural menopause and on the basis of completed records. Uniform criteria of six months from the termination of menstruation have been adopted in both WOBASZ studies in accordance with the study manual. The exclusion criteria were pregnancy and surgical menopause. Then, a group of 916 women with medically diagnosed cardiovascular disease (CVD) was extracted from the general study group of postmenopausal women. The remaining women without past history of CVD have served as control. The flow chart of the study participants is provided in Figure 1.

2.2. Data Collection. Data on marital status, level of education, menopause status, family history of CVD, history of myocardial infarction or stroke, medications and supplements used, leisure-time physical activity, alcohol intake, and smoking habit were collected from a self-reported standardized questionnaire designed for the WOBASZ study. Cardiovascular disease (CVD) classification was adopted in accordance with the WHO [15]. Hypertension has been recognized if systolic BP $\geq$ 140 mmHg and/or diastolic BP $\geq$ 90 mmHg and/or when antihypertensive drugs were

Table 1: General description of the studied postmenopausal women with and without CVD.

Trait	Women diagnosed with CVD $N = 916$ Mean ± SD or %	Women without CVD $N = 1683$ Mean ± SD or %	Significance level p
Age (years)	65.5 ± 9.2	60.98 ± 8.4	<0.0001
Marital status (%)			
Married	57.2	63.9	0.001
Single	42.8	36.1	
Level of education (%)			
Under middle	61.0	55.2	0.009
Middle	31.9	35.4	
Academic	7.1	9.5	
Age of natural menopause (years), mean, SD, range	49.9 ± 4.4	50.0 ± 4.1	0.875
Duration of menopause (years), mean, SD, range	15.6 ± 9.6	11.0 ± 8.6	<0.0001
Family history of CVD (%)	39.4	36.1	0.098
Diseases (%)			
Hypertension	73.1	57.3	<0.0001
Myocardial infarction	12.6	0	<0.0001
Stroke	7.9	0	<0.0001
Diabetes	19.8	13.4	<0.0001
Medication			
Menopause hormone therapy (%)	3.8	4.9	0.178
Hypotensive drugs (%)	61.0	34.9	<0.0001
Cholesterol-lowering therapy (%)	29.3	13.5	<0.0001
Antidiabetic medication or insulin (%)	15.6	8.2	<0.0001
Smoking status (%)			
Current smokers	12.5	20.9	<0.0001
Past smokers	18.6	17.7	
Never smokers	68.9	61.4	
Leisure-time physical activity (%)			
Low level	52.1	47.8	0.107
Middle level	16.5	18.4	
High level	31.3	33.8	
BMI (kg/m^2) (%)			
Underweight (BMI < 18.5)	0.3	0.7	<0.0001
Normal (BMI 18.5–24.99)	28.2	27.8	
Overweight (BMI 25–29.99)	35.2	39.2	
Obesity (BMI > 30)	44.3	32.2	
Abdominal obesity (%)	71.1	62.5	<0.0001
Supplementation with antioxidant vitamins (A, C, and E) (%)	9.9	8.9	0.391
Alcohol intake (g pure ethanol/day), mean, SD	0.7 ± 2.7	1.0 ± 2.9	<0.0001
Fasting glucose (mmol/l), mean, SD	5.7 ± 1.9	5.5 ± 1.6	0.531
Total cholesterol (mmol/l), mean, SD	5.4 ± 1.2	5.8 ± 1.3	<0.0001
LDL cholesterol (mmol/l), mean, SD	3.2 ± 1.1	3.6 ± 1.1	<0.0001
HDL cholesterol (mmol/l), mean, SD	1.4 ± 0.4	1.5 ± 0.4	<0.0001
Triglyceride (mmol/l), mean, SD	1.6 ± 1.0	1.5 ± 0.8	0.1006

used. Criteria for diabetes were glucose level ≥ 7.0 mmol/L and/or the use of glucose-lowering drugs. Two categories of the marital status were included: married and singles, while "singles" were widows, unmarried women, divorced, and separated. Education level was given in three categories: (1) under middle—no education, partial or completed education for primary level, vocational lower secondary education, and partial secondary education; (2) middle—secondary

education and partial academic education; and (3) academic education. Smoking status was assessed in three categories: current smokers, past smokers, and never smokers, depending on a habit of smoking at least one cigarette a day. Physical activity at leisure was assessed at a low level—when there was no such physical activity, for example, jogging, cycling, swimming, and gardening for at least 30 minutes a day or only occasional activity (once a week, several times a month, and several times a year); middle level—physical activity, for example, jogging, cycling, swimming, and gardening was for at least 30 minutes a day every second or third day; and high level—physical activity as given above every day or almost every day. Body measurements, such as height, body mass, and waist circumference were taken by the personnel trained in standard procedures. The body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Abdominal obesity has been recognized if waist circumference was >88 cm. Supplementation with at least one of the antioxidant vitamins (A, C, and E) was collected from the self-reported WOBASZ questionnaire. Blood pressure (BP) was measured three times on the right arm after 5 minutes of resting in a sitting position in one-minute intervals, and the final BP was calculated as an average of the second and third measurements. Biochemical analyses, as fasting glucose, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, were carried out at the Central Laboratory of the National Institute of Cardiology, Warsaw. General description of the study group, stratified by CVD status, is given in Table 1.

The WOBASZ study protocol was approved by the Bioethics Committee of the National Institute of Cardiology (WOBASZ, number 708) and (WOBASZ II, number 1344).

2.3. Dietary Assessment. Nutritional data were collected by qualified interviewers using a single 24-hour dietary recall. On the basis of the recalls, it was found that 367 dishes, food items, and beverages consumed by the participants were sources of polyphenol intakes. These products were grouped into 5 food categories: beverages (alcoholic and nonalcoholic)—coffee, tea, wine, beer, fruit drinks, and juices; cereals—bread (wheat, rye, and mixed), groats, and macaroni; fruit (fresh, frozen, dried, and jam); vegetables (fresh, frozen, pickled, and legumes); and other food products—cocoa products (chocolates—semisweet chocolate, bitter chocolate, and white chocolate; cocoa), nuts and seeds (almonds, peanut, coconut, hazelnuts, pistachio, walnuts, poppy seeds, sesame seeds, sunflower seeds, and pumpkin seeds), cookies and pastry, and other sweets (candies, etc.). The meal preparation techniques were taken into consideration as factors affecting the polyphenol contents of food items. Individual components of complex dishes have been extracted using dish recipes from the Polish Food Composition Tables [16]. These recipes give the amounts of food items required for 100 g dish portion, with consideration of yield factors. A quantitative composition of plant components of ready-to-eat foods was taken according to information given on food labels. For fruit yoghurt, for example, a typical amount of 5% respective added fruit has been taken for the calculations.

2.4. Estimation of Dietary Antioxidant Activity and Polyphenol Intake. Antioxidant activity of food products was mostly taken from our own databases of ferric-reducing antioxidant potential (FRAP) of food [17, 18], which represent mean values of antioxidant activity for 69 food products. Some missing values were complemented with other databases [19]. Dietary antioxidant activity per day as well as daily intakes of polyphenols was determined by multiplying the daily consumption of individual food items by antioxidant activity and polyphenol contents in these food items. The intake of total polyphenols was estimated using our own total polyphenol databases [17, 18] as well as the online Phenol-Explorer database [20]. For the calculation of dietary polyphenol intake (DPI) and the dietary total antioxidant capacity (DTAC) by different databases, the same food items and the same culinary techniques were taken into account.

2.5. Statistical Analyses. Descriptive data for the study population characteristics of women diagnosed with CVD and of women without history of CVD were calculated as means and SDs for the continuous variables and as percentages for the categorical variables. For comparison purposes of dietary intakes, Student's *t*-test was used. DTAC and DPI are given unadjusted and adjusted to 1000 kcal to correct for total energy intake. Additionally, all postmenopausal women were grouped into quartiles based on DTAC and DPI total values. Percentages of women diagnosed with CVD were calculated across quartiles of DTAC and DPI. For nonnormally distributed data, such as contributions of food categories to DTAC and DPI, Kruskal-Wallis test was used. Odds ratios (ORs) with the corresponding 95% confidence intervals (CIs) for CVD by DTAC and DPI, unadjusted and adjusted for age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy, were calculated using logistic regression. Due to a wide span between the lowest and the highest value of polyphenol intake, OR for DPI was calculated by 100 units (100 mg/d). All tests of statistical significance were two-sided. The SAS software version 9.2 (SAS Institute Inc., Cary, NC) was used for all the statistical calculations.

3. Results

Table 2 displays the mean dietary intakes in the studied postmenopausal women, stratified by the CVD status. It was found that CVD women had statistically lower energy input as well as essential nutrient intakes (protein, carbohydrates, total fat, saturated fat, and cholesterol) compared to the women without CVD. Energy from food was lower in CVD women approximately by 8%, protein by 6%, carbohydrate by 7%, total fat and saturated fat by 11%, and cholesterol by 11.5%. Table 2 also provides the consumption of the most important groups of food products that contain polyphenols, which are vegetables, fruit, tea, and coffee [11]. There were no significant differences in the intake of vegetables, fruits, and tea between the groups of women. However, it was found that women with CVD consumed significantly less coffee. DPI

Table 2: Mean dietary intakes in the postmenopausal women by prevalence of CVD.

Consumption	Women diagnosed with CVD $N = 916$ Mean ± SD	Women without CVD $N = 1683$ Mean ± SD	p
Energy from food (kcal/d), mean, SD	1516.9 ± 579.5	1652.9 ± 628.1	<0.0001
Protein (g/d), mean, SD	55.6 ± 22.7	58.9 ± 23.6	<0.0001
Carbohydrate (g/d), mean, SD	209.1 ± 86.2	224.3 ± 90.1	<0.0001
Total fat (g/d), mean, SD	57.7 ± 28.7	64.9 ± 31.9	<0.0001
Saturated fat (g/d), mean, SD	21.7 ± 11.9	24.4 ± 13.0	<0.0001
Cholesterol (mg/d), mean, SD	207.1 ± 140.0	234.2 ± 153.7	<0.0001
Vegetables (g/d)	215.0 ± 156.0	214.0 ± 155.0	0.850
Fruits (g/d)	214.0 ± 226.0	216.0 ± 227.0	0.989
Tea (g/d)	360.9 ± 251.7	348.1 ± 250.6	0.180
Coffee (g/d)	129.6 ± 165.5	175.3 ± 171.9	<0.0001
DPI (mg/d/1000 kcal)	1243.0 ± 654.0	1236.0 ± 528.0	0.493
DTAC (mmol/d/1000 kcal)	7.7 ± 5.6	7.7 ± 4.6	0.099

and DTAC, standardized for 1000 kcal of energy, were not significantly different.

Taking into account the raw data, the variables tested in this study, such as DPI and DTAC, were associated with reduced odds of CVD in postmenopausal women (Table 3). Analysis of this data indicates that the women with higher DPI (per 100 units = 100 mg/d) and higher DTAC had 2.3% reduced odds of CVD. Adjustments for multiple variables such as age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy, however, reduced the odds ratio of CVD to 1.1% in the case of DPI, while DTAC was no longer associated with the reduced odds of CVD.

Table 4 shows the percentages of the women with CVD in the individual quartiles of DTAC and DPI, unadjusted as well as adjusted for age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy. The mean values for the lowest versus highest quartiles were as follows: for DPI, it was 948 mg/d versus 2975.8 mg/d and for DTAC, it was 5.05 mmol/d versus 19.87 mmol/l. In the case of both DPI and DTAC, the increasing percent of CVD women within the decreasing DPI and DTAC quartiles was observed. Given the unadjusted data, the least proportion of the women with CVD (29.54%) was observed in the highest quartile of DPI (14.28–191.82 mmol/d) and the largest proportion of the women with CVD (42.06%) was in the lowest quartile of DTAC (range 0.22–7.18 mmol/d). In the case of unadjusted data concerning DPI, the largest percentage of the women with CVD (42.84%) was found in the lowest quartile of DPI (range 1120–1263 mg/d) and the least proportion of the women with CVD (28.15%) was found in the highest quartile of DPI (2303–8793 mg/d). Also in this case, a significant decreasing trend toward the lower percentage of CVD women within the individual increasing DPI quartiles was observed, which was still significant even after the adjustment for age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy. The percentage of CVD women within the individual DTAC quartiles was gradually decreasing. This trend was statistically significant. However, in a multiple-adjustment model, this tendency was no longer significant for DTAC.

Table 3: Odds ratios (ORs) and 95% confidence intervals (CIs) for prevalence of CVD by dietary total antioxidant capacity (continuous) and dietary polyphenol intake (per 100 mg/d).

Variables	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)
DPI (mg/d)	0.977 (0.968; 0.987)	0.989 (0.978; 0.999)
DTAC (mmol/d)	0.977 (0.964; 0.990)	0.992 (0.979; 1.005)

[a]Unadjusted. [b]Adjusted for age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy.

Table 5 shows the contributions of individual food categories to the DPI value in the women with and without CVD. The total DPIs in the individual groups were 1766.39 mg/d in the CVD women and 1920.57 mg/d in the women without CVD. These values were statistically significant at $p < 0.0001$. The total DPIs in both study groups were mostly affected by the consumption of beverages, which contributed to the total DPI in 41.81% in the case of the CVD women and in 44.19% in the women without CVD. These differences were statistically significant at $p < 0.001$. In the both groups of women, the coffee and tea consumption accounted for about 98% of the beverages' DPI value. The remaining food categories contributed less to the total DPI value. They were listed according to the decreasing impact: fruits (26.31% in the CVD women and 24.05% in the women without CVD), cereals (14.10% and 13.34%, resp.), vegetables (12.12% and 11.88%, resp.), and other food categories (5.66% and 6.54%, resp.). DPI values from vegetables ($p = 0.044$) and other foods ($p = 0.0002$) were significantly lower in the women diagnosed with CVD. Taking into account the individual food products, given in a descending order, consumption of tea (21.34%), coffee (19.56%), apples (12.79%), potato (5.51%), and mixed bread (4.87%) had the largest impact on DPI in CVD women, as measured by the

Table 4: Percentages of CVD women, odds ratios (ORs), and 95% confidence intervals (CIs) for prevalence of CVD by quartiles of DTAC and DPI.

Parameters		Quartile 1	Quartile 2	Quartile 3	Quartile 4	p
DPI (mg/d)	Mean ± SD	948.2 ± 236	1523.2 ± 142	2016.3 ± 154	2975.8 ± 724	
	Range	(1120–1263)	(1264–1759)	(1760–2302)	(2303–8793)	
	% CVD[a]	42.84	36.00	34.00	28.15	<0.0001
	% CVD[b]	40.60	35.04	34.50	31.80	0.011
DTAC (mmol/d)	Mean ± SD	5.05 ± 1.50	8.84 ± 0.96	12.20 ± 1.05	19.87 ± 9.74	
	Range	(0.22–7.18)	(7.19–10.48)	(10.49–14.27)	(14.28–191.82)	
	% CVD[a]	42.06	36.31	33.08	29.54	<0.0001
	% CVD[b]	39.72	34.87	34.12	33.17	0.073

[a]Unadjusted. [b]Adjusted for age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy.

Table 5: Contributions of food categories to DPI in the women with and without CVD.

Food categories		Women with CVD $N = 916$	Women without CVD $N = 1683$	p value
Beverages	mg/d (mean ± SD)	738.47 ± 471	848.61 ± 492	<0.001
	Contribution to DTAC (%)	41.81	44.19	
	Major sources (% contribution)*	Tea (21.34), coffee (19.56)	Coffee (24.34), tea (18.94)	
Cereals	mg/d (mean ± SD)	249.01 ± 150	256.17 ± 157	0.188
	Contribution to DTAC (%)	14.10	13.34	
	Major sources (% contribution)*	Mixed bread (4.87), rye bread (3.36), wheat bread (3.20)	Mixed bread (5.59), pastry (2.83), rye bread (2.91)	
Fruit	mg/d (mean ± SD)	464.72 ± 519	461.86 ± 499	0.735
	Contribution to DTAC (%)	26.31	24.05	
	Major sources (% contribution)*	Apples (12.79), plums (3.61), strawberries (2.25)	Apples (12.33), plums (2.75), strawberries (2.12)	
Vegetables	mg/d (mean ± SD)	214.09 ± 147	228.23 ± 156	0.044
	Contribution to DTAC (%)	12.12	11.88	
	Major sources (% contribution)*	Potato (5.51), tomato (1.49), cabbage (1.39)	Potato (5.56), cabbage (1.33), tomato (1.30)	
Other food categories	mg/d (mean ± SD)	100.10 ± 199	125.70 ± 208	0.0002
	Contribution to DTAC (%)	5.66	6.54	
	Major sources (% contribution)*	Cookies and pastry (2.50), cocoa products (2.08), nuts and seeds (0.81)	Cookies and pastry (2.83), cocoa products (2.53), nuts and seeds (0.86)	
Total	mg/d (mean ± SD)	1766.39 ± 865	1920.57 ± 825	<0.0001
	Contribution to DTAC (%)	100	100	

*In each food category, individual food products with the strongest impact on the DPI were only listed.

percentage of the DPI value. Similarly, among women without CVD, the most important foods that influenced DPI were also (in a descending order) coffee (24.34%), tea (18.94%), apples (12.33%), mixed bread (5.59%), and potato (5.56%).

The total and the partial (for each food category) DTAC values for CVD women and the women without CVD are given in Table 6. The total DTAC of 10.84 mmol/d in the women diagnosed with CVD was significantly lower in comparison to that of 11.85 mmol/d in the women without CVD. Beverages were the main food category which contributed in more than a half to the DTAC total value: 53.87% and 57.13% in the women diagnosed with CVD and in the women without CVD, respectively. These differences were statistically significant at $p < 0.001$. Among the beverages, which included alcoholic and nonalcoholic drinks, the consumption of coffee and tea affected the DTAC in the respective groups of women (with

Table 6: Contributions of food categories to DTAC in the women with and without CVD and major dietary sources that impact DTAC within each food category.

Food categories		Women with CVD $N = 916$	Women without CVD $N = 1683$	p value
Beverages	mmol/d (mean ± SD)	5.84 ± 3.84	6.77 ± 4.03	<0.001
	Contribution to DTAC (%)	53.87	57.13	
	Major sources (% contribution)*	Coffee (26.57), tea (26.11)	Coffee (32.83), tea (23.04)	
Cereals	mmol/d (mean ± SD)	0.38 ± 0.29	0.39 ± 0.27	
	Contribution to DTAC (%)	3.51	3.29	0.206
	Major sources (% contribution)*	Mixed bread (1.11), rye bread (0.83), wheat bread (0.74)	Mixed bread (1.27), rye bread (0.68), wheat bread (0.59)	
Fruit	mmol/d (mean ± SD)	2.19 ± 3.48	2.11 ± 3.05	
	Contribution to DTAC (%)	20.20	17.81	0.427
	Major sources (% contribution)*	Apples (5.81), strawberries (3.69), plums (2.03), grapes (1.94)	Apples (5.57), strawberries (3.38), plums (1.6), grapes (1.43)	
Vegetables	mmol/d (mean ± SD)	1.67 ± 1.58	1.77 ± 1.62	
	Contribution to DTAC (%)	15.41	14.94	0.124
	Major sources (% contribution)*	Potato (5.63), beetroot (2.77), cabbage (1.85), tomato (1.11), broccoli and cauliflower (0.92)	Potato (5.65), beetroot (2.70), cabbage (1.94), broccoli and cauliflower (1.01), tomato (0.93)	
Other foods	mmol/d (mean ± SD)	0.76 ± 5.82	0.81 ± 3.46	
	Contribution to DTAC (%)	7.01	6.83	<0.0001
	Major sources (% contribution)*	Nuts and seeds (3.41), cocoa products (1.85), cookies and pastry (0.73)	Nuts and seeds (2.87), cookies and pastry (2.87), cocoa products (1.94)	
Total	mmol/d (mean ± SD)	10.84 ± 8.53	11.85 ± 6.66	<0.0001
	Contribution to DTAC (%)	100	100	

*In each category of food products, only those that had the greatest impact on the DTAC were listed.

CVD and without CVD) in the same extent of 97.8%. The other food categories ranked in terms of impact on the DTAC in the women with CVD versus the women without CVD were fruits, vegetables, other food categories, and cereals. DTAC from the foods grouped in the category of other foods was significantly lower in the CVD women ($p < 0.0001$). It was found that within each of the categories of food, similar foods affected partial DTACs in the both groups of women. For example, apples, strawberries, plums, and grapes delivered most of the DTAC in the fruit food category both in the women with CVD and in the women without CVD. Similar observations were made for cereals, vegetables, and other food categories. With regard to individual food products, a consumption of coffee (26.57%), tea (26.11%), apples (5.81%), potato (5.63%), strawberries (3.69%), and nuts and seeds (3.41%) had the greatest impact on the DTAC in CVD women. Among the women without CVD, the food products, which in the largest percentage influenced on DTAC, were coffee (32.83%), tea (23.04%), potato (5.65%), apples (5.57%), strawberries (3.38%), and nuts and seeds (2.87%).

4. Discussion

The results of the present study indicated that polyphenol intake and the antioxidant activity in postmenopausal CVD women were significantly lower and accounted for 1766.39 mg/d and 10.84 mmol/d, respectively, versus 1920.57 mg/d and 11.85 mmol/d in the women without CVD. However, lower energy intake among women with CVD compared to the women without history of CVD led us to make adjustments for energy. After the adjustment to 1000 kcal, both DPI and DTAC did not differ between the groups. Interestingly, in contrast to the current research, in an earlier analysis based on the WOBASZ (2003–2005) study

concerning CVD women of different ages, an increase in dietary antioxidant activity and a greater consumption of polyphenols than those in the healthy women were observed, which was explained by healthier dietary choices by CVD women [21]. In contrast, the present study shows dietary modifications made by postmenopausal CVD women in terms of reduced calorie and coffee intakes, but the consumption of vegetables and fruits was at a similar level. These modifications were reflected in the DPI and DTAC values. At the same time, the study indicates (after the adjustment of model for multiple variables as age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy) that there was a small reduction observed with regard to DPI, but no decline in the odds of CVD in the women according to DTAC. In addition, it was found that coffee and tea were the main sources of DPI and DTAC in the studied postmenopausal women, which is consistent with other observations in Polish urban population [22].

Senescence is characterized by increased rates of hypertension, diabetes, CVD, and cancer [23]. Several studies found an inverse relationship between dietary total antioxidant capacity (DTAC) and the risk of chronic diseases in aging people. A meta-analysis of those data concluded that DTAC has a great potential for clinical applications and public health [24]. Previously, we found a diminished DTAC value in an elderly population, and particularly in the elderly women, compared to that in the young and middle-aged subjects [25]. DTAC, among other things, corresponds with polyphenol content in the diet. It has not been established, however, whether the effect of polyphenols on cardiovascular health is directly related to their antioxidant activity [26]. Several other mechanisms of polyphenol action in humans, which are not related to direct antioxidant activity, have been suggested, including microbiota transformation or the enhancement of internal mechanisms of antioxidant protection [27, 28]. Regardless of the mechanisms of action, beneficial polyphenol effects on the heart health were observed. In the PREDIMED study, a 46% reduction in the risk of CVD during 4.3 years of a follow-up period was observed, comparing Q5 with Q1 of total polyphenol intake [29]. Recently, we found that the existing databases of dietary polyphenols are partially incomplete and should be further expanded to better reflect the dietary polyphenol contents in foods [11]. Therefore, on the basis of our own dietary database [17, 18] as well as of other available published databases [20] and taking into account the amounts of individual food consumed, we calculated dietary polyphenol intakes (DPI) for postmenopausal women with CVD and the non-CVD women. Likewise, dietary total antioxidant capacity (DTAC) was calculated using our own [17, 18] and other commonly accessible databases [19], which are based on the FRAP (ferric-reducing antioxidant potential) assay. At this point, it should be mentioned that the research methodology of dietary TAC is varied. The FRAP assay is the most widely used experimental method to assess TAC in foods, although several methods have been developed for measuring total antioxidant capacity [27]. Several studies demonstrate that higher TAC from diet and supplements beneficially alters lipid and glucose profiles [30, 31], improves endothelial function [32], and reduces systemic inflammation [33]. Recent clinical studies have shown that higher TAC from diet and supplements can be associated with a decrease in inflammatory markers in overweight/obese postmenopausal women [34]. Supplements in this study largely contributed to the TAC value. Conversely, reduced rates of CVD were not observed for a single antioxidant vitamin supplement (beta-carotene, vitamin C, and vitamin E) users in randomized controlled trials [35, 36]. In contrast to this research, supplementation in our study was not calculated, with reference to the purpose of the study, which was to demonstrate a dietary impact on TAC. In addition to this, in various studies, DTAC has been calculated with different methods. DTAC in our own study was determined by using the experimental FRAP method that measures combined TACs of food samples [27]. It is common to use the FRAP assay in order to assess the relationship between the dietary TAC and the occurrence of diseases. In several studies, a negative association between the dietary TAC and the occurrence of cancers has been found [37, 38], although not all studies support the existence of such dependency [39]. Another approach is to determine DTAC on the basis of theoretical calculations of individual antioxidants (polyphenols, carotenoids, and vitamins C and E) in food products. In our study, however, TAC value of individual food products results from a complex interaction of many of the components included in one food sample. We found, however, that diets with higher TAC, after adjustment for potential confounders for CVD in postmenopausal women such as age, smoking, alcohol intake, education, physical activity, and menopause hormone therapy, in contrast to DPI, were not associated with the reduced odds of CVD. Possibly, the content of antioxidants in the diet is insufficient to significantly affect the level of antioxidants in the human body. Generally, despite polyphenol dietary abundance, their bioavailability is rather low [26]. Therefore, other mechanisms for their actions are suggested, as it was mentioned before. In addition, bioavailability of some other food ingredients, such as carotenoids, for example, can be negatively affected by the digestion and absorption process [40].

Quartile analysis provides more information on the relationship between DTAC and DPI and the prevalence of CVD. Despite that DTAC in the upper quartile is almost four times higher than that in the bottom quartile, the number of women with CVD in the upper quartile is only about 17% lower in the model of multiple adjustments than that in the bottom quartile, and this trend is not significant. A similar situation applies to DPI, which in the upper quartile is three times higher than that in the lower quartile, while the number of women with CVD in the upper quartile is only ~22% lower than that in the bottom quartile. However, in the case of DPI, this trend is statistically significant, showing that the total polyphenol intake may affect the incidence of CVD only to a small extent, while other factors such as lifestyle-related factors, may be relevant to CVD risk in postmenopausal women. It is also possible that only an in-depth analysis of individual polyphenols could give the answer to the argument raised in this paper, but this requires further analysis.

By comparing the composition of the diets in the both groups of women, we found that the supply of energy, protein, carbohydrates, and fats was significantly lower in the women with CVD. Lower energy and nutrient intakes might have resulted somewhat from both an older age of the CVD women and possibly due to the nutritional restrictions because of the increased body mass as well as larger prevalence of diabetes in the group of CVD women. CVD women in this study were characterized by a higher proportion of obesity cases [44.3%) and diabetes (19.8%) compared to non-CVD women (32.2% and 13.4%, resp.). The prevalence of overweight and obesity is common in postmenopausal women in the Polish population [41], and an excessive body weight is associated with oxidative stress [42]. We also found that despite the lower energy and nutrient intakes from the diet, the supply of vegetables and fruits in the postmenopausal CVD women remained similar to that in the women without CVD. Vegetables and fruit are together a group of food that was consumed in the highest amounts (together, they represent the amount of 430 g/d). They were one of the main sources of antioxidants and polyphenols in the both groups of women. Also, the structure of food consumed by the participants was similar in the respective groups of women. The main beverages in either case were coffee and tea. The tea intake did not differ significantly between the two groups of women, while the consumption of coffee was significantly lower among CVD women. Some alcoholic beverages, such as wine and beer, which normally are polyphenolic supplies for the general population, had no impact on the DPI and DTAC values in the studied group of postmenopausal women.

The results concerning the intake of coffee and the risk of CVD are conflicting. One of the most important risk factors of CVD is high blood pressure. In hypertensive elderly populations, a habitual coffee drinking may lead to uncontrolled blood pressure (BP) [43]. However, several cohort studies and a meta-analysis do not confirm an association between longer-term coffee consumption and increased BP and also between habitual coffee consumption and the increased risk of CVD in hypertensive subjects [44]. In other study in Poland, it was indicated that the risk of hypertension was lower in persons consuming moderate amount of coffee (3-4 cups/day) and higher intake of coffee was not protective [45]. A literature search shows that moderate intake of coffee appears to be protective against CVD [46–49] and coffee consumption can confer benefits, particularly for postmenopausal women, by reducing inflammation and the risk of CVD [50]. Recent studies demonstrate that drinking 1-2 cups of coffee per day in women reduces the risk of mortality due to CVD, and this effect was observed in nonsmokers only [46]. Interestingly, DPI and DTAC from the remaining food categories did not differ in principle, with the exception of the category of other foods (for DPI and DTAC) and vegetables (for DPI). But overall, the beverages had a determining influence on the total values of the DPI and DTAC. In addition, the analysis of the structure of the DTAC and DPI in various food categories indicates similar selection of food products in the both groups of women (with CVD and without CVD).

This study has its strengths and limitations. The advantage of this population-based study is a large group of postmenopausal women; however, the participation rate in the WOBASZ II study was rather low. Also, this study provides data regarding the relationship between DTAC and the prevalence of CVD in postmenopausal women, which was not so far studied. Among the limitations, first is the cross-sectional nature of the study which does not allow to present time sequence between the disease and exposure. The results may be biased by the reverse causality; persons after the diagnosis of CVD could have been advised to change the diet and to decrease the consumption of coffee. Next, a single 24-hour recall does not reflect the long-term consumption. Therefore, small dietary variations of the examined women might have affected the results obtained. However, there is no satisfactory tool to evaluate food consumption, and each of these methods has its strengths and weaknesses [51]. The single 24-hour recall method was used in WOBASZ and WOBASZ II studies, because in contrast to other methods, it is simple, inexpensive, and relatively brief, with less potential to interfere with respondent's dietary behavior and usually with a better response rate which translates into increased representativeness of the population. For these reasons, it is often used in epidemiological studies. Possibly, the use of multiday recalls would be more beneficial, because they give better estimates of the population's dietary intake [51], but there is more burden on the respondents. Another alternative for the 24-hour recall is a food frequency questionnaire. This latter, however, does not measure many details of dietary intake, which is essential for the good interpretation of dietary intake of polyphenols, which are present in a variety of foods. For the above reasons, a single 24-hour recall method was selected.

5. Conclusion

In this study, higher dietary polyphenol intake, but not the dietary total antioxidant capacity, was found to be inversely associated with CVD in postmenopausal women, which points to the health benefits of increased polyphenol intake from food sources for these women.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

This study was supported by the National Institute of Cardiology (Grant no. 2.17/I/16) and by the Medical University of Bialystok (Grant no. N/ST/ZB/16/001/3317).

References

[1] P. Collins, C. M. Webb, T. J. de Villiers, J. C. Stevenson, N. Panay, and R. J. Baber, "Cardiovascular risk assessment in women - an update," *Climacteric*, vol. 19, no. 4, pp. 329–336, 2016.

[2] I. Tzoulaki, P. Elliott, V. Kontis, and M. Ezzati, "Worldwide exposures to cardiovascular risk factors and associated health effects: current knowledge and data gaps," *Circulation*, vol. 133, no. 23, pp. 2314–2333, 2016.

[3] R. H. Eckel, J. M. Jakicic, J. D. Ard et al., "2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines," *Circulation*, vol. 129, no. 25 Supplement 2, pp. S76–S99, 2014.

[4] S. H. Kim, M. Y. Sim, and S. B. Park, "Association between duration of reproductive lifespan and Framingham risk score in postmenopausal women," *Maturitas*, vol. 82, no. 4, pp. 431–435, 2015.

[5] T. Rahman, I. Hosen, M. M. T. Islam, and H. U. Shekhar, "Oxidative stress and human health," *Advances in Bioscience and Biotechnology*, vol. 3, no. 7A, pp. 997–1019, 2012.

[6] E. Birben, U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci, "Oxidative stress and antioxidant defense," *World Allergy Organization Journal*, vol. 5, no. 1, pp. 9–19, 2012.

[7] D. Pastori, P. Pignatelli, A. Farcomeni et al., "Aging-related decline of glutathione peroxidase 3 and risk of cardiovascular events in patients with atrial fibrillation," *Journal of American Heart Association*, vol. 5, no. 9, article e003682, 2016.

[8] E. J. Lesnefsky, Q. Chen, and C. L. Hoppel, "Mitochondrial metabolism in aging heart," *Circulation Research*, vol. 118, no. 10, pp. 1593–1611, 2016.

[9] G. Bjørklund and S. Chirumbolo, "Role of oxidative stress and antioxidants in daily nutrition and human health," *Nutrition*, vol. 33, pp. 311–321, 2017.

[10] K. B. Pandey and S. I. Rizvi, "Plant polyphenols as dietary antioxidants in human health and disease," *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 5, pp. 270–278, 2009.

[11] A. M. Witkowska, M. E. Zujko, A. Waśkiewicz, K. M. Terlikowska, and W. Piotrowski, "Comparison of various databases for estimation of dietary polyphenol intake in the population of Polish adults," *Nutrients*, vol. 7, no. 11, pp. 9299–9308, 2015.

[12] G. Broda and S. Rywik, "Multicenter national Polish population health status tests – WOBASZ project with defined problems and treatment goals," *Kardiologia Polska*, vol. 63, no. 6, supplement 4, pp. S601–S604, 2005.

[13] S. Rywik, W. Kupść, W. Piotrowski et al., "Multicenter national Polish population health status tests – WOBASZ project. Establishment of methods and logistics," *Kardiologia Polska*, vol. 63, no. 6, supplement 4, pp. 605–613, 2005.

[14] W. Drygas, A. A. Niklas, A. Piwońska et al., "Multi-center National Population Health Examination Survey (WOBASZ II study): assumptions, methods and implementation," *Kardiologia Polska*, vol. 74, no. 7, pp. 681–690, 2016.

[15] S. Mendis, P. Puska, and B. Norrving, "World Health Organization (2011). Global atlas on cardiovascular disease prevention and control (PDF)," in *World Health Organization in Collaboration with the World Heart Federation and the World Stroke Organization*, pp. 3–18, World Health Organization, Geneva, 2011.

[16] H. Kunachowicz, I. Nadolna, B. Przygoda, and K. Iwanow, *Food Composition Tables*, PZWL, Warsaw, 2005.

[17] M. E. Zujko and A. M. Witkowska, "Antioxidant potential and polyphenol content of selected food," *International Journal of Food Properties*, vol. 14, no. 2, pp. 300–308, 2011.

[18] M. E. Zujko and A. M. Witkowska, "Antioxidant potential and polyphenol content of beverages, chocolates, nuts, and seeds," *International Journal of Food Properties*, vol. 17, no. 1, pp. 86–92, 2014.

[19] M. H. Carlsen, B. L. Halvorsen, K. Holte et al., "The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide," *Nutrition Journal*, vol. 9, p. 3, 2010.

[20] V. Neveu, J. Perez-Jiménez, and F. Vos, "Phenol-Explorer: an online comprehensive database on polyphenol contents in foods," *Database: The Journal of Biological Databases and Curation*, vol. 2010, 2010. bap024.

[21] M. E. Zujko, A. M. Witkowska, A. Waśkiewicz, W. Piotrowski, and K. M. Terlikowska, "Dietary antioxidant capacity of the patients with cardiovascular disease in a cross-sectional study," *Nutrition Journal*, vol. 14, no. 1, p. 26, 2015.

[22] G. Grosso, U. Stepaniak, A. Micek, D. Stefler, M. Bobak, and A. Pająk, "Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study," *European Journal of Nutrition*, vol. 25, pp. 1-2, 2016.

[23] B. G. Childs, M. Durik, D. J. Baker, and J. M. van Deursen, "Cellular senescence in aging and age-related disease: from mechanisms to therapy," *Nature Medicine*, vol. 21, no. 12, pp. 1424–1435, 2015.

[24] M. A. Nascimento-Souza, P. G. Paiva, H. S. Martino, and A. Q. Ribeiro, "Dietary total antioxidant capacity as a tool in health outcomes in middle-aged and older adults: a systematic review," *Critical Reviews in Food Science and Nutrition*, vol. 19, no. article 27646047, 2016.

[25] M. E. Zujko, A. M. Witkowska, A. Waśkiewicz, and I. Mirończuk-Chodakowska, "Dietary antioxidant and flavonoid intakes are reduced in the elderly," *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 843173, 8 pages, 2015.

[26] P. C. Hollman, A. Cassidy, B. Comte et al., "The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established," *Journal of Nutrition*, vol. 141, no. 5, pp. 989S–1009S, 2011.

[27] I. F. F. Benzie and S. W. Choi, "Chapter one – antioxidants in food: content, measurement, significance, action, cautions, caveats, and research needs," *Advances in Food and Nutrition Research*, vol. 71, pp. 1–53, 2014.

[28] H. Sies, "Total antioxidant capacity: appraisal of a concept," *Journal of Nutrition*, vol. 137, no. 6, pp. 1493–1495, 2007.

[29] A. Tresserra-Rimbau, E. B. Rimm, A. Medina-Remón et al., "Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 24, no. 6, pp. 639–647, 2014.

[30] K. Kim, T. M. Vance, and O. K. Chun, "Greater total antioxidant capacity from diet and supplements is associated with a less atherogenic blood profile in U.S. adults," *Nutrients*, vol. 8, no. 1, p. 15, 2016.

[31] H. H. Hermsdorff, B. Puchau, A. C. Volp et al., "Dietary total antioxidant capacity is inversely related to central adiposity as well as to metabolic and oxidative stress markers in healthy young adults," *Nutrition & Metabolism*, vol. 8, no. 59, 2011.

[32] L. Franzini, D. Ardigo, S. Valtuena et al., "Food selection based on high total antioxidant capacity improves endothelial function in a low cardiovascular risk population,"

Nutrition Metabolism and Cardiovascular Diseases, vol. 22, no. 1, pp. 50–57, 2012.

[33] S. Valtuena, N. Pellegrini, L. Franzini et al., "Food selection based on total antioxidant capacity can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress," *American Journal of Clinical Nutrition*, vol. 87, no. 5, pp. 1290–1297, 2008.

[34] Y. Wang, M. Yang, S. G. Lee et al., "Diets high in total antioxidant capacity improve risk biomarkers of cardiovascular disease: a 9-month observational study among overweight/obese postmenopausal women," *European Journal of Nutrition*, vol. 53, no. 6, pp. 1363–1369, 2014.

[35] C. H. Hennekens, J. E. Buring, J. E. Manson et al., "Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease," *The New England Journal of Medicine*, vol. 334, no. 18, pp. 1145–1149, 1996.

[36] N. R. Cook, C. M. Albert, J. M. Gaziano et al., "A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study," *Archives of Internal Medicine*, vol. 167, no. 15, pp. 1610–1618, 2007.

[37] A. Pantavos, R. Ruiter, E. F. Feskens et al., "Total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk: the Rotterdam Study," *International Journal of Cancer*, vol. 136, no. 9, pp. 2178–2186, 2015.

[38] A. L. Lucas, C. Bosetti, P. Boffetta et al., "Dietary total antioxidant capacity and pancreatic cancer risk: an Italian case-control study," *British Journal of Cancer*, vol. 115, no. 1, pp. 102–107, 2016.

[39] K. M. Russnes, E. Möller, K. M. Wilson et al., "Total antioxidant intake and prostate cancer in the Cancer of the Prostate in Sweden (CAPS) study. A case control study," *BMC Cancer*, vol. 16, p. 438, 2016.

[40] R. E. Kopec, B. Gleize, P. Borel, C. Desmarchelier, and C. Caris-Veyrat, "Are lutein, lycopene, and β-carotene lost through the digestive process?" *Food & Function*, vol. 8, no. 4, pp. 1494–1503, 2017.

[41] U. Stepaniak, A. Micek, A. Waśkiewicz et al., "Prevalence of general and abdominal obesity and overweight among adults in Poland. Results of the WOBASZ II study (2013-2014) and comparison with the WOBASZ study (2003-2005)," *Polskie Archiwum Medycyny Wewnętrznej*, vol. 126, no. 9, pp. 662–671, 2016.

[42] C. Duggan, J. de Dieu Tapsoba, C. Y. Wang et al., "Dietary weight loss, exercise, and oxidative stress in postmenopausal women: a randomized controlled trial," *Cancer Prevention Research (Philadelphia)*, vol. 9, no. 11, pp. 835–843, 2016.

[43] E. Lopez-Garcia, E. Orozco-Arbeláez, L. M. Leon-Muñoz et al., "Habitual coffee consumption and 24-h blood pressure control in older adults with hypertension," *Clinical Nutrition*, vol. 35, no. 6, pp. 1457–1463, 2016.

[44] A. E. Mesas, L. M. Leon-Muñoz, F. Rodriguez-Artalejo, and E. Lopez-Garcia, "The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis," *American Journal of Clinical Nutrition*, vol. 94, no. 4, pp. 1113–1126, 2011.

[45] G. Grosso, U. Stepaniak, M. Polak et al., "Coffee consumption and risk of hypertension in the Polish arm of the HAPIEE cohort study," *European Journal of Clinical Nutrition*, vol. 70, no. 1, pp. 109–115, 2016.

[46] E. Mostofsky, M. B. Johansen, S. Lundbye-Christensen, A. Tjønneland, M. A. Mittleman, and K. Overvad, "Risk of atrial fibrillation associated with coffee intake: findings from the Danish Diet, Cancer, and Health study," *European Journal of Preventive Cardiology*, vol. 23, no. 9, pp. 922–930, 2016.

[47] G. Grosso, U. Stepaniak, A. Micek, D. Stefler, M. Bobak, and A. Pajak, "Coffee consumption and mortality in three Eastern European countries: results from the HAPIEE (Health, Alcohol and Psychosocial factors in Eastern Europe) study," *Public Health Nutrition*, vol. 20, no. 1, pp. 82–91, 2017.

[48] M. Ding, S. N. Bhupathiraju, A. Satija, R. M. van Dam, and F. B. Hu, "Long-term coffee consumption and risk of cardiovascular disease: a systematic review and a dose-response meta-analysis of prospective cohort studies," *Circulation*, vol. 129, no. 6, pp. 643–659, 2014.

[49] A. Crippa, A. Discacciati, S. C. Larsson, A. Wolk, and N. Orsini, "Coffee consumption and mortality from all causes, cardiovascular disease, and cancer: a dose-response meta-analysis," *American Journal of Epidemiology*, vol. 180, no. 8, pp. 763–775, 2014.

[50] L. F. Andersen, D. R. Jacobs Jr, M. H. Carlsen, and R. Blomhoff, "Consumption of coffee is associated with reduced risk of death attributed to inflammatory and cardiovascular diseases in the Iowa Women's Health Study," *American Journal of Clinical Nutrition*, vol. 83, no. 5, pp. 1039–1046, 2006.

[51] F. E. Thompson and A. F. Subar, "Dietary assessment methodology," in *Nutrition in the Prevention and Treatment of Disease 3rd Ed., pp. 3–46*, A. M. Coulston, C. J. Boushey and M. G. Ferruzzi, Eds., Academic Press, USA, 2013.

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2017, Article ID 8536910, 12 pages
https://doi.org/10.1155/2017/8536910

Research Article

Paradoxical Effect of Nonalcoholic Red Wine Polyphenol Extract, Provinols™, in the Regulation of Cyclooxygenases in Vessels from Zucker Fatty Rats (*fa/fa*)

Abdelali Agouni,[1,2] Hadj Ahmed Mostefai,[1] Anne-Hélène Lagrue,[1] Martina Sladkova,[1,3] Philippe Rouet,[4] Franck Desmoulin,[5] Olga Pechanova,[3] Maria Carmen Martínez,[1,6] and Ramaroson Andriantsitohaina[1,6]

[1]*INSERM U1063, Stress Oxydant et Pathologies métaboliques, Université d'Angers, Université Bretagne Loire, Angers, France*
[2]*Pharmaceutical Science Section, College of Pharmacy, Qatar University, Doha, Qatar*
[3]*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia*
[4]*UMR UT3 CNRS 5288, Team Evolutionary Medicine, Obesity and Heart Failure: Molecular and Clinical Investigations, Avenue Jean Poulhes, Toulouse, France*
[5]*ToNIC, Toulouse NeuroImaging Center, Université de Toulouse, Inserm, UPS, Toulouse, France*
[6]*CHU, Angers, France*

Correspondence should be addressed to Ramaroson Andriantsitohaina; ramaroson.andriantsitohaina@univ-angers.fr

Received 27 February 2017; Accepted 13 April 2017; Published 4 June 2017

Academic Editor: Anna V. Queralt

The aim of this work was to study the vascular effects of dietary supplementation of a nonalcoholic red wine polyphenol extract, Provinols, in Zucker fatty (ZF) obese rats. ZF or lean rats received diet supplemented or not with Provinols for 8 weeks. Vasoconstriction in response to phenylephrine (Phe) was then assessed in small mesenteric arteries (SMA) and the aorta with emphasis on the contribution of cyclooxygenases (COX). Although no difference in vasoconstriction was observed between ZF and lean rats both in SMA and the aorta, Provinols affected the contribution of COX-derived vasoconstrictor agents. The nonselective COX inhibitor, indomethacin, reduced vasoconstriction in vessels from both groups; however, lower efficacy was observed in Provinols-treated rats. This was associated with a reduction in thromboxane-A2 and 8-isoprostane release. The selective COX-2 inhibitor, NS398, reduced to the same extent vasoconstriction in aortas from ZF and Provinols-treated ZF rats. However, NS398 reduced response to Phe only in SMA from ZF rats. This was associated with a reduction in 8-isoprostane and prostaglandin-E release. Paradoxically, Provinols decreased COX-2 expression in the aorta, while it increased its expression in SMA. We provide here evidence of a subtle and paradoxical regulation of COX pathway by Provinols vessels from obese rats to maintain vascular tone within a physiological range.

1. Introduction

The incidence of obesity has increased significantly worldwide in the past decade, reaching epidemic proportions. Obesity is commonly associated with comorbidities (such as hypertension, dyslipidaemia, and diabetes) and with an increased risk of premature death [1]. Obesity, in particular abdominal obesity, was shown to be a primary contributor to acquired insulin resistance, as increasing adiposity is correlated with impaired insulin action [2, 3]. Obesity is also associated with a broad inflammatory response, particularly, but not exclusively, in vessels [4, 5].

There are several inflammatory mediators that impact the vascular function in different animal models of obesity and metabolic syndrome, such as cyclooxygenase- (COX-) derived prostanoid derivatives [4–6]. Arachidonic acid is released by membrane phospholipids through phospholipase A2 cleavage; it can be metabolized by COX pathway into prostaglandins and thromboxane A2 (TXA2) [7]. COX exists in two major isoforms (COX-1 and COX-2) and one variant

(COX-3) [8]. COX-1 is found in all tissues, while COX-2 is known as an inducible enzyme that produces, in most cases, large amounts of prostaglandins. COX-2 is constitutively expressed in only a few sites, such as parts of the kidney and central nervous system, but is highly upregulated and active at the sites of inflammation including vascular smooth muscle cells and adipose tissue. It is known that many prostanoid derivatives have specific vasoactive properties, thereby contributing to the local regulation of vascular function [9].

Polyphenols constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, present in all plants that are commonly consumed in the Mediterranean diet, including grains, legumes, fruits, vegetables, extra virgin olive oil, and red wine [10, 11]. Epidemiological studies report an inverse association between dietary polyphenol consumption and mortality from cardiovascular diseases [12, 13]. Furthermore, red wine-derived polyphenols have been reported to improve cardiovascular function in various animal models of cardiovascular diseases and metabolic disturbances. For instance, red wine polyphenols were reported to improve endothelial function and normalise blood pressure in NO-deficient hypertensive rats [14], in spontaneously hypertensive rats [15] and deoxycorticosterone acetate- (DOCA-) salt-induced hypertensive rats [16]. In addition, red wine polyphenols were found to protect against cardiac ischemia [17], stroke [18], and improved cardiac function and endothelial function in obese rats [19]. Dietary polyphenols have also been found to inhibit cellular enzymes, such as phospholipase A2 and COX, in order to reduce arachidonic acid and prostaglandin production, thus exerting an important anti-inflammatory action. Polyphenolic compounds extracted from red wine were able to modulate COX-2 activity and gene expression in different cell types [20–22].

We have previously reported that dietary red wine polyphenol extract, Provinols, improves endothelial function in the obese Zucker fatty (ZF) rat, a common animal model of obesity and metabolic syndrome, by reducing oxidative stress and enhancing nitric oxide (NO) bioavailability [19]. However, the effects of Provinols on vascular inflammation and vasoreactivity have not yet been studied. The aim of the present work was therefore to investigate the vascular effects of a dietary supplementation of Provinols in ZF rats, with respect to the COX pathway regulation.

2. Materials and Methods

2.1. Animals and Experimental Protocol. All animal studies were carried out using approved institutional protocols and conform the *Guide for the Care and Use of Laboratory animals* published by US National Institutes of Health (NIH Publication number 85-23, revised 1996). The study involved 12 Zucker fatty (ZF) rats and 12 of their lean nonobese rats (Charles River, L'Arbresle, France); all of which received, for eight weeks, either a control diet or a diet containing a dose of 20 mg/kg/day of Provinols (Société Française de Distilleries; Vallon Pont d'Arc, France), a nonalcoholic red wine polyphenol extract. The dose of Provinols given to animals was described to be able to induce several cardiovascular protective effects, such as the improvement of endothelial function [23], the prevention of the increase in blood pressure in NO-deficient hypertensive rats [14], and the protection against cardiac ischemia [17], stroke [18], and improved cardiac function and endothelial function in obese rats [19]. The eight-week treatment has been reported to protect against the deleterious effects of hypertension [24] and chronic stress exposure [25] and obesity [19].

2.2. Vascular Reactivity. Aortic and small mesenteric arteries (SMA) were obtained from ZF or lean rats having or having not received Provinols, which were then cleaned from connective tissue and cut into rings of 1.5–2 mm long. Vessel rings were then mounted on a wire myograph (Danish MyoTechnology, Aarhus, Denmark) filled with physiological salt solution (PSS) as described previously [19, 26, 27]. The concentration-response curves were constructed by cumulative application of phenylephrine (Phe, 1 nmol/L to 10 μmol/L; Sigma-Aldrich, St. Quentin, Fallavier, France) to vessels with functional endothelium in the presence or absence of a given inhibitor: the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L; Sigma-Aldrich), the selective COX-2 inhibitor N-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonamide (NS398, 10 μmol/L; Sigma-Aldrich), or indomethacin, the nonselective COX inhibitor (INDO, 100 μmol/L). All inhibitors were used at their maximal active concentrations at which they inhibit the release of either NO from all isoforms of NO synthases (L-NAME), metabolites from COX-2 isoforms (NS398), or metabolites from COX (INDO) in blood vessels, as reported in our previous studies [26].

2.3. Determination of Prostanoid Production. Vessels with endothelium were treated with Phe (10 μmol/L, 30 minutes, 37°C). The medium was next collected. Then, TXA2, prostaglandin E metabolites, prostacyclin, and total 8-isoprostane were measured by enzyme immunoassays kits (Cayman Chemicals, Ann Arbor, Michigan). The concentration of prostanoids was expressed as pg/mL/mg of dry weight (dw) tissue [28].

2.4. Western Blotting. Aortas and SMA vessels were dissected, homogenized, and lysed. Proteins (80 μg) were separated on 10% SDS-PAGE. Blots were probed with anticyclooxygenase-(COX-) 1 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-COX-2 (BD Biosciences, San Jose, CA), and anti-NF-κB p65 (Abcam, Paris, France) antibodies. A monoclonal mouse β-actin antibody (Sigma-Aldrich) was used at 1/5000 dilution for visualization of protein gel loading. The membranes were then washed at least three times in Tris buffer solution containing 0.05% Tween and incubated for 1 hour per wash at room temperature, with the appropriate horseradish peroxidase- (HRP-) conjugated secondary antibody (Amersham). The protein antibody complexes were detected by ECL plus (Amersham) according to the protocol of the manufacturer as previously done [29, 30].

2.5. Staining and Imaging by Confocal Microscopy. Vessels with endothelium were frozen and cut into 7 μm sections.

Fixed sections were incubated (2 hours) in blocking buffer (5% nonfat dry milk in phosphate-buffered saline). After three washes, tissue sections were incubated overnight with a polyclonal NF-κB p65 antibody (1 : 100). After three washes, sections were incubated (1 hour) with murine Alexa fluor-488-labeled antibody (1 : 100). After washes, vessel sections were mounted on glass slides and a Solamere confocal equipment with a DLS-300 laser mounted on a Nikon Eclipse TE 2000-S inverted microscope was used for the optical sectioning of the tissue. Digital image recording was performed using the QED in vivo software.

2.6. Carotid Tissue Dual Phase Metabolite Extraction

2.6.1. Extraction Protocol. Frozen sections of carotids ($n = 10$, 25 ± 8 mg) were powdered in liquid N_2 with a mortar and pestle and, then, immediately extracted using a dual phase extraction method [31]. Briefly, frozen tissue fragments were homogenized for 20 seconds in ice-cold solvents (purex-analytical-grade) methanol and chloroform in a ratio of 2 : 1 (1.5 mL) by using an Ultra-Turrax homogenizer. After 5 minutes in contact with the first solvents at 4°C, 0.5 mL chloroform and 0.5 mL distilled water were added and homogenized. The samples were then centrifuged at 2000 g for 30 minutes. The organic fraction was collected. Vials were sealed under a flow of Ar and maintained at −20°C. Prior analysis, lipid extracts were dried off and dissolved in 400 μL of chloroform.

2.6.2. Phospholipid Analysis. The lipid extract (200 μL from the total 400 μL) was dried and dissolved in 50 μL of chloroform/methanol (1 : 1, v/v); 20 μL was analysed by HPLC on a Summit DIONEX system using an Uptisphere 6OH (5 μmol/L) 250×2 mm column. The flow rate was 0.25 mL/minute, and the column temperature was kept at 25°C during all runs. A light-scattering detector was used for the detection (Polymer Laboratories PL-ELS2100, Church Stretton, United Kingdom). The evaporator and nebulisator temperature was kept, respectively, at 50 and 80°C, and the nitrogen flow was 1.8 mL/minute. A binary solvent system was used with (A): hexane/isopropanol (82 : 18, v/v) and (B): isopropanol/water (85 : 15, v/v) in the presence of triethylamine (0.014%, v/v) and acetic acid (0.5%, v/v). The gradient profile was started at 5% B for 5 minutes, moved to 35% B in 25 minutes, and then to 85% B in 8 minutes. Finally, the gradient was returned to the starting conditions in 10 minutes. The column was equilibrated for 10 minutes before the next run.

2.6.3. Fatty Acid Analysis. Glyceryl triheptadecanoate (2 μg) as internal standard and hexane (1 mL) was added to the lipid extract (80 μL from the total 400 μL). Transmethylation was performed for 1 hour in boron trifluoride methanol solution 14% (1 mL, Sigma-Aldrich) at 55°C. After the addition of water (1 mL) to the crude, fatty acid methyl esters (FAME) were extracted with hexane (3 mL), dried by evaporation, and dissolved in ethyl acetate (20 μL). FAME (1 μL) were analysed by gas-liquid chromatography [32] on a 5890 Hewlett Packard system using a Famewax RESTEK-fused silica capillary columns ($30\,\text{m} \times 0.32$ mm i.d., 0.25 mm film thickness). Oven temperature was programmed from 110°C to 220°C at a rate of 2°C per min, and the carrier gas was hydrogen (0.5 bar). The injector and the flame ionization detector were at 225°C and 245°C, respectively.

2.6.4. Neutral Lipid Molecular Analysis. Internal standards were added to the lipid extract (100 μL from the total 400 μL): 3 mg of stigmasterol, 1 mg of 1,3-dimyristine, 1 mg of cholesteryl heptadecanoate, and 5 mg of glyceryl triheptadecanoate. Neutral lipids were separated using a SPE column (Strata SI-1 Silica 55 μm, 70 Å, 100 mg), washed with 2 mL of chloroform, crudely dissolved in 20 μL of chloroform, applied on the cartridge, and eluted with 2 mL of chloroform. The organic phases were evaporated until dry and dissolved in 20 μL of ethyl acetate. One μL of the lipid extract was analysed by gas-liquid chromatography on a FOCUS Thermo Electron system using a Zebron-1 Phenomenex-fused silica capillary column ($5\,\text{m} \times 0.32$ mm i.d., 0.50 mm film thickness). Oven temperature was programmed from 200°C to 350°C at a rate of 5°C per min, and the carrier gas was hydrogen (0.5 bar). Profiles of neutral lipid molecular species were determined according to total acyl carbon number [32]. The injector and the flame ionization detector were operating at 315°C and 345°C, respectively.

2.7. Data Analysis. Data are expressed as mean ± SEM, and n represents the number of experiences. Statistical analyses were performed using a one-way analysis of variance (ANOVA) and Mann–Whitney U tests or ANOVA for repeated measures and subsequent Bonferroni's post hoc test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Provinols Did Not Affect Contractile Response to Phe in both Aortas and SMA from ZF Rats. The contractile response to a vasoconstrictor agonist (Phe) was investigated in both conductance (aorta) and resistance (SMA) arteries from ZF and lean rats with or without Provinols treatment. As shown in Figure 1, there was no significant difference in the contractile response to Phe between all the groups in both the aorta and SMA. Due to the absence of differences in overall contraction between lean and ZF rats, the rest of the experiments were carried out on ZF rats only.

3.2. Effect of NO Inhibition on Vascular Reactivity in Aortas and SMA from ZF Rats. To assess the contribution of NO levels in vasoconstriction of vessels from ZF and Provinols-treated ZF rats, NO synthases (NOS) were inhibited using L-NAME and then contraction in response to Phe was assessed in both the aorta and SMA. While the nonselective blockade of NOS did not modify contraction in response to Phe in SMA from both groups of rats (Figures 2(a), 2(b), and 2(c)), it potentiated to the same extent the contractile response to Phe in aortas from both ZF and Provinols-treated ZF rats (Figures 2(d), 2(e), and 2(f)).

3.3. Provinols Reduced the Involvement of COX-Derived Vasoconstrictor Metabolites in SMA from Obese Rats. To figure out the relative contribution of vasoactive prostanoid

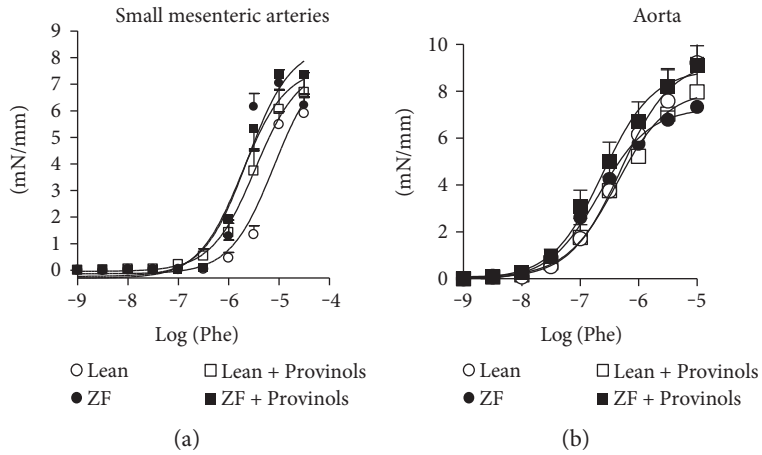


Figure 1: Provinols did not affect contractile response to Phe in aortas and SMA from obese ZF rats. Shown are concentration-response curves to phenylephrine (Phe) cumulative concentrations (M) of small mesenteric arteries (SMA, (a)) and rat aortic rings (b) from lean and ZF rats treated or not with Provinols. Values are expressed as mean ± SEM of millinewtons (mN) per mm of vessel length. $N = 6$ in each group.

derivatives in the contractile response to Phe in SMA, COX isoforms were blocked using selective and non-selective inhibitors. In ZF rats, both COX-2 (NS398) (Figure 3(a)) and nonselective COX (INDO) (Figure 3(b)) inhibitors reduced to the same extent the contractile response to Phe in SMA, suggesting the involvement of COX-2-derived vasoconstrictor metabolites in this contractile response. In Provinols-treated ZF rats, NS398 (Figure 3(c)) failed to affect the contractile response to Phe; however, indomethacin (Figure 3(d)) reduced contraction in SMA, suggesting the implication of non-COX-2-derived vasoconstrictor metabolites in this contractile response. Interestingly, when we compared the contractile response in the presence of inhibitors between the two groups, we observed that contraction was greater in vessels from Provinols-treated ZF rats in the presence of COX-2 inhibitor (Figures 3(e) and 3(f)), indicating a smaller contribution of COX-2-derived vasoconstrictor metabolites in the contractile response to Phe in SMA obtained from ZF rats which received Provinols compared to ZF rats.

3.4. Provinols Reduced the Involvement of COX-Derived Vasoconstrictor Metabolites in Aortas from Obese ZF Rats. The relative contribution of vasoactive prostanoid derivatives in the contractile response to Phe was assessed in aortas by inhibiting COX isoforms. Both the nonselective inhibition of COX and the selective blockade of COX-2 reduced the contractile response to Phe in aortas from ZF rats; however, the reduction in contractile response was higher with INDO (Figures 4(a) and 4(b)), suggesting the participation of both COX-2- and non-COX-2-derived vasoconstrictor metabolites in the contractile response to Phe in ZF rats. Aortas from ZF rats which received Provinols exhibited a reduction in the contractile response to Phe in the presence of NS398 or INDO; however, the reduction was slightly higher in the presence of INDO, suggesting the involvement of mainly COX-2-derived metabolites in the aortas from the polyphenol group (Figures 4(c) and 4(d)).

The comparison of vascular contraction between aortas from both experimental groups in the presence of inhibitors showed no major differences in the presence of NS398, although vessels from the polyphenol group had a trend for a higher contractile response (Figure 4(e)); however, in the presence of INDO, the contraction in vessels from the polyphenol group was significantly greater (Figure 4(f)), suggesting a reduced involvement of non-COX-2-derived vasoconstrictor metabolites in aortas from Provinols-treated ZF rats.

3.5. Provinols Reduced COX-Derived Vasoconstrictor Metabolite Release in Aortas and SMA from Obese ZF Rats. The functional studies suggest a reduced participation of COX-derived vasoconstrictor metabolites in both conductance and resistance arteries from Provinols-treated ZF rats. Indeed, in the aorta, the measurement of COX-derived metabolite production in vessels showed that the Provinols reduced the release of 8-isoprostane and TXA2, both vasoconstrictor metabolites, without affecting that of prostacyclin and prostaglandin E metabolites (Figures 5(a)–5(d)). In SMA, the Provinols reduced the production of vasoconstrictor metabolites 8-isoprostane and prostaglandin E, without modifying the release of prostacyclin and TXA2 metabolites (Figures 5(e)–5(h)).

Altogether, these data indicate that the Provinols reduced the participation of COX-derived vasoconstrictor agents in the contractile response to Phe in both aortas and SMA.

3.6. Provinols Modulated the Expression of COX Isoforms and NF-κB in Vessels from Obese ZF Rats. Since Provinols could modulate the contribution of COX-derived agents in the contractile response of vessels from obese rats, the expression of COX-1 and COX-2 was therefore assessed. Results showed that Provinols did not affect the expression of COX-1, neither in the aorta nor in SMA (Figures 6(a) and 6(b)); however, it paradoxically affected the expression of COX-2 in aortas and SMA. While Provinols reduced

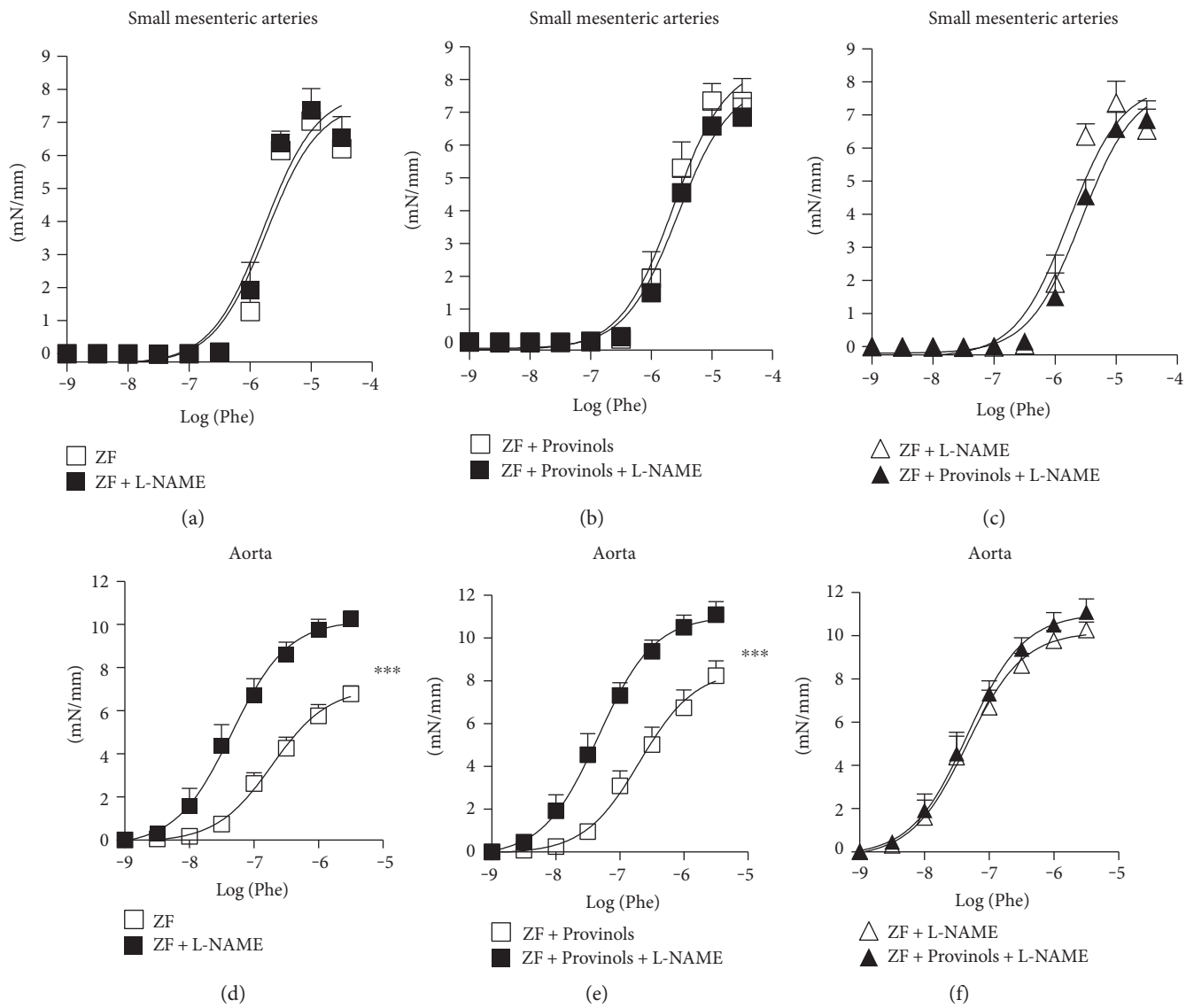


Figure 2: Effect of NO inhibition on vasoreactivity in aortas and SMA from obese ZF rats. Shown are concentration-response curves to phenylephrine (Phe) cumulative concentrations (M) in the presence of NO-synthase nonselective inhibitor (L-NAME) of small mesenteric arteries (SMA) (a–c) and rat aortic rings (d–f) from ZF rats treated or not with Provinols. Values are expressed as mean ± SEM of millinewtons (mN) per mm of vessel length. $^{***}P < 0.001$ versus ZF rats.

COX-2 expression in the aorta, it enhanced its expression in SMA, (Figures 6(c) and 6(d)).

The transcription factor NF-κB controls the transcription of proinflammatory enzymes such as COX-2; hence, its expression was assessed. The expression of NF-κB was significantly reduced by Provinols within the aorta (Figures 6(e) and 6(f)); however, its expression did not change in SMA (Figure 6(g)).

3.7. Provinols Altered Phospholipid Profile in Carotids from Obese ZF Rats. An alteration in the activity of phospholipase A2, primarily responsible of prostanoid derivatives generation, may lead to an alteration of the lipid composition in vessels from ZF obese rats. Hence, the effects of Provinols on the distribution of membrane neutral lipids and fatty acids were analysed in carotid artery from ZF rats.

As shown in Table 1, the neutral lipid composition and fatty acid composition of total lipids from carotid tissues remained similar in Provinols-treated and control groups. Neither concentrations nor percent distribution of neutral lipid molecular species of triacylglycerides (TAG), diacylglycerides D (AG), nor cholesteryl esters were modified by Provinols (Table 1). Moreover, Provinols did not modify neither the concentration nor the distribution of fatty acid molecular species of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Table 2). However, even though phospholipid contents were similar in the two groups, Provinols led to an alteration of the phospholipid's profile. As shown in Table 3, Provinols significantly increased the contribution of phosphatidylethanolamine (PE) to total

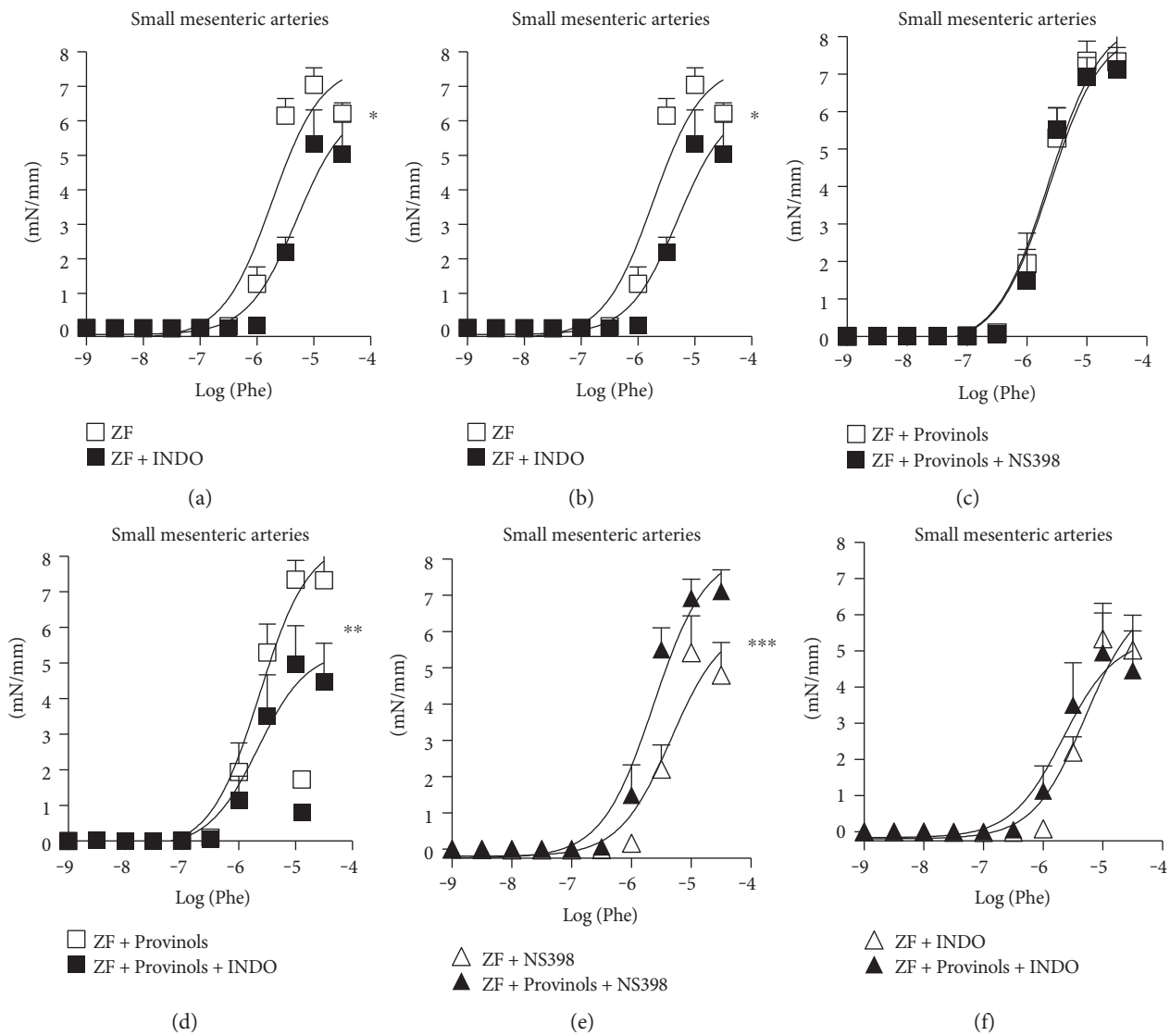


Figure 3: Provinols reduced the involvement of COX-derived vasoconstrictor metabolites in SMA from obese ZF rats. Concentration-response curves to phenylephrine (Phe) cumulative concentrations (M) in the presence of COX-2 inhibitor (NS398) or the nonselective COX inhibitor (indomethacin, INDO) of small mesenteric arteries (SMA) from ZF rats which received or not Provinols. $N = 6$ in each group. Values are expressed as mean ± SEM of millinewtons (mN) per mm of vessel length. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus ZF rats.

phospholipids (41.6 ± 6.4 nmol/mg and 50 ± 2.7 nmol/mg from nontreated and Provinols-treated ZF rats, respectively).

4. Discussion

Our findings reported in this study indicated that red wine polyphenols modulated the relative contribution of COX-derived metabolites in vasoreactivity in both conductance and resistance arteries from ZF rats, although without affecting the global contractile response of vessels. Indeed, Provinols could reduce the release of COX-derived vasoconstrictor agents from both types of arteries by affecting the expression and/or activity of COX enzymes as well as NF-κB transcription factor. In addition, Provinols treatment altered the phospholipid molecular species distribution in carotid arteries from ZF rats. These data underscore the beneficial role of red wine polyphenols in modulating the contribution of inflammatory agents in the control of vascular tone in obese rats.

In a previous study, we showed that ZF rats had an impaired endothelium-dependent relaxation to acetylcholine compared to their lean controls [19]; however, the present study demonstrated that there was no difference in the contractile response to Phe between control and obese rats. In a study by Mingorance et al. [5], authors found that aortas and SMA from ZF rats had a lower response to Phe than lean animals, whereas ZF rats showed a diminished response to acetylcholine only in the aorta. The discrepancy between our findings and those from Mingorance et al. may be due to the age of animals used, 15 weeks in our study and 31 weeks of age in the other one. In addition, an exaggerated adrenergically mediated vascular reactivity

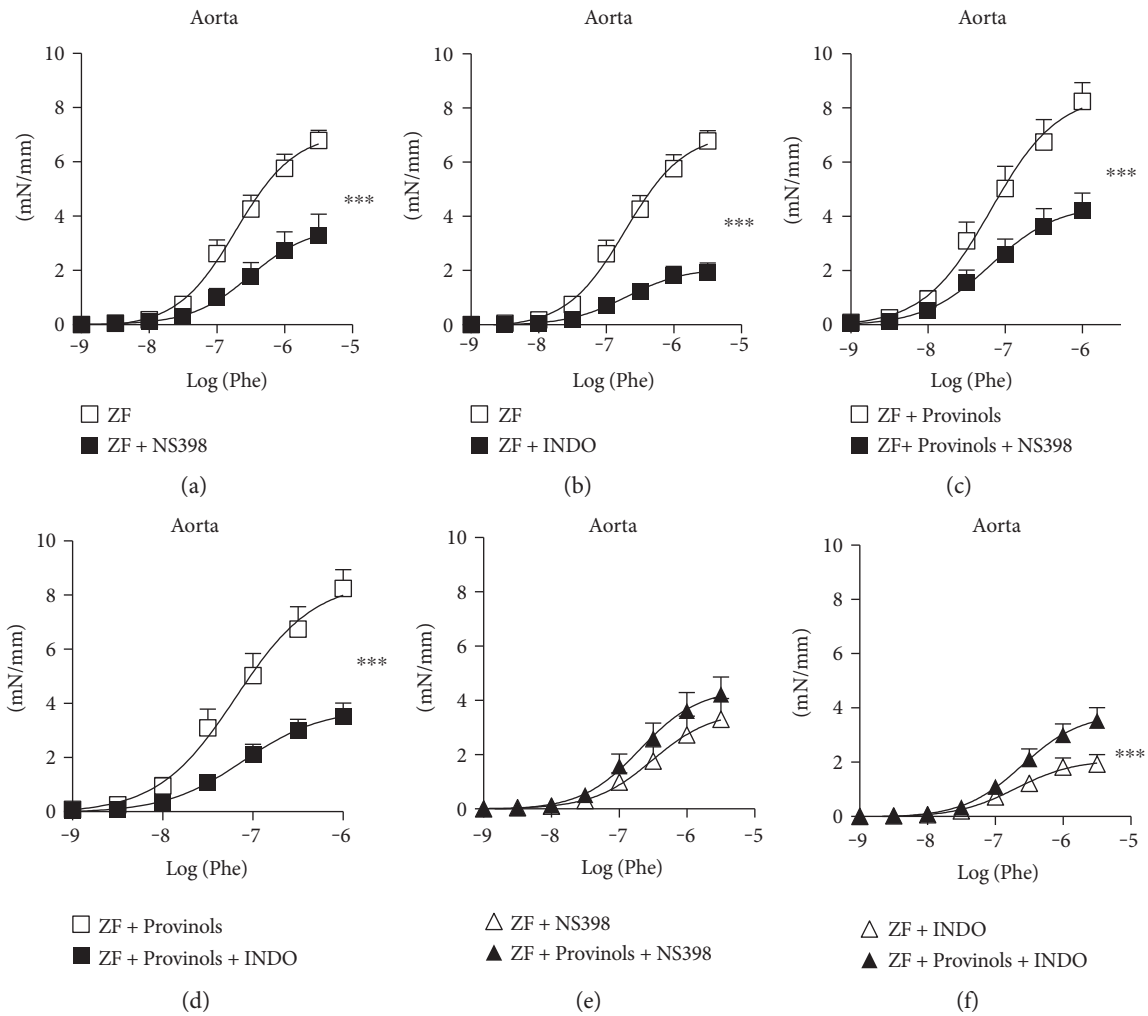


Figure 4: Provinols reduced the involvement of COX-derived vasoconstrictor metabolites in the aorta from obese ZF rats. Concentration-response curves to phenylephrine (Phe) cumulative concentrations (M) in the presence of COX-2 inhibitor (NS398) or the nonselective COX inhibitor (indomethacin, INDO) of aorta rings from ZF rats which received or not Provinols. Values are expressed as mean ± SEM of millinewtons (mN) per mm of vessel length. $N = 6$ in each group. $^{***}P < 0.001$ versus ZF rats.

has been also documented in aortic and SMA rings from obese rats [33, 34].

Although no difference was observed in the contractile response to Phe, we observed that Provinols treatment significantly modulated the relative contribution of COX-derived metabolites in vasoreactivity in both the aorta and SMA. Of note, SMA from ZF rats had more contribution from COX-2-derived vasoconstrictor metabolites in the contractile response to Phe compared to vessels from ZF rats fed a diet containing Provinols. Aortas from ZF rats had, however, more contribution from non-COX-2-derived vasoconstrictor agents in the contractile response to Phe in comparison to aortas from the polyphenol group. Consistent with these findings, the evaluation of prostanoid derivative release revealed that vasoconstrictor COX-derived agents were reduced in the Provinols group (8-isoprostane/TXA2 in the aorta and 8-isoprostane/prostanglandin E in SMA). Interestingly, previous studies showed an increased participation of COX-derived vasoconstrictor agents (i.e., TXA2) in vessels from ZF rats compared to lean controls [4, 5]. Both the aorta and SMA from the Provinols group had reduced levels of 8-isoprostane production, indicating a reduction of oxidative stress. Indeed, in contrast to classic prostaglandins, isoprostanes are formed by free radical-catalysed lipid peroxidation of arachidonic acid and cell membrane phospholipids and constitute thus a good marker for oxidative stress level. These observations are in line with our previous results which indicated that Provinols reduced superoxide anion release in aortas and SMA from ZF rats [19].

Findings from our study support previous reports demonstrating that polyphenols can regulate COX-2 expression and secretion [35, 36]. In this study, we observed that Provinols did not affect the expression of the constitutive COX-1 isoform in both the aorta and SMA; paradoxically, however, Provinols reduced COX-2 expression in the aorta and enhanced its protein expression in SMA. These results are not in apparent line with our functional findings or those of biochemical analysis of COX-derived metabolites release. The reduction in COX-2 expression in aortas may explain the reduction of the release of COX-derived metabolites. This

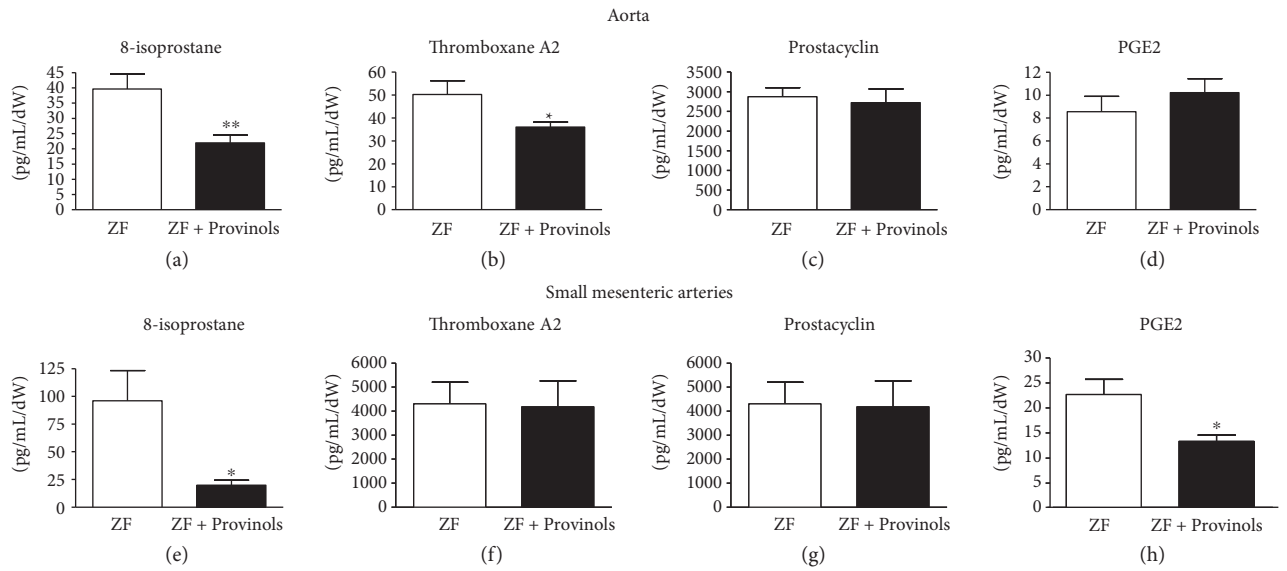


Figure 5: Provinols reduced COX-derived vasoconstrictor metabolite release in aortas and SMA from obese ZF rats. Concentration of COX derivatives thromboxane A2, prostaglandin E2 (PGE2), 8-isoprostane, and prostacyclin in the supernatants of the rat aorta and SMA from ZF rats treated or not with Provinols and stimulated with Phe ($n = 6$). The concentration of prostanoids is expressed as pg/mL/mg of dry weight (dw) tissue. $^{*}P < 0.05$; $^{**}P < 0.01$ versus ZF rats.

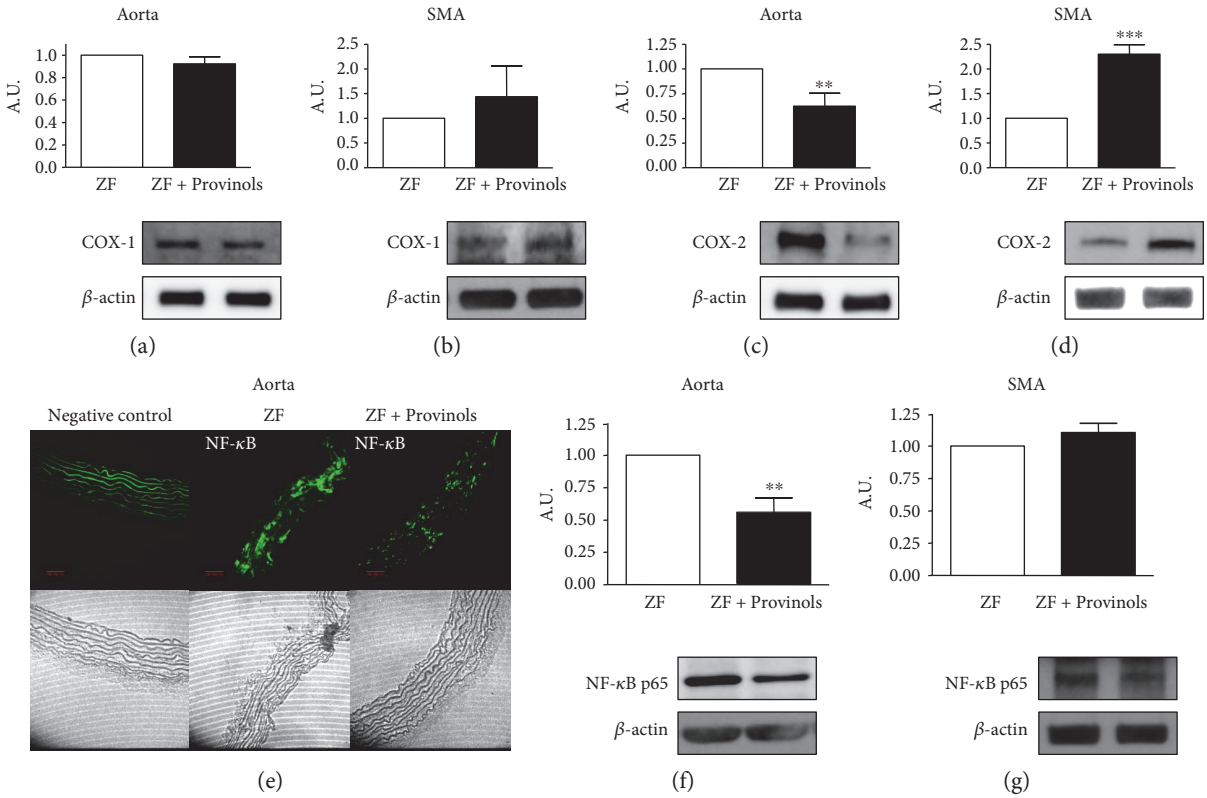


Figure 6: Provinols modulated the expression of COX isoforms and NF-κB in vessels from obese ZF rats. Western blots showing the expression of cyclooxygenase- (COX-) 1 (a, b) and COX-2 (c, d) in the aorta and SMA from ZF rats treated or not with Provinols. NF-κB expression in the rat aorta was assessed by confocal imaging (e) and western blot analysis (f). In (c) is shown NF-κB expression in SMA by western blot analysis. Quantification of immunoblots' signal was done by densitometric analysis; β-actin loading control was included. Data are representative of four separate blots, and the densitometry values are expressed in arbitrary units (A.U.) as mean ± SEM. $^{**}P < 0.01$; $^{***}P < 0.001$ versus ZF rats.

Table 1: Comparison between neutral lipid molecular species from carotids in control and Provinols-treated rats.

Neutral lipids	ZF	ZF + Provinols
Percent distribution of TAG		
TAG C49	4.7 ± 0.3	3.8 ± 0.6
TAG C51	13.5 ± 0.8	12.1 ± 1.3
TAG C53	32 ± 0.9	31.4 ± 1
TAG C55	34.6 ± 1	35.77 ± 1.2
TAG C57	13.3 ± 0.7	14.6 ± 1.2
TAG C59	1.8 ± 0.4	2.4 ± 0.6
Total TAG (nmol/mg)	**4.68 ± 1.72**	**4.36 ± 1.35**
Percent distribution of DAG		
DAG 14–16	16.9 ± 11.6	12 ± 2.5
DAG 16-16	19.6 ± 6.7	14.4 ± 4.4
DAG 16–18	34 ± 9.2	43 ± 5.3
DAG 18-18	17 ± 14.7	19 ± 11.2
DAG18–20	12 ± 2.5	14 ± 0.3
DAG18–22	Nd	Nd
Total DAG (nmol/mg)	**0.10 ± 0.03**	**0.08 ± 0.03**
Percent distribution of cholesteryl esters		
C14 cholesteryl ester	Nd	Nd
C16 cholesteryl ester	15.1 ± 1.7	17.3 ± 3.8
C18 cholesteryl ester	17.9 ± 6	17.6 ± 5.1
C20:4 cholesteryl ester	67 ± 6.1	65.1 ± 8.3
C22 cholesteryl ester	Nd	Nd
Total cholesteryl esters (nmol/mg)	**1.8 ± 0.6**	**0.13 ± 0.03**
Cholesterol (nmol/mg)	**0.12 ± 0.04**	**1.6 ± 0.3**

Data are means ± SEM. Nd: not detected. Concentrations are expressed as nanomoles per mg of tissue wet weight (nmol/mg). Triacylglycerides (TAG) and diacylglycerides (DAG) families were defined according to their total number of carbon atoms (as described in Materials and Methods).

Table 2: Comparison between fatty acid (FA) molecular species from carotids in control and Provinols-treated rats.

FA	ZF	ZF + Provinols
Total FA (nmol/mg)	**14.59 ± 4.58**	**19.74 ± 6.43**
Percent distribution of FA		
SAFA	41.5 ± 2.4	40.9 ± 1.3
MUFA	38.1 ± 4.9	39.7 ± 1.4
PUFA	20.4 ± 2.6	19.4 ± 2.1
Percent distribution of SAFA		
14 : 0	3.3 ± 0.7	3.4 ± 1.3
16 : 0	72.6 ± 5.9	72.5 ± 2.7
18 : 0	22 ± 4.4	22.9 ± 3.6
20 : 0	1.1 ± 0.5	0.6 ± 0.2
24 : 0	1.1 ± 0.8	0.6 ± 0.2
Percent distribution of MUFA		
16 : 1 n-7	17.8 ± 0.8	17 ± 2.4
18 : 1 n-9	78.5 ± 4.3	82 ± 2
20 : 1 n-9	0.4 ± 0.1	0.4 ± 0.2
22 : 1 n-9	Nd	Nd
24 : 1 n-9	3.3 ± 4.5	0.6 ± 0.3
Percent distribution of PUFA		
18 : 2 n-6	53.1 ± 11.7	57.9 ± 9.3
18 : 3 n-6	Nd	Nd
18 : 3 n-6	2.1 ± 0.8	2.3 ± 0.6
20 : 2 n-6	1.3 ± 0.3	1.4 ± 0.4
20 : 3 n-6	Nd	Nd
20 : 3 n-6	1.7 ± 0.4	1.6 ± 0.4
20 : 4 n-6	38.5 ± 12	33.2 ± 9.2
20 : 5 n-3	Nd	Nd
22 : 5 n-3	Nd	Nd
22 : 2 n-6	Nd	Nd
22 : 6 n-3	3.3 ± 1	3.4 ± 1

Data are means ± SEM. Total fatty acids (FA) are expressed as nanomoles per mg of tissue wet weight. SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; Nd: not detected.

is further corroborated by the reduction of NF-κB transcription factor expression in aortas from the polyphenol group. The activation of NF-κB is upstream of the synthesis of acute phase inflammatory mediators. Among the genes known to be positively regulated by NF-κB is *COX-2* [37]. In the present work, we found that Provinols reduced NF-κB in the aorta, but not in SMA, accounting potentially for reducing COX-2 expression. Previously, it has been shown that tea polyphenols inactivated phosphorylated forms of nuclear NF-κB and reduced COX-2 expression in rat mammary tumours [38]. Furthermore, procyanidin extracts, a mixture of polyphenols, inhibited NF-κB (p65) translocation in lipopolysaccharide-stimulated macrophages [39].

In SMA from rats treated with polyphenols, we observed an increase in COX-2 expression even though functional data and biochemical analysis indicated that these vessels have less contribution of COX-2-derived metabolites towards the contractile response to Phe. This paradoxical effect could be explained by a subtle effect of Provinols on the activity of COX enzymes and/or phospholipase A2 activity, which would contribute to the reduced release of COX-derived

Table 3: Comparison between phospholipids (PL) from carotids in control and Provinols-treated rats.

Percent distribution of PL	ZF	ZF + Provinols
PE	41.6 ± 6.4	50 ± 2.7*
PC	38 ± 5.7	33.9 ± 1.9
SM	13 ± 4.4	9.4 ± 1
PS	4.8 ± 1.2	4.3 ± 0.6
PI	2.6 ± 1.6	2.5 ± 0.4
PG	Nd	Nd
Total PL (nmol/mg)	**3.8 ± 1.3**	**3.6 ± 0.9**

Data are means ± SEM. Total phospholipids (PL) are expressed as nanomoles per mg of tissue wet weight. *Difference between groups at $P < 0.05$ ($n = 5$). PE: phosphatidylethanolamine; PC: phosphatidylcholine; SM: sphigomyeline; PS: phosphatidylserine; PI: phosphatidylinositol, PG: phosphatidylglycerol; Nd: not detected.

metabolites. The increase in COX-2 protein expression may therefore be a compensatory mechanism to the decrease in the enzyme's activity. Although the reduction in COX-derived metabolites caused by Provinols may suggest a reduction in COX activity, further studies are warranted to ascertain the effects of Provinols on COX enzymes' activity. Consistent with our findings, a previous study from Kane et al. [40] showed that red wine polyphenols reduced angiotensin II-induced COX-2 overexpression in rat vessels. Previous publications have also reported an inhibitory effect of red wine extract and tea polyphenols on COX-2 activity [20, 38]. However, a recent study evaluated the effect of several food polyphenols, including resveratrol, a component of red wine polyphenols, on the activity of COX-2 and reported that although these compounds could elicit the inhibition of COX–2, their potency was 100- to 1000-fold lower compared to known pharmacological inhibitors of COX-2 such as celecoxib and indomethacin [41].

Finally, we have shown that one focal effect of Provinols treatment was an alteration of phospholipid molecular species in carotid arteries from ZF rats. The increase in the PE contribution to total phospholipids suggested that Provinols treatment had a specific effect on phospholipid turnover because neutral lipids and fatty acid molecular species remain unchanged. Indeed, a reduction in PE degradation has been previously observed in platelets and liver microsomes due to inhibition of the secretory phospholipase A2 [42]. This result is suggestive of a reduction in the activity of phospholipase A2 in vessels from Provinols-treated rats especially when taken together with the reduction in the secretion of bioactive prostanoids.

In conclusion, we showed in this study that the global response to vasoconstrictor agonists was not altered in ZF compared to lean rats; however, red wine polyphenols could modulate COX expression and/or activity via a mechanism involving NF-κB pathway. The present study provided evidence of a subtle regulation of vasocontractility both in conductance and resistance arteries by Provinols to maintain vascular tone within a physiological range. Such effect is linked to a paradoxical effect of Provinols on COX pathway. These data highlight further the beneficial role of red wine polyphenols in correcting cardiovascular disturbances associated with obesity and metabolic syndrome.

Conflicts of Interest

The authors have nothing to declare.

Acknowledgments

We thank A. Dequesne (technical platform of Toulouse Midi-Pyrénées Genopole® IFR31) and J. Bertrand-Michel (lipidomics technical platform IFR30) for technical assistance in biochemistry and lipid analysis, respectively. We also thank Dr. Mohamed Lamine Freidja and Dr. Mamadou Sarr for their support in this work. This work is partially supported by the National Institute for Medical Research (INSERM, France) and Université d'Angers (France).

References

[1] K. F. Adams, A. Schatzkin, T. B. Harris et al., "Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old," *The New England Journal of Medicine*, vol. 355, no. 8, pp. 763–778, 2006.

[2] R. A. DeFronzo and E. Ferrannini, "Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease," *Diabetes Care*, vol. 14, no. 3, pp. 173–194, 1991.

[3] M. Bastien, P. Poirier, I. Lemieux, and J. P. Despres, "Overview of epidemiology and contribution of obesity to cardiovascular disease," *Progress in Cardiovascular Diseases*, vol. 56, no. 4, pp. 369–381, 2014.

[4] P. Arvola, X. Wu, M. Kahonen et al., "Exercise enhances vasorelaxation in experimental obesity associated hypertension," *Cardiovascular Research*, vol. 43, no. 4, pp. 992–1002, 1999.

[5] C. Mingorance, M. Alvarez de Sotomayor, F. J. Jimenez-Palacios et al., "Effects of chronic treatment with the CB1 antagonist, rimonabant on the blood pressure, and vascular reactivity of obese Zucker rats," *Obesity (Silver Spring)*, vol. 17, no. 7, pp. 1340–1347, 2009.

[6] S. Dal-Ros, C. Bronner, C. Schott et al., "Angiotensin II-induced hypertension is associated with a selective inhibition of endothelium-derived hyperpolarizing factor-mediated responses in the rat mesenteric artery," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 328, no. 2, pp. 478–486, 2009.

[7] J. H. Yoon and S. J. Baek, "Molecular targets of dietary polyphenols with anti-inflammatory properties," *Yonsei Medical Journal*, vol. 46, no. 5, pp. 585–596, 2005.

[8] V. Stangl, H. Dreger, K. Stangl, and M. Lorenz, "Molecular targets of tea polyphenols in the cardiovascular system," *Cardiovascular Research*, vol. 73, no. 2, pp. 348–358, 2007.

[9] M. Feletou, R. Kohler, and P. M. Vanhoutte, "Endothelium-derived vasoactive factors and hypertension: possible roles in pathogenesis and as treatment targets," *Current Hypertension Reports*, vol. 12, no. 4, pp. 267–275, 2010.

[10] L. Bravo, "Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance," *Nutrition Reviews*, vol. 56, no. 11, pp. 317–333, 1998.

[11] M. A. Russo, L. Sansone, L. Polletta et al., "Sirtuins and resveratrol-derived compounds: a model for understanding the beneficial effects of the Mediterranean diet," *Endocrine, Metabolic & Immune Disorders Drug Targets*, vol. 14, no. 4, pp. 300–308, 2014.

[12] E. Middleton Jr., C. Kandaswami, and T. C. Theoharides, "The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer," *Pharmacological Reviews*, vol. 52, no. 4, pp. 673–751, 2000.

[13] G. Chiva-Blanch, S. Arranz, R. M. Lamuela-Raventos, and R. Estruch, "Effects of wine, alcohol and polyphenols on cardiovascular disease risk factors: evidences from human studies," *Alcohol and Alcoholism*, vol. 48, no. 3, pp. 270–277, 2013.

[14] I. Bernatova, O. Pechanova, P. Babal, S. Kysela, S. Stvrtina, and R. Andriantsitohaina, "Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 282, no. 3, pp. H942–H948, 2002.

[15] R. Lopez-Sepulveda, R. Jimenez, M. Romero et al., "Wine polyphenols improve endothelial function in large vessels of female spontaneously hypertensive rats," *Hypertension*, vol. 51, no. 4, pp. 1088–1095, 2008.

[16] R. Jimenez, R. Lopez-Sepulveda, M. Kadmiri et al., "Polyphenols restore endothelial function in DOCA-salt hypertension: role of endothelin-1 and NADPH oxidase," *Free Radical Biology & Medicine*, vol. 43, no. 3, pp. 462–473, 2007.

[17] H. Ralay Ranaivo, M. Diebolt, and R. Andriantsitohaina, "Wine polyphenols induce hypotension, and decrease cardiac reactivity and infarct size in rats: involvement of nitric oxide," *British Journal of Pharmacology*, vol. 142, no. 4, pp. 671–678, 2004.

[18] M. F. Ritz, Y. Curin, A. Mendelowitsch, and R. Andriantsitohaina, "Acute treatment with red wine polyphenols protects from ischemia-induced excitotoxicity, energy failure and oxidative stress in rats," *Brain Research*, vol. 1239, pp. 226–234, 2008.

[19] A. Agouni, A. H. Lagrue-Lak-Hal, H. A. Mostefai et al., "Red wine polyphenols prevent metabolic and cardiovascular alterations associated with obesity in Zucker fatty rats (Fa/Fa)," *PLoS One*, vol. 4, no. 5, article e5557, 2009.

[20] C. Luceri, G. Caderni, A. Sanna, and P. Dolara, "Red wine and black tea polyphenols modulate the expression of cycloxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced f344 rat colon tumors," *The Journal of Nutrition*, vol. 132, no. 6, pp. 1376–1379, 2002.

[21] G. de Gaetano, A. De Curtis, A. di Castelnuovo, M. B. Donati, L. Iacoviello, and S. Rotondo, "Antithrombotic effect of polyphenols in experimental models: a mechanism of reduced vascular risk by moderate wine consumption," *Annals of the New York Academy of Sciences*, vol. 957, no. 1, pp. 174–188, 2002.

[22] A. B. Santhakumar, A. C. Bulmer, and I. Singh, "A review of the mechanisms and effectiveness of dietary polyphenols in reducing oxidative stress and thrombotic risk," *Journal of Human Nutrition and Dietetics*, vol. 27, no. 1, pp. 1–21, 2014.

[23] M. Diebolt and R. Andriantsitohaina, "Wine polyphenols modulate calcium handling in rat aorta: involvement of nitric oxide pathway," *Fundamental & Clinical Pharmacology*, vol. 16, no. 4, pp. 289–296, 2002.

[24] O. Pechanova, I. Bernatova, P. Babal et al., "Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension," *Journal of Hypertension*, vol. 22, no. 8, pp. 1551–1559, 2004.

[25] A. Puzserova, Z. Csizmadiova, R. Andriantsitohaina, and I. Bernatova, "Vascular effects of red wine polyphenols in chronic stress-exposed Wistar-Kyoto rats," *Physiological Research*, vol. 55, Supplement 1, pp. S39–S47, 2006.

[26] H. A. Mostefai, F. Meziani, M. L. Mastronardi et al., "Circulating microparticles from patients with septic shock exert protective role in vascular function," *American Journal of Respiratory and Critical Care Medicine*, vol. 178, no. 11, pp. 1148–1155, 2008.

[27] A. Agouni, A. H. Lagrue-Lak-Hal, P. H. Ducluzeau et al., "Endothelial dysfunction caused by circulating microparticles from patients with metabolic syndrome," *The American Journal of Pathology*, vol. 173, no. 4, pp. 1210–1219, 2008.

[28] A. Agouni, P. H. Ducluzeau, T. Benameur et al., "Microparticles from patients with metabolic syndrome induce vascular hypo-reactivity via Fas/Fas-ligand pathway in mice," *PLoS One*, vol. 6, no. 11, article e27809, 2011.

[29] H. Maamoun, M. Zachariah, J. H. McVey, F. R. Green, and A. Agouni, "Heme oxygenase (HO)-1 induction prevents endoplasmic reticulum stress-mediated endothelial cell death and impaired angiogenic capacity," *Biochemical Pharmacology*, vol. 127, pp. 46–59, 2017.

[30] Z. Safiedeen, I. Rodriguez-Gomez, L. Vergori et al., "Temporal cross talk between endoplasmic reticulum and mitochondria regulates oxidative stress and mediates microparticle-induced endothelial dysfunction," *Antioxidants & Redox Signaling*, vol. 26, no. 1, pp. 15–27, 2017.

[31] E. G. Bligh and W. J. Dyer, "A rapid method of total lipid extraction and purification," *Canadian Journal of Biochemistry and Physiology*, vol. 37, no. 8, pp. 911–917, 1959.

[32] A. Barrans, X. Collet, R. Barbaras et al., "Hepatic lipase induces the formation of pre-beta 1 high density lipoprotein (HDL) from triacylglycerol-rich HDL2. A study comparing liver perfusion to in vitro incubation with lipases," *The Journal of Biological Chemistry*, vol. 269, no. 15, pp. 11572–11577, 1994.

[33] E. M. Brooks-Asplund, A. A. Shoukas, S. Y. Kim, S. A. Burke, and D. E. Berkowitz, "Estrogen has opposing effects on vascular reactivity in obese, insulin-resistant male Zucker rats," *Journal of Applied Physiology*, vol. 92, no. 5, pp. 2035–2044, 2002.

[34] Y. Ouchi, S. Z. Han, S. Kim et al., "Augmented contractile function and abnormal Ca^{2+} handling in the aorta of Zucker obese rats with insulin resistance," *Diabetes*, vol. 45, Supplement 3, pp. S55–S58, 1996.

[35] E. Scoditti, N. Calabriso, M. Massaro et al., "Mediterranean diet polyphenols reduce inflammatory angiogenesis through MMP-9 and COX-2 inhibition in human vascular endothelial cells: a potentially protective mechanism in atherosclerotic vascular disease and cancer," *Archives of Biochemistry and Biophysics*, vol. 527, no. 2, pp. 81–89, 2012.

[36] H. Taleb, R. K. Morris, C. E. Withycombe, S. E. Maddocks, and A. D. Kanekanian, "Date syrup-derived polyphenols attenuate angiogenic responses and exhibits anti-inflammatory activity mediated by vascular endothelial growth factor and cyclooxygenase-2 expression in endothelial cells," *Nutrition Research*, vol. 36, no. 7, pp. 636–647, 2016.

[37] E. H. Kim, H. K. Na, D. H. Kim et al., "15-Deoxy-Delta 12,14-prostaglandin J2 induces COX-2 expression through Akt-driven AP-1 activation in human breast cancer cells: a potential role of ROS," *Carcinogenesis*, vol. 29, no. 4, pp. 688–695, 2008.

[38] P. Roy, J. George, S. Srivastava, S. Tyagi, and Y. Shukla, "Inhibitory effects of tea polyphenols by targeting cyclooxygenase-2 through regulation of nuclear factor kappa B, Akt and p53 in rat mammary tumors," *Investigational New Drugs*, vol. 29, no. 2, pp. 225–231, 2009.

[39] X. Terra, J. Valls, X. Vitrac et al., "Grape-seed procyanidins act as antiinflammatory agents in endotoxin-stimulated RAW 264.7 macrophages by inhibiting NFkB signaling pathway," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 11, pp. 4357–4365, 2007.

[40] M. O. Kane, N. Etienne-Selloum, S. V. Madeira et al., "Endothelium-derived contracting factors mediate the Ang II-induced endothelial dysfunction in the rat aorta: preventive effect of red wine polyphenols," *Pflügers Archiv*, vol. 459, no. 5, pp. 671–679, 2009.

[41] I. Willenberg, A. K. Meschede, F. Gueler, M. S. Jang, N. Shushakova, and N. H. Schebb, "Food polyphenols fail to cause a biologically relevant reduction of COX-2 activity," *PLoS One*, vol. 10, no. 10, article e0139147, 2015.

[42] T. C. Moon, H. S. Hwang, Z. Quan et al., "Ochnaflavone, naturally occurring biflavonoid, inhibits phospholipase A2 dependent phosphatidylethanolamine degradation in a CCl4-induced rat liver microsome," *Biological & Pharmaceutical Bulletin*, vol. 29, no. 12, pp. 2359–2361, 2006.