UNIVERSITAT DE BARCELONA

Dietary (poly)phenols intake and cardiometabolic health in adolescents from Spain

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FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

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DIETARY (POLY)PHENOL INTAKE AND CARDIOMETABOLIC HEALTH IN ADOLESCENTS FROM SPAIN.

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Dra. Rosa M. Lamuela Raventós i Dra. Anna Tresserra Rimbau (directores)

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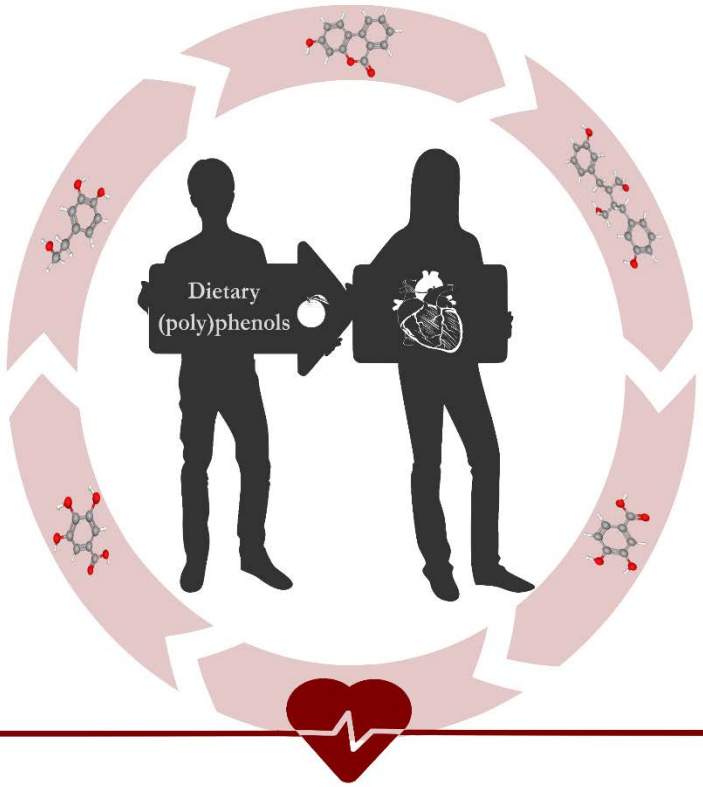
Abbreviations and acronyms

B	Regression coefficient
BG	Blood glucose
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
CVDs	Cardiovascular diseases
CVH	Cardiovascular health
CVRF	Cardiovascular risk factors
DBP	Diastolic blood pressure
ESI	Electrospray
FFAs	Free fatty acids
FFQ	Food frequency questionnaires
FM	Fat mass
FMI	Fat mass index
GAE	Gallic acid equivalent
HDL-c	High-density lipoprotein cholesterol
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
IL-6	Interleukin 6
LC	Liquid chromatography
LDL-c	Low-density lipoproteins cholesterol
LOD	Limit of detection
LOQ	Limit of quantification
MAP	Mean arterial blood pressure
MetS	Metabolic syndrome
MS/MS	Tandem mass spectrometry
MSn	Multi-stage mass analysis
OR	Odds ratio
PAI1	Plasminogen activator inhibitor-1
QqQ	Triple quadrupole
QTrap	Triple-quadrupole ion trap
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Structural equation model
SI!	Salud integral!
T2DM	Type 2 diabetes mellitus

TC	Total cholesterol
TG	Triglycerides
TNF	Tumor necrosis factor
TPE	Total (poly)phenol excretion
UHPLC	Ultra high-performance liquid chromatography
UM-0	Urolithin nonproducers
UM-A	Urolithin metabotypes A
UM-B	Urolithin metabotypes B
VLDL	Very low-density lipoprotein
WC	Waist circumference
WHtR	Waist-to-height ratio



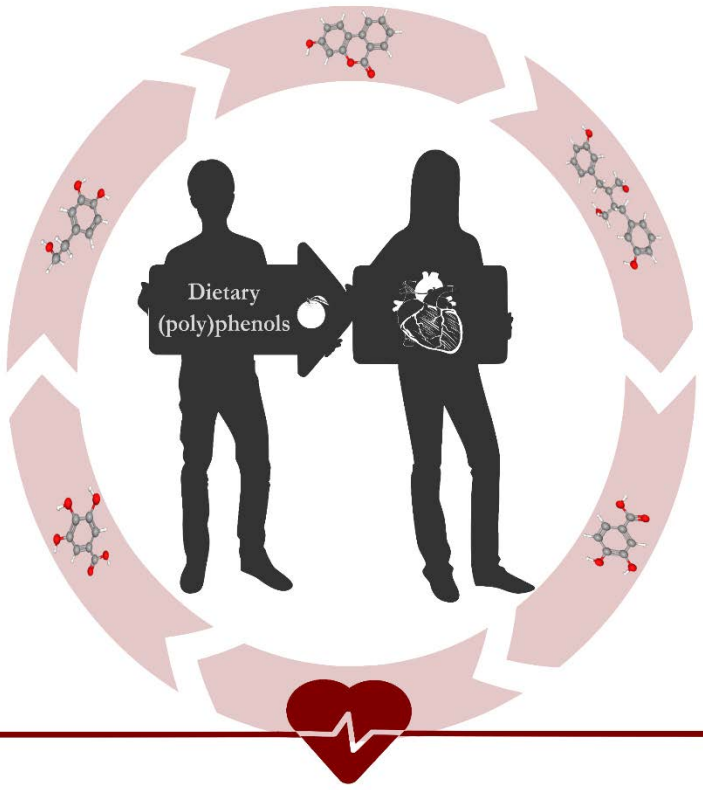
ABSTRACT

Abstract

The rising of cardiometabolic risk factors like obesity, higher blood pressure, and alteration in the metabolism of lipids and glucose during adolescence increase the risk of type 2 diabetes mellitus, cardiovascular diseases, and all-cause mortality in adulthood. Several observational studies and clinical trials conducted in adults have shown the role of (poly)phenols improving cardiometabolic health parameters, even in individuals with high risk of cardiovascular disease. To date, there are very few studies about (poly)phenols and cardiometabolic health in adolescents, one of them is the HELENA study. Additionally, the diversity and the complexity of (poly)phenols lead to difficulties in their dietary estimation in epidemiological studies. Most of the studies evaluated dietary (poly)phenols in adolescents based on dietary tools and rarely used biomarkers in biological samples.

This thesis aimed to study the role of dietary (poly)phenols, estimated using different approaches, on cardiometabolic health parameters in a cohort of 1326 adolescents aged 11 to 14 years old enrolled at baseline in the SI! Program for secondary schools in Spain. Overall, our findings suggested that among adolescents: i) dietary (poly)phenols estimated by food frequency questionnaire are related to improved cardiometabolic and adiposity parameters, ii) Total (poly)phenols in urine analyzed by the Folin-Ciocalteu spectrophotometric method and used as a marker of (poly)phenol intake, was inversely associated with blood pressure and lipid profile, and with healthier cardiovascular health., iii) A novel and accurate analytical method to identify and quantify 54 urinary phenolic metabolites (mainly microbial phenolic metabolites) even aglycones and phase II metabolites through liquid chromatography coupled to mass spectrometer was developed enterolignans (enterodiol, enterolactone, and their derivatives) and urolithins (A and B and their derivatives) were identified as candidate markers of whole grains and nuts intake, respectively, iv) some phenolic metabolites (gallic acid, urolithin A and B) were associated with better cardiometabolic health and with lower probability of having metabolic syndrome.

The results of this thesis suggest that higher dietary (poly)phenols measure through food frequency questionaries or in urine were associated with better cardiometabolic health, specifically body weight/composition, blood pressure, blood glucose, and lipid profile; in adolescents from a Spanish cohort.



INTRODUCTION

1. INTRODUCTION

1.1. Cardiometabolic health in adolescents

Cardiovascular diseases (CVDs), particularly ischemic heart disease and stroke, are the leading cause of global death, contributing to disability and increasing health care costs (1). The number of CVDs deaths is rising alarmingly up from 12.1 million in 1990 to 18.6 million in 2019 (1). In Europe, CVDs are still a major cause of morbidity and mortality, reaching 4 million deaths each year, accounting for 45% of all deaths in adults (2). The main causes of CVD can be classified into non-modifiable risk factors such as age, sex, family history of CVD risk, and modifiable risk factors. According to European Heart Association, the main causal and modifiable CVD risk factors included dyslipidemia, high blood pressure (BP), cigarette smoking, diabetes mellitus, and adiposity (3).

It is documented that metabolic syndrome (MetS) in adults is a predictor of CVD (2- to 4-fold higher), type 2 diabetes mellitus (T2DM), and all-cause mortality (4–7). MetS consists of a combination of cardiovascular risk factors including impaired glucose metabolism, abdominal obesity, dyslipidemia (increased triglycerides (TG) and decreased high-density lipoprotein cholesterol (HDL-c)), and/or elevated BP (8,9). The pathogenesis of MetS is complex and many aspects are still not fully understood, but the most accepted hypothesis to describe pathology of MetS is insulin resistance. The molecular pathways of insulin resistance are explained by defects between the union of insulin with its receptor, where obesity and sedentary lifestyles combined with an unhealthy diet and still unknown genetic factors interact to produce this metabolic condition (10) (**Figure 1**).

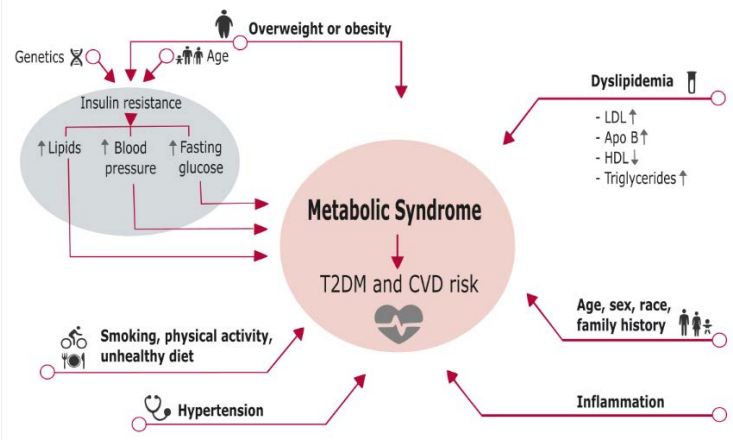


Figure 1. Factors contributing to MetS. Adapted from Kahn *et al.* (11)

Because of the increasing rates of overweight and obesity, unhealthy eating behavior, and sedentary lifestyle worldwide, the MetS is raising in adults but also in young populations as adolescents (12,13). According to a recent study published in the Lancet, around 35.5 million (5%) adolescents aged 13 to 18 years old from 44 countries in 13 regions worldwide had MetS in 2020 (12). Although the prevalence of MetS in adolescents is low compared to adults (up to 31%), having MetS at early life stage has serious metabolic consequences in adulthood. Thus, youth with MetS and some combination of MetS components could have 2-3 times higher risk of developing T2DM and subclinical atherosclerosis in adulthood (14–16). The lack of a consensus about the MetS definition is the main problem of MetS in the pediatric population (17). Thus, at this time, more than thirty definitions are described in the literature with substantial different MetS diagnostic criteria (18), and the most reported are described in **Table 1**. Depending on each definition used, the prevalence of MetS in children and adolescents has a large variation, from 0.3 to 26.4% (19).

Table 1. MetS diagnostic criteria in children and adolescents. Adapted from Reisinger *et al.* (19)

Ref.	Definition	Abdominal obesity (WC, cm)	High BP (mm Hg)	Dyslipidemia (mg/dL)	High BG (mg/dL)
Zimmet *et al.* (20)	Abdominal obesity plus 2 of 4 criteria	**10–15 years of age** WC ≥ 90th percentile **>15 years of age** WC ≥94 (♂) WC ≥ 80 (♀)	SBP ≥ 130 or DBP ≥ 85	TG ≥150 HDL-c <40 10–15 years of age HDL < 40 (♂) <50 (♀)	≥100 or diagnosis of T2DM
Cook *et al.* (21)	3 or more of 5 criteria	WC ≥ 90th percentile	≥90th percentile	TG ≥ 110 HDL-c ≤ 40	≥110
Ford *et al.* (22)	3 or more of 5 criteria	WC ≥ 90th percentile	≥90th percentile	TG ≥ 110 HDL-c ≤ 40	≥110
de Ferrantei *et al.* (23)	3 or more of 5 criteria	WC ≥ 75th percentile	≥90th percentile	TG ≥ 100 HDL-c ≤ 50	≥110

♂ boys, ♀ girls, BG blood glucose, BP blood pressure, DBP diastolic blood pressure, HDL-c high-density lipoprotein cholesterol, SBP systolic blood pressure, T2DM type 2 diabetes mellitus, TG triglycerides, WC waist circumference.

Due to the increase and the large variation in the prevalence of MetS in children and adolescents, some epidemiological researchers have proposed evaluating continuous MetS score (cMetS) as an index for modeling MetS in pediatric population (24). Several statistical approaches have been applied to calculate cMetS, like principal components analysis, centiles rankings, and so on (25–27). Hesmant *et al.* validated a cMetS, like the sum of standardized residuals of cardiometabolic risk variables: WC, mean arterial blood pressure (MAP), HDL-c, TG, and blood glucose (BG), resulting in an accurate predictor of MetS in 3843 Iranian children and adolescents aged 7-18 years old (24).

1.1.1. Obesity and cardiometabolic complications

As mentioned before, obesity in adolescence, especially abdominal obesity, is the main contributor to the severity of MetS, T2DM, and CVD (14). Obesity is characterized by an excess of adipose mass. The excess of visceral adipose mass increases the growth and the number of adipocytes, disrupting the normal lipid accumulation in tissues such as muscle and liver, and elevating free fatty acids (FFAs). Higher FAAs levels in plasma reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. The high circulating glucose and FFAs increase pancreatic insulin secretion resulting in hyperinsulinemia. Thus, when insulin resistance develops, it facilitates the flow of FFAs from adipose tissue to the liver, there is a reduction in glucose transformation to glycogen, and elevation of synthesis of TG, very low-density lipoprotein (VLDL), and apolipoproteins (C-II, C-III, and B-100). The higher and abnormal production of TG increases lipid accumulation in the liver and muscle, but also elevates circulating TG levels. Hypertriglyceridemia modifies the composition of lipoproteins, decreases HDL-c, and increases low-density lipoproteins cholesterol (LDL-c) levels. LDL-c is transformed into a smaller particle, with a higher atherogenic capacity. The adipocyte is also an endocrine cell, that releases many molecules like cytokines and hormones. In obesity, adipose tissue exhibits abnormalities in the production of these molecules affecting insulin resistance. For example, there is a high production of inflammatory cytokines such as tumor necrosis factor alfa (TNF α), interleukin 6 (IL-6), C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI1), and lower production of protective hormones like adiponectin, which regulates glucose levels and fatty acid metabolism. Additionally, obesity is associated with changes in adipocyte gen expression spanning, for example, it promotes the overexpression of angiotensinogen gen increasing angiotensin II, and probably contributing to the appearance of hypertension (28) (**Figure 2**).

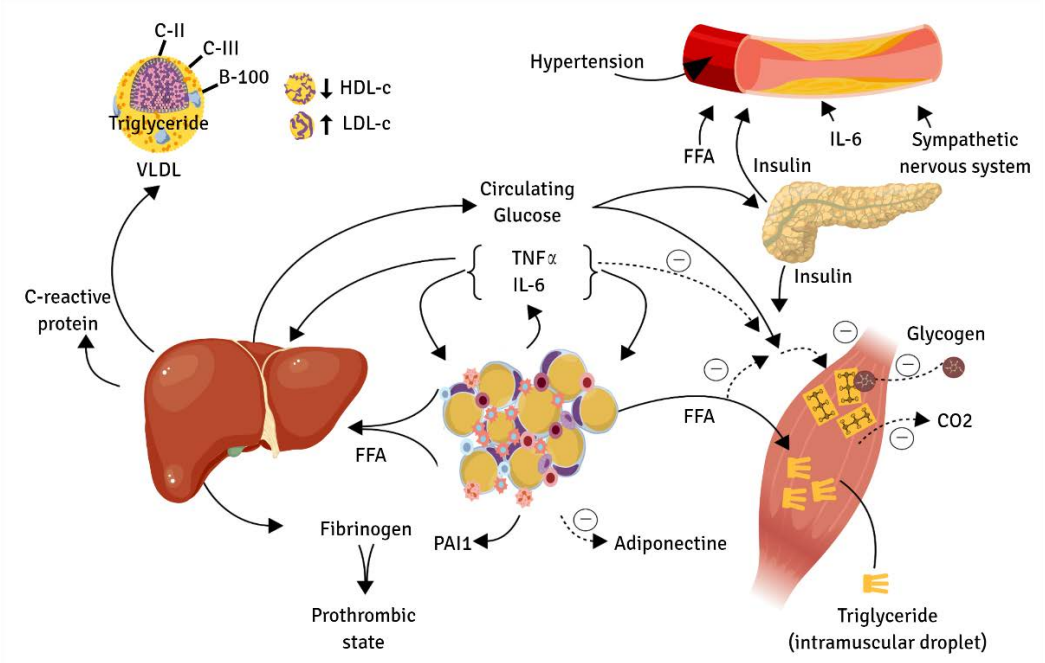


Figure 2. Physiopathology of obesity and MetS. The excess adipose tissue elevates free fatty acids (FFAs), inflammatory cytokines such as tumor necrosis factor alfa (TNF α), interleukin 6 (IL-6), C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI1), and decreases adiponectin. In the muscle, a higher circulating FAAs reduces insulin sensitivity. Elevated FFAs increase pancreatic insulin secretion resulting in hyperinsulinemia. Thus, when insulin resistance develops, there is a high flow of FFAs from adipose tissue to the liver, reducing glucose transformation into glycogen, and increasing the synthesis of triglycerides, very-low-density lipoprotein (VLDL), apolipoproteins (C-II, C-III, and B-100), reducing high-density lipoprotein cholesterol (HDL-c) and elevating low-density lipoprotein cholesterol (LDL-c). Adapted from Eckel *et al.* (28).

From 1975 to 2016, obesity in children and adolescents, measured by body mass index (BMI), has increased worldwide, from 5 to 50 million in girls and from 6 to 74 million in boys (29). In Spain, a recent study showed that the prevalence of overweight and obesity trends in 2.5 million children and adolescents decreased from 2005 to 2017 (30). However, this trend varies depending on sex, age, and region. In general, boys have a higher prevalence of obesity than girls. However, regarding change prevalence, in adolescents aged from 12 to 17 years, the prevalence of obesity increased in girls (mainly in Aragon, Cantabria, Catalonia, and Navarra) and decreased in boys, as observed in **Figure 3.**

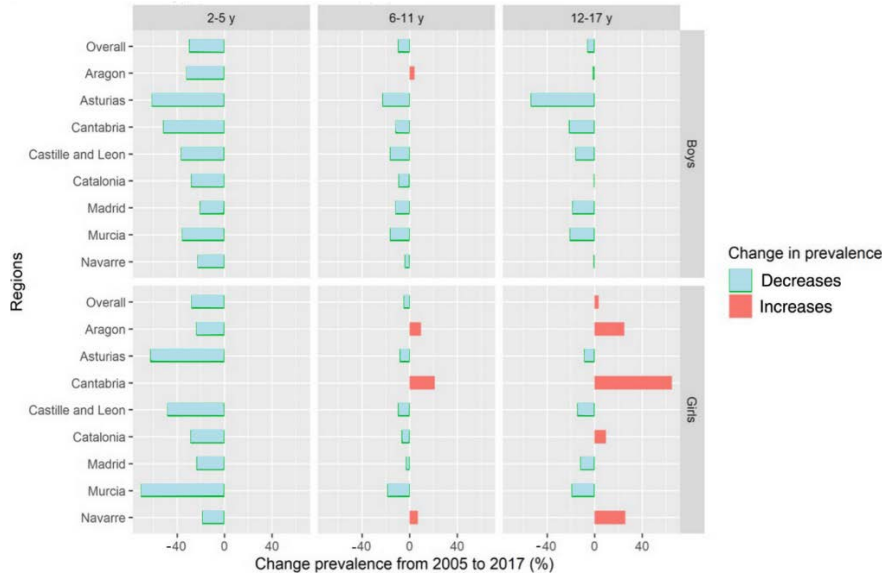


Figure 3. Percent change in the prevalence of obesity in Spain from 2005 to 2017 according to age, sex, and region. Bont *et al.* (30).

Lifestyles are acquired in early life stages, and they persist from childhood to adulthood (31). For this reason, childhood and adolescence are critical periods for the adoption of food preferences and other key behaviors associated with a healthy diet and physical activity, to promote better cardiovascular health in adulthood (31,32). Preliminary evidence reports an inadequate dietary pattern amongst adolescents in Spain, characterized by lower intake of fruits and vegetables resulting in lower Mediterranean diet adherence, higher intake of high-fat meals, sugar, in addition to unhealthy choices such as breakfast skipping, eating at fast-food restaurants, intake of processed foods and sweets (32,33).

1.1.2. Strategies to improve cardiometabolic health in adolescents

It is well documented that interventions based on healthy dietary behaviors and active physical activity are the best way to reduce the prevalence of obesity and improve cardiometabolic health in a young population (34). Adolescence is a key period of biological, social, and behavioral changes that will be essential for adopting a healthy lifestyle and eating habits in adulthood (35). However, dietary behaviors depend on multiple complex factors which need to be considered, like food preferences, parental education, family income, and nutritional knowledge (36). Lifestyle habits, such as watching television for more than two hours, also influence food consumption patterns. Each of these individual, environmental, and sociocultural factors represent a potential barrier, but also an opportunity to

promote healthy habits to improve cardiometabolic health (36). Therefore, identifying each of these determinants will be important in the implementation of programs to improve eating habits and lifestyle in the community (36).

In this context, during the last years, Spanish public schools have promoted the implementation of health education systems to increase physical activity and improve eating patterns in children and adolescents, as well as their families and school environment, through the SI! (Salud Integral) Program (31,37). The interventions in schools are probably the best way to promote healthy lifestyles at a low cost and with greater effectiveness (31,35,37). The SI! Program is an educational intervention that aims to establish healthy lifestyles at an early age stage, intervening in four basic interrelated components: the human body, physical activity, diet, and the management of emotions, the latter aimed at preventing the use of drugs like tobacco and alcohol (31,37). Based on scientific evidence, the SI Program! seeks to promote physical activity, healthy eating, reducing sedentary leisure time, and avoiding drug or alcohol consumption, through increased knowledge and changing attitudes and habits together with the control of emotions, with the aim of reducing body weight and visceral adiposity parameters, as well as improving metabolic profiles and cardiovascular health (31,37) (**Figure 4**). This intervention is aimed at secondary school students and is based on the adolescent's potential to have a positive impact on their health, which will be reinforced by the family and school environment (37). The design of this intervention is based on the success of this strategy implemented in preschool children aged 3 to 5 years in Spain, proving to be effective in adopting healthy habits (31).

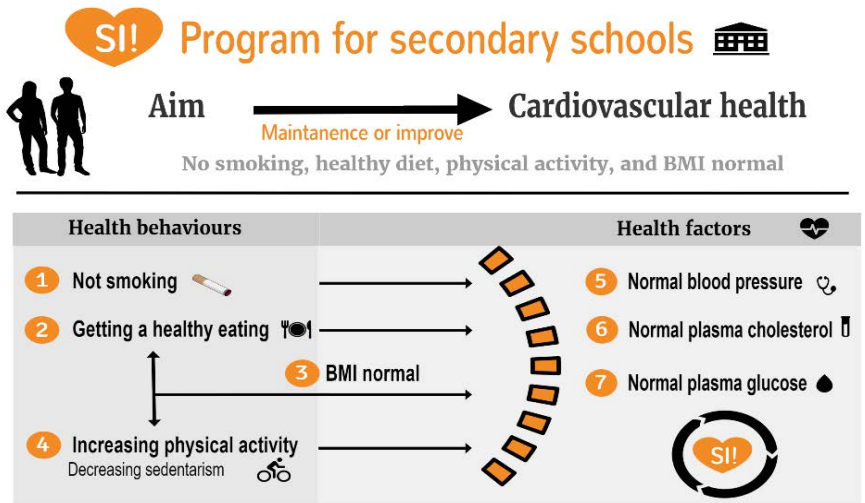


Figure 4. Design of the Si! Program for secondary schools in Spain. Adapted from Fernández-Jiménez *et al.* (37)

The implementation of the SI! Program in adolescents from secondary schools in Madrid and Barcelona ended in 2021, and currently, the effect of the intervention is ongoing publish. Details of the data recruitment are described by Fernandez-Jimenez, R. et al (37), and in the method section of the present thesis.

1.2. Dietary (poly)phenols

(Poly)phenols constitute a group of bioactive compounds or phytochemicals of very great structural diversity and are largely widespread in the plant kingdom. (Poly)phenols are secondary metabolites produced by plants, through complex shikimic acid and malonic acid pathways, as a response to environmental and ecological challenges (38).

1.2.1. Structure and classification of dietary (poly)phenols

(Poly)phenols represent the largest group of phytochemicals, with several thousand different phenolic structures identified in plants to date. (Poly)phenols are characterized by having at least one phenyl aromatic hydrocarbon ring carrying one or more hydroxyl groups. According to aglycone chemical structure, (poly)phenols can be classified into five main groups: flavonoids, phenolic acids, stilbenes, lignans, and "others" such as coumarins, tyrosols, etc. (38). The basic chemical structure of (poly)phenol is an aglycone, and it is rarely found in foods. Generally, (poly)phenols in foods are linked to glycosides (glucose, rhamnose, rutin), esters or in their polymeric form.

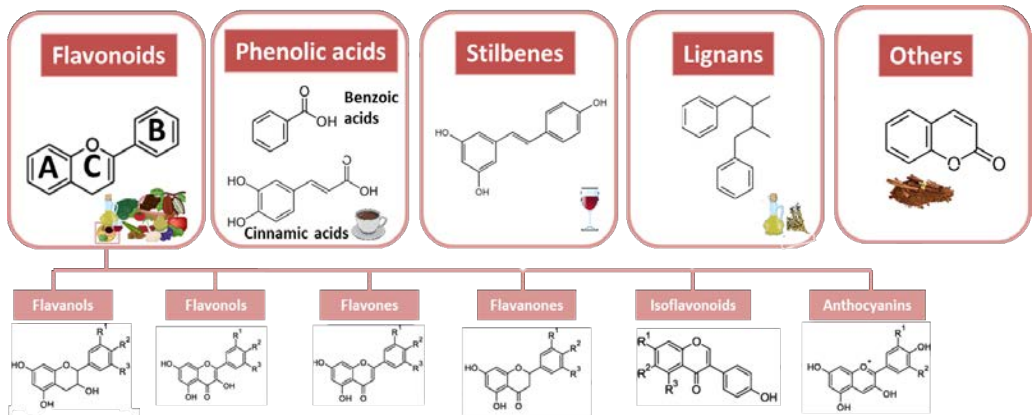


Figure 5. Classification of dietary (poly)phenols. Adapted from Hano *et al.* (39)

Flavonoids are the main group of (poly)phenols, with more than 4000 different compounds identified in plants (38). The basic chemical structure of flavonoids consists of two six-carbon phenyl aromatic hydrocarbon rings (ring A and ring B)

that are bound by one heterocyclic ring of three-carbon (ring C) (**Figure 5**). According to the hydroxylation pattern and variation in the heterocyclic ring, flavonoids can be divided into six subclasses: anthocyanins, flavanols or flavan-3-ols, flavonols, flavones, flavanones, and isoflavonoids. In plants, generally, flavonoids are found in their glycosylated form which are formed by an aglycone linked to glycosidic sugars (glucose, galactose, or rhamnose) (38,40). Anthocyanins are compounds responsible for the red, blue, and purple pigments in fruit (berries, pomes, and red wine) and vegetables (onion, leaf vegetables, purple sweet potato), and cyanidin, delphinidin, and pelargonidin are the most identified in foods. Flavanols or flavan-3-ols are a very complex group of (poly)phenols, which include monomers of flavanols (catechin, epicatechin, and their gallates) or polymers of flavanols (proanthocyanidins). Flavones, such as luteolin, apigenin, and baicalein have similar chemical structures to flavonols, except for the lack of oxygenation at ring C. In general, flavones are present in leaf vegetables like celery and parsley. Flavanols are mainly present in tea, berries, red wine, and cocoa-based products. Among flavonols, quercetin, myricetin, isorhamnetin, and kaempferol are the most found in citrus fruits, grapes, onions, and lettuce. Hesperidin and naringenin are the most representative of flavanones, and they are present in citrus fruits. Isoflavonoids such as genistein and daidzein are present in soy and soy products and are classified as phytoestrogens due to their similar chemical structure to estrogen (**Table 2**) (41).

Phenolic acids are the most abundant non-flavonoids in plants and are divided into hydroxybenzoic acids (e.g., gallic acid, vanillic, and syringic acids) and hydroxycinnamic acids (e.g., *m*-coumaric, *o*-coumaric, *p*-coumaric, ferulic, chlorogenic acids), both characterized by having at least one phenyl aromatic hydrocarbon ring and organic carboxylic acid in their chemical structure, as can be seen in **Figure 5** (38,39). Depending on their soluble or bound form, phenolic acids can be found in the endoplasmic reticulum and preserved in the vacuoles of cell plants or found in the cell wall conjugated with glycosides and ester bonds (38,39). These phenolic compounds are widely distributed in fruits (apples, cherries, berries, wine), vegetables (broccoli, lettuce, and tomatoes), grains, seeds, legumes, coffee, etc. (**Table 2**).

There are other non-flavonoids relevant to human health that can be found in plants, although they are not widespread in plant foods. Among these, stilbenes and lignans are the most representative. Stilbenes, being resveratrol the main contributor, are found in grapes and red wine but in low quantities. Lignans are recognized as phytoestrogens and are found in the bound forms in flax, sesame, and

many grains and tyrosols are mainly present in olive and extra virgin olive oil (**Table 2**).

Table 2. (Poly)phenol food sources.

(Poly)phenol subclasses	Food sources
Flavonoids	
• **Anthocyanins**	Berries, drupes, pomes, fig, fruit juices, nuts, pulses, olives, leaf vegetables, onion, red wine, purple sweet potato.
• **Flavanols or Flavan-3-ols**	
• **Catechins**	Cocoa and cocoa products, pomes, cereals, berries, gourds, fruit juices, nuts, pulses, wine, beer, ciders, tea.
• **Proanthocyanidins**	Cocoa and cocoa products, cereals, berries, cherries, pomes, drupes, tropical fruits, nuts, red wine.
• **Theaflavin**	Tea
• **Flavanones**	Citrus fruit
• **Flavones**	Herbs, spices, whole-grain cereals, citrus fruit (orange), olive.
• **Flavonols**	Spices, herbs, nuts, berries, olive, leaf vegetables, pulses, cabbages, onion, whole-grain cereals, tea (black).
• **Isoflavonoids**	Soy and soy products.
Phenolic acids	
• **Hydroxybenzoic acids**	Nuts, berries, tropical fruit, leaf vegetables, cocoa, soy products, herbs, tea.
• **Hydroxycinnamic acids**	Herbs, berries, cereals, dried fruits, coffee beverage, drupes, pomes, berries, tropical fruits, drupes, tuber (potato).
• **Hydroxyphenylacetic acids**	Olives, olive oil
• **Hydroxyphenylpropanoic acids**	Olives
Stilbenes	Red wine, berries, nuts
Lignans	Seeds oils, cabbages (kale, broccoli), nuts, drupes
Other (poly)phenols	
• **Alkylmethoxyphenols**	Seed oils (rape seed), beer, coffee beverages
• **Alkylphenols**	Cereals and cereals products, coffee beverages, and cocoa
• **Furanocoumarins**	Stalk vegetables (Celery), herbs (parsley), citrus juice
• **Hydroxybenzaldehydes**	Nuts, red wine, cocoa powder, olives.
• **Naphtoquinones**	Nuts
• **Tyrosols**	Olives, olive oil
• **Curcuminoids**	Spices

Data obtained from Phenol Explorer version 3.6

1.2.2.Factors that could influence (poly)phenol content in foods

Agricultural and technological factors have shown to influence the content of (poly)phenols in plants. The main agricultural factors include genetic diversity, environment (seasonal variability, water availability, sunlight, and temperature), soil fertilization, irrigation system, pest/microbial stress, and ripening stage (42). Although most of these factors are controlled by the grower, climate change has an impact on the phenolic content. For example, studies show that sun exposure can increase anthocyanins and flavonols (like quercetin) in some plants (40,42).
Technological factors include all post-harvesting treatments, such as cleaning, storage, antimicrobial treatments, and food processing. During the storage, there is a gas generation that can modulate the synthesis of some (poly)phenol subclasses. Food processing, mainly mechanical, thermal, and non-thermal treatments, affects the content and profile of dietary (poly)phenols (42,43). Drying, grinding, and heat treatments cause (poly)phenol losses in foods. Meanwhile, encapsulation enhances (poly)phenol bioavailability. (Poly)phenols are susceptible to changes during cooking techniques (boiling, frying, steaming, baking, stewing, roasting, and microwave), mainly by the degradation by heat, oxidation, isomerization, and release of (poly)phenols by rupture of cell walls (44).

1.2.3. Bioavailability of dietary (poly)phenols and the role of the gut microbiota

The human biological health effect of dietary (poly)phenols depends on the quantity consumed and their absorption, distribution, metabolism, and elimination (45). Briefly, (poly)phenol bioavailability involved the following processes (**Figure 6**): 1) release of (poly)phenols from the food matrix; 2) changes in (poly)phenols during gastric/small intestinal digestion; 3) cellular uptake of aglycons and some conjugated (poly)phenols by enterocytes; 4) microbial phenolic metabolism of non-absorbed (poly)phenols; 5) phase I/II enzyme modification in the small intestine or colon; 6) transport of (poly)phenols in the bloodstream and tissue redistribution; 7) (poly)phenol excretion via the kidney, mammalian glands, or re-excretion into the gut via bile (46,47).

In foods, most of the (poly)phenols are present as glycosides, esters, and polymers, the latest characterized by their high molecular weight and poor bioavailability (48,49). Digestion begins in the oral cavity with the reduction of particle size and the enzymatic action of amylase, enhancing the process during the following stages of digestion. Due to the short interaction time, the impact of enzymatic digestion on (poly)phenol release is low (46,47).

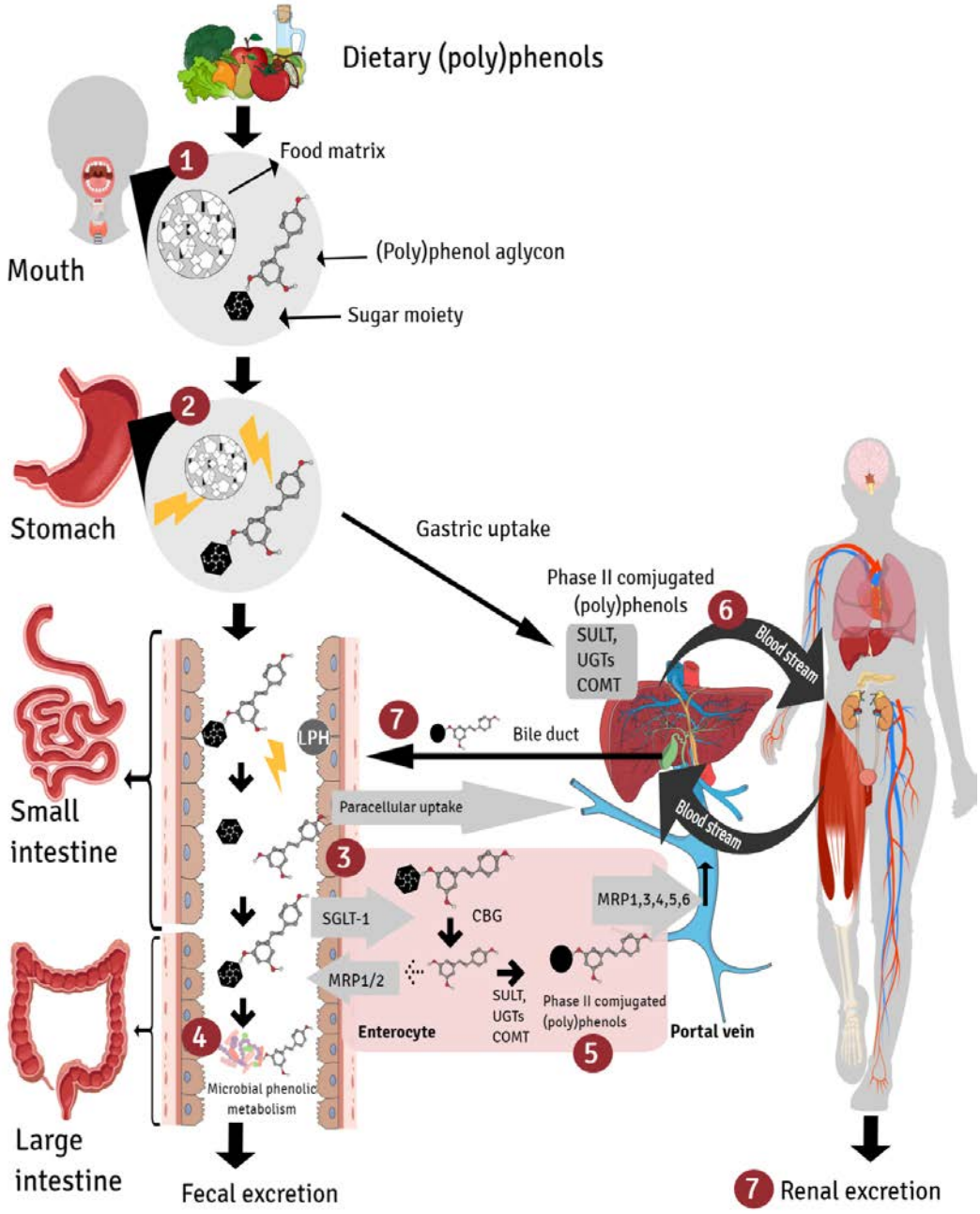


Figure 6. (Poly)phenol bioavailability. Adapted from Bohn and Del Rio *et al.* (41,47)

CBG, cytosolic β-glucosidase; COMT, catechol-O-methyl transferase; LPH, lactase-phlorizin hydrolase; MRP, multidrug resistance proteins; SGLT-1, sodium-glucose linked transporter 1.

In the stomach, pepsin reaction, peristaltic movement, and the low pH decrease particle size, promoting the release of (poly)phenols (47). According to some authors, anthocyanins, isoflavonoids aglycones, and some phenolic acids (caffeic acid, gallic acid, and chlorogenic acid) can be absorbed in this gastric phase, due to the rapid postprandial appearance in plasma (47,50–52).

In the small intestine, aglycones can be absorbed. However, more than 80% of dietary (poly)phenols (mainly in their glycoside form) are poorly absorbed in the small intestine and pass to the large intestine, which are metabolized by gut microbiota through enzymatic reactions (deglycosylation, dehydroxylation, demethylation, deconjugation, epimerization, ring fission, hydrolysis, and chain-shortening reaction) and transformed into lower molecular weight compounds, namely microbial phenolic metabolites. During the absorption, (poly)phenols are conjugated in the small intestine and later in the liver, following phase I (e.g. reduction/oxidation, methylation, hydroxylation, hydrolysis, e.g. via cytochrome P450-dependent mixed-function oxidases and catechol-O-methyl-transferase) and phase II metabolism (e.g. glucuronidation by uridine-5'-diphosphate glucuronosyltransferase, and sulfation via sulfotransferases). This process depends on the nature of the substrate and dose of each phenolic. When (poly)phenols reach the systematic circulation, they are rapidly eliminated from plasma, distributed to different target tissues, or excreted through biological fluids, like urine, bile, and breast milk. Excretion of phenolic compounds varies according to molecular weight; thus, heavy compounds are usually eliminated through bile, meanwhile, molecules with lower weight are eliminated frequently through urine (53). Since dietary (poly)phenols are extensively modified, and the forms (mainly glucuronide and sulfate) that appear in human samples are usually different from the precursor (poly)phenol found in foods, greater attention should be focused on exploring the potential biological activity of (poly)phenol metabolites, especially metabolites derived from gut microbiota. Some microbial phenolic metabolites could have more bioactive effects than their native or parental (poly)phenols, as is the case of lignans, which are metabolized to enterolactone and enterodiol, or ellagitannins metabolized in urolithins, which act as stronger phytoestrogens than their parental compound (45,49,54,55). Given the above, details of the bioavailability of (poly)phenols and the role of gut microbiota on human health are available in **Article 1**.

The bioavailability of dietary (poly)phenols differs among individuals. This interindividual variation could be explained by different factors: the composition of gut microbiota, genetic polymorphisms in the expression of enzymes implicated in phase I/II metabolism, intake of alcohol, smoking, age, sex, and the synergistic

effects of the mixture of (poly)phenols and other nutrients present in the diet, as well as the food matrix (56,57). A positive effect of sugars on (poly)phenol glucoside uptake has been suggested (46). Thus, a green tea formulation with sucrose, ascorbic acid, or citrus juices protected catechins degradation and improved their bioavailability (58). Similarly, chocolate products with sucrose increased plasmatic levels of catechins and epicatechins (59). On another hand, dietary lipids enhance apolar (poly)phenol absorption by the formation of mixed micelles, thus, enhancing its transepithelial transport (46,47). Tuck *et al.* showed that absorption of tyrosol and hydroxytyrosol increased after the administration of olive oil (lipid-rich matrix) (60). By contrast, proteins can negative effect on (poly)phenols bioavailability. However, is difficult to judge due to some contradictions in the studies. Studies with blueberries showed that milk addition reduces the recovery of anthocyanins having a negative effect on the antioxidant activity of blueberries (61). However, studies with cacao powder and milk showed an increase of urinary phenolic acids such as vanillic and phenylacetic acid; and a reduction of protocatechuic, hydroxybenzoic, hydroxyhippuric, hippuric, caffeic, and ferulic acids after the consumption of cacao powder dissolved in milk (62). It is probable that this protein effect depends on type of protein and (poly)phenols (46,47). Dietary fiber increases the intestinal bulk (soluble fiber) and reduces transit time (insoluble fiber), both action that reduces (poly)phenol bioavailability. However, positive effect on (poly)phenol bioavailability has been observed with fermentable fiber such as fructo-oligosaccharides, inulin, and resistant starches, increasing the microbial phenolic metabolism (e.g. quercetin and isoflavones) (63–65). Although, the effect of mineral intake on (poly)phenol bioavailability has not ever been described, (poly)phenols reduce the absorption of iron, zinc, copper, and sodium, due to chelation (47,66).

Health status also has implications on the bioavailability of (poly)phenols. Some (poly)phenols as epigallocatechin-3-*O*-gallate have a high affinity to plasma proteins extending their half-life in the blood (67).

1.2.4.Methods of estimation and quantification of (poly)phenol intake

In epidemiological studies, different methods to assess phenolic intake are used to evaluate the relationship between these compounds and human health, and they can be divided into two groups: dietary tools and biomarker analysis using analytical approaches (68,69).

1.2.4.1. Dietary tools to estimate (poly)phenol intake

The diversity and the complexity of (poly)phenols lead to difficulties to estimate dietary intakes. The most reliable tools for (poly)phenol intake are 24-h or 48-h diet recall, food records/diaries, diet history questionnaires, weight food records, or food frequency questionnaires (FFQ). Each one, including its strengths and limitations, is described in **Table 3**. Among them, FFQ is the most widely dietary assessment tool used to measure food sources of (poly)phenol intake, because of its ability to measure long-term exposure to dietary factors (68,70). These methods are then cross-referenced using available (poly)phenol food composition databases (68). In this thesis, we used the Phenol-Explorer database because it is the most comprehensive database that includes flavonoid and non-flavonoids subclasses of (poly)phenols from more than 400 foods (71). However, in the literature, other databases are also used such as the USDA database for flavonoids, the UK food standards Agency Food composition database on phytoestrogens, etc. (68).

Table 3. Strengths and limitations of (poly)phenol intake commonly used in epidemiological studies. Adapted from Xu *et al.* (70)

Characteristics	Strengths	Limitations	Ability to capture food sources of (poly)phenols
FFQs			
A list with specific food items (10–200+), able to assess long-term intake exposure.	Easy to conduct or self-reported, capture past-long-term intake exposure.	Less able to capture day-to-day variability in diet; lack of specificity foods; prone to misreporting and memory bias.	Able to capture long-term (poly)phenol intake.
24-h/48-h Recall			
Recall food intake in the previous 24 h or 48 h. To evaluate habitual diet, two or more recalls are needed to apply during a long period of time.	Easy to conduct; not restricted to a predefined food list.	High participant burden if conducted multiple times; prone to misreport and memory bias.	More specificity (poly)phenol content; repeat recalls are needed to capture habitual dietary (poly)phenol.
Food records or diaries			
Record of intake for 3, 7 days, etc.; usually assisted with pictures of portion sizes.	Able to identify day-to-day variability after 2 or more records, not restricted to a predefined food list.	Quality of data may decline with the increasing number of days reported. Hand-written food records are inexpensive to collect	More specificity (poly)phenol content; able to capture intake of less common foods.

	It can capture infrequently consumed foods.	but expensive to code.	
Diet history questionnaire			
Structured questionnaire/interview on food intake during a specific period with open-ended questions.	Not restricted to a predefined list of foods; capture past long-term intake exposure.	Standard protocol and training are needed for the interview and coding; prone to misreporting and memory bias.	Able to capture (poly)phenol intake from less common food or infrequently consumed foods.
Weighed food records			
Weigh and record the portion of every food intake for a consecutive period of time.	Accurate in portion size and less memory bias; not restricted to a predefined food list.	High participant burden (need weighing tools and instructions); standard protocol and training are needed for coding.	Able to capture (poly)phenol intake from less common foods; repeat measurement will increase the ability to capture infrequently consumed foods.

1.2.4.2. Analytical method to analyse (poly)phenols in biological samples

Dietary (poly)phenol intake can also be determined by quantifying biomarkers using analytical approaches, which include individual (poly)phenol and their derivatives found in human biological samples (68). Due to the lack of general and standard methods to determine (poly)phenol biomarkers in biological samples, researchers must develop and validate their own method (68). Spectrophotometry, chromatography, and mass spectrometry are the approaches widely used to quantify (poly)phenols in human samples. In this thesis, analytical methods to identify and quantify (poly)phenol in urine were used and therefore explained in this section.

1.2.4.2.1. Spectrophotometric method to quantify of total (poly)phenols in urine

The spectrophotometric Folin-Ciocalteu method adapted to urine is the most rapid and simple method widely used to estimate total (poly)phenol content, which can be used as a biomarker of (poly)phenol intake in large epidemiological studies (72). However, the lack the specificity for individual phenolic compounds is a limitation of this method (38). The Folin-Ciocalteu method is based on the reduction in alkaline medium of a mixture of phosphomolybdic and phosphotungstic acid (Folin-Ciocalteu reagent) by (poly)phenols. In this redox reaction, a series of tungsten and molybdenum oxides are formed giving a blue color that is proportional to the concentration of (poly)phenols. The Folin-Ciocalteu reagent

does not react specifically with (poly)phenols but also with sugars, aromatic amines, sulfur dioxide, ascorbic acid, organic acids, Fe (II), and other non-phenolic but oxidizable organic substances. Therefore, previous urine treatment is needed to remove interference to avoid false readings and thus lead to erroneous results.

1.2.4.2.2. Chromatographic methods to quantify individual (poly)phenol

The development and application of new technologies to determine phenolic compounds and their metabolites in biological samples are increasing. Thus reverse-phase liquid chromatography (LC) coupled with mass spectrometry (LC-MS/MS) with differences in the separation modes have been scarcely applied for the rapid and accurate targeted identification and quantification of individual (poly)phenols in urine, blood, and feces (38,45,73). However, some flavonoids like isoflavones are derivatized to methyl esters and analyzed by gas chromatography (GC), or a normal phase column is applied for the separation and quantification of procyanidins (38). Triple Quadrupole (QqQ) or Triple-Quadrupole Ion Trap (QTrap) with reverse phase chromatography is the most frequently mass spectrometer detectors used in these targeted analyses, due to their high sensitivity and selectivity (73). Otherwise, it has been well-shown that high-resolution mass spectrometry (HRMS), like liquid chromatography coupled with - linear ion trap quadrupole-Orbitrap HRMS (LC-LTQ-Orbitrap HRMS), is extended used for identification of (poly)phenols in nutrimetabolomics studies due to provide exact mass information, two-stage mass analysis (MS/MS), and multi-stage mass analysis (MS^n), facilitates the elucidation of the structures of known and unknown compounds (73–75). In addition to this, with the information obtained in full-scan mode from HRMS, researchers can identify interferences coming from the matrix and prevent false results (76). According to the results of different publications, HRMS is a fully appropriate approach for quantitative LC-MS analysis due to its versatility, speed of data processing, and robustness characteristics (77,78). In this thesis, LC-LTQ-Orbitrap-HRMS approach was used to detect and quantify (poly)phenols in urine, and details of the chromatographic method will be explained in the Method and result section.

1.3. Dietary (poly)phenols and microbial phenolic metabolites and their implication in cardiometabolic health

As mentioned before, dietary intervention is an important strategy to improve cardiometabolic health. In this context, evidence suggests that the consumption of plant-derived foods, characterized by a high content of bioactive compounds like (poly)phenols, is associated with the prevention of CVDs in adults (79–81).

Although (poly)phenol concentration is different in each food, it does not mean that their activity in the body is proportional once ingested (54,55). (Poly)phenols have favorable effects in different ways, including the reduction of adiposity and BP, inhibition of platelet activation, improvement of the lipid profile, and anti-inflammatory effects (inhibition of cytokines production and reducing of adhesion molecule expression), all of them contribute to the reduction of CVD risk and mortality (41,80–85). In a recent meta-analysis based on thirty-nine studies, Micek *et al.* showed that a higher intake of anthocyanins and flavanols was associated with a lower risk of CVD, and flavonols and flavones were associated with a lower risk of coronary heart disease (85). Moreover, a panel of experts in (poly)phenols has developed for the first time a guideline recommendation for dietary flavanols, highlighting the importance of these phenolic compounds in the prevention of cardiometabolic diseases (86). Mechanisms of action of dietary (poly)phenol are related to its capacity to modulate lipid metabolism, stimulating "browning", which is the development of beige adipocytes. Moreover, (poly)phenol could inhibit gluconeogenesis and stimulate the release of insulin by pancreatic β-cells. All the above could explain the link between total dietary (poly)phenol and cardiometabolic parameters found in the literature (41,85,87,88). Additionally, phenolic compounds produced by gut microbiota (like urolithins, enterolignans, phenolic acids (hydroxycinnamic acids, hydroxybenzoic acids, hydroxy-phenylpropionic acids, hydroxyphenylacetic acids), and stilbenes (dihydroresveratrol)) have implications on human health (45,89–95). It is the case of urolithins, molecules closely related to endothelial function, blood pressure, inflammation, insulin resistance, platelet aggregation, and oxidative stress (92,96–99). Enterolactone, an enterolignan, is related to low odds of presenting obesity, high C-reactive protein, high triglycerides, low HDL-c, and MetS (95).

Nevertheless, up to date, only a few studies have explored the relationship between (poly)phenol intake and cardiometabolic health in adolescents (100,101). Moreover, there are no studies that explore microbial phenolic metabolites and cardiometabolic conditions in this target population. Since adolescence is a critical period for later cardiometabolic complications, and dietary (poly)phenols protect cardiometabolic and cardiovascular health in adults, studying this relationship in adolescents is needed to promote early healthy eating behavior interventions.

nutrients

MDPI

Review

Microbial Phenolic Metabolites: Which Molecules Actually Have an Effect on Human Health?

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Abstract: The role of gut microbiota in human health has been investigated extensively in recent years. The association of dysbiosis, detrimental changes in the colonic population, with several health conditions has led to the development of pro-, pre- and symbiotic foods. If not absorbed in the small intestine or secreted in bile, polyphenols and other food components can reach the large intestine where they are susceptible to modification by the microbial population, resulting in molecules with potentially beneficial health effects. This review provides an overview of studies that have detected and/or quantified microbial phenolic metabolites using high-performance liquid chromatography as the separation technique, followed by detection through mass spectrometry. Both in vitro experimental studies and human clinical trials are covered. Although many of the microbial phenolic metabolites (MPM) reported in in vitro studies were identified in human samples, further research is needed to associate them with clinical health outcomes.

Keywords: microbiota; health; polyphenols; mass spectrometry; liquid chromatography; plasma; urine

1. Introduction

The human gut is home to a diverse community of microorganisms, whose involvement in metabolism, disease, immunity, nutrition and an expanding number of other health-related issues is becoming increasingly evident. Their important role in the maintenance of the health-disease equilibrium is supported by studies reporting that germ-free animals are more likely to suffer infections and unbalanced nutrient uptake, and these animals only regain their health status when colonised by normal microbiota [1].

Each human has a unique gut microbiota that changes throughout life. The modifications begin at birth, when the microbiome is affected by the delivery method, and then by whether breast or formulated milk is given [2]. In subsequent years, the environment, diet and lifestyle all influence the microbiome [3,4]. Alterations in the microbiota can cause dysbiosis, which has been associated with a variety of health issues ranging from gastrointestinal diseases [5] to mental health [6] and aging [7,8]. The importance of maintaining a healthy microbiota has led to the development of a whole industry around pro-, pre- and even symbiotic foods [9,10].

It is not only the composition of the gut bacterial community, however, that is responsible for these changes in health. Food components that pass through the small intestine without absorption reach the colon, where they can be transformed by the microbiota into more easily absorbed metabolites with varying benefits [11]. The metabolic capacity of microorganisms in the gut has proven greater than that of the host [12,13] to the extent that the microbiota has been postulated to function as a separate organ [14].

Among the molecules that undergo microbial modification in the intestine are polyphenols, plant secondary metabolites that provide beneficial health effects through their interaction with the human body [15,16]. The main site of absorption of polyphenols is the small intestine, but when these molecules bear a glycoside group, they need to be hydrolysed prior to entering circulation [17]. This occurs through two possible mechanisms: the action of lactase-phlorizin hydrolase (LPH) or the cytosolic β-glucosidase (CBG). LPH, located at the edge of the enterocyte, deglycosylates the polyphenols, increasing their lipophilicity and allowing them to enter the enterocyte by passive diffusion. CBG is found inside the enterocyte and metabolises the more polar glycosides after they have been introduced through transporters such as sodium-dependent glucose transporter 1 SGLT1 [17].

Some polyphenols can reach the colon intact, or after being secreted in bile after following the enterohepatic cycle. In the gut, the microbiota can hydrolyse the glycosides and also transform the aglycones into new molecules [18]. Their transformation into simpler components involves different reactions, such as ring fission, hydrolysis, demethylation, reduction, decarboxylation, dehydroxylation and isomerisation [19,20]. There is evidence of bacterial presence in the small intestine; thus, microbial phenolic metabolites (MPM) could possibly be generated there [21,22]. However, investigations around microbial modification of food components have been mainly performed in colonic samples. This might be due to the high invasivity of small intestine sampling, but also because the food chyme spends less time there than in the large intestine, thus, microbes have less time to act on the polyphenols.

The phenolic compounds and metabolites absorbed in both the small intestine and the colon can cross the basolateral membrane of the enterocyte, where they can be locally conjugated into phase II metabolites (where they are transformed into glucuronides, sulphates and other conjugate forms), enter the bloodstream and reach the liver, where they undergo further conjugation [17,23]. Once they enter the systemic circulation, polyphenols can be distributed to most tissues, reaching even breast milk and the brain [24]. Finally, circulating polyphenols and their metabolites derived from human modification alone or after microbial modification are primarily excreted in urine [25,26]. Those polyphenols that have not been absorbed or reabsorbed are excreted through the faeces [23]. The cumulative excretion of MPM in urine provides a more accurate assessment of absorption than plasma pharmacokinetics. Treated as xenobiotics, MPM are rapidly removed from the bloodstream into the urine and their presence in the circulatory system is transitory [27].

All these processes are shown in Figure 1.

The analysis of MPM in biological samples is complex due to their structural diversity, their low and varied concentrations, the limited availability of standards, and the complexity of the biological matrix [24,28]. Plasma and urine are the most widely used human biological samples, although faeces, tissues, ileal fluid and breastmilk have also been studied [24].

MPM in plasma and urine samples have generally been studied using mass spectrometry (MS) methods, with differences in the separation mode [28]. Some studies have used gas chromatography (GC) or high-performance liquid chromatography (HPLC) and even ultra-high-performance liquid chromatography (UHPLC). Examples of these separation methods are found throughout this text. When the target compounds are conjugates, liquid chromatography (LC) is the preferred separation technique, because their low volatility renders GC unsuitable [28]. Additionally, due to the complexity of the matrix, some cleaning and extraction are sometimes performed to minimise noise [29].

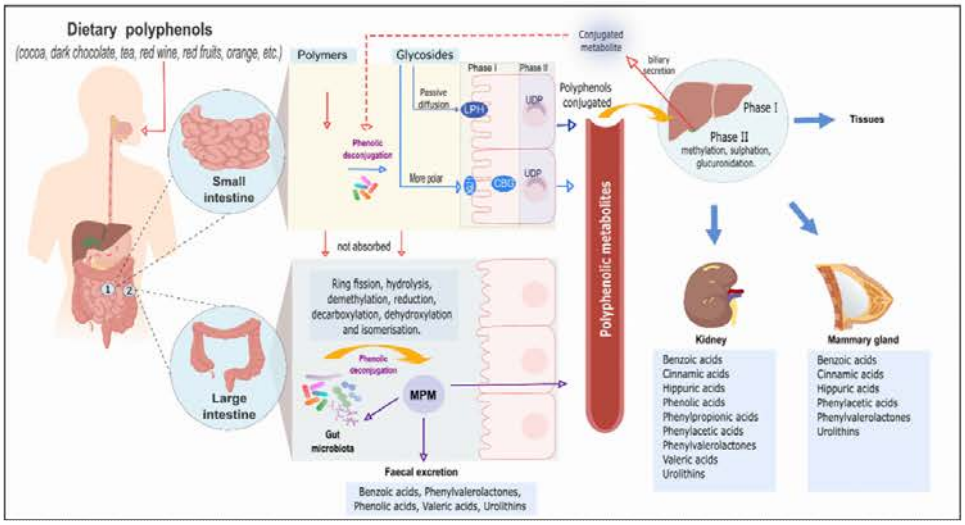

Figure 1. Metabolic pathways of polyphenols produced by the human gut microbiota. LPH: lactase-phlorizin hydrolase; CBG: cytosolic β-glucosidase; UDP: uridine 5′-diphospho-glucuronosyltransferase; MPM: microbial phenolic metabolites.

The growing interest in microbiota and phenolic compounds has prompted researchers to explore their possible interactions. Moreover, phenolic compounds can act as prebiotics, modifying the growth of bacterial colonies, an effect mainly studied in vitro [30]. Conversely, the microbiota is able to modify phenolic compounds, leading to production of new molecules that can pass through the membrane of the enterocyte and impact human health [31].

These results could help researchers evaluate exposure to specific foods in clinical and epidemiological studies. However, because multiple molecules are tested at the same time, it is not possible to differentiate which metabolite comes from what molecule.

This review aims to summarise the findings of studies of polyphenol gut microbiota interactions using HPLC-MS to identify and/or quantify metabolites. The results of in vitro and/or in vivo studies are reported by phenolic group.

2. Human Interventions

2.1. Phenolic Acids

Coffee is one of the most popular beverages worldwide, and several epidemiological studies have linked its consumption to a decreased risk of type 2 diabetes, some cancers and cardiovascular disease. These health effects have been partly attributed to a high concentration of chlorogenic acids (CGAs). The low bioavailability of CGAs means their impact on health relies on metabolism by the gut microbiota, which has prompted investigations into this process.

Mills et al. [30] found that coffee with the highest levels of CGAs increased the growth of *Bifidobacterium spp.* 10 h after exposure. CGAs also increased *Clostridium coccoides–Eubacterium*, associated with the prevention of obesity and related diseases [32]. Ludwig et al. [33], after incubating human faecal samples with espresso coffee, identified and quantified 11 metabolites arising from CGA degradation by HPLC-MS and GC-MS. Caffeic and ferulic acids were the first to be formed after one hour of incubation. The major end products after six hours were dihydrocaffeic, dihydroferulic, and 3-(3′-hydroxyphenyl)propionic acids. The rate and extent of the breakdown were influenced by the faecal microbiota composition of the participants. These results were in accordance with a previous study, in which caffeic, chlorogenic and caftaric acids were incubated with human faecal microbiota

and the metabolites identified by HPLC coupled to triple quadrupole MS. All free acids (caffeic, quinic and tartaric) were mainly metabolised to 3-hydroxyphenylpropionic and to a lesser extent to benzoic acid [34].

Gómez-Juaristi et al. [35] studied the absorption and metabolism of hydroxycinnamates after the consumption of 3.5 g of an instant soluble green and roasted coffee blend in 250 mL of hot water. Dihydrohydroxycinnamoylquinic acids (3-, 4- and 5-dihydrocaffeoylquinic acids, 3-, 4- and 5-dihydroferuloylquinic acids and dihydrocoumaroylquinic acid) were identified in urine for the first time after green coffee ingestion. Feruloylglycine and isoferuloylglycine were also detected, as well as phase II derivatives of lactones, namely three sulphated derivatives of caffeoylquinic lactone; one glucuronidated derivative of feruloylquinic lactone; 11 metabolic derivatives of hydroxyphenylpropionic, hydroxyphenylacetic, and hydrophenylbenzoic acids; and hydroxyhippuric acid and phloroglucinol.

The impact of colonic microbiota on the bioavailability of polyphenols from orange juice has also been studied [36]. After mixing and incubating target polyphenols with human faeces under anaerobic conditions, the phenolic acid metabolites were analysed by MS. Colonic microbiota had an impact on the production of hydroxy- and methoxyphenylpropionic acids, which are subsequently converted into hippuric acid and its hydroxylated counterparts in the liver. The bioactivity of such colon-derived metabolites is attracting growing interest. In vitro studies have indicated that protocatechuic, syringic, gallic and vanillic acids may play a protective role against atherosclerosis [37] and that 3-(3-methoxy-4-hydroxyphenyl)propionic acid has attenuating effects on diabetes [38].

2.2. Stilbenes

Resveratrol, the main stilbene found in grapes and red wine, is known for having anti-inflammatory effects, and an ability to block human platelet aggregation and promote eicosanoid synthesis [39]. Bode et al. [19] studied *trans*-resveratrol metabolism by the human gut microbiota in 12 healthy men. Twenty-four hours after of a single supplement of 0.5 mg trans-resveratrol/kg body weight, the microbial metabolites: dihydroresveratrol, lunularin and 3,4′-dihydroxy-*trans*-stilbene were found in urine.

2.3. Flavanones

The consumption of oranges or orange juice has been inversely correlated with ischemic stroke and acute coronary events, effects mainly attributed to the flavanone content of the fruit. Pereira-Caro et al. [26,27] identified 33 MPM in urine after the ingestion of orange juice, mainly cinnamic acids, phenylhydracrylic acids, phenylpropionic acids, phenylacetic acids, benzoic acids, mandelic acids, benzenetriols and hippuric acids. Maximum excretion was observed between 5–10 and 10–24 h after orange juice administration, indicating that all these compounds were derived from colonic microflora mediated flavanone metabolism. Ordóñez et al. [28] obtained similar results in a study where three healthy participants ingested 500 mL of orange juice, and urine was collected over a 24 h period. A total of 22 free phenolic acids, one benzenetriol and 35 phase II metabolites were identified and quantified. The MPM found in the study are listed in Table S1.

2.4. Flavan-3-ols

Recent clinical trials have reported beneficial effects of cocoa polyphenols on cardiovascular health [40,41]. These include flavon-3-ols, present in significant amounts in cocoa mainly as monomers and polymers, and epicatechin and procyanidins [42]. However, the biological effects of flavon-3-ols depend on bioavailability. Epicatechin is easily absorbed in the small intestine and quickly metabolised, whereas polymeric procyanidins reach the colon, where they are transformed into hydroxyphenylvalerolactones by the gut microbiota. These metabolites are easily absorbed in the colonocyte and could have greater biological activity than the parent form [41,43].

In a feeding study by Martin et al. [44], 10 participants who regularly ate dark chocolate and 10 who rarely did so were given 2 × 25 g of dark chocolate daily and morning spot urine samples were collected each day. Metabolites of epicatechin (5-(3,4,dihydroxyphenyl)-γ-valerolactone, 2S-1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)-propan-2-ol and 4-hydroxy-5-(3´,4´-dihydroxyphenyl)-valeric acid)) were identified in both groups, but only participants who regularly ate chocolate saw an increase of sulphated, glucuronided and methyl-sulphated conjugates of 5-(3,4-dihydroxyphenyl)-valeric acid (Table S1). These results suggest that gut microbial metabolism of cocoa phenolics differs according to the history of chocolate ingestion.

Urpí-Sardà et al. [42,45] also evaluated the consumption of cocoa. In this case, 42 healthy participants consumed 40 g/day of cocoa for four weeks. A total of 19 metabolites were identified and quantified in 24 h of urine collection, predominantly hydroxyphenylacetic acids and hydroxybenzoic acids. The principal groups of MPM in plasma were hydroxyphenylvalerolactones, hydroxyphenylpropionic acids, hydroxycinnamic acids and hydroxybenzoic acid (Table S1).

Wiese et al. [43] described similar outcomes, where 5-(3',4'-dihydroxyphenyl)-γ-valerolactone was the principal gut metabolite in plasma, and thus, the most relevant gut-mediated metabolite of procyanidin B1. The authors also confirmed interesting pathways of polymeric procyanidin degradation by gut microbiota. They suggested that some MPM arising from procyanidins, namely 5-(3´,4´-dihydroxyphenyl)-γ-valerolactone and 4-hydroxy-5(3',4'-dihydroxyphenyl)valeric acid, may eventually be metabolised into conjugated 5-(3´,4´-dihydroxyphenyl)-γ-valerolactone and conjugated phenylvaleric acids (Table S1).

Interestingly, Khymenets et al. [46] identified epicatechin metabolites derived from host and microbiota in human milk samples of nursing mothers after intake of dark chocolate. Human milk has a wide range of bioactive compounds interesting for human health [47]. The principal phase II host metabolites were methyl epicatequin, epicatechin glucuronide and sulphate. Dihydroxyphenylvalerolactone sulphate was the principal gut-mediated epicatechin metabolite detected in human milk.

Some studies have focused on flavanols from tea. After the consumption of 500 mL of green tea, several valerolactones were detected in urine in their conjugated form, linked to glucuronide, sulphate and methyl groups [25]. Duynhoven et al. [48] performed a study with black tea supplementation, and identified that host-conjugated metabolites derived from catechins, kaempferol and gallic acid appeared most rapidly in plasma (before one hour), compared to conjugated MPM such as valerolactones, valeric acids, phenols and phenolic acids (after two to four hours). However, due to the rapid appearance of MPM in plasma, the authors suggested that microbial activity could begin in the ileum and not in the colon. Likewise, while catechin conjugates continued in circulation for up to six hours, 5-(3',4'-dihydroxyphenyl)-γ-valerolactone glucuronide and pyrogallol sulphate circulated at higher concentrations for up to almost 30 h. Schantz et al. [49] found relevant evidence that metabolism of green tea catechins and gallic acid may take place inside the ileum, similar to Duynhoven et al. [48]. Using an exvivo ileostomy model, (−)-epicatechin gallate was rapidly degraded to gallic acid and (−)-epicatechin during the first two hours of incubation. Gallic acid was further degraded into pyrogallol after 24 h of incubation. Likewise, 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone and 5-(3',4'-dihydroxyphenyl)-γ-valerolactone were identified as metabolites of catechins and (−)-epicatechin, and the only metabolite of epicatechin gallate was 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (Table S1).

Inter-individual variation is another relevant factor to consider when assessing MPM bioavailability, as it may directly affect the biological response to polyphenol intake. Duynhoven et al. [48] reported that participants showed more variation in MPM such as valerolactones and valeric acids than in components directly absorbed in the small intestine such as catechins, after a single dose of black tea. Moreover, intake of polyphenol-containing foods can affect the gut microbiota profile and numbers, thereby increasing individual variability in metabolism [50]. Plasma levels of MPM can also be

affected by long periods of supplementation. Clarke et al. [51] observed an increased concentration of valerolactones after three months of green tea supplementation.

Regarding the conjugate forms of MPM, it seems that the human metabolism has a stronger tendency towards sulphatation than glucuronidation. Van Duynhoven et al. [48] reported that plasma levels of sulphated pyrogallol and 5-(3′,5′-dihydroxyphenyl)-y-valerolactone were higher than the glucuronide forms. Similarly, Clarke et al. [51] found that the plasma valerolactone sulphate concentration was higher compared to other conjugated forms after 12 weeks of 450 mg green tea and 25 mg vitamin C supplementation. In contrast, Zhang et al. [24] identified only glucuronide forms of phenylvalerolactones and phenylcinnamic acids in plasma.

In a study by Zhao et al. [52], a drink supplemented with catechin and epicatechin was inoculated with gut microbiota from healthy human participants and incubated at 37 °C for 24 h under anaerobic conditions. After this treatment, all catechin and epicatechin was metabolised and significant production of gallic, 3,4-dihydroxybenzoic and homovanillic acids was detected. A single bacterial isolate was also able to convert catechin and epicatechin into several phenolic acid metabolites. This activity could potentially enhance the bioefficacy of polyphenols that are poorly absorbed in the upper gastrointestinal tract, generating phenolic metabolites with greater bioavailability and an extended half-life.

2.5. Anthocyanins, Proanthocyanidin and Ellagitannins

Raspberry, strawberry and pomegranate contain a wide variety of polyphenols, including anthocyanins and the poorly absorbed ellagitannins, which are transformed by the gut microbiota into urolithins. After consumption of red raspberry purée, three urolithins (urolithin A, urolithin A glucuronide and urolithin B glucuronide) were identified in urine. Microbial metabolites derived from anthocyanins and proanthocyanidins (benzoic acids, phenyl acids, phenylcinnamic acids and phenylvalerolactones) were also detected [24]. An increase in urolithins and the derivative urolithin A glucuronide was found after the consumption of fresh strawberries or the equivalent dose of strawberry purée [53]. The urolithins were found in urine for up to 92 h after consumption, indicating long persistence in the body after only a single dose of an ellagitannin-rich food.

Nuñez-Sánchez et al. [54] identified urolithin derivatives in plasma from 33 colorectal cancer patients after 15 days of 900 mg/day of pomegranate extract consumption. The same authors also identified 23 metabolites in urine, including ellagic acid, four methyl ellagic acid derivatives, gallic acid, two isomers of valoneic acid dilactone, gallagic acid dilactone and 15 urolithin derivatives (Table S1).

In their pioneering work in this field, Zhang et al. [24] identified polyphenol microbial metabolites in human breast milk after chronic consumption of 125 g/day of red raspberry purée. The most relevant MPM belonged to the group of urolithins (Table S1).

Other investigations with polyphenols have associated anthocyanins [55] and high-flavanol cocoa [56] with enhanced growth of the beneficial bacteria *Lactobacillus and Enterococcus spp*. After entering the colon, anthocyanins are fermented by the intestinal microbiota and the resulting metabolites (quercetin and phloretin derivatives; caffeoylquinic, caffeic and coumaroylquinic acids; procyanidins and catechins) may be responsible for the observed health effects in vivo [57].

Ellagitannins and ellagic acid undergo microbial metabolism in the gastrointestinal tract leading to urolithins [58], which have potential use as intake biomarkers for foods rich in those polyphenols, such as pomegranates, berries, nuts and oak-aged red wines [59]. Their promising application as functional foods depends on determination of the bacteria responsible for urolithin production, as well as elucidating the urolithin production pathways. García-Villalba et al. [60] studied the time course of urolithin production from ellagic acid by human faecal microbiota from two individuals with different urinary excretion patterns, excreting either urolithin A or isourolithin A. They found that bacteria from the *Clostridium coccoides* group played a crucial role in the production of both urolithins and the metabolic intermediates were urolithins M-5, M-6, M-7, C and E.

To further investigate the production of urolithins from ellagic acid and ellagitannins, Selma et al. [58] performed an in vitro study with a newly described species, *Gordonibacter urolithinfaciens*,

isolated from the human faeces of a healthy participant. In pure cultures under anaerobic conditions, these intestinal bacteria sequentially produced urolithins M-5, M-6 and C, whereas urolithins A and B and isourolithin A were not detected. A more complete understanding of the metabolism of urolithins A and B and isourolithin A requires more investigation under the physiological conditions found in vivo.

2.6. Flavonols

Some studies have suggested that the clinical effects of some traditional herbal remedies might be caused by the metabolites of isorhamnetin glucoside an abundant polyphenol. Du et al. [61] supplemented a mixture of preculture bacteria and general anaerobic medium broth with isorhamnetin glucoside, and after 48 h of incubation five metabolites (isorhamnetin, kaempferol, quercetin, kaempferol glucoside and acetylated isorhamnetin glucoside) were tentatively identified. Kaempferol is widely reported to possess a range of pharmacological properties including anticancer, cardioprotective, neuroprotective, antioxidant, anti-inflammatory, antimicrobial and antiallergic activity [62]. The health benefits of quercetin include protection against several diseases such as osteoporosis, pulmonary and cardiovascular diseases and cancer [63]; antiproliferative, antiatherosclerotic and neuroprotective effects are also described [64].

3. Other Food Intervention Studies with Mixed Polyphenols

Grapes and grape-derived products such as wine are rich in phenolic compounds, particularly flavonoids, anthocyanins, proanthocyanidins, procyanidins, phenolic acids and stilbenes. The various beneficial health effects associated with their consumption seem due to this wide variety of bioactive compounds [65].

Boto-Ordóñez et al. [20] designed an open, randomised, crossover, controlled intervention trial where nine healthy men followed three 20-day interventions with dealcoholised red wine, red wine or gin. The aim was to evaluate the association between changes in the concentration of intestinal bacteria and urinary microbial phenolic acids. *Bifidobacterium* was significantly correlated with differences in 4-hydroxybenzoic, syringic, *p*-coumaric and homovanillic acids, which are metabolic products of anthocyanin degradation. Although the correlation was statistically significant, the authors highlight the difficulty of establishing whether these metabolites are derived only from anthocyanin or also from other sources. Homovanillic acid, for instance, could also be derived from ferulic acid, *p*-coumaric acid from dehydroxylation of caffeic acid, and syringic acid from gallic acid.

Functional beverages based on grape extracts were used to observe modifications in the urinary metabolome. A study by Khymenets et al. [65,66] involved 31 healthy participants who consumed a drink containing grape skin extract or a placebo control for 15 days. Urine was collected on the first day during the first four postprandial hours, and for 24 h on the last day of the intervention. Among 18 metabolites identified after consumption of grape skin extract, 4-hydroxyhippuric acid was excreted in a high concentration, due to both *p*-hydroxybenzoic acid glycination and microbial metabolic activities. Hydroxydimethoxybenzoic acid glucuronide, a conjugate of syringic and dimethylgallic acids, was also identified. Other metabolites found were syringic acid, 3-methylgallic acid, vanillic acid glucuronide and vanilloylglycine, which could derive directly from the grape extract or have been produced by the microbiota from hydroxycinnamates and flavan-3-ols. Dihydrosinapic acid glucuronide was identified as a metabolite of sinapic acid, the most abundant hydroxycinnamate found in red grapes and derived products. Finally, the authors found eight metabolites of first-stage flavan-3-ol microbial metabolism, namely derivatives of hydroxy(dihydroxyphenyl)valeric acid (two glucuronides and sulphate), dihydroxyphenyl (two glucuronides), hydroxymethoxyphenyl (two glucuronides) and hydroxyphenylvalerolactones (one glucuronide) [65] (Table S1).

In a study by Sasot et al. [66], a beverage made with grape pomace was administered to 12 healthy participants, with urine collected before intake and 24 h afterwards. Seventy phenolic metabolites were identified through LC-ESI-LTQ-Orbitrap-MS analysis (see Table S1).

Some of these metabolites hydroxybenzoic acids, syringic acid, hydroxyhippuric acid and hydroxyphenylpropionic acids were also found by Khymenets et al. [65], who also identified two phenolic compounds derived from intestinal microflora, 4-hydroxy-5-(3,4-dihydroxyphenyl)valeric acid and 5-(3´,4´-dihydroxyphenyl)-γ-valerolactone. The former had previously been described as a gut microbiota-derived metabolite produced after grape intake, while the latter is the most abundant metabolite of (epi)catechin and procyanidin B degradation and can be metabolised into simple phenolic acids such as 3-hydroxyphenylpropionic acid, 3-hydroxyhippuric acid and 3-hydroxybenzoic acid.

Most clinical trials in this area have focused on the impact of red wine on gut microbiota, likely because of available evidence for its preventive effects against chronic diseases [50,67,68]. Red wine polyphenols may modulate the profile and activity of gut microbiota, increasing the growth of probiotics bacteria such as *Bifidobacterium* and decreasing non-beneficial bacteria. This could be due to the antimicrobial characteristics of red wine, which affect host microbiota interaction [69]. In line with this, studies have reported changes in the profile of MPM in human faeces after moderate intake of red wine polyphenols [50,67,68]. In a targeted analysis by liquid chromatography coupled to mass spectometry (LC-MS), an increase in MPM including 3,5-dihydroxybenzoic acid, 3-methylgallic acid, *p*-coumaric acid, phenyl propionic acid, protocatechuic acid, vanillic acid, syringic acid and 4-hydroxy-5-(phenyl)valeric acid was observed after moderate wine intake [50,68] (Table S1). Final products of MPM may derive from the metabolism of oligomers and polymers of flavan-3-ols and procyanidins in the colon [68]. Furthermore, a non-targeted analysis after wine intake revealed the intra and inter-individual variability of faecal polyphenol metabolites [68].

Tomato and tomato-based products are rich in phenolic acids (homovanillic acid hexoside, 5-caffeoylquinic acid, caffeic acid hexoside-I and ferulic acid), flavanones (naringenin) and flavonols (rutin). In an open, controlled, randomised and crossover feeding trial with 40 healthy participants, MPM were analysed in urine after consumption of tomato and tomato sauces. A wide variety of metabolites and their glucuronide and sulphate conjugates were detected, namely 3-(4-hydroxyphenylpropionic) acid, 3-(3-hydroxyphenyl)propionic acid, 4-hydroxyphenylpropionic acid, 3-phenylpropionic acid, 4-hydroxyhippuric acid, 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, and phenylacetic acid (see Table S1). Higher concentrations were obtained for the glucuronide and sulphate metabolites than for their aglycones [70].

After supplying an almond skin extract to 24 healthy participants, Llorach et al. [71] identified 34 metabolites in urine, classified as conjugates of flavonoids, hydroxyphenylvalerolactone, 4-hydroxy-5-(phenyl)-valeric acid, hydroxyphenylpropionic acid, hydroxyphenylacetic acid and other phenolic acids.

Table 1 summarises the main families of MPM found in biological fluids. An extended version of the table is available in the Supplementary Material (Table S1).

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References

1. O'Hara, A.M.; Shanahan, F. The gut flora as a forgotten organ. *EMBO Rep.* **2006**, *7*, 688–693. [CrossRef] [PubMed]
2. Azad, M.; Konya, T.; Persaud, R.; Guttman, D.; Chari, R.; Field, C.; Sears, M.; Mandhane, P.; Turvey, S.; Subbarao, P.; et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: A prospective cohort study. *BJOG An. Int. J. Obstet. Gynaecol.* **2016**, *123*, 983–993. [CrossRef] [PubMed]
3. Zmora, N.; Suez, J.; Elinav, E. You are what you eat: Diet, health and the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 35–56. [CrossRef] [PubMed]
4. Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G.; Gasbarrini, A.; Mele, M. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* **2019**, *7*, 14. [CrossRef]
5. Tamboli, C.P.; Neut, C.; Desreumaux, P.; Colombel, J.F. Dysbiosis in inflammatory bowel disease. *Gut* **2004**, *53*, 1–4. [CrossRef]
6. Inserra, A.; Rogers, G.B.; Licinio, J.; Wong, M.-L. The microbiota-inflammasome hypothesis of major depression. *BioEssays* **2018**, *40*, e1800027. [CrossRef]
7. Kim, S.; Jazwinski, S.M. The gut microbiota and healthy aging: A mini-review. *Gerontology* **2018**, *64*, 513–520. [CrossRef]
8. Ottaviani, E.; Ventura, N.; Mandrioli, M.; Candela, M.; Franchini, A.; Franceschi, C. Gut microbiota as a candidate for lifespan extension: An ecological/evolutionary perspective targeted on living organisms as metaorganisms. *Biogerontology* **2011**, *12*, 599–609. [CrossRef]
9. Fuller, R.; Gibson, G.R. Modification of the intestinal microflora using probiotics and prebiotics. *Scand. J. Gastroenterol. Suppl.* **1997**, *32*, 28–31. [CrossRef]
10. Markowiak, P.; Śliżewska, K. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients* **2017**, *9*, 1021. [CrossRef]
11. Yadav, M.; Verma, M.K.; Chauhan, N.S. A review of metabolic potential of human gut microbiome in human nutrition. *Arch. Microbiol.* **2018**, *200*, 203–217. [CrossRef] [PubMed]
12. Gill, S.R.; Pop, M.; DeBoy, R.T.; Eckburg, P.B.; Turnbaugh, P.J.; Samuel, B.S.; Gordon, J.I.; Relman, D.A.; Fraser-Liggett, C.M.; Nelson, K.E. Metagenomic analysis of the human distal gut microbiome. *Science* **2006**, *312*, 1355–1359. [CrossRef] [PubMed]
13. Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K.S.; Manichanh, C.; Nielsen, T.; Pons, N.; Levenez, F.; Yamada, T.; et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **2010**, *464*, 59–65. [CrossRef] [PubMed]
14. Clarke, G.; Stilling, R.M.; Kennedy, P.J.; Stanton, C.; Cryan, J.F.; Dinan, T.G. Minireview: Gut microbiota: The neglected endocrine organ. *Mol. Endocrinol.* **2014**, *28*, 1221–1238. [CrossRef]
15. Grosso, G. Effects of polyphenol-rich foods on human health. *Nutrients* **2018**, *10*, 1089. [CrossRef]
16. Vauzour, D.; Rodriguez-Mateos, A.; Corona, G.; Oruna-Concha, M.J.; Spencer, J.P.E. Polyphenols and human health: Prevention of disease and mechanisms of action. *Nutrients* **2010**, *2*, 1106–1131. [CrossRef]
17. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid. Redox Signal.* **2013**, *18*, 1818–1892. [CrossRef]
18. Mosele, J.; Macià, A.; Motilva, M.-J. Metabolic and microbial modulation of the large intestine ecosystem by non-absorbed diet phenolic compounds: A review. *Molecules* **2015**, *20*, 17429–17468. [CrossRef]
19. Bode, L.M.; Bunzel, D.; Huch, M.; Cho, G.-S.; Ruhland, D.; Bunzel, M.; Bub, A.; Franz, C.M.; Kulling, S.E. In vivo and in vitro metabolism of trans-resveratrol by human gut microbiota. *Am. J. Clin. Nutr.* **2013**, *97*, 295–309. [CrossRef]

20. Boto-Ordóñez, M.; Urpi-Sarda, M.; Queipo-Ortuño, M.I.; Tulipani, S.; Tinahones, F.J.; Andres-Lacueva, C. High levels of bifidobacteria are associated with increased levels of anthocyanin microbial metabolites: A randomized clinical trial. *Food Funct.* **2014**, *5*, 1932–1938. [CrossRef]
21. Kleerebezem, M. Microbial metabolic gatekeeping in the jejunum. *Nat. Microbiol.* **2018**, *3*, 650–651. [CrossRef] [PubMed]
22. Sundin, O.H.; Mendoza-Ladd, A.; Zeng, M.; Diaz-Arévalo, D.; Morales, E.; Fagan, B.M.; Ordoñez, J.; Velez, P.; Antony, N.; McCallum, R.W. The human jejunum has an endogenous microbiota that differs from those in the oral cavity and colon. *BMC Microbiol.* **2017**, *17*, 160. [CrossRef] [PubMed]
23. Marín, L.; Miguélez, E.M.; Villar, C.J.; Lombó, F. Bioavailability of dietary polyphenols and gut microbiota metabolism: Antimicrobial properties. *Biomed Res. Int.* **2015**, *2015*, 1–18. [CrossRef] [PubMed]
24. Zhang, X.; Sandhu, A.; Edirisinghe, I.; Burton-Freeman, B. An exploratory study of red raspberry (*Rubus idaeus* L.) (poly)phenols/metabolites in human biological samples. *Food Funct.* **2018**, *9*, 806–818. [CrossRef] [PubMed]
25. Calani, L.; Del Rio, D.; Luisa Callegari, M.; Morelli, L.; Brighenti, F. Updated bioavailability and 48 h excretion profile of flavan-3-ols from green tea in humans. *Int. J. Food Sci. Nutr.* **2012**, *63*, 513–521. [CrossRef] [PubMed]
26. Pereira-Caro, G.; Polyviou, T.; Ludwig, I.-A.; Nastase, A.M.; Moreno-Rojas, J.M.; Garcia, A.L.; Malkova, D.; Crozier, A. Bioavailability of orange juice (poly)phenols: The impact of short-term cessation of training by male endurance athletes. *Am. J. Clin. Nutr.* **2017**, *106*, 791–800. [CrossRef] [PubMed]
27. Pereira-Caro, G.; Borges, G.; van der Hooft, J.; Clifford, M.N.; Del Rio, D.; Lean, M.E.; Roberts, S.A.; Kellerhals, M.B.; Crozier, A. Orange juice (poly)phenols are highly bioavailable in humans. *Am. J. Clin. Nutr.* **2014**, *100*, 1378–1384. [CrossRef]
28. Ordóñez, J.L.; Pereira-Caro, G.; Ludwig, I.; Muñoz-Redondo, J.M.; Ruiz-Moreno, M.J.; Crozier, A.; Moreno-Rojas, J.M. A critical evaluation of the use of gas chromatography- and high performance liquid chromatography-mass spectrometry techniques for the analysis of microbial metabolites in human urine after consumption of orange juice. *J. Chromatogr. A* **2018**, *1575*, 100–112. [CrossRef]
29. Feliciano, R.P.; Mecha, E.; Bronze, M.R.; Rodriguez-Mateos, A. Development and validation of a high-throughput micro solid-phase extraction method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid identification and quantification of phenolic metabolites in human plasma and urine. *J. Chromatogr. A* **2016**, *1464*, 21–31. [CrossRef]
30. Mills, C.E.; Tzounis, X.; Oruna-Concha, M.-J.; Mottram, D.S.; Gibson, G.R.; Spencer, J.P.E. In vitro colonic metabolism of coffee and chlorogenic acid results in selective changes in human faecal microbiota growth. *Br. J. Nutr.* **2015**, *113*, 1220–1227. [CrossRef]
31. Gowd, V.; Bao, T.; Wang, L.; Huang, Y.; Chen, S.; Zheng, X.; Cui, S.; Chen, W. Antioxidant and antidiabetic activity of blackberry after gastrointestinal digestion and human gut microbiota fermentation. *Food Chem.* **2018**, *269*, 618–627. [CrossRef] [PubMed]
32. Neyrinck, A.M.; Possemiers, S.; Verstraete, W.; De Backer, F.; Cani, P.D.; Delzenne, N.M. Dietary modulation of clostridial cluster xiva gut bacteria (*Roseburia* spp.) by chitin–glucan fiber improves host metabolic alterations induced by high-fat diet in mice. *J. Nutr. Biochem.* **2012**, *23*, 51–59. [CrossRef] [PubMed]
33. Ludwig, I.A.; Paz de Peña, M.; Concepción, C.; Alan, C. Catabolism of coffee chlorogenic acids by human colonic microbiota. *BioFactors* **2013**, *39*, 623–632. [CrossRef] [PubMed]
34. Gonthier, M.-P.; Remesy, C.; Scalbert, A.; Cheynier, V.; Souquet, J.-M.; Poutanen, K.; Aura, A.-M. Microbial metabolism of caffeic acid and its esters chlorogenic and caftaric acids by human faecal microbiota in vitro. *Biomed. Pharmacother.* **2006**, *60*, 536–540. [CrossRef]
35. Gómez-Juaristi, M.; Martínez-López, S.; Sarria, B.; Bravo, L.; Mateos, R. Bioavailability of hydroxycinnamates in an instant green/roasted coffee blend in humans. Identification of novel colonic metabolites. *Food Funct.* **2018**, *9*, 331–343. [CrossRef]
36. Pereira-Caro, G.; Borges, G.; Ky, I.; Ribas, A.; Calani, L.; Del Rio, D.; Clifford, M.N.; Roberts, S.A.; Crozier, A. In vitro colonic catabolism of orange juice (poly)phenols. *Mol. Nutr. Food Res.* **2015**, *59*, 465–475. [CrossRef]

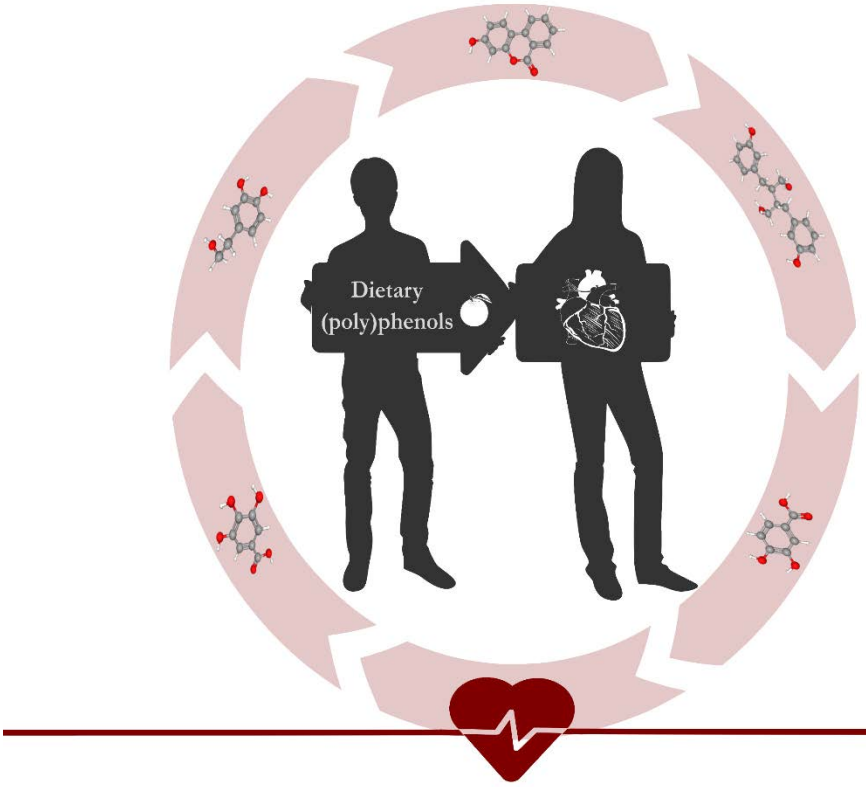
37. Hidalgo, M.; Martin-Santamaria, S.; Recio, I.; Sanchez-Moreno, C.; de Pascual-Teresa, B.; Rimbach, G.; de Pascual-Teresa, S. Potential anti-inflammatory, anti-adhesive, anti/estrogenic, and angiotensin-converting enzyme inhibitory activities of anthocyanins and their gut metabolites. *Genes Nutr.* **2012**, *7*, 295–306. [CrossRef]
38. Verzelloni, E.; Pellacani, C.; Tagliazucchi, D.; Tagliaferri, S.; Calani, L.; Costa, L.G.; Brighenti, F.; Borges, G.; Crozier, A.; Conte, A.; et al. Antiglycative and neuroprotective activity of colon-derived polyphenol catabolites. *Mol. Nutr. Food Res.* **2011**, *55*, S35–S43. [CrossRef]
39. Ramírez-Garza, S.L.; Laveriano-Santos, E.P.; Marhuenda-Muñoz, M.; Storniolo, C.; Tresserra-Rimbau, A.; Vallverdú-Queralt, A.; Lamuela-Raventós, R.M. Health Effects of Resveratrol: Results from Human. *Nutrients* **2018**, *10*, 1892. [CrossRef]
40. Monagas, M.; Khan, N.; Andrés-Lacueva, C.; Casas, R.; Urpí-Sardà, M.; Llorach, R.; Lamuela-Raventós, R.M.; Estruch, R. Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. *Am. J. Clin. Nutr.* **2009**, *90*, 1144–1150. [CrossRef]
41. Arranz, S.; Valderas-Martínez, P.; Chiva-Blanch, G.; Casas, R.; Urpí-Sardà, M.; Lamuela-Raventós, R.M.; Estruch, R. Cardioprotective effects of cocoa: Clinical evidence from randomized clinical intervention trials in humans. *Mol. Nutr. Food Res.* **2013**, *57*, 936–947. [CrossRef] [PubMed]
42. Urpí-Sardà, M.; Monagas, M.; Khan, N.; Lamuela-Raventós, R.M.; Santos-Buelga, C.; Sacanella, E.; Castell, M.; Permanyer, J.; Andrés-Lacueva, C. Epicatechin, procyanidins, and phenolic microbial metabolites after cocoa intake in humans and rats. *Anal. Bioanal. Chem.* **2009**, *394*, 1545–1556. [CrossRef] [PubMed]
43. Wiese, S.; Esatbeyoglu, T.; Winterhalter, P.; Kruse, H.-P.; Winkler, S.; Bub, A.; Kulling, S.E. Comparative biokinetics and metabolism of pure monomeric, dimeric, and polymeric flavan-3-ols: A randomized cross-over study in humans. *Mol. Nutr. Food Res.* **2015**, *59*, 610–621. [CrossRef] [PubMed]
44. Martin, F.-P.J.; Montoliu, I.; Nagy, K.; Moco, S.; Collino, S.; Guy, P.; Redeuil, K.; Scherer, M.; Rezzi, S.; Kochhar, S. Specific dietary preferences are linked to differing gut microbial metabolic activity in response to dark chocolate intake. *J. Proteome Res.* **2012**, *11*, 6252–6263. [CrossRef]
45. Urpí-Sardà, M.; Monagas, M.; Khan, N.; Llorach, R.; Lamuela-Raventós, R.M.; Jáuregui, O.; Estruch, R.; Izquierdo-Pulido, M.; Andrés-Lacueva, C. Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 7258–7267. [CrossRef]
46. Khymenets, O.; Rabassa, M.; Rodríguez-Palmero, M.; Rivero-Urgell, M.; Urpi-Sarda, M.; Tulipani, S.; Brandi, P.; Campoy, C.; Santos-Buelga, C.; Andrés-Lacueva, C. Dietary epicatechin is available to breastfed infants through human breast milk in the form of host and microbial metabolites. *J. Agric. Food Chem.* **2016**, *64*, 5354–5360. [CrossRef]
47. Ballard, O.; Morrow, A.L. Human milk composition: Nutrients and bioactive factors. *Pediatr. Clin. North Am.* **2013**, *60*, 49–74. [CrossRef]
48. Van Duynhoven, J.; van der Hooft, J.J.J.; van Dorsten, F.A.; Peters, S.; Foltz, M.; Gomez-Roldan, V.; Vervoort, J.; de Vos, R.C.H.; Jacobs, D.M. Rapid and sustained systemic circulation of conjugated gut microbial catabolites after single-dose black tea extract consumption. *J. Proteome Res.* **2014**, *13*, 2668–2678. [CrossRef]
49. Schantz, M.; Erk, T.; Richling, E. Metabolism of green tea catechins by the human small intestine. *Biotechnol. J.* **2010**, *5*, 1050–1059. [CrossRef]
50. Jiménez-Girón, A.; Queipo-Ortuño, M.I.; Boto-Ordóñez, M.; Muñoz-González, I.; Sánchez-Patán, F.; Monagas, M.; Martín-Álvarez, P.J.; Murri, M.; Tinahones, F.J.; Andrés-Lacueva, C.; et al. Comparative study of microbial-derived phenolic metabolites in human feces after intake of gin, red wine, and dealcoholized red wine. *J. Agric. Food Chem.* **2013**, *61*, 3909–3915. [CrossRef]
51. Clarke, K.A.; Dew, T.P.; Watson, R.E.B.; Farrar, M.D.; Osman, J.E.; Nicolaou, A.; Rhodes, L.E.; Williamson, G. Green tea catechins and their metabolites in human skin before and after exposure to ultraviolet radiation. *J. Nutr. Biochem.* **2016**, *27*, 203–210. [CrossRef] [PubMed]
52. Zhao, D.; Yuan, B.; Carry, E.; Pasinetti, G.M.; Ho, L.; Faith, J.; Mogno, I.; Simon, J.; Wu, Q. Development and validation of an ultra-high performance liquid chromatography/triple quadrupole mass spectrometry method for analyzing microbial-derived grape polyphenol metabolites. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **2018**, *1099*, 34–45. [CrossRef] [PubMed]

53. Truchado, P.; Larrosa, M.; García-Conesa, M.T.; Cerdá, B.; Vidal-Guevara, M.L.; Tomás-Barberán, F.A.; Espín, J.C. Strawberry processing does not affect the production and urinary excretion of urolithins, ellagic acid metabolites, in humans. *J. Agric. Food Chem.* **2012**, *60*, 5749–5754. [CrossRef] [PubMed]
54. Nuñez-Sánchez, M.A.; García-Villalba, R.; Monedero-Saiz, T.; García-Talavera, N.V.; Gómez-Sánchez, M.B.; Sánchez-Álvarez, C.; García-Albert, A.M.; Rodríguez-Gil, F.J.; Ruiz-Marín, M.; Pastor-Quirante, F.A.; et al. Targeted metabolic profiling of pomegranate polyphenols and urolithins in plasma, urine and colon tissues from colorectal cancer patients. *Mol. Nutr. Food Res.* **2014**, *58*, 1199–1211. [CrossRef] [PubMed]
55. Hidalgo, M.; Oruna-Concha, M.J.; Kolida, S.; Walton, G.E.; Kallithraka, S.; Spencer, J.P.E.; Gibson, G.R.; de Pascual-Teresa, S. Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. *J. Agric. Food Chem.* **2012**, *60*, 3882–3890. [CrossRef]
56. Salminen, S.; Bouley, C.; Boutron-Ruault, M.C.; Cummings, J.H.; Franck, A.; Gibson, G.R.; Isolauri, E.; Moreau, M.C.; Roberfroid, M.; Rowland, I. Functional food science and gastrointestinal physiology and function. *Br. J. Nutr.* **1998**, *80*, S147–S171. [CrossRef]
57. Kahle, K.; Kraus, M.; Scheppach, W.; Ackermann, M.; Ridder, F.; Richling, E. Studies on apple and blueberry fruit constituents: Do the polyphenols reach the colon after ingestion? *Mol. Nutr. Food Res.* **2006**, *50*, 418–423. [CrossRef]
58. Selma, M.V.; Beltrán, D.; García-Villalba, R.; Espín, J.C.; Tomás-Barberán, F.A. Description of urolithin production capacity from ellagic acid of two human intestinal *Gordonibacter* species. *Food Funct.* **2014**, *5*, 1779–1784. [CrossRef]
59. Cerdá, B.; Tomás-Barberán, F.A.; Espín, J.C. Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: Identification of biomarkers and individual variability. *J. Agric. Food Chem.* **2005**, *53*, 227–235. [CrossRef]
60. García-Villalba, R.; Beltrán, D.; Espín, J.C.; Selma, M.V.; Tomás-Barberán, F.A. Time course production of urolithins from ellagic acid by human gut microbiota. *J. Agric. Food Chem.* **2013**, *61*, 8797–8806. [CrossRef]
61. Du, L.; Zhao, M.; Xu, J.; Qian, D.; Jiang, S.; Shang, E.; Guo, J.; Duan, J. Analysis of the metabolites of isorhamnetin 3 -o -glucoside produced by human intestinal flora in vitro by applying ultraperformance liquid chromatography/quadrupole time-of-flight mass spectrometry. *J. Agric. Food Chem.* **2014**, *62*, 2489–2495. [CrossRef] [PubMed]
62. Calderón-Montaño, J.M.; Burgos-Morón, E.; Pérez-Guerrero, C.; López-Lázaro, M. A review on the dietary flavonoid kaempferol. *Mini Rev. Med. Chem.* **2011**, *11*, 298–344. [CrossRef] [PubMed]
63. Boots, A.W.; Haenen, G.R.M.M.; Bast, A. Health effects of quercetin: From antioxidant to nutraceutical. *Eur. J. Pharmacol.* **2008**, *585*, 325–337. [CrossRef] [PubMed]
64. Kleemann, R.; Verschuren, L.; Morrison, M.; Zadelaar, S.; van Erk, M.J.; Wielinga, P.Y.; Kooistra, T. Anti-Inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models. *Atherosclerosis* **2011**, *218*, 44–52. [CrossRef] [PubMed]
65. Khymenets, O.; Andrés-Lacueva, C.; Urpí-Sardà, M.; Vázquez-Fresno, R.; Mart, M.M.; Reglero, G.; Torres, M.; Llorach, R. Metabolic fingerprint after acute and under sustained consumption of a functional beverage based on grape skin extract in healthy human subjects. *Food Funct.* **2015**, *6*, 1288–1298. [CrossRef]
66. Sasot, G.; Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Mercader-Martí, M.; Estruch, R.; Lamuela-Raventós, R.M. Identification of phenolic metabolites in human urine after the intake of a functional food made from grape extract by a high resolution LTQ-Orbitrap-MS approach. *Food Res. Int.* **2017**, *100*, 435–444. [CrossRef]
67. Jiménez-Girón, A.; Ibáñez, C.; Cifuentes, A.; Simó, C.; Muñoz-González, I.; Martín-Álvarez, P.J.; Bartolomé, B.; Moreno-Arribas, M.V. Faecal metabolomic fingerprint after moderate consumption of red wine by healthy subjects. *J. Proteome Res.* **2015**, *14*, 897–905. [CrossRef]
68. Jiménez-Girón, A.; Muñoz-González, I.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Bartolomé, B. Towards the fecal metabolome derived from moderate red wine intake. *Metabolites* **2014**, *4*, 1101–1118. [CrossRef]
69. Queipo-Ortuño, M.I.; Boto-Ordóñez, M.; Murri, M.; Gomez-Zumaquero, J.M.; Clemente-Postigo, M.; Estruch, R.; Cardona Diaz, F.; Andrés-Lacueva, C.; Tinahones, F.J. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am. J. Clin. Nutr.* **2012**, *95*, 1323–1334. [CrossRef]

70. Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Di Lecce, G.; Valderas-Martínez, P.; Tulipani, S.; Jáuregui, O.; Escribano-Ferrer, E.; Estruch, R.; Illan, M.; Lamuela-Raventós, R.M. Bioavailability of tomato polyphenols is enhanced by processing and fat addition: Evidence from a randomized feeding trial. *Mol. Nutr. Food Res.* **2016**, *60*, 1578–1589. [CrossRef]
71. Llorach, R.; Garrido, I.; Monagas, M.; Urpí-Sardà, M.; Tulipani, S.; Bartolomé, B.; Andrés-Lacueva, C. Metabolomics study of human urinary metabolome modifications after intake of almond (*Prunus dulcis* (Mill.) D.A. Webb) skin polyphenols. *J. Proteome Res.* **2010**, *9*, 5859–5867. [CrossRef] [PubMed]

Key messages:

- Adolescence is an important stage characterized by physiological, behavioral, and emotional changes, all of them implicated in their cardiometabolic health.
- Obesity is one the most important modifiable factor associated with the increase of MetS, and the higher prevalence of obesity in adolescents compromise their cardiometabolic health in adulthood.
- Dietary (poly)phenols improve cardiometabolic health and prevent CVD risk and mortality in adults, but up to date, scare studies have been conducted in adolescents.
- The diversity and the complexity of (poly)phenol plant-foods lead to difficulties in their dietary evaluation. FFQ and total (poly)phenol content in urine are used to estimate dietary (poly)phenols. Considering that several factors are implicated in the availability of phenolic compounds, the quantification of phenolic metabolites is also necessary to evaluate which specific compounds may have a health effect.
- Microbial phenolic metabolites have an implication for human health, and some of them could have more biological effects than their phenolic precursor found in plant foods. However, most of the evidence is based on adults, but not on young populations like adolescents.
- Evidence about the cardiometabolic health implication of dietary (poly)phenols and their metabolites needed to be investigated in adolescents to promote early healthy eating intervention and establish dietary recommendations of (poly)phenol intake.



HYPOTHESIS AND OBJECTIVES

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

Dietary (poly)phenols are related to better cardiometabolic health at baseline in adolescents enrolled from the SI! Program for secondary schools in Spain.

2.2. Objectives

2.2.1. General

To study the relationship between dietary (poly)phenols, using different approaches, and cardiometabolic health parameters at baseline in adolescents enrolled from the SI! Program for a secondary school in Spain.

2.2.2. Specific

1. To study the relationship between dietary (poly)phenols, estimated by food frequency questionnaire, and cardiometabolic health parameters. (**Article 2 and 3**)
2. To study the relationship between total (poly)phenols excreted in urine, as a biomarker of (poly)phenol intake, and cardiometabolic health parameters. (**Article 4 and 5**)
3. To validate an analytical method to identify and quantify urinary phenolic metabolites (**Article 6**)
4. To study the relationship between urinary phenolic metabolites and cardiometabolic health parameters (**Article 7**)

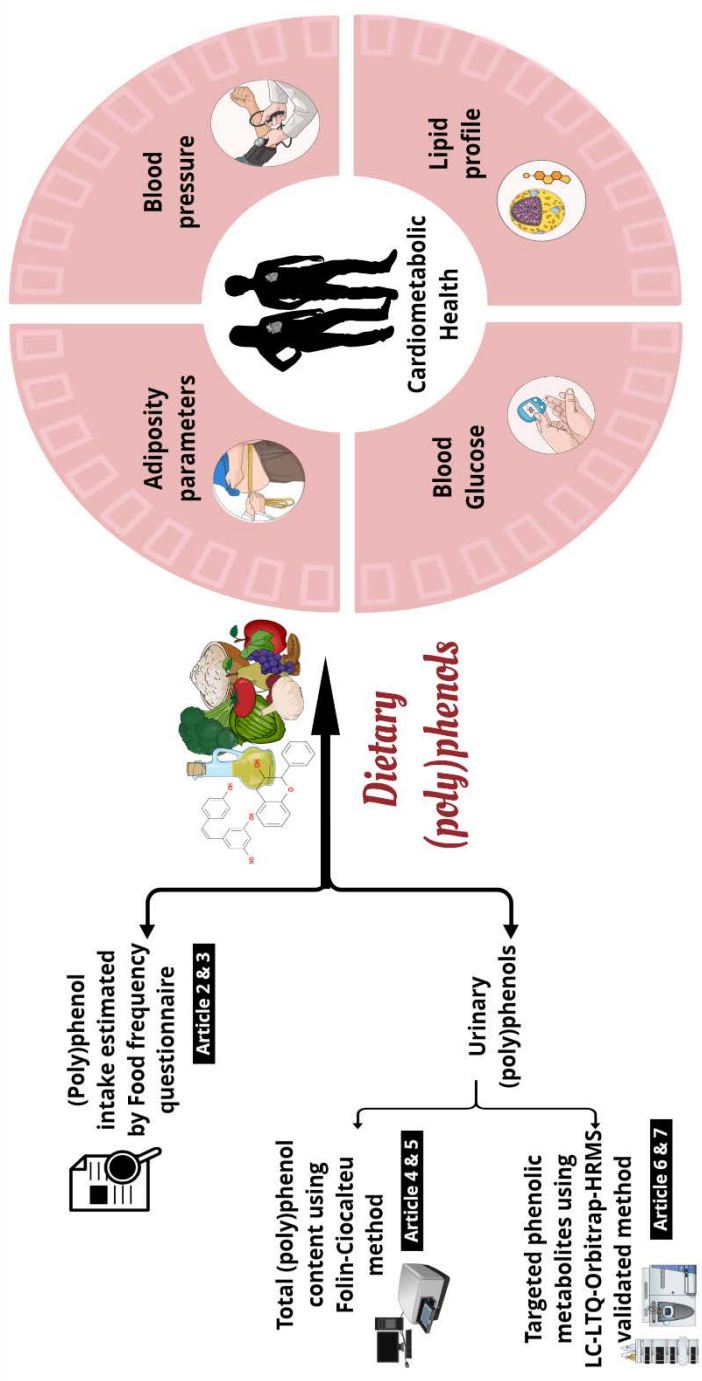
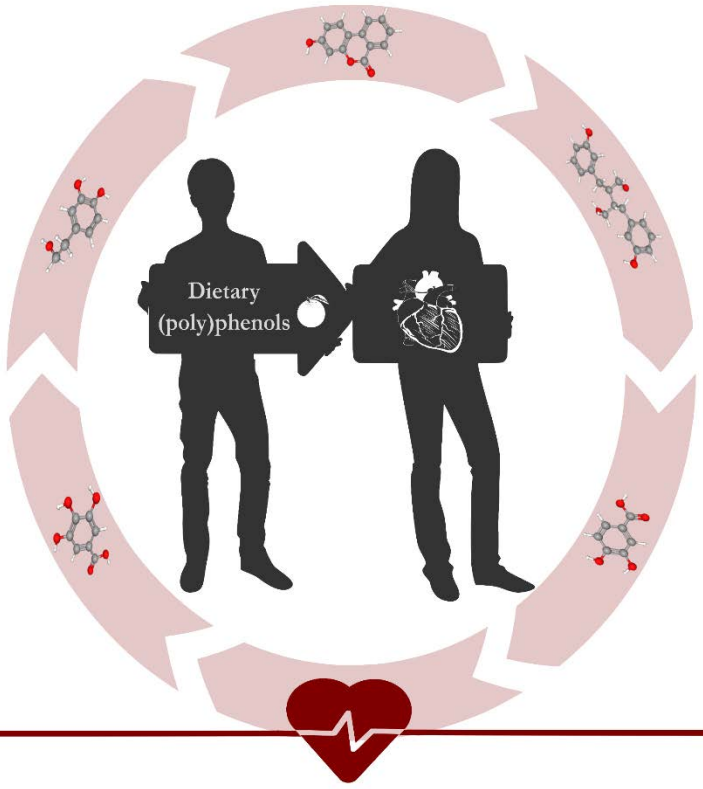


Figure 7. Dietary (poly)phenols, using different approaches, and cardiometabolic health in adolescents aged 11 to 14 years old.



METHODOLOGY

3. METHODOLOGY

3.1. Study design

This thesis has been carried out as a baseline cross-sectional analysis within the SI! Program for Secondary Schools study, a cluster-randomized controlled intervention trial (NCT03504059) aiming to evaluate the effect of a short-term (2-year) or a long-term (4-year) lifestyle educational program on cardiometabolic health in adolescents aged from 12 to 16 years from 24 secondary public schools in Spain (17 in Barcelona and 7 in Madrid). Clusters were schools with the following inclusion criteria: public schools located in Barcelona or Madrid providing education from 1st through 4th grade of Secondary School, with 3 to 5 classes in 1st grade. The local government education agencies from both municipalities invited all the eligible schools to a presentation of the study. The schools that agreed to participate were randomly allocated 1:1:1 to receive the educational intervention through a short-term (2-year) or a long-term (4-year) intervention or to receive the standard curriculum (control). The trial was initiated in 2017 and finalized in 2021. A total of 1326 participants aged between 11 to 14 years old were recruited at baseline (**Figure 8**). Details of the study design and data collection have been previously described by Jiménez-Rodríguez *et al.* (37). The study protocol was approved by the Joint Commission on Ethics of the Instituto de Salud Carlos III in Madrid (CEI PI 35_2016), the Fundació Unió Catalana d'Hospitals (CEI 16/41), and the University of Barcelona (IRB00003099) and conducted in compliance with the Declaration of Helsinki. Parents or caregivers provided assent and written informed consent at the beginning of the study.

3.1. Methods

3.1.1. Evaluation of anthropometric and biochemistry markers

Body weight was measured with an electronic scale (OMRON BF511), and height with a portable stadiometer (SECA 213), with adolescents wearing light clothes and without shoes. Body fat percentage was obtained by bioelectric impedance. Waist circumference (WC) was measured in triplicate using a flexible and non-elastic Holtain tape (HOLTAIN of 0.1cm precision). From the data of these anthropometric variables, diverse indexes were calculated and described in the **articles (from 2 to 7)** presented in this thesis in the **Result section**.

BP was measured with an OMRON M6. Two measurements were taken at two- or three-minutes intervals after the children relaxed and became familiar with the device. In case there was a difference of more than 10 mmHg in the systolic blood pressure (SBP) and/or more than 5mmHg in the diastolic blood pressure (DBP) a third measurement was taken. BG and lipid profile levels (TG, total cholesterol (TC), HDL-c, LDL-c) were analyzed using a portable analyzer CardioCheck Plus device in capillary blood samples, in the morning and fasting.

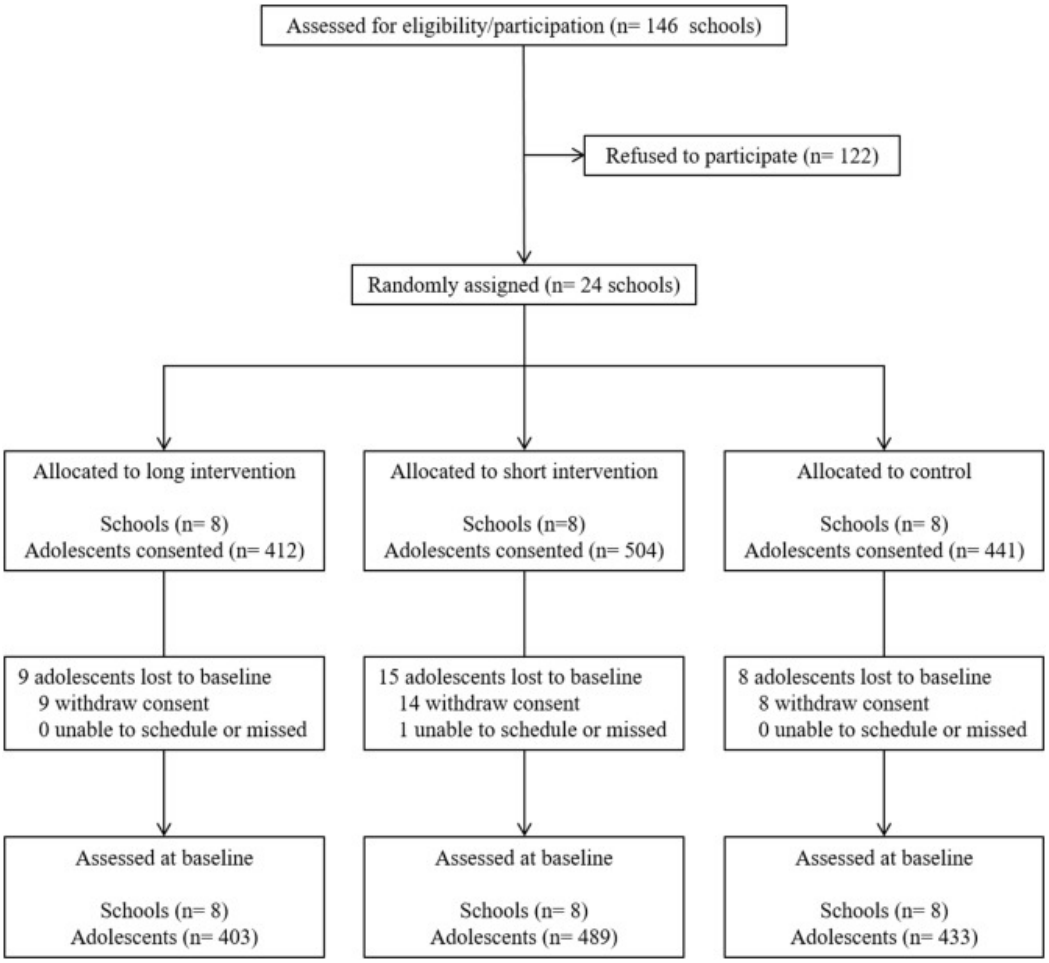


Figure 8. SI! Program for Secondary Schools flow-chart. Jiménez-Rodríguez *et al.* (37).

3.1.2. Evaluation of dietary (poly)phenols

The estimated intake of (poly)phenols and their subclasses (flavonoids, phenolic acids, lignans, stilbenes, and others) are obtained by a validated FFQ. Data on the (poly)phenol content in foods are obtained from the Phenol-Explorer 3.6 database (www.phenol-explorer.eu) (71) following a standardized procedure described by Tresserra-Rimbau *et al.* and Castro-Barquero *et al.* (79,80). Details of the estimation of dietary (poly)phenols are described in **Article 2.**

3.1.3. Evaluation of (poly)phenols in urine

3.1.3.1. Total (poly)phenols by the Folin-Ciocalteu method

Spot urine samples were collected in the morning and stored at −80°C until analyses. For all the urine samples, total (poly)phenols and creatinine were analyzed as described by Medina-Remón A. et al (72,102). The validated Folin-Ciocalteu (F-C) spectrophotometric method was applied to determine the content of total (poly)phenol excretion (TPE), previously a solid phase extraction to avoid interferences with other plausible component presence in urine was carried out. TPE was expressed by mg gallic acid equivalent (GAE)/g creatinine (**Figure 9**). **Articles 4** and **5** described the methodology used.

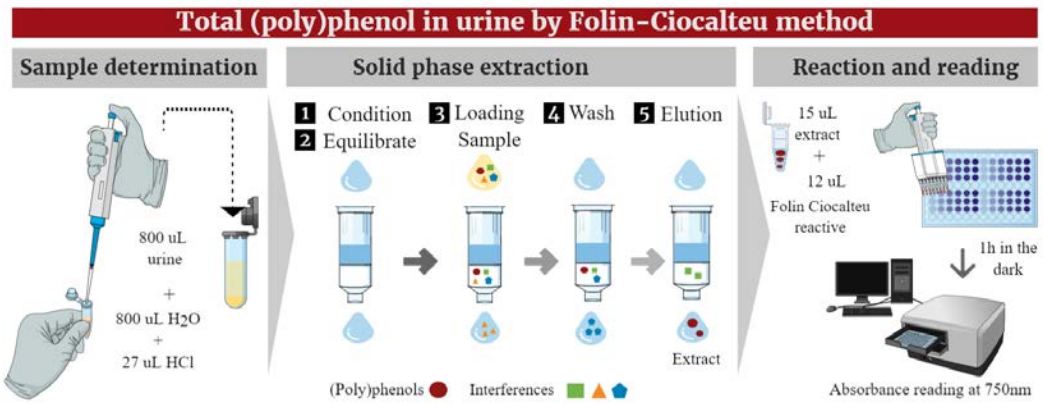


Figure 9. Method to estimate total (poly)phenols in urine by Folin-Ciocalteu.

3.1.3.2. Urinary phenolic metabolites by LC-LTQ-Orbitrap-HRMS

Identification and quantification of phenolic compounds from phenolic metabolites, especially gut microbiota phenolic metabolites, were performed by using liquid chromatography coupled to a linear trap quadrupole high resolution

mass spectrometer (LC-LTQ-Orbitrap-HRMS). This analytical method was validated, developed, and explained in **Articles 6** (74).

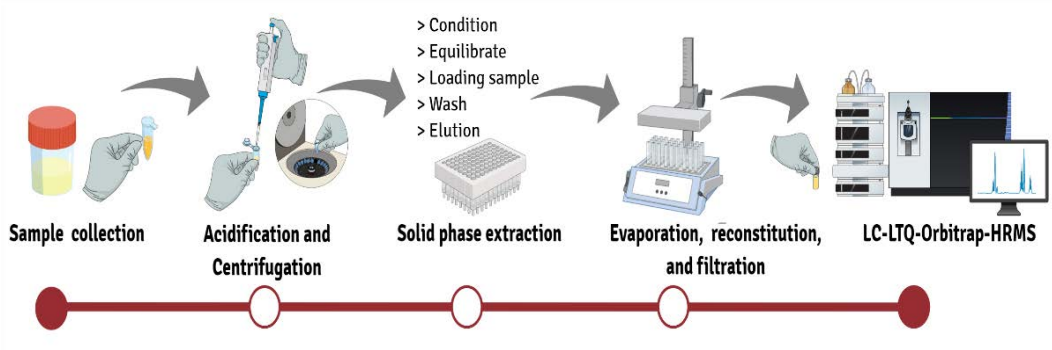


Figure 10. Schematic representation of the experimental analysis of urinary phenolic metabolites by LC-LTQ-Orbitrap-HRMS.

3.1.3.3. Evaluation of physical activity

Physical activity was estimated by accelerometry (Actigraph wGT3X-BT). Chandler' cut-off points were applied for the calculation of time spent in different physical activity intensities (103). Additionally, a validated physical activity questionnaire was applied and described in the protocol design of the original study (37).

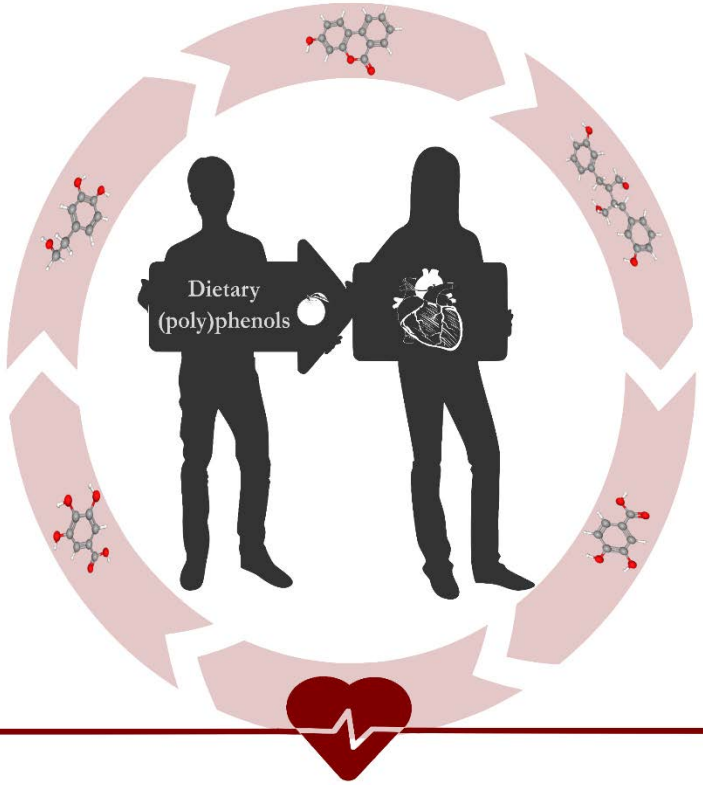
3.1.3.4. Evaluation of sociodemographic variables and other covariates

Information about parental education and household income was obtained from a questionnaire answered by the parents of the participants (37). Educational parents' educational levels were categorized following the cut-off described by UNESCO (104). Household incomes were categorized as low, medium, and high based on salary in Spain (105). Sexual maturation status was determined according to Tanner & Whitehouse method, using pictograms (106).

3.1.3.5. Statistical analyses

Data analyses were carried out using Stata statistical software package version 16.0 (StataCorp., College Station, TX, USA) and R software (R Foundation for Statistical Computing, Vienna, Austria) in most of the articles presented in this thesis. To estimate (poly)phenol intake, SPSS version 27.0 (Chicago, IL, USA) was applied. A descriptive analysis of the population is presented as means ± SD and frequency distribution, for continuous and categorical variables, respectively. Association between dietary (poly)phenols even urinary (poly)phenols and cardiometabolic

health parameters in adolescents were evaluated using mixed-effect multiple regression models. No imputation was applied in case of missing data in some variables. These statistical analyses have been extensively explained in all the articles presented in this thesis.



RESULTS

4. RESULTS

This section presents the results obtained during the doctoral thesis. These results have been classified into four sections, according to the specific objectives mentioned before, as it is shown:

1. Dietary (poly)phenol intake and cardiometabolic health in adolescents (**Articles 2 and 3**)
2. Total urinary (poly)phenols and cardiometabolic health in adolescents (**Articles 4 and 5**)
3. Validation of an analytical method to identify and quantified urinary microbial phenolic metabolites (**Article 6**)
4. Urinary phenolic metabolites and cardiometabolic health parameters in adolescents (**Article 7**)

4.1. Dietary (poly)phenol intake and cardiometabolic health in adolescents

4.1.1. Article 2: Dietary (poly)phenol intake is associated with some cardiometabolic health parameters in adolescents.

Emily P. Laveriano-Santos, Sara Castro-Barquero, Camila Arancibia-Riveros, Anna Tresserra-Rimbau, Ana María Ruiz-León, Rosa Casas, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Jesús Martínez-Gómez, Gloria Santos-Beneit, Juan M. Fernández-Alvira, Rodrigo Fernández-Jiménez, and Rosa M. Lamuela-Raventós

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Abstract

Introduction
Adolescence is a critical period for later cardiometabolic complications. Several studies about the protective role of (poly)phenol on metabolic disorders in adults are reported, but little explored in adolescents.

Objective
To explorer associations between intake of (poly)phenol and its subclasses with cardiometabolic health parameters in adolescents

Methods
A cross-sectional study was conducted on 944 individuals aged 11 to 14 years from the Si! Program for secondary school trial, with available semi-quantitative food frequency questionnaires. (Poly)phenol intake was assessed using Phenol-Explorer database. Cardiometabolic parameters included waist circumference (WC), blood pressure (BP), blood glucose (BG), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c). Multilevel mixed effect linear regression analysis was used to assess quintiles of (poly)phenol intake and cardiometabolic parameters.

Results
The mean intake of total (poly)phenol in this population was 683.2 ± 379.4 mg/day. Compared to the lowest quintile, adolescents in the highest quintile of total (poly)phenol intake had lower WC z-score (β=-0.09, 95%CI= -0.09; -0.08, *P-for*

trend=0.002), mean arterial pressure (MAP) z-score (β =-0.11, 95% CI=-0.14; -0.08, *P-for* trend=0.247) and HDL-c (β=-1.44, 95% CI=-2.59; -0.29, *P-for* trend=0.086), after multivariable adjustment. WC z- score (β=-0.11, 95%CI= -0.18; -0.04, *P-for trend*=0.007), diastolic BP z-score (β=-0.12, 95% CI= -0.22; -0.01, *P-for* trend=0.001), and HDL-c (β=-2.11, 95% CI= -3.61; -0.60, *P-for* trend=0.038) were lower in the highest quintile of flavonoids intake as compared to lowest quintile. The highest quintile of phenolic acids intake was associated with lower WC z-score (β=-0.15, 95% CI= -0.28; -0.01, P-for trend=0.016), BG (β=-0.37, 95% CI= -0.73; -0.01, *P-for* trend=0.793), TG (β=-9.91, 95% CI= -14.82; -5.00, *P-for* trend <0.001), and highest quintile of stilbenes intake had associated with lower BG (β=-1.82, 95% CI= -3.11; -0.52, *P-for* trend <0.001), TG (β=-3.55, 95% CI= -6.60: -0.51, *P-for* trend=0.108) and higher HDL-c (β=2.33, 95% CI= 1.31; 3.35, *P-for* trend=0.446) compared to the lowest quintile.

Conclusion

In summary, a higher intake of (poly)phenol is associated with better cardiometabolic parameters. Clinical studies are needed to confirm this effect and establish dietary recommendations.

Dietary (poly)phenol intake is associated with some cardiometabolic health parameters in adolescents.

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Conflict of interest

R.M.L-R reports receiving lecture fees from Cerveceros de España and receiving lecture fees and travel support from Adventia and Idilia Foods SL. RE reports grants from Fundación Dieta Mediterránea, Spain and Cerveza y Salud, Spain. Also, personal fees for given lectures from Brewers of Europe, Belgium, Fundación Cerveza y Salud, Spain, Pernaud-Ricard, Mexico, Instituto Cervantes, Alburquerque, USA; Instituto Cervantes, Milan, Italy, Instituto Cervantes, Tokyo, Japan, Lilly Laboratories, Spain, and Wine and Culinary International Forum, Spain, and non-financial support to organize a National Congress on Nutrition. Also feeding trials with products from Grand Fountain and Uriach Laboratories, Spain. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

The other authors declare that they have no competing interests.

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ANOVA: Analysis of variance

β: unstandardized coefficient

BG: blood glucose

CI: confidence interval

DBP: diastolic blood pressure

FFQ: food frequency questionnaire

HDL-c: high-density lipoprotein-choleste

MAP: mean arterial pressure

SBP: systolic blood pressure

SD: standard deviation

TG: triglycerides

TPI: total (poly)phenol intake

WC: waist circumference

ABSTRACT

Background: Adolescence is a critical period for later cardiometabolic complications. Several studies about the protective role of (poly)phenol on metabolic disorders in adults have been reported but little explored in adolescents.

Objective: To explore associations between (poly)phenol intake and its subclasses with cardiometabolic health parameters in adolescents.

Methods: A cross-sectional study was conducted on 944 individuals aged 11 to 14 years enrolled in the SI! Program for Secondary School trial (NCT03504059, http://www.clinicaltrials.gov), with available semi-quantitative food frequency questionnaires. (Poly)phenol intake was assessed using the Phenol-Explorer database. Cardiometabolic parameters included waist circumference (WC) age-sex z-scores, blood pressure age-sex z-scores, blood glucose (BG), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c). Multilevel mixed effect linear regression analysis was used to assess the relationship between quintiles of (poly)phenol intake and cardiometabolic parameters.

Results: The mean intake of total (poly)phenol in this population was 683.2 ± 379.4 mg/day. Compared to the lowest quintile, adolescents in the highest quintile of total (poly)phenol intake had lower WC z-score (β=-0.09, 95%CI= -0.09; -0.08, *P-for* trend=0.002), mean arterial pressure (MAP) z-score (β =-0.11, 95% CI=-0.14; -0.08, *P-for* trend=0.247) and HDL-c (β=-1.44, 95% CI=-2.59; -0.29, *P-for* trend=0.086), after multivariable adjustment. WC z- score (β=-0.11, 95%CI= -0.18; -0.04, *P-for* trend=0.007), diastolic BP z-score (β=-0.12, 95% CI= -0.22; -0.01, *P-for* trend=0.001), and HDL-c (β=-2.11, 95% CI= -3.61; -0.60, *P-for* trend=0.038) were lower in the highest quintile of flavonoids intake as compared to lowest quintile. The highest quintile of phenolic acids intake was associated with lower WC z-score (β=-0.15, 95% CI= -0.28; -0.01, *P-for* trend=0.016), BG (β=-0.37, 95% CI= -0.73; -0.01, *P-for* trend=0.793), TG (β=-9.91, 95% CI= -14.82; -5.00, *P-for* trend <0.001), and highest quintile of stilbenes intake had associated with lower BG (β=-1.82, 95% CI= -3.11; -0.52, *P-for* trend <0.001), TG (β=-3.55, 95% CI= -6.60: -0.51, *P-for* trend=0.108) and higher HDL-c (β=2.33, 95% CI= 1.31; 3.35, *P-for* trend=0.446) compared to the lowest quintile.

106 Conclusion: Higher intake of (poly)phenols, especially flavonoids, phenolic acids, and
107 stilbenes is associated with healthier cardiometabolic parameters. Clinical studies are
108 needed to confirm this effect and establish dietary recommendations.

109 Keywords: phytochemical, stilbene, resveratrol, lignan, bioactive compounds,
110 cardiovascular health, teenagers, pediatrics

Introduction

Adolescence is a critical period of physiological and social changes. The presence of cardiometabolic risk factors like obesity, higher blood pressure, and alteration in the metabolism of lipids and glucose during adolescence, increases the risk of cardiometabolic complications in adulthood [1,2]. Behavioral strategies, based on an active lifestyle and healthy dietary patterns, are considered the best way to improve cardiometabolic health [3,4].

In terms of dietary patterns, a diet based on plant-derived foods, characterized by a high content of (poly)phenols, has gained importance due to its protective role on cardiometabolic health parameters [5–7]. The health effect of dietary (poly)phenol is attributable to anti-inflammatory and prebiotic properties involved in different pathways which include reduction of adiposity marker, blood pressure (BP), improving lipid profile, and glucose metabolism [8–11]. (Poly)phenols represent the largest group of phytochemicals, with more than 8,000 different compounds described, and can be classified depending on their chemical structure into flavonoids, phenolic acids, stilbenes, and lignans [11]. The diversity and complexity of phenolic compounds lead to difficulties in their dietary estimation. The most reliable tools for (poly)phenol intake are 24-h dietary recall, food diaries, or food frequency questionnaires (FFQ). FFQ is the most popular dietary assessment tool used to measure food sources of (poly)phenol intake, because of its ability to measure long-term exposure to dietary factors [12].

To date, there are only very few studies about dietary (poly)phenols and cardiometabolic health in adolescents, therefore the aim of the present study was to evaluate the cross-sectional association of (poly)phenol intake with cardiometabolic health parameters in adolescents enrolled in the SI! (*Salud Integral-Comprehensive Health*) Program for Secondary Schools in Spain.

Materials and methods

Design of the study and participants

The present cross-sectional study was conducted within the framework of the baseline data of the SI! (*Salud Integral-Comprehensive Health*) Program for Secondary Schools trial (NCT03504059) collected at participants' enrollment. A detailed description of the

original study design, recruitment methods, and data collection processes has been previously published by Fernandez-Jimenez et al. [13]. The SI! Program trial was conducted following the ethical guidelines of the Declaration of Helsinki, and the protocol was approved by institutional ethics committees. Participants were informed about the methodology of the study, and parents or caregivers provided written informed consent before joining the study.

A total of 1,326 adolescents from 24 Secondary Schools in Spain were recruited at baseline of the SI! Program trial. Of the total sample, 382 participants were excluded from the present analysis (**Figure 1**): 141 without or incomplete FFQ data at baseline, and 241 participants who under- or over-reported dietary energy intake (less than 803 or above 4,013 Kcal/day for male, and less than 502 or above 3,511 Kcal/day for female) [14,15].

Assessment of cardiometabolic health parameters

Cardiometabolic parameters were considered according to the variables evaluated by the International Diabetes Federation: waist circumference (WC), BP, blood glucose (BG), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) [16].

All the anthropometric, blood pressure, and biochemistry analyses were performed in the morning and fasting by trained staff, details of the data collection methodology are described by Fernandez-Jimenez et al. [13]. Weight and height were measured to the nearest 0.1 kg scale and 0.1 cm stadiometer, respectively. WC was measured to the nearest 0.1 cm using a flexible Holtain tape [13]. Body mass index (BMI) was obtained by dividing weight in kilograms by the square of height in meters. Age-and sex-specific BMI and WC z-scores were calculated using Centers for Disease Control standard references [17,18].

Systolic and diastolic blood pressure (SBP and DBP) was measured in a sitting position using a digital automatic monitor (Omron M6, OMROM Health are Co., Kyoto, Japan) [13]. In this analysis, the lowest reading of SBP and DBP separately was considered. Age- and sex-specific SBP and DBP z-scores were calculated according to the High Blood Pressure Working Group of the National Blood Pressure Education Program for children and adolescents [19]. Additionally, mean arterial pressure (MAP) was also considered in the analysis because is a useful indicator of blood pressure, using the following formula:

MAP = DBP + 1/3(SBP–DBP) [20,21]. Age-and sex-specific MAP z-scores were calculated based on our population.

Blood parameters such as BG, TG, and HDL-c concentrations were analyzed using a portable whole blood analyzer, CardioCheck Plus, in capillary blood samples [13].

Assessment of dietary, socio-demographic, and lifestyle information

Dietary intake was assessed using a semiquantitative FFQ consisting of 151 items [15]. Standard units or portion size was specified for each food item, and participants were asked about the average frequency of food consumption (daily, weekly, or monthly) during the previous year. The daily food portion size was converted into grams to estimate energy and nutrient intake, using values from Spanish food composition tables [22,23].

Information about parental education and household income was evaluated by a self-reported questionnaire [13]. The parental education level was defined according to the International Standard Classification of Education into low (no studies, primary or secondary education), medium (post-secondary non-tertiary education or short-cycle tertiary education), and high (university education) levels [24]. For the statistical analysis, parental education was categorized into high and non-high (low and medium). Household income was categorized into three categories: low (under 29.132 €), medium (approximately 29.132 €), and high (above 29.132 €), based on the cut-off of the mean salary in Spain [25].

Physical activity was estimated by accelerometry (Actigraph wGT3X-BT, ActiGraph, Pensacola, USA) [13], and moderate-to-vigorous physical activity (min/day) was categorized according to the cut points of Chandler *et al.* [26]. Physical activity information was additionally obtained by a self-reported questionnaire, including a list, frequency, and amount of time of daily physical activities [13,27]. Pubertal status was determined according to Tanner and Whitehouse's method, using pictograms based on a scale [28].

Estimation of dietary (poly)phenol intake

The total (poly)phenol and its subclasses intake were assessed using an online Phenol-Explorer database (version 3.6) [29], following a standardized procedure previously described [7,30]. (Poly)phenol content from food items containing more food components

was estimated by taking into account individual portion ingredients from traditional Spanish recipes. Food items of the FFQ with traces or no content of (poly)phenols were excluded, due to lack of contribution to (poly)phenol intake. For the present analysis, individual (poly)phenol intake was estimated by multiplying its individual phenolic content in each food by the daily consumption of each food. TPI was calculated as the sum of individual subclasses of (poly)phenol: flavonoids (sum of anthocyanins, chalcones, flavanols or flavan-3-ols, flavanones, flavones, flavonols, and isoflavonoids), phenolic acids, lignans, stilbenes, and other polyphenols (including alkylphenols, alkylmethoxyphenols, furanocoumarins, hydroxybenzaldehydes, naphtoquinones, tyrosols, curcuminoids, among others). Individual (poly)phenols were obtained by chromatography and chromatography after hydrolysis (in the case of lignans and some hydroxybenzoic and hydroxycinnamic acids). In the case of flavonoids data on proanthocyanidins was obtained by normal-phase high-performance liquid chromatography.

Statistical analysis

Data analyses were carried out using Stata statistical software package version 16.0 (StataCorp., College Station, TX, USA), SPSS version 27.0 (Chicago, IL, USA), and R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). A descriptive analysis of the population is presented as means ± standard deviation (SD) and frequency distribution, for continuous and categorical variables, respectively. One-way analysis of variance (ANOVA) test or chi-squared test was used for comparison of characteristics of participants among quintiles of energy-adjusted TPI. Additionally, a T-test or ANOVA was used to compare differences between sociodemographic and lifestyle categorical variables and TPI and its subclasses (continuous). Bonferroni test was used for the pairwise multiple comparisons in independent groups.

For the statistical analysis, total and (poly)phenol subclasses were adjusted by energy intake (kcal/day) using the residual method described by Willet et al. [14] and categorized into quintiles.

Association between quintiles of energy-adjusted TPI with cardiometabolic health parameters (WC z-score, DBP z-score, SBP z-score, MAP z-score, HDL-c, TG, BG, continuous) was performed using multiple multilevel mixed effect linear regression, with

robust variance. We compared quintiles using as reference the lowest quintile of (poly)phenol. Results of the regression models were expressed as unstandardized ß coefficients and their 95% confidence intervals (CI). Fixed effects models were as follows: (model 1) was adjusted for sex (female/male) and age (continuous); (model 2) included variables in model 1 plus pubertal status (score I to V), physical activity (moderate-vigorous physical activity min/day, quintiles), BMI z-score (continuous, this variable did not consider in the analysis with WC), fasting (yes/no), high parental educational level (yes/no), and household income (low/medium/high); and (model 3) included model 2 plus energy intake (kcal/day, continuous), fiber (g/day, continuous), added sugar (g/day, continuous), and protein (g/day, continuous). SBP, DBP, and MAP were additionally adjusted by sodium (mg/day, continuous). Municipalities and schools were considered random effects. Linear regression analyses were also performed to explore plausible associations between specific quintiles of energy-adjusted (poly)phenol subclasses and cardiometabolic health parameters, adjusted by the full regression model mentioned before (model 3). Test for trends across energy-adjusted quintiles of (poly)phenol and its subclasses intake was calculated by post-estimation contrast test.

Additionally, a multilevel mixed-effect linear regression analysis was conducted to explore associations between energy-adjusted flavonoid subclasses intake and cardiometabolic parameters, all of them as continuous variables. To avoid skewed distribution, flavonoid subclasses data was normalized and scaled in 1-SD. Due to the cardiometabolic health implications of flavanols or flavan-3-ols [31], a sub-analysis was conducted to observe the relationship between categories of flavanol intake and cardiometabolic parameters in our sample. To this, flavonoid intake was categorized according to the guideline recommendation proposed by Crowe-White K. et al. as follows: low (<400mg/day), recommended (400-600 mg/day), and high (>600 mg/day) [31]. In those analyses, fixed effects included variables in model 3 as described earlier and random effects included municipalities and schools.

The level of significance reported was set at a two-sided test and compared to a significance level of 5%.

Results

General characteristics

Table 1 presents the participants' characteristics. Among the 944 adolescents (female 48%), the mean age was 12.0 ± 0.4 years. No significant differences were observed between physical activity, parental education level, and household income between quintiles of energy-adjusted TPI.

Dietary characteristics

Regarding dietary (poly)phenol, the mean of energy-adjusted TPI was 683.2 ± 379.4 mg/day, and the most consumed classes were flavonoids (530.1 ± 331.3 mg/day) and phenolic acids (97.8 ± 64.5 mg/day) which represented 77.6% and 14.3% of TPI, respectively (**Table 2**). The main contributor of (poly)phenol subclasses were proanthocyanidins, followed by hydroxycinnamic acids, and flavanones (**Table 2**). The main food sources of (poly)phenols were fruits and their derivatives, such as natural juices or juice from concentrate; chocolate and chocolate products; vegetal oils, especially extra virgin olive oil and olives; vegetables, such as spinach, lettuce, cabbage, among others, and nuts, mainly walnuts.

Dietary energy-adjusted (poly)phenols intake and their subclasses according to sociodemographic characteristics are presented in **Supplementary Table 1.** TPI, flavonoids, and phenolic acids were higher in females than males. Participants with parents with primary/secondary school education levels had a higher intake of phenolic acids. Adolescents with a high household income had a low intake of phenolic acids, lignans, and stilbenes.

Participants in the highest quintile of energy-adjusted TPI had a significantly higher consumption of fruits, vegetables, whole-grain cereals, and nuts; and lower consumption of refined cereals, dairy products, meat, and cookies, compared to the lowest quintile. Additionally, lower intake of proteins, total fat, even saturated and monounsaturated fatty acids, carbohydrates, and sodium; and higher intake of added sugars and fiber were observed in the highest quintile of energy-adjusted TPI than in the lowest quintile (**Supplementary Table 2**).

Dietary (poly)phenols and cardiometabolic health parameters

Associations between quintiles of energy-adjusted TPI and cardiometabolic health parameters are described in **Table 3**. In the full-adjusted model, participants in the highest

quintile of energy-adjusted TPI had lower values in some cardiometabolic health parameters as compared to participants in the lowest quintile: WC z-score (β=-0.09, 95% CI= -0.09; -0.08, *P-for* trend=0.002), MAP z-score (β =-0.11, 95% CI=-0.14;-0.08, *P-for* trend=0.247), and HDL-c (β=-1.44, 95% CI=-2.59; -0.29, *P-for* trend=0.086).

Due to the heterogeneity of (poly)phenols, the main groups were studied separately (total flavonoids, total phenolic acids, lignans, stilbenes, and other (poly)phenols) as shown in **Figures 2, 3,** and **Supplementary Table 3.** Compared to lowest quintile of energy-adjusted flavonoids intake, lower WC z-score (β=-0.11, 95% CI= -0.18; -0.04, *P-for* trend=0.007), DBP z-score (β=-0.12, 95% CI= -0.22; -0.01, *P-for* trend=0.001), and HDL-c (β=-2.11, 95% CI= -3.61; -0.60, *P-for* trend=0.038) were found in the participants in the highest quintile. Details of analyses between subclasses of flavonoids and cardiometabolic parameters are described in **Supplementary Table 4**. Briefly, a higher intake of anthocyanins was associated with lower BG (β=-0.28, 95% CI= -0.49; -0.07, *P* =0.009) and TG (β=-2.77, 95% CI= -5.10; -0.45, *P* =0.019). Flavonols were inversely associated with WC z-score (β=-0.02, 95% CI=-0.05; -0.002, *P* =0.032) and BG (β=-0.24, 95% CI= -0.30; -0.18, *P* <0.001). Flavanols were directly associated with BG (β=0.27, 95% CI= 0.11; 0.44, *P* =0.001) and inversely with HDL-c (β=0.76, 95% CI= -0.91; -0.61, *P* <0.001). However, when flavanol intake was categorized, participants with a flavanol intake <400mg/day had higher values of WC z-score compared to those with a recommended intake (400-600 mg/day) (β=0.03, 95% CI= 0.01;0.05, *P-for* trend=0.390). Additionally, participants with a high flavanol intake (> 600mg/day) had higher values of BG (β=2.31, 95% CI= 1.30;3.33, *P-for* trend=0.705) and lower HDL-c (β=-1.91, 95% CI= -1.26;-1.13, *P-for* trend=0.002) compared to those with a recommended intake (**Supplementary Table 5)**.

Participants in the highest quintile of energy-adjusted phenolic acids intake showed lower WC z-score (β=-0.15, 95% CI= -0.28; -0.01, *P-for* trend=0.016), BG (β=-0.37, 95% CI= -0.73; -0.01, *P-for* trend=0.793), TG (β=-9.91, 95% CI= -14.82; -5.00, *P-for* trend <0.001) compared to the lowest quintile. Additionally, the highest quintile of energy-adjusted stilbenes intake was associated with lower BG (β=-1.82, 95% CI= -3.11; -0.52, *P-for* trend <0.001) and TG (β=-3.55, 95% CI= -6.60; -0.51, *P-for* trend=0.108), and higher HDL-c (β=2.33, 95% CI= 1.31; 3.35, *P-for* trend=0.446) compared to the lowest quintile. Finally, regarding other polyphenols, participants in the highest quintile of this

(poly)phenols intake presented lower values of BG (β=-1.60, 95% CI= -3.13; -0.08, *P-for* trend=0.040) compared to the lowest quintile (**Figures 2, 3,** and **Supplementary Table 3)**.

Discussion

The present cross-sectional study shows inverse associations between certain dietary (poly)phenols, especially flavonoids, phenolic acids, stilbenes, and "other (poly)phenol" and WC z-score, MAP z-score, DBP z-score, BG, TG, and HDL-c (except for stilbenes, for which a direct association was observed).

Dietary (poly)phenol intake in adolescents

There are few studies about the estimation of dietary (poly)phenols intake in adolescents. One of the most important in Europe is the HELENA study, which estimated dietary (poly)phenol intake using 24-h recall in 2,428 adolescents aged 12.5 to 17.5 years old in 9 European countries. In the HELENA study, a median of (poly)phenol intake of 326 mg/day was reported [32], being considerably lower than the median of 598.6 mg/day found in our study. In another study, a survey conducted on 2,045 adolescents aged 11 to 18 years old in the UK, the mean intake of total (poly)phenols was 455 ± 263.2 mg/day [33]. The higher values of total (poly)phenols showed in our study compared to the two cohorts, could be to the difference in flavonoid intake. Flavonoids and phenolic acid were the main (poly)phenol subclasses in our study, representing 77.6% and 14.3% of TPI. Similar results were found in the HELENA study, where flavonoids and phenolic acid represented 75-76% and 17-19% respectively [32], and in the UK Cohort, represented approximately 78% and 22% of total (poly)phenols intake [33]. Flavanols or flavan-3-ols was the main contributor to total flavonoid intake

Lignans and stilbenes had a lower contribution to TPI, below 0.6%, likewise to a previous study in European adolescents [32]. These similarities could also be attributable that both studies used the Phenol-Explorer database. In the case of the contribution of different food groups to the daily (poly)phenol intake, the values found in this study are similar to those reported in other studies, where fruits, vegetables, cocoa-based products, and nuts are one of the main contributors to (poly)phenols intake [32,33]. Regarding "other (poly)phenols", in our study, olive oil and olives were the main contributors to tyrosol intake, as in the HELENA study [32].

Dietary TPI and cardiometabolic health parameters

Phenolic intake shows a positive influence on cardiovascular health [30]. Estimating habitual (poly)phenol intake through FFQs or other dietary instruments is important to understand the protective effect of long-term dietary (poly)phenol exposure on health [34].

In our study, we found that higher TPI was associated with lower values of WC z-score, MAP z-score, and HDL-c, but no association with other cardiometabolic health parameters also evaluated as SBP z-score, DBP z-score, BG, and TG after the adjustment of anthropometric, lifestyle, sociodemographic and dietary covariates. Wisnuwardani et al., showed no differences between quartiles of (poly)phenol intake in 657 European adolescents with cardiometabolic variables (the sub study from HELENA cohort), only BMI z-score was lower in the group of high (poly)phenol intake after the adjustment for potential confounders [35]. Although both studies are conducted in adolescents, there are some differences in polyphenol intake (the median (poly)phenol intake in the HELENA study was 347.2 mg/day, lower than in our study: 683.2 ± 379.4 mg/day), probably by differences in dietary patterns. In the HELENA study, about 70% of the adolescents were from non-Mediterranean countries [35]. Other studies conducted in adult populations with higher metabolic syndrome risk and higher dietary (poly)phenol intake support some of our findings. In the HAPIEE study, high TPI was inversely associated with WC, SBP, DBP (only in women), and TG (only in women) in 8,821 adults aged 45-69 years old in Poland. The TOSCA.IT cohort, conducted in 2,573 people with type 2 diabetes in Italy, showed that a higher intake of TPI was associated with lower values of SBP and TG, and with higher HDL-c levels. In the PREDIMED-Plus cohort, higher TPI was directly associated with HLD-c (β=0.37, 95% CI= 0.10; 0.64) in 6,633 adults aged 65.0 ± 4.9 years old with Metabolic Syndrome in Spain [7]. By contrast, we found an inverse association with HDL-c, probably because our sample population is young and healthy.

Mechanisms of action of dietary (poly)phenol are related to its capacity to improve endothelial function and antioxidant status and modulate adipose tissue by inhibiting adipocyte differentiation, modulating lipolysis, and activating ß-oxidation [36]. Moreover, (poly)phenol could inhibit gluconeogenesis and stimulate the release of insulin by pancreatic β-cells. It could explain the link between total dietary (poly)phenol and some cardiometabolic parameters found in our study.

Dietary (poly)phenol subclasses intake and cardiometabolic health parameters

Due to the heterogeneity in the chemical structure of dietary (poly)phenol subclasses, we studied by separate: flavonoids, phenolic acids, lignan, stilbene, and others. The intake of (poly)phenol and subclasses at certain doses might have an impact on cardiometabolic health, by decreasing body weight, BP, and improving the metabolism of glucose and lipids [7,30,37–39].

Flavonoids were the most consumed phenolic compounds in our sample population, and their highest energy-adjusted intake was associated with a lower WC z-score, DBP z-score, and HDL-c compared to the lowest intake. Similar findings were described in the TOSCA.IT cohort, where adults with type 2 diabetes with high flavonoid intake had lower levels of TG, SBP, and DBP [40]. In the PREDIMED-Plus cohort, adults with metabolic syndrome and with high flavonoid intake had low WC [7]. By contrast, in the HELENA study, higher flavonoid intake was not associated with WC z-score, only with a lower BMI z-score [35]. Among studies conducted outside Europe, Sohrab et al. showed that flavonoid intake was inversely associated with SBP and DBP in Iranian adults (n=2,618) aged 19 to 64 years old [41]. Out of flavonoid subclasses, flavanols or flavan-3-ols are one of the most important due to their implications in cardiometabolic outcomes, improving blood pressure, cholesterol levels, and blood sugar [31]. Therefore, an Expert Panel has recently proposed a guideline recommendation highlighting 400-600 mg/day of flavanol intake protects cardiometabolic health [31]. Our findings are in agreement with this guideline recommendation, thus we found that adolescents with a flavanol intake <400mg/day had 0.03 higher values of WC z-score compared to those with a recommended intake (400-600 mg/day), and adolescents with a high flavanol intake (> 600mg/day) had 2.31 higher values of BG and 1.91 lower HDL-c compared to those with a recommended intake. Our results could be explained because flavonoids are implicated in several molecular pathways related to cardiometabolic health. Flavonoids regulate nitric oxide (NO) levels in the vascular endothelial cell by increasing the activation of NO synthase and endothelial NO synthase, thus regulating BP [42]. Additionally, the well-described antioxidant and anti-inflammatory properties of flavonoids, could explain their action on mechanisms related to lipid metabolism and weight status like the inhibition of the differentiation of adipocytes, increase of fatty acid oxidation, decrease

419 in the synthesis of fatty acids, increase in thermogenesis and energy expenditure [43].
420 Flavanoids have also prebiotic properties that may affect microbiota metabolism [44].

421 Phenolic acids were the second (poly)phenol subclass most widely consumed in our
422 adolescent population, and we found that the highest quintile of energy-adjusted phenolic
423 acid intake was associated with lower values of WC z-score, BG, and TG compared to
424 the lowest quintile. Similar findings were reported in two studies conducted in adults,
425 where a higher intake of phenolic acids was associated with a better profile of lipid and
426 glucose metabolism [38,40]. In the HELENA study, ferulic acid, a hydroxycinnamic acid,
427 was inversely associated with WC in European adolescents [35]. Results from an *in vitro*
428 study conducted by Fujimaki et al. concluded that phenolic acids could suppress lipid
429 accumulation and regulate the mRNA expression of adipogenic transcription factors, both
430 mechanisms implicated in obesity [45]. Phenolic acid also can regulate metabolic
431 hormone secretion, including insulin, leptin, adiponectin, and proinflammatory cytokines,
432 implicated in the metabolism of glucose and lipids [46].

433 Regarding stilbenes, resveratrol is the most relevant and it is implicated in several
434 mechanisms related to metabolic health [9]. In our study, although its dietary contribution
435 was lower (less than 0.05%), higher energy-adjusted stilbenes intake was associated with
436 lower values of BG and with a better lipid profile (lower TG and higher HDL-c levels).
437 Higher levels of HDL-c are an indicator of better cardiometabolic health. In our study we
438 found some contradictions with (poly)phenols subclasses, while higher flavonoid intake
439 was associated with lower HDL-c levels, stilbenes intake was positively associated with
440 this lipoprotein, maybe because our sample population is young and healthy, and
441 associations are not clinical significance. Further longitudinal studies are needed to clarify
442 the true direction of these associations.

443 We did not find significant associations between lignans, phytoestrogens usually found
444 in fiber-rich foods, and any cardiometabolic health parameters, although, in a similar
445 population, lignans were inversely associated with BMI z-score after the adjustment of
446 age, sex, European region, and Tanner stage, but not when it was adjusted by dietary
447 factors [35].

448 As we observed, not all phenolic compounds have the same association with all
449 cardiometabolic health parameters evaluated. Therefore, promoting a healthy dietary

pattern in adolescents [47], based on a higher intake of food-rich polyphenols (like fruits, vegetables, nuts, cocoa-based products, and virgin olive oil), could be an effective public health strategy to improve cardiometabolic health in adolescents. Additionally, future

Limitations and strengths

There are some limitations to be considered in the present study. First, is its cross-sectional design, which precludes causal assumptions about dietary (poly)phenol intake and differences in cardiometabolic health parameters. Although we have adjusted for multiple potential confounders in the present analysis, we cannot completely rule out the residual confounding. Second, data derived from FFQ are prone to bias because misreporting is common in dietary self-assessments. Adolescents with high values of BMI tend to report lower energy intake, and this is more likely in adolescents with self-image dissatisfaction [48]. Therefore, BMI z-score was considered a fixed effect in the regression analyses. Third, although (poly)phenol intake was estimated through an updated comprehensive database (Phenol-explorer), information about some foods widely consumed in Spain or specific areas in Spain is still scarce because they have not been characterized or are poorly characterized, resulting in large variation in the estimation of dietary of some phenolic compounds. In addition to this, (poly)phenol content in specific foods can differ according to growth, harvesting, and processing conditions [34]. Fourth, as participants did not use accelerometers in water sports or during sports competitions, the physical activity levels could have been underestimated. Related to BG measurement, it could be overestimated since 2.3% (n=22) of the participants were in non-fasting conditions.

Strengths of the present study include the large sample size and the standardization of measures performed in the SI! Program for Secondary Schools trial. Regarding (poly)phenol rich-food intake, it could be altered by seasonality, for this reason, the FFQ used in this study capture seasonal variation data collection during the entire year, thus reducing bias. Finally, we have used a complete and comprehensive database (Phenol-Explorer) which includes flavonoid and non-flavonoids subclasses of (poly)phenols from more than 400 foods [29,49].

Conclusion

In summary, a higher dietary (poly)phenol intake was associated with better cardiometabolic health parameters. Clinical studies are needed to confirm the effects of dietary (poly)phenol intake and its subclasses on cardiometabolic health in this target population. Additionally, our work helps to collect dietary data supporting the development of nutritional guidelines including safe doses of (poly)phenol, as well as the development of educational and health policies targeting the dietary habits of adolescents.

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Authors' contributions

R.M.L.-R. was responsible for the conception and design of the study. R.M.L.-R., R.F.-J., J.M.F.-A., G.S.-B., M.d.M., P.B., A.d.C.-G., E.P.L.-S., C.A.-R., A.T.-R., A.M.R.-L., R.C., and R.E. conducted the study and participated in data collection of the SI! Program for Secondary Schools trial. E.P.L.-S., S.C.-B., and A.T.-R. were responsible for statistical considerations in the analysis. E.P.L.-S., S.C.-B., A.T.-R., and R.M.L.-R. drafted the manuscript. C.A.-R., R.F.-J., J.M.F.-A., G.S.-B., M.d.M., P.B., A.d.C.-G., J.M.-G., A.M.R.-L., R.C., and R.E. participated in critically reviewing and interpreting the data for the manuscript. All authors had full access to all the study data and share the final responsibility for the decision to submit this report for publication. A.T.-R. and R.M.L.-R. were the guarantors of this work and, as such, had full access to all the study data, and take responsibility for the integrity of the data and accuracy of the data analysis.

Data and resource availability

The data sets generated and/or analyzed during the current study are not publicly available. Requestors wishing to access the database used in this study can request the Steering Committee (SC) chair of the SI! Program trial: gsantos@fundacionshe.org, rodrigo.fernandez@cnic.es, juanmiguel.fernandez@cnic.es, RESTRUCH@clinic.cat, lamuela@ub.edu, bibanez@cnic.es, vfuster@cnic.es.

REFERENCES

[1] J. Steinberger, S.R. Daniels, N. Hagberg, C.R. Isasi, A.S. Kelly, D. Lloyd-Jones, R.R. Pate, C. Pratt, C.M. Shay, J.A. Towbin, E. Urbina, L. v. van Horn, J.P. Zachariah, Cardiovascular Health Promotion in Children: Challenges and Opportunities for 2020 and Beyond: A Scientific Statement From the American Heart Association, Circulation. 134 (2016) e236–e255. https://doi.org/10.1161/CIR.0000000000000441.

[2] J.A. Morrison, L.A. Friedman, P. Wang, C.J. Glueck, Metabolic Syndrome in Childhood Predicts Adult Metabolic Syndrome and Type 2 Diabetes Mellitus 25 to 30 Years Later, J Pediatr. 152 (2008) 201–206. https://doi.org/10.1016/j.jpeds.2007.09.010.

[3] G.E. Vincent, S.M. Jay, C. Sargent, C. Vandelanotte, N.D. Ridgers, S.A. Ferguson, Improving Cardiometabolic Health with Diet, Physical Activity, and Breaking Up Sitting: What about Sleep?, Front Physiol. 8 (2017) 865. https://doi.org/10.3389/fphys.2017.00865.

[4] A.N. Funtikova, E. Navarro, R.A. Bawaked, M. Fíto, H. Schröder, Impact of diet on cardiometabolic health in children and adolescents, Nutr J. 14 (2015) 118. https://doi.org/10.1186/s12937-015-0107-z.

[5] K.J. Penczynski, C. Herder, D. Krupp, J. Rienks, S. Egert, S.A. Wudy, M. Roden, T. Remer, A.E. Buyken, Flavonoid intake from fruit and vegetables during adolescence is prospectively associated with a favourable risk factor profile for type 2 diabetes in early adulthood, Eur J Nutr. 58 (2019) 1159–1172. https://doi.org/10.1007/s00394-018-1631-3.

[6] A. Tresserra-Rimbau, E.B. Rimm, A. Medina-Remón, M.A. Martínez-González, R. de la Torre, D. Corella, J. Salas-Salvadó, E. Gómez-Gracia, J. Lapetra, F. Arós, M. Fiol, E. Ros, L. Serra-Majem, X. Pintó, G.T. Saez, J. Basora, J.V. Sorlí, J.A. Martínez, E. Vinyoles, V. Ruiz-Gutiérrez, R. Estruch, R.M. Lamuela-Raventós, Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study, Nutrition, Metabolism and Cardiovascular Diseases. 24 (2014) 639–647. https://doi.org/10.1016/j.numecd.2013.12.014.

[7] S. Castro-Barquero, A. Tresserra-Rimbau, F. Vitelli-Storelli, M. Doménech, J. Salas-Salvadó, V. Martín-Sánchez, M. Rubín-García, P. Buil-Cosiales, D. Corella, M. Fitó, D. Romaguera, J. Vioque, Á. Alonso-Gómez, J. Wärnberg, J. Martínez, L. Serra-Majem, F. Tinahones, J. Lapetra, X. Pintó, J. Tur, A. Garcia-Rios, L.

García-Molina, M. Delgado-Rodriguez, P. Matía-Martín, L. Daimiel, J. Vidal, C. Vázquez, M. Cofán, A. Romanos-Nanclares, N. Becerra-Tomas, R. Barragan, O. Castañer, J. Konieczna, S. González-Palacios, C. Sorto-Sánchez, J. Pérez-López, M. Zulet, I. Bautista-Castaño, R. Casas, A. Gómez-Perez, J. Santos-Lozano, M. Rodríguez-Sanchez, A. Julibert, N. Martín-Calvo, P. Hernández-Alonso, J. Sorlí, A. Sanllorente, A. Galmés-Panadés, E. Cases-Pérez, L. Goicolea-Güemez, M. Ruiz-Canela, N. Babio, Á. Hernáez, R. Lamuela-Raventós, R. Estruch, Dietary Polyphenol Intake is Associated with HDL-Cholesterol and A Better Profile of other Components of the Metabolic Syndrome: A PREDIMED-Plus Sub-Study, Nutrients. 12 (2020) 689. https://doi.org/10.3390/nu12030689.

[8] A. Scalbert, C. Manach, C. Morand, C. Rémésy, L. Jiménez, Dietary Polyphenols and the Prevention of Diseases, Crit Rev Food Sci Nutr. 45 (2005) 287–306. https://doi.org/10.1080/1040869059096.

[9] S. Ramírez-Garza, E. Laveriano-Santos, M. Marhuenda-Muñoz, C. Storniolo, A. Tresserra-Rimbau, A. Vallverdú-Queralt, R. Lamuela-Raventós, Health Effects of Resveratrol: Results from Human Intervention Trials, Nutrients. 10 (2018) 1892. https://doi.org/10.3390/nu10121892.

[10] A. Rana, M. Samtiya, T. Dhewa, V. Mishra, R.E. Aluko, Health benefits of polyphenols: A concise review, J Food Biochem. 00 (2022) e14264. https://doi.org/10.1111/jfbc.14264.

[11] N. Kardum, M. Glibetic, Polyphenols and Their Interactions With Other Dietary Compounds: Implications for Human Health, in: Adv Food Nutr Res, Adv Food Nutr Res, 2018: pp. 103–144. https://doi.org/10.1016/bs.afnr.2017.12.001.

[12] Y. Xu, M. le Sayec, C. Roberts, S. Hein, A. Rodriguez-Mateos, R. Gibson, Dietary Assessment Methods to Estimate (Poly)phenol Intake in Epidemiological Studies: A Systematic Review, Advances in Nutrition. 12 (2021) 1781–1801. https://doi.org/10.1093/ADVANCES/NMAB017.

[13] R. Fernandez-Jimenez, G. Santos-Beneit, A. Tresserra-Rimbau, P. Bodega, M. de Miguel, A. de Cos-Gandoy, C. Rodríguez, V. Carral, X. Orrit, D. Haro, I. Carvajal, B. Ibañez, C. Storniolo, M. Domènech, R. Estruch, J.M. Fernández-Alvira, R.M. Lamuela-Raventós, V. Fuster, Rationale and design of the school-based SI! Program to face obesity and promote health among Spanish adolescents: A cluster-randomized controlled trial, Am Heart J. 215 (2019) 27–40. https://doi.org/10.1016/j.ahj.2019.03.014.

[14] W.C. Willett, G.R. Howe, L.H. Kushi, Adjustment for total energy intake in epidemiologic studies, Am J Clin Nutr. 65 (1997) 1220S-1228S. https://doi.org/10.1093/ajcn/65.4.1220S.

[15] C. Juton, S. Castro-Barquero, R. Casas, T. Freitas, A.M. Ruiz-León, F. Crovetto, M. Domenech, F. Crispi, E. Vieta, E. Gratacós, R. Estruch, H. Schroder, Reliability and Concurrent and Construct Validity of a Food Frequency Questionnaire for Pregnant Women at High Risk to Develop Fetal Growth Restriction, Nutrients. 13 (2021) 1629. https://doi.org/10.3390/nu13051629.

[16] P. Zimmet, G. Alberti, F. Kaufman, N. Tajima, M. Silink, S. Arslanian, G. Wong, P. Bennett, J. Shaw, S. Caprio, The metabolic syndrome in children and adolescents, The Lancet. 369 (2007) 2059–2061. https://doi.org/10.1016/S0140-6736(07)60958-1.

[17] A.K. Sharma, D.L. Metzger, C. Daymont, S. Hadjiyannakis, C.J. Rodd, LMS tables for waist-circumference and waist-height ratio Z-scores in children aged 5–19 y in NHANES III: association with cardio-metabolic risks, Pediatr Res. 78 (2015) 723–729. https://doi.org/10.1038/pr.2015.160.

[18] C.L. Ogden, R.J. Kuczmarski, K.M. Flegal, Z. Mei, S. Guo, R. Wei, L.M. Grummer-Strawn, L.R. Curtin, A.F. Roche, C.L. Johnson, Centers for Disease Control and Prevention 2000 Growth Charts for the United States: Improvements to the 1977 National Center for Health Statistics Version, Pediatrics. 109 (2002) 45–60. https://doi.org/10.1542/peds.109.1.45.

[19] National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents., Pediatrics. 114 (2004) 555–76.

[20] D.G. Rowland, H.P. Gutgesell, Use of mean arterial pressure for noninvasive determination of left ventricular end-systolic wall stress in infants and children, Am J Cardiol. 74 (1994) 98–99. https://doi.org/10.1016/0002-9149(94)90503-7.

[21] S.S. Franklin, V.A. Lopez, N.D. Wong, G.F. Mitchell, M.G. Larson, R.S. Vasan, D. Levy, Single Versus Combined Blood Pressure Components and Risk for Cardiovascular Disease, Circulation. 119 (2009) 243–250. https://doi.org/10.1161/CIRCULATIONAHA.108.797936.

[22] O. Moreiras, H. Carvajal, L. Cabrera, C. Cuadrado, Tablas de composición de alimentos, 2019.

[23] J. Mataix, L. García, M. Mañas, E. Martínez, J. Llopis, Tabla de composición de alimentos, Granada, 2003.

[24] UNESCO, International Standard Classification of Education (ISCED) 2011, UNESCO Institute for Statistics, 2012. https://doi.org/10.15220/978-92-9189-123-8-en.

[25] INE: Instituto Nacional de Estadística, Encuesta anual de estructura salarial. , (n.d.). https://www.ine.es/jaxiT3/Datos.htm?t=9948 (accessed June 19, 2022).

[26] J.L. Chandler, K. Brazendale, M.W. Beets, B.A. Mealing, Classification of physical activity intensities using a wrist-worn accelerometer in 8-12-year-old children, Pediatr Obes. 11 (2016) 120–127. https://doi.org/10.1111/ijpo.12033.

[27] N. Barbosa, C.E. Sanchez, J.A. Vera, W. Perez, J.-C. Thalabard, M. Rieu, A physical activity questionnaire: reproducibility and validity., J Sports Sci Med. 6 (2007) 505–18.

[28] J.M. Tanner, R.H. Whitehouse, Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty., Arch Dis Child. 51 (1976) 170–179. https://doi.org/10.1136/adc.51.3.170.

[29] J.A. Rothwell, J. Perez-Jimenez, V. Neveu, A. Medina-Remon, N. M'Hiri, P. Garcia-Lobato, C. Manach, C. Knox, R. Eisner, D.S. Wishart, A. Scalbert, 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. 2013 (2013) bat070–bat070. https://doi.org/10.1093/database/bat070.

[30] A. Tresserra-Rimbau, A. Medina-Remón, J. Pérez-Jiménez, M.A. Martínez-González, M.I. Covas, D. Corella, J. Salas-Salvadó, E. Gómez-Gracia, J. Lapetra, F. Arós, M. Fiol, E. Ros, L. Serra-Majem, X. Pintó, M.A. Muñoz, G.T. Saez, V. Ruiz-Gutiérrez, J. Warnberg, R. Estruch, R.M. Lamuela-Raventós, Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: The PREDIMED study, Nutrition, Metabolism and Cardiovascular Diseases. 23 (2013) 953–959. https://doi.org/10.1016/j.numecd.2012.10.008.

[31] K.M. Crowe-White, L.W. Evans, G.G.C. Kuhnle, D. Milenkovic, K. Stote, T. Wallace, D. Handu, K.E. Senkus, Flavan-3-ols and Cardiometabolic Health: a Guideline Recommendation by the Academy of Nutrition and Dietetics, Advances in Nutrition. 10 (2022) S308–S319. https://doi.org/10.1093/advances/nmac105.

643 [32] R.W. Wisnuwardani, S. de Henauw, O. Androutsos, M. Forsner, F. Gottrand, I.
644 Huybrechts, V. Knaze, M. Kersting, C. le Donne, A. Marcos, D. Molnár, J.A.
645 Rothwell, A. Scalbert, M. Sjöström, K. Widhalm, L.A. Moreno, N. Michels,
646 Estimated dietary intake of polyphenols in European adolescents: the HELENA
647 study, Eur J Nutr. 58 (2019) 2345–2363. https://doi.org/10.1007/s00394-018-
648 1787-x.
649 [33] N. Ziauddeen, A. Rosi, D. del Rio, B. Amoutzopoulos, S. Nicholson, P. Page, F.
650 Scazzina, F. Brighenti, S. Ray, P. Mena, Dietary intake of (poly)phenols in
651 children and adults: cross-sectional analysis of UK National Diet and Nutrition
652 Survey Rolling Programme (2008–2014), Eur J Nutr. 58 (2019) 3183–3198.
653 https://doi.org/10.1007/s00394-018-1862-3.
654 [34] Y. Probst, V. Guan, K. Kent, A systematic review of food composition tools used
655 for determining dietary polyphenol intake in estimated intake studies, Food Chem.
656 238 (2018) 146–152. https://doi.org/10.1016/j.foodchem.2016.11.010.
657 [35] R.W. Wisnuwardani, S. de Henauw, M. Forsner, F. Gottrand, I. Huybrechts, V.
658 Knaze, M. Kersting, C. le Donne, Y. Manios, A. Marcos, D. Molnár, J.A. Rothwell,
659 A. Scalbert, M. Sjöström, K. Widhalm, L.A. Moreno, N. Michels, Polyphenol
660 intake and metabolic syndrome risk in European adolescents: the HELENA study,
661 Eur J Nutr. 59 (2020) 801–812. https://doi.org/10.1007/s00394-019-01946-1.
662 [36] X. Wang, Y. Qi, H. Zheng, Dietary Polyphenol, Gut Microbiota, and Health
663 Benefits, Antioxidants. 11 (2022) 1212. https://doi.org/10.3390/antiox11061212.
664 [37] K. Liu, M. Luo, S. Wei, The Bioprotective Effects of Polyphenols on Metabolic
665 Syndrome against Oxidative Stress: Evidences and Perspectives, Oxid Med Cell
666 Longev. 2019 (2019) 1–16. https://doi.org/10.1155/2019/6713194.
667 [38] G. Grosso, U. Stepaniak, A. Micek, D. Stefler, M. Bobak, A. Pająk, Dietary
668 polyphenols are inversely associated with metabolic syndrome in Polish adults of
669 the HAPIEE study, Eur J Nutr. 56 (2017) 1409–1420.
670 https://doi.org/10.1007/s00394-016-1187-z.
671 [39] M. Kosmalski, A. Pękala-Wojciechowska, A. Sut, T. Pietras, B. Luzak, Dietary
672 Intake of Polyphenols or Polyunsaturated Fatty Acids and Its Relationship with
673 Metabolic and Inflammatory State in Patients with Type 2 Diabetes Mellitus,
674 Nutrients. 14 (2022) 1083. https://doi.org/10.3390/nu14051083.
675 [40] M. Vitale, O. Vaccaro, M. Masulli, E. Bonora, S. del Prato, C.B. Giorda, A.
676 Nicolucci, S. Squatrito, S. Auciello, A.C. Babini, L. Bani, R. Buzzetti, E. Cannarsa,

M. Cignarelli, M. Cigolini, G. Clemente, S. Cocozza, L. Corsi, F. D'Angelo, E. Dall'Aglio, G. di Cianni, L. Fontana, G. Gregori, S. Grioni, C. Giordano, R. Iannarelli, C. Iovine, A. Lapolla, D. Lauro, L. Laviola, C. Mazzucchelli, S. Signorini, L. Tonutti, R. Trevisan, C. Zamboni, G. Riccardi, A.A. Rivellese, Polyphenol intake and cardiovascular risk factors in a population with type 2 diabetes: The TOSCA.IT study, Clinical Nutrition. 36 (2017) 1686–1692. https://doi.org/10.1016/j.clnu.2016.11.002.

[41] G. Sohrab, S. Hosseinpour-Niazi, J. Hejazi, E. Yuzbashian, P. Mirmiran, F. Azizi, Dietary polyphenols and metabolic syndrome among Iranian adults, Int J Food Sci Nutr. 64 (2013) 661–667. https://doi.org/10.3109/09637486.2013.787397.

[42] P. Mena, R. Domínguez-Perles, A. Gironés-Vilaplana, N. Baenas, C. García-Viguera, D. Villaño, Flavan-3-ols, anthocyanins, and inflammation, IUBMB Life. 66 (2014) 745–758. https://doi.org/10.1002/iub.1332.

[43] L.M. Cercato, J.P. Oliveira, M.T.S. Souza, N. Andrade, F. Martel, E.A. Camargo, Effect of flavonoids in preclinical models of experimental obesity, PharmaNutrition. 16 (2021) 100260. https://doi.org/10.1016/j.phanu.2021.100260.

[44] C.L. Cheatham, D.C. Nieman, A.P. Neilson, M.A. Lila, Enhancing the Cognitive Effects of Flavonoids With Physical Activity: Is There a Case for the Gut Microbiome?, Front Neurosci. 16 (2022) 96. https://doi.org/10.3389/FNINS.2022.833202/BIBTEX.

[45] T. Fujimaki, C. Sato, R. Yamamoto, S. Watanabe, H. Fujita, H. Kikuno, M. Sue, Y. Matsushima, Isolation of phenolic acids and tannin acids from *Mangifera indica* L. kernels as inhibitors of lipid accumulation in 3T3-L1 cells, Biosci Biotechnol Biochem. 86 (2022) 665–671. https://doi.org/10.1093/bbb/zbac030.

[46] O.B. Ibitoye, T.O. Ajiboye, Dietary phenolic acids reverse insulin resistance, hyperglycaemia, dyslipidaemia, inflammation and oxidative stress in high-fructose diet-induced metabolic syndrome rats, Arch Physiol Biochem. 124 (2018) 410–417. https://doi.org/10.1080/13813455.2017.1415938.

[47] Arcila-Agudelo, Ferrer-Svoboda, Torres-Fernàndez, Farran-Codina, Determinants of Adherence to Healthy Eating Patterns in a Population of Children and Adolescents: Evidence on the Mediterranean Diet in the City of Mataró (Catalonia, Spain), Nutrients. 11 (2019) 854. https://doi.org/10.3390/nu11040854.

[48] L. Jones, A. Ness, P. Emmett, Misreporting of Energy Intake From Food Records Completed by Adolescents: Associations With Sex, Body Image, Nutrient, and Food Group Intake, Front Nutr. 8 (2021) 970. https://doi.org/10.3389/fnut.2021.749007.

[49] E.B. Hill, A.J. Kennedy, K.M. Roberts, K.M. Riedl, E.M. Grainger, S.K. Clinton, Considerations for Use of the Phenol-Explorer Database to Estimate Dietary (Poly)phenol Intake, J Acad Nutr Diet. 121 (2021) 833–834. https://doi.org/10.1016/j.jand.2021.02.010.

720 Table 1. Baseline characteristics of the study population according to quintiles of energy-
721 adjusted of TPI (mg/day).

Characteristics	Total	Q1 (<438.0)	Q2 (438.0-570.1)	Q3 (570.2-699.9)	Q4 (700.0-875.0)	Q5 (>875.0)	*P*	*P-for* trend
N (total)	944	189	189	189	189	188		
Female, *n* (%)	455 (48)	76 (40)	88 (47)	91 (48)	98 (52)	102 (54)	0.066	**0.004**
Age (years)	12.0 ± 0.4	12.0 ± 0.4	12.0 ± 0.5	12.0 ± 0.4	12.0 ± 0.4	12.0 ± 0.4	0.620	0.543
WC (cm)	71.9 ± 10.1	72.0 ± 10.5	71.8 ± 9.7	72.9 ± 10.6	71.1 ± 9.5	71.7 ± 10.3	0.527	0.546
SBP (mm Hg)	104.9 ± 10.7	103.7 ± 11.0	104.4 ± 10.7	105.5 ± 11	105.7 ± 10.4	105.1 ± 10.3	0.334	0.086
DBP (mm Hg)	61.8 ± 8.6	61.6 ± 8.5	61.3 ± 8.5	62.9 ± 8.7	61.6 ± 8.7	61.7 ± 8.8	0.392	0.798
MAP (mm Hg)	76.2 ± 8.3	75.6 ± 8.1	75.7 ± 7.7	77.1 ± 8.2	76.3 ± 8	76.1 ± 8.1	0.433	0.345
BG (mg/dL)	102.9 ± 11.9	102.5 ± 12.2	103.7 ± 11.5	103.1 ± 11.4	101.9 ± 12.9	103.3 ± 11.3	0.221	0.908
TG (mg/dL)	77.9 ± 37.5	74.8 ± 38.8	76.4 ± 35.1	80.5 ± 38.8	80.0 ± 36.8	78.1 ± 38	0.508	0.237
HDL-c (mg/dL)	62.9 ± 16.2	65.3 ± 18.2	63.0 ± 16.3	61.6 ± 15.1	62.1 ± 15.9	62.8 ± 15.1	0.229	0.113
Moderate-to-vigorous physical activity (min/day)	74.4 ± 25.5	75.4 ± 23.6	74.3 ± 24.8	73.6 ± 25.4	76.5 ± 28.4	72.3 ± 24.9	0.559	0.495
Parental educational level, *n* (%)							0.374	0.374
Low and medium	676 (74)	135 (73)	134 (73)	129 (71)	136 (73)	142 (78)		
High	240 (26)	49 (27)	49 (27)	52 (29)	50 (27)	40 (22)		
Household income							0.488	0.153
Low	257 (29)	51 (29)	44 (25)	51 (29)	55 (30)	56 (31)		
Medium	267 (30)	45 (26)	61 (35)	50 (29)	51 (28)	60 (34)		
High	366 (41)	80 (45)	71 (40)	73 (42)	79 (42)	63 (35)		

722 Values are expressed as frequencies and percentages for categorical variables or means ±
723 SDs for continuous variables, except for the median (min-max) polyphenol intake. Blood
724 glucose (BG), diastolic blood pressure (DBP), high-density lipoprotein-cholesterol
725 (HDL-c), mean arterial pressure (MAP), systolic blood pressure (SBP), standard
726 deviation (SD), triglycerides (TG), and waist circumference (WC). Analysis of variance
727 one factor (ANOVA) was used for continuous variables and the $\chi 2$ test for categorical
728 variables. *P* and *P-for* trend values <0.05 are considered statistically significant.

729

Table 2. Contribution (%) of polyphenol subclasses to total polyphenol intake and food sources

Polyphenol subclasses	Mean *(mg/day)* ± *SD, (% of total polyphenols)*	Median (25th, 75th percentile)	Food sources (% of contribution)
Total polyphenols	683.2 ± 379.4	598.6 (441.7; 854.8)	
Flavonoids	530.1 ± 331.3, (77.6)	459.7 (314.9; 664.2)	
• **Anthocyanins**	57.6 ± 75.9, (8.4)	31.9 (19.5; 96.0)	Cherries (43.3), strawberries (40.3), grape (12.0), olives (2.6), and other foods (1.8)
• **Dihydrochalcones**	1.2 ± 1.14 (0.2)	1.2 (0.4; 1.4)	Apples (79.5), fruit juices from concentrate (20.4)
• **Flavanols**	328.0 ± 245.7 (48.0)	275.2 (162.6; 410.6)	
• **Catechins**	27.5 ± 19.0, (4.0)	23.6 (15.9; 34.7)	Cocoa powder (30.5), apples (11.4), strawberries (7.8), peaches (7.1), cherries (6.7), fruit juices from concentrate (6.1), dark chocolate >70% cocoa (5.0), grapes (5.1), chocolate (5.0), other foods (15.3)
• **Proanthocyanidins**	300.5± 230.6, (44.0)	252.0 (147.4; 380.4)	Cocoa powder (36.8), strawberries (15.4), dark chocolate >70% cocoa (13.8), cherries (9.0), apples (8.4), chocolates (5.8), grapes (4.6), other foods (4.1)
• **Theaflavins**	0.11 ± 0.82, (0.02)	0.0 (0;0)	Tea (100)

• **Flavanones**	70.4 ± 76.9, (10.3)	53.9 (20.6; 92.9)	Oranges (72.9), fruit juices from concentrate (14.2), natural orange juice (10.9), other foods (2.0)
• **Flavones**	41.5 ± 28.5, (6.1)	35.5 (21.7; 51.7)	Bread (27.6), oranges (26.9), whole-grain bread (17.8), cookies, pastries, and bakery products (12.9), natural orange juice (5.8), artichoke (3.8), other foods (5.2)
• **Flavonols**	29.5 ± 20.8, (4.3)	25.3 (15.1; 38.0)	Spinach (27.9), onions (23.5), lettuce (12.8), green beans (4.3), cabbage (3.8), asparagus (3.6), apples (3.5), other foods (17.5)
• **Isoflavonoids**	1.8 ± 8.5, (0.3)	0.0 (0; 0)	Soy milk (100)
Phenolic acids	97.8 ± 64.5, (14.3)	81.9 (59.8; 118.4)	
• **Hydroxybenzoic acids**	6.7 ± 4.8, (1.0)	5.7 (3.7; 8.3)	Strawberries (27.1), olives (15.2), walnuts (12.8), chickpeas (11.7), banana (4.8), Swiss chard (4.3), other foods (22.6)
• **Hydroxycinnamic acids**	90.7 ± 62.0, (13.3)	75.6 (54.9; 109.6)	Potatoes (13.5), plums (11.2), cherries (11.0), seeds (9.0), apples (7.4), coffee (6.1), white bread (5.6), fruit juices from concentrate (5.2), other foods (21.4)
• **Hydroxyphenylacetic acids**	0.3 ± 0.3, (<0.01)	0.1 (0.0; 0.2)	Olives (97.2), extra virgin olive oil (1.8), other foods (1.0)
• **Hydroxyphenylpropanoic acids**	0.2 ± 0.2, (<0.01)	0.1 (0.0; 0.2)	Olives (100)
Stilbenes	0.2 ± 0.3, (<0.01)	0.1 (0.0; 0.3)	Strawberries (55.0), grapes (29.7), dark chocolate >70% cocoa (1.0), other foods (14.2)

Lignans	3.8 ± 5.1, (0.6)	2.4 (1.2; 4.1)	Seeds (71.5), oranges (2.8), peaches (2.3), extra virgin olive oil (1.9), other foods (21.4)
Other polyphenols	51.3 ± 34.4, (7.5)	598.6 (441.5; 854.8)	
• **Alkylphenols**	22.8 ± 27.3, (3.3)	18.9 (4.7; 35.5)	Breakfast cereals (74.5), whole grain bread (10.4), cookies, pastries, and bakery products (6.6), pasta (4.4), other foods (4.1)
• **Furanocoumarins**	0.4 ± 0.4, (0.1)	0.3 (0.1; 0.5)	Celery stalks (60.6), parsley (36.1), grapefruit juice (3.3)
• **Hydroxybenzaldehydes**	0.1 ± 0.2, (<0.01)	0.0 (0.01; 0.05)	Walnuts (25.5), cocoa powder (4.1), olives (2.4), other foods (67.5)
• **Naphtoquinones**	0.3 ± 0.5, (<0.01)	0.2 (0; 0.2)	Walnuts (100)
• **Tyrosols**	21.5 ± 14.6, (3.1)	17.5 (11.6; 27.6)	Olives (40.0), extra virgin olive oil (24.6), refined olive oil (11.7), other foods (23.7)
• **Curcuminoids**	4.1 ± 12.2, (0.6)	0.0 (0; 3.2)	Spices (100)
• **Other**	1.8 ± 2.8, (0.3)	0.8 (0.4; 2.2)	Orange juice (51.6), other fruit juices (34.7), pears (6.8), other foods (6.9)

SD standard deviation

Table 3. Cardiometabolic parameters according to quintiles of energy-adjusted TPI.

		Q1 (<438.0 mg/day)	Q2 (438.0-570.1 mg/day)	Q3 (570.2-699.9 mg/day)	Q4 (700.0-875.0 mg/day)	Q5 (>875.0 mg/day)	*P-for* Trend
WC (z-score)	Model 1, β (95% CI)	Reference	0.05 (-0.03;0.14)	0.13 (0.02;0.24)*	0.02 (-0.01;0.05)	0.05 (0.04;0.07)***	0.284
	Model 2, β (95% CI)	Reference	0.10 (-0.01;0.20)	0.12 (0.06;0.18)***	0.06 (0.04;0.09)***	0.03 (-0.01;0.06)	0.491
	Model 3, β (95% CI)	Reference	0.04 (-0.02;0.16)	0.01 (-0.14;0.16)	-0.04 (-0.15;0.07)	-0.09 (-0.09;-0.08)***	**0.002**
SBP (z-score)	Model 1, β (95% CI)	Reference	-0.001 (-0.18;0.18)	0.09 (-0.05;0.23)	0.12 (0.02;0.23)*	0.07 (-0.03;0.18)	**<0.001**
	Model 2, β (95% CI)	Reference	-0.02 (-0.23;0.18)	0.05 (-0.22;0.33)	0.13 (-0.09;0.34)	0.06 (-0.15;0.27)	0.225
	Model 3, β (95% CI)	Reference	0.01 (-0.20;0.22)	0.10 (-0.19;0.39)	0.15 (-0.08;0.37)	0.04 (-0.19;0.28)	0.371
DBP (z-score)	Model 1, β (95% CI)	Reference	-0.06 (-0.12;0.00)	0.05 (-0.08;0.19)	-0.04 (-0.28;0.20)	-0.03 (-0.06;0.00)	0.532
	Model 2, β (95% CI)	Reference	-0.09 (-0.11;-0.06)***	0.02 (-0.16;0.20)	-0.05 (-0.31;0.21)	-0.06 (-0.09;-0.03)***	0.348
	Model 3, β (95% CI)	Reference	-0.10 (-0.12;-0.07)***	-0.01 (-0.15;0.13)	-0.10 (-0.29;0.08)	-0.14 (-0.27;0.001)	**<0.001**
MAP (z-score)	Model 1, β (95% CI)	Reference	-0.04 (-0.17;0.10)	0.16 (-0.02;0.35)	-0.07 (-0.25;0.40)	0.05 (0.03;0.07)***	0.060
	Model 2, β (95% CI)	Reference	-0.06 (-0.18;0.05)	0.11 (-0.17;0.40)	0.06 (-0.33;0.45)	0.01 (-0.10;0.12)	0.573
	Model 3, β (95% CI)	Reference	-0.06 (-0.18;0.06)	0.10 (-0.16;0.35)	-0.001 (-0.32;0.32)	-0.11 (-0.14;-0.08)***	0.247
BG (mg/dL)	Model 1, β (95% CI)	Reference	1.74 (0.07;3.42)*	0.91 (0.64;1.18)***	0.58 (-2.06;3.22)	1.33 (-0.95;3.61)	<0.001
	Model 2, β (95% CI)	Reference	1.31 (-1.11;3.72)	1.19 (0.92;1.46)***	0.43 (-2.02;2.88)	1.47 (-1.70;4.64)	0.006
	Model 3, β (95% CI)	Reference	1.31 (-0.49;3.12)	1.15 (0.55;1.76)***	0.16 (-2.78;3.10)	0.90 (-2.17;3.96)	0.362

TG (mg/dL)	Model 1, β (95% CI)	Reference	0.95 (0.86;1.04)***	5.22 (2.77;7.68)***	4.82 (4.27;5.38)***	2.63 (-6.86;12.12)	0.358
	Model 2, β (95% CI)	Reference	0.06 (-3.33;3.44)	2.69 (-2.62;8.00)	4.19 (4.00;4.38)***	1.60 (-9.53;12.73)	0.451
	Model 3, β (95% CI)	Reference	-0.48 (-2.07;1.12)	1.57 (-1.39;4.54)	2.67 (0.19;5.15)*	-0.30 (-8.70;8.09)	0.696
HDL-c (mg/dL)	Model 1, β (95% CI)	Reference	-1.88 (-5.26;1.49)	-3.19 (-5.99;-0.39)*	-2.04 (-3.14;-0.94)***	-1.68 (-2.10;-1.26)***	**<0.001**
	Model 2, β (95% CI)	Reference	-1.56 (-2.80;-0.32)*	-3.16 (-5.16;-1.15)**	-1.86 (-3.87;0.15)	-1.51 (-1.55;-1.48)***	**<0.001**
	Model 3, β (95% CI)	Reference	-1.38 (-2.23;-0.53)**	-2.99 (-5.62;-0.35)*	-1.66 (-3.82;0.49)	-1.44 (-2.59;-0.29)*	0.086

Unstandardized coefficient (β), blood glucose (BG), confidence interval (CI), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDL-c), mean arterial pressure (MAP), systolic blood pressure (SBP), triglycerides (TG), waist circumference (WC). Multilevel linear mixed-effect models. Model 1: sex and age. Model 2: model 1 plus pubertal status, physical activity, body mass index z-score (except for WC), fasting, high parental educational level, and household income. Model 3: model 2 plus energy, fiber, added sugars, and protein intake. Municipality and schools were considered random effects. SBP, DBP, and MAP were additionally adjusted by sodium intake. *P<0.05, **P <0.01, and ***P<0.001. P and *P-for* Trend across quintiles of TPI <0.05 are considered significant.

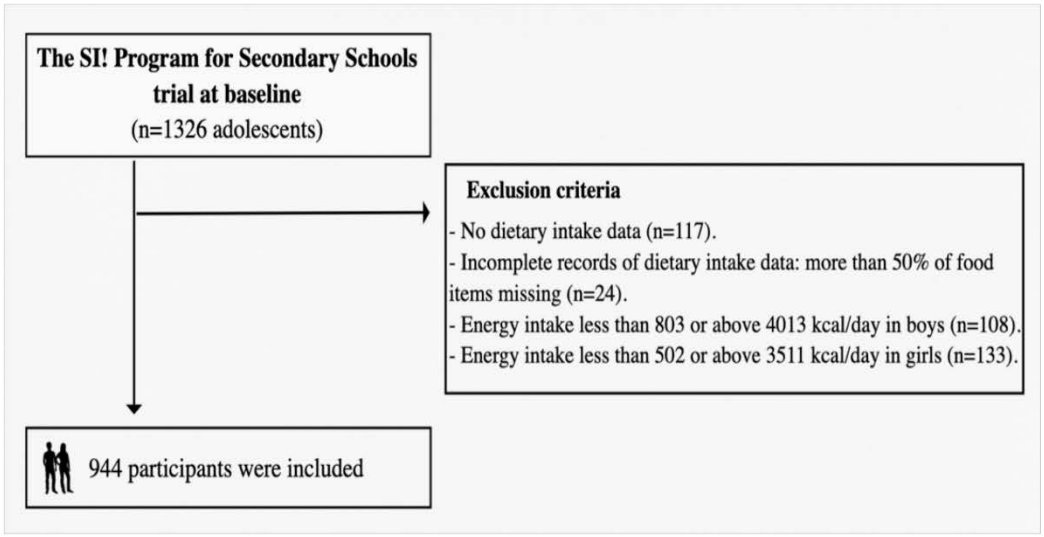

Figure 1. Flowchart of participant' selection

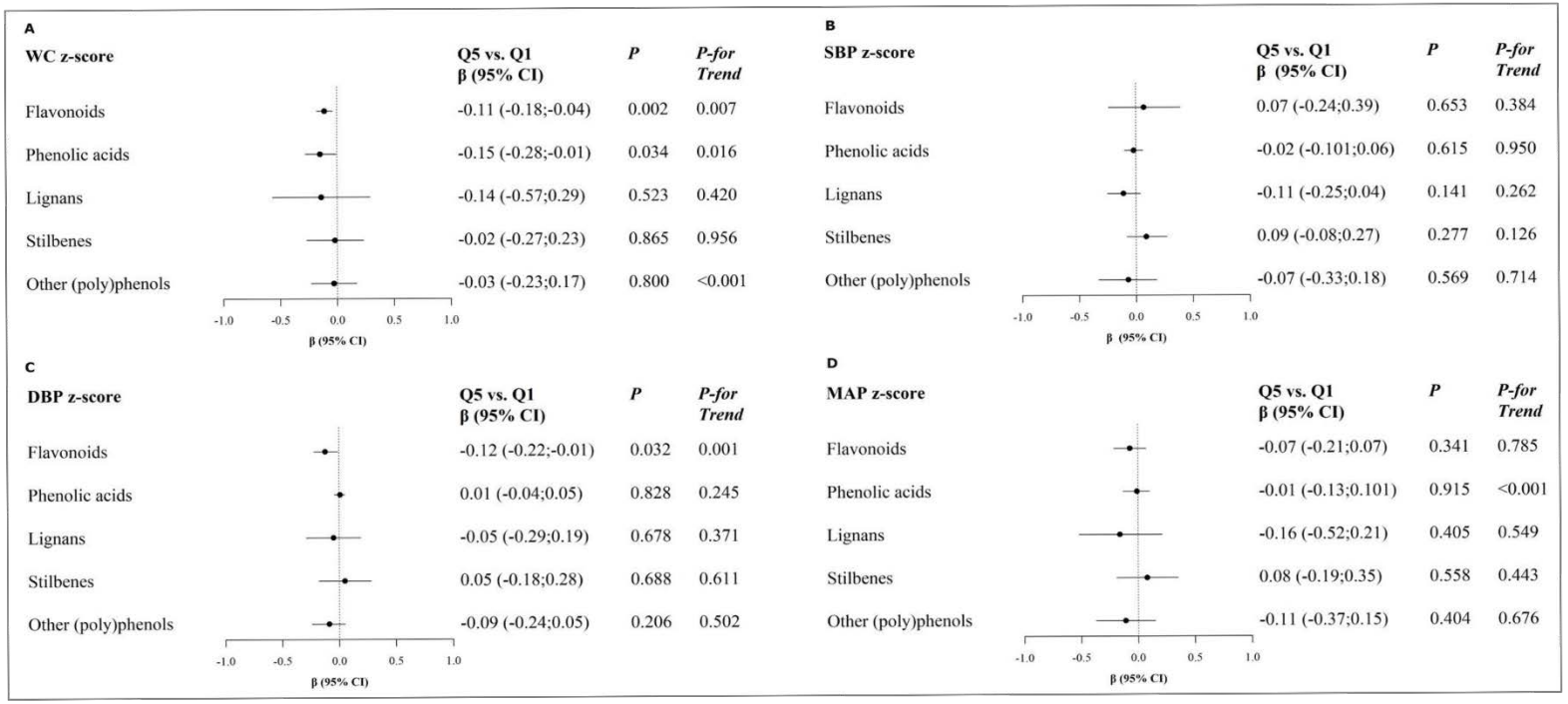

Figure 2. Energy-adjusted (poly)phenol intake subclasses by waist circumference and blood pressure.

A: WC z-score, B: SBP z-score, C: DBP z-score, D: MAP z-score. Confidence interval (CI), diastolic blood pressure (DBP), systolic blood pressure (SBP), unstandardized coefficient (β), mean arterial pressure (MAP), waist circumference (WC). Multilevel linear mixed-effect model. Fixed variables were sex, age, pubertal status, physical activity, body mass index z-score (except for WC), fasting, high parental educational level, household income, energy, fiber, added sugars, and protein intake. SBP, DBP, MAP was additionally adjusted by sodium intake. Municipality and schools were considered random effects. *P* and *P-for* Trend across quintiles of (poly)phenol intake subclasses <0.05 are considered significant.

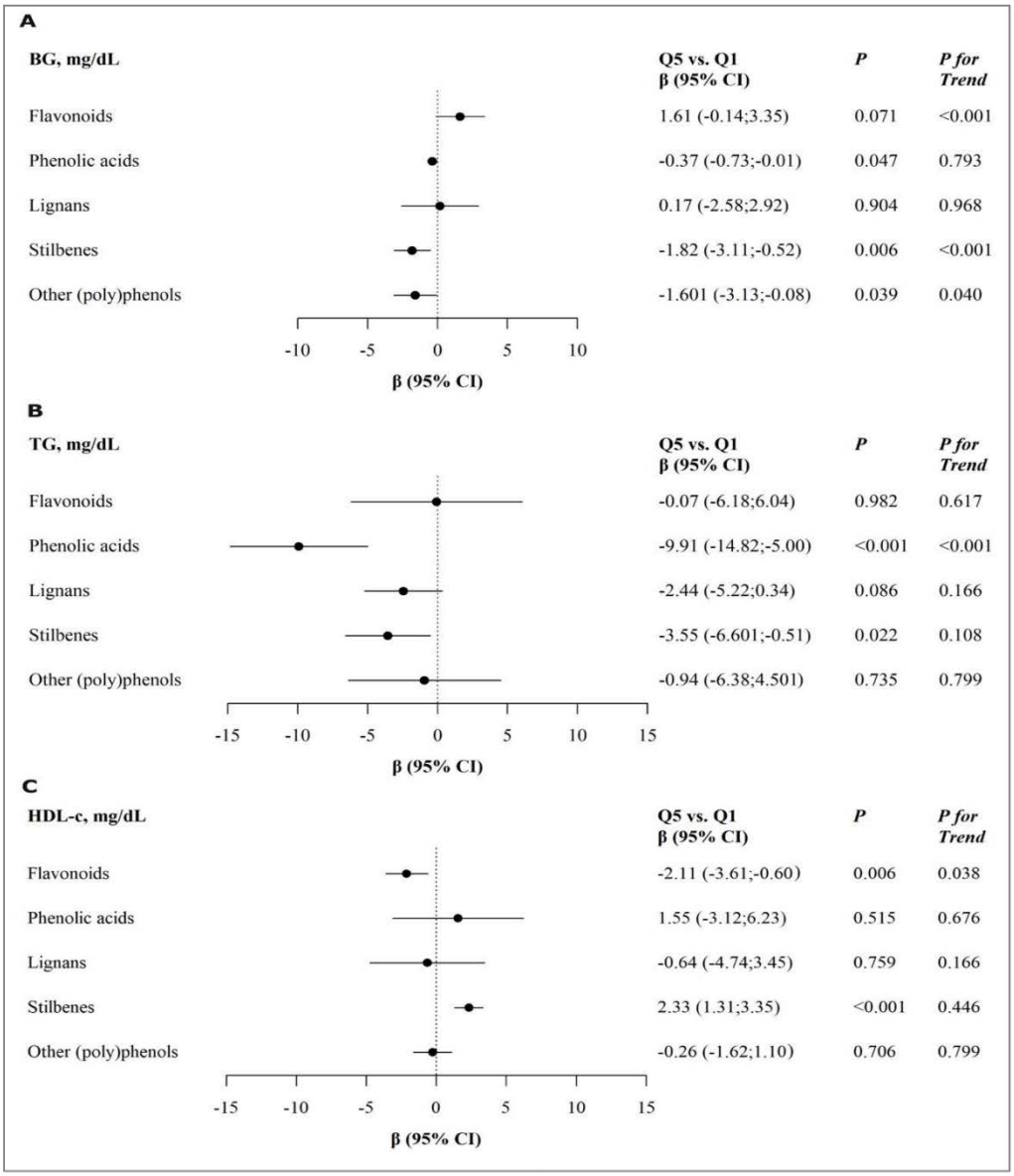

Figure 3. . Energy-adjusted of (poly)phenol intake subclasses by biochemistry parameters

A: BG, B: TG, C: HDL-c. Blood glucose (BG), confidence interval (CI), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), unstandardized coefficient (β). Multilevel linear mixed-effect model. Fixed variables were sex, age, pubertal status, physical activity, body mass index z-score, fasting, high parental educational level, household income, energy, fiber, added sugars, and protein intake. Municipality and schools were considered random effects. *P* and *P-for* Trend across quintiles of (poly)phenol intake subclasses <0.05 are considered significant.

On-line Supplementary Materials

Dietary (poly)phenol intake is associated with some cardiometabolic health parameters in adolescents

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Supplementary Table 1. Mean energy-adjusted intake of total and polyphenol subclasses according to socio-demographic characteristics in adolescents

Characteristics	N	Total (poly)phenol (mg/day)		Flavonoids (mg/day)		Phenolic acids (mg/day)		Lignans (mg/day)		Stilbenes (mg/day)		Otros	
		Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*
Sex			**0.006**		**0.010**		**0.001**		0.976		0.450		0.059
Boys	489	654.6 ± 328.7		505.8 ± 298.3		91.6 ± 57.1		3.8 ± 5.7		0.19 ± 0.35		53.2 ± 36.4	
Girls	455	714.0 ± 336.0		556.2 ± 301.3		104.5 ± 60.0		3.8 ± 4.0		0.21 ± 0.32		49.3 ± 24.8	
Parental education			0.084		0.122		**0.005**		0.099		0.122		0.056
Low and Medium	676	693.1 ± 349.7		538.9 ± 316.1		100.0 ± 61.9		3.8 ± 4.4		0.21 ± 0.33		50.2 ± 30.6	
High	240	650.5 ± 255.2		504.4 ± 233.5		87.9 ± 40.9		3.3 ± 3.8		0.17 ± 0.36		54.7 ± 34.5	
Household income			0.113		0.226		**0.001**		**<0.001**		**0.017**		0.169
Lower	257	711.0 ± 357.7[a]		553.0 ± 323.3[a]		102.8 ± 55.9[a]		4.4 ± 4.6[a]		0.24 ± 0.37[a]		50.6 ± 28.9[a]	
Medium	267	692.6 ± 344.2[a]		538.3 ± 311.3[a]		101.0 ± 68.1[a]		4.0 ± 4.6[a]		0.20 ± 0.31[ab]		49.1 ± 31.3[a]	
High	366	658.0 ± 276.3[a]		513.0 ± 255.8[a]		88.2 ± 43.4[b]		3.0 ± 3.4[b]		0.17 ± 0.31[b]		53.7 ± 33.2[a]	

Comparison between subcategories was performed using T-Test or ANOVA. Bonferroni test was used for the pairwise multiple comparisons in independent groups. [a,b] Means of groups that do not share a letter are significantly different. *P* values <0.05 are considered significant.

Supplementary Table 2. Daily dietary food and nutrient intake according to quintiles of energy-adjusted of total (poly)phenol intake in adolescents

	Q1 (<438.0 mg/day)	Q2 (438.0-570.1 mg/day)	Q3 (570.2-699.9 mg/day)	Q4 (700.0-875.0 mg/day)	Q5 (>875.0 mg/day)	*P*	*P-for* trend
N (total)	189	189	189	189	188		
Food items							
Fruits, g	205.0 ± 134.7^{a}	241.4 ± 144.8^{ab}	276.0 ± 142.3^{b}	375.5 ± 190.4^{c}	576.7 ± 361.8^{d}	**<0.001**	**<0.001**
Vegetables, g	164.1 ± 118.3^{a}	186.0 ± 155.7^{ab}	201.5 ± 147.0^{ab}	226.1 ± 157.0^{bc}	248.8 ± 155.9^{c}	**<0.001**	**<0.001**
Whole grain cereals. g	12.8 ± 23.0^{a}	14.7 ± 26.9^{a}	21.2 ± 36.7^{ab}	19.7 ± 34.6^{ab}	24.3 ± 36.5^{b}	**0.003**	**<0.001**
Refined cereals, g	143.9 ± 84.6^{c}	119.0 ± 64.1^{b}	98.6 ± 58.5^{a}	102.9 ± 67.4^{ab}	101.6 ± 59.5^{ab}	**<0.001**	**<0.001**
Legumes, g	60.7 ± 47.7	59.8 ± 38.4	62.4 ± 58.5	59.3 ± 35.5	60.8 ± 37.1	0.970	0.965
Dairy products, g	495.6 ± 299.9^{b}	384.7 ± 226.1^{a}	370.0 ± 235.5^{a}	363.1 ± 212.8^{a}	419.9 ± 264.5^{a}	**<0.001**	**0.003**
Meat, g	18.7 ± 7.5^{c}	16.3 ± 6.2^{b}	13.9 ± 5.3^{a}	13.3 ± 6.2^{a}	14.9 ± 7.2^{ab}	**<0.001**	**<0.001**
Olive oil, g	18.7 ± 16.1	17.4 ± 15.3	15.5 ± 15.3	15.4 ± 11.0	17.1 ± 14.1	0.372	0.324
Fish and shellfish, g	94.7 ± 74.9	81.8 ± 48.8	82.5 ± 44.5	85.5 ± 46.3	88.8 ± 54.7	0.138	0.530
Nuts, g	9.0 ± 9.8^{a}	9.9 ± 12.0^{ab}	11.4 ± 14.6^{ab}	12.6 ± 16.2^{ab}	13.8 ± 18.0^{b}	**0.008**	**<0.001**
Cookies, pastries, and sweets, g	83.3 ± 66.9^{b}	71.1 ± 48.8^{ab}	58.6 ± 45.4^{a}	66.5 ± 46.5^{a}	65.7 ± 49.3^{a}	**<0.001**	**0.001**
Soft drinks, g	65.5 ± 124.2	59.4 ± 94.1	45.0 ± 58.9	51.4 ± 92.6	52.6 ± 93.8	0.267	0.123
Energy and Nutrients							
Energy, kcal	2799.4 ± 546.4^{d}	2500.4 ± 559.0^{bc}	2311.0 ± 579.8^{a}	2426.7 ± 595.6^{ab}	2658.9 ± 606.6^{cd}	**<0.001**	**0.008**
Proteins, g	136.9 ± 33.2^{c}	119.6 ± 28.7^{ab}	111.7 ± 31.9^{a}	112.7 ± 32.0^{a}	124.0 ± 34.2^{b}	**<0.001**	**<0.001**
Protein, (% energy)	19.6 ± 3.3^{b}	19.3 ± 2.9^{ab}	19.4 ± 3.4^{ab}	18.6 ± 3.0^{a}	18.7 ± 3.0^{ab}	**0.005**	**0.007**
Total fat, g	139.9 ± 31.3^{c}	115.5 ± 31.6^{b}	104.4 ± 33.2^{a}	106.8 ± 30.8^{ab}	114.5 ± 33.8^{b}	**<0.001**	**<0.001**
Total fat (% energy)	41.7 ± 5.6^{c}	41.4 ± 6.1^{c}	40.3 ± 6.4^{bc}	39.5 ± 5.2^{ab}	38.5 ± 5.7^{a}	**<0.001**	**<0.001**
SFA, g	43.4 ± 11.2^{c}	37.2 ± 10.4^{b}	33.6 ± 11.2^{a}	33.8 ± 10.7^{a}	36.2 ± 11.3^{ab}	**<0.001**	**<0.001**
SFA (% energy)	14.0 ± 2.3^{d}	13.4 ± 2.2^{cd}	12.9 ± 2.3^{bc}	12.5 ± 2.1^{ab}	12.2 ± 2.2^{a}	**<0.001**	**<0.001**
MUFA, g	54.6 ± 15.5^{c}	49.2 ± 16.0^{b}	44.5 ± 16.4^{a}	45.5 ± 14.2^{ab}	48.8 ± 16.7^{ab}	**<0.001**	**<0.001**
MUFA (% energy)	17.5 ± 3.6^{b}	17.6 ± 3.9^{b}	17.2 ± 4.1^{ab}	16.8 ± 3.1^{ab}	16.4 ± 3.5^{a}	**0.006**	**<0.001**
PUFA, g	21.8 ± 6.7^{b}	19.9 ± 6.7^{ab}	18.0 ± 6.7^{a}	18.8 ± 6.6^{a}	19.9 ± 7.0^{ab}	**<0.001**	**0.002**

PUFA (% energy)	7.0 ± 1.6	7.1 ± 1.7	7.0 ± 1.7	7.0 ± 1.7	6.7 ± 1.5	0.101	**0.047**
Carbohydrates, g	157.0 ± 55.0^{c}	136.6 ± 45.0^{b}	120.8 ± 42.4^{a}	125.7 ± 48.0^{ab}	127.3 ± 41.5^{ab}	**<0.001**	**<0.001**
Carbohydrates (% energy)	22.5 ± 6.9^{c}	22.1 ± 6.8^{bc}	21.2 ± 6.7^{bc}	20.6 ± 5.5^{ab}	19.3 ± 5.1^{a}	**<0.001**	**<0.001**
Added sugars, g	112.0 ± 38.5^{a}	106.9 ± 39.5^{a}	108.1 ± 38.2^{a}	126.0 ± 38.6^{c}	153.2 ± 51.3^{d}	**<0.001**	**<0.001**
Added sugar (% energy)	15.9 ± 4.3^{a}	16.9 ± 4.2^{a}	18.7 ± 4.7^{b}	20.9 ± 4.6^{c}	23.1 ± 5.4^{d}	**<0.001**	**<0.001**
Fiber, g	26.9 ± 8.9^{a}	26.8 ± 9.7^{a}	27.8 ± 10.3^{ab}	30.5 ± 9.8^{b}	35.1 ± 12.1^{c}	**<0.001**	**<0.001**
Na, mg	3900.8 ± 1093.1^{c}	3333.5 ± 989.6^{ab}	3095.7 ± 961.6^{a}	3249.1 ± 1063.1^{ab}	3460.4 ± 1063.2^{b}	**<0.001**	**<0.001**

Values are expressed as means ± SDs. Sodium (Na), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA). Analysis of variance one factor (ANOVA) was performed, and Bonferroni test was used for the pairwise multiple comparisons in independent groups. ^{a,b,c,d} Means of groups that do not share a letter are significantly different. *P* and *P-for* trend values <0.05 are considered significant.

Supplementary Table 3. Cardiometabolic parameters according to quintiles of energy-adjusted of (poly)phenol subclasses intake in adolescents.

	Q1	Q2	Q3	Q4	Q5	*P-for* Trend
Flavonoids (mg/day)	**(<315.6)**	**(315.6-426.2)**	**(426.3-530.2)**	**(530.3-708.4)**	**(>708.4)**	
WC z-score, β (95% CI)	Reference	-0.05 (-0.16;0.06)	-0.01 (-0.14;0.12)	-0.10 (-0.28;0.07)	-0.11 (-0.18;-0.04)**	**0.007**
SBP z-score, β (95% CI)	Reference	-0.05 (-0.21;0.11)	0.06 (-0.17;0.30)	0.13 (-0.14;0.41)	0.07 (-0.24;0.39)	0.384
DBP z-score, β (95% CI)	Reference	-0.07 (-0.10;-0.05)***	-0.03 (-0.14;0.08)	-0.09 (-0.22;-0.01)***	-0.12 (-0.22;-0.01)*	**0.001**
MAP z-score, β (95% CI)	Reference	-0.08 (-0.16;0.01)	0.06 (-0.16;0.28)	-0.001 (-0.25;0.25)	-0.07 (-0.21;0.07)	0.785
BG (mg/dL), β (95% CI)	Reference	1.35 (1.15;1.54)***	1.70 (0.20;3.19)*	0.63 (-2.75;4.01)	1.61 (-0.14;3.35)	**<0.001**
TG (mg/dL), β (95% CI)	Reference	-2.03 (-4.92;0.85)	0.439 (-7.30;8.15)	2.11 (1.50;2.73)***	-0.07 (-6.18;6.04)	0.617
HDL-c (mg/dL), β (95% CI)	Reference	-1.72 (-2.52;-0.93)***	-2.76 (-2.97;-2.55)***	-1.94 (-3.91;0.02)	-2.11 (-3.61;-0.60)**	**0.038**
Phenolic acids (mg/day)	**(<60.9)**	**(60.9-77.5)**	**(77.6-95.5)**	**(95.6-124.7)**	**(>124.7)**	
WC z-score, β (95% CI)	Reference	-0.06 (-0.31;0.20)	-0.07 (-0.16;0.03)	-0.19 (-0.51;0.14)	-0.15 (-0.28;-0.01)*	**0.016**
SBP z-score, β (95% CI)	Reference	-0.05 (-0.08;-0.03)***	0.001 (-0.15;0.15)	-0.02 (-0.23;0.18)	-0.02 (-0.10;0.06)	0.950
DBP z-score, β (95% CI)	Reference	0.07 (-0.10;0.24)	-0.02 (-0.02;-0.01)***	0.03 (0.02;0.04)***	0.01 (-0.04;0.05)	0.371
MAP z-score, β (95% CI)	Reference	0.04 (-0.12;0.20)	-0.02 (-0.05;0.02)	0.02 (-0.02; 0.07)	-0.01 (-0.13;0.10)	**<0.001**
BG (mg/dL), β (95% CI)	Reference	-0.37 (-1.35;2.10)	-1.64 (-3.13;-0.15)*	0.53 (-1.32;2.38)	-0.37 (-0.73;-0.01)*	0.793
TG (mg/dL), β (95% CI)	Reference	-9.44 (-9.69;-6.20)***	-9.80 (-11.68;-7.92)***	-5.90 (-8.52;-3.29)***	-9.91 (-14.82;-5.00)***	**<0.001**
HDL-c (mg/dL), β (95% CI)	Reference	0.04 (-4.62;4.70)	1.40 (-6.05;8.85)	-1.08 (-5.71;3.56)	1.55 (-3.12;6.23)	0.676
Lignans (mg/day)	**(<1.2)**	**(1.2-2.1)**	**(2.2-3.1)**	**(3.2-4.7)**	**(>4.7)**	
WC z-score, β (95% CI)	Reference	0.02 (-0.03;0.07)	-0.03 (-0.08;0.03)	-0.17 (-0.49;0.15)	-0.14 (-0.57;0.29)	0.420
SBP z-score, β (95% CI)	Reference	-0.02 (-0.04;0.01)	-0.07 (-0.20;0.06)	0.04 (0.02;0.06)***	-0.11 (-0.25;0.04)	0.262
DBP z-score, β (95% CI)	Reference	-0.01 (-0.12;0.09)	-0.04 (-0.24;0.17)	-0.001 (-0.11;0.10)	-0.05 (-0.29;0.19)	0.797
MAP z-score, β (95% CI)	Reference	-0.07 (-0.15;0.02)	-0.11 (-0.39;0.17)	-0.05 (-0.19;0.09)	-0.16 (-0.52;0.21)	0.549
BG (mg/dL), β (95% CI)	Reference	0.21 (-0.42;0.84)	0.45 (-2.45;3.34)	0.001 (-1.67;1.68)	0.17 (-2.58;2.92)	0.968
TG (mg/dL), β (95% CI)	Reference	-2.57 (-7.95;2.81)	3.24 (-6.12;12.60)	-4.58 (-14.17;5.00)	-2.44 (-5.22;0.34)	0.166
HDL-c (mg/dL), β (95% CI)	Reference	0.81 (-4.16;5.78)	-0.03 (-1.55;1.50)	1.12 (-1.71;3.95)	-0.64 (-4.74;3.45)	0.752
Stilbenes (mg/day)	**(<0.03)**	**(0.03-0.09)**	**(0.10-0.16)**	**(0.17-0.30)**	**(>0.30)**	
WC z-score, β (95% CI)	Reference	-0.01 (-0.17;0.17)	-0.01 (-0.18;0.17)	0.06 (-0.35;0.48)	-0.02 (-0.27;0.23)	0.956
SBP z-score, β (95% CI)	Reference	-0.16 (-0.34;0.02)	-0.11 (-0.23;0.002)	0.02 (-0.30;0.35)	0.09 (-0.08;0.27)	0.126

DBP z-score, β (95% CI)	Reference	-0.07 (-0.13;-0.01)*	-0.13 (-0.31;0.04)	-0.03 (-0.15;0.09)	0.05 (-0.18;0.28)	0.611
MAP z-score, β (95% CI)	Reference	-0.14 (-0.22;-0.05)**	-0.18 (-0.39;0.04)	-0.02 (-0.27;0.22)	0.08 (-0.19;0.35)	0.443
BG (mg/dL), β (95% CI)	Reference	-1.43 (-0.16;0.31)	-0.66 (-2.73;1.41)	-0.79 (-0.99;-0.60)***	-1.82 (-3.11;-0.52)**	**<0.001**
TG (mg/dL), β (95% CI)	Reference	-3.45 (-4.36;-2.54)***	3.65 (3.52;3.79)***	-1.16 (-2.28;-0.03)*	-3.55 (-6.60;-0.51)*	0.108
HDL-c (mg/dL), β (95% CI)	Reference	1.34 (-0.62;3.29)*	0.80 (-4.92;6.53)	-0.49 (-3.77;2.79)	2.33 (1.31;3.35)***	0.446
Others (poly)phenols (mg/day)	**(<29.3)**	**(29.3-40.7)**	**(40.8-52.3)**	**(52.4-67.6)**	**(>67.6)**	
WC z-score, β (95% CI)	Reference	-0.01 (-0.20;0.17)	-0.10 (-0.16;-0.04)**	-0.12 (-0.26;0.02)	-0.03 (-0.23;0.17)	**<0.001**
SBP z-score, β (95% CI)	Reference	0.04 (-0.15;0.23)	-0.02 (-0.15;0.10)	0.08 (-0.21;0.36)	-0.07 (-0.33;0.18)	0.714
DBP z-score, β (95% CI)	Reference	-0.04 (-0.19;0.11)	0.002 (-0.10;0.11)	0.02 (-0.22;0.61)	-0.09 (-0.24;0.05)	0.502
MAP z-score, β (95% CI)	Reference	0.01 (-0.17;0.19)	-0.001 (-0.13;0.13)	0.07 (-0.35;0.48)	-0.11 (-0.37;0.15)	0.676
BG (mg/dL), β (95% CI)	Reference	0.68 (-0.05;1.40)	0.79 (0.11;1.47)*	0.43 (-0.04;0.90)	-1.60 (-3.13;-0.08)*	**0.040**
TG (mg/dL), β (95% CI)	Reference	0.45 (-7.89;8.79)	0.04 (-8.24;8.31)	2.97 (2.17;3.77)***	-0.94 (-6.38;4.50)	0.475
HDL-c (mg/dL), β (95% CI)	Reference	-1.06 (-4.65;2.53)	-1.35 (-6.21;3.51)	-0.78 (-3.57;2.00)	-0.26 (-1.62;1.10)	0.799

Unstandardized coefficient (β), blood glucose (BG), confidence interval (CI), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDL-c), mean arterial pressure (MAP), systolic blood pressure (SBP), triglycerides (TG), waist circumference (WC). Multilevel linear mixed-effect model. Fixed variables were sex, age, pubertal status, physical activity, body mass index z-score (it did not consider as a fixed effect in the analysis with WC), fasting, high parental educational level, household income, energy, fiber, added sugars, and protein intake. SBP, DBP, and MAP were additionally adjusted by sodium intake. Municipality and schools were considered random effects. *P<0.05, **P <0.01, and ***P<0.001. P and *P-for* Trend across quintiles of energy-adjusted (poly)phenol subclasses <0.05 are considered significant.

Supplementary Table 4. Cardiometabolic parameters according to quintiles of energy-adjusted flavonoids subclasses intake in adolescents.

		WC z-score	SBP z-score	DBP z-score	MAP z-score	BG (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)
Anthocyanins	β (95% CI)	-0.02 (-0.06;0.02)	0.05 (-0.05;0.15)	0.01 (-0.06;0.07)	0.03 (-0.06; 0.12)	-0.28 (-0.49;-0.07)	-2.77 (-5.10;-0.45)	0.17 (-0.13;0.46)
	P	0.290	0.339	0.855	0.502	**0.009**	**0.019**	0.261
Flavanols	B (95% CI)	-0.03 (-0.07;0.01)	0.02 (-0.08;0.11)	-0.03 (-0.06; 0.002)	-0.001 (-0.04; 0.03)	0.27 (0.11; 0.44)	-0.31 (-1.58;0.97)	-0.76 (-0.91;-0.61)
	P	0.052	0.719	0.065	0.879	**0.001**	0.637	**<0.001**
Flavanones	β (95% CI)	-0.004 (-0.12;0.11)	0.02 (-0.001;0.04)	-0.01 (-0.06; 0.04)	-0.01 (-0.04;0.03)	0.50 (-0.06; 1.07)	1.98 (-4.21; 8.18)	-0.26 (-1.44;0.92)
	P	0.947	0.106	0.777	0.760	0.080	0.531	0.663
Flavones	β (95% CI)	0.05 (-0.006; 0.11)	0.03 (-0.04;0.09)	0.01 (-0.01; 0.03)	0.02 (-0.01; 0.04)	0.38 (-1.37; 2.14)	0.92 (-2.91; 4.74)	-0.36 (-0.74; 0.01)
	P	0.080	0.443	0.257	0.233	0.670	0.639	0.058
Flavonols	β (95% CI)	-0.02 (-0.05; -0.002)	-0.02 (-0.05;0.01)	-0.04 (-0.08; 0.01)	-0.04 (-0.10;0.03)	-0.24 (-0.30;-0.18)	-0.19 (-2.88;2.50)	0.02 (-0.91; 0.95)
	P	**0.032**	0.107	0.111	0.310	**<0.001**	0.890	0.972

Unstandardized coefficient (β), blood glucose (BG), confidence interval (CI), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDL-c), mean arterial pressure (MAP), systolic blood pressure (SBP), triglycerides (TG), waist circumference (WC). Multilevel linear mixed-effect model. Fixed variables were sex, age, pubertal status, physical activity, body mass index z-score (it did not consider as a fixed effect in the analysis with WC), fasting, high parental educational level, household income, energy, fiber, added sugars, and protein intake. SBP, DBP, and MAP were additionally adjusted by sodium intake. Municipality and schools were considered random effects. $P < 0.05$ are considered significant.

Supplementary Table 5. Cardiometabolic parameters according to flavanol intake recommendation.

Outcome	Flavanol intake			
	Low (<400mg/day) vs. Recommended n=697 (74%)	**Recommended (400-600mg/day)** n=143 (15%)	**High (>600mg/day) vs. Recommended** n=104 (11%)	*P-for* Trend
	β (95% CI)	β (95% CI)	β (95% CI)	
WC z-score	0.03 (0.01-0.05)*	Reference	-0.07 (-0.33; 0.18)	0.390
SBP z-score	0.02 (-0.16;0.21)	Reference	0.01 (-0.29;0.31)	0.878
DBP z-score	0.05 (-0.04;0.14)	Reference	-0.03 (-0.34;0.28)	0.473
MAP z-score	0.04 (-0.13;0.20)	Reference	-0.01 (-0.37;0.34)	0.598
BG, mg/dL	2.00 (-0.61;4.61)	Reference	2.31 (1.30;3.33)***	0.705
TG, mg/dL	-0.87 (-3.75;2.02)	Reference	-1.37 (-7.58;4.83)	0.764
HDL-c, mg/dL	0.72 (-0.57;2.01)	Reference	-1.91 (-1.26;-1.13)***	**0.002**

Unstandardized coefficient (β), blood glucose (BG), confidence interval (CI), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDL-c), mean arterial pressure (MAP), systolic blood pressure (SBP), triglycerides (TG), waist circumference (WC). Multilevel linear mixed-effect model. Fixed variables were sex, age, pubertal status, physical activity, body mass index z-score (it did not consider as a fixed effect in the analysis with WC), fasting, high parental educational level, household income, energy, fiber, added sugars, and protein intake. SBP, DBP, and MAP were additionally adjusted by sodium intake. Municipality and schools were considered random effects. *P<0.05 and ***P<0.001. P and *P-for* Trend across flavanol intake categories <0.05 are considered significant.

4.1.2. Article 3: Flavonoid Intake from Cocoa-Based Products and Adiposity Parameters in Adolescents in Spain.

Emily P. Laveriano-Santos, Camila Arancibia-Riveros, Anna Tresserra-Rimbau, Sara Castro-Barquero, Ana María Ruiz-León, Ramón Estruch, Rosa Casas, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Jesús Martínez-Gómez, Carla Rodríguez, Gloria Santos-Beneit, Juan M. Fernández-Alvira, Rodrigo Fernández-Jiménez, and Rosa M. Lamuela-Raventós

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Abstract

Introduction
Cocoa-based products are a good source of flavonoids, which may have beneficial effects on metabolic health.

Objective
To assess the relationship between flavonoids from cocoa-based products and adiposity parameters in adolescents.

Methods
A cross-sectional study was conducted involving 944 adolescents aged 11-14 years enrolled in the SI! Program for Secondary Schools trial in Spain with available baseline data from food frequency questionnaires and anthropometric measurements [weight, height, waist circumference (WC), and fat mass percentage (% FM) by bioimpedance analysis]. Fat mass index (FMI) and waist-to-height ratio (WHtR) were obtained by dividing fat mass by height and WC by height, respectively. Body mass index (BMI), WC, and FMI for age and gender z-score were calculated. Overweight/obesity was defined as BMI ≥ 85th percentile and excess adiposity as %FM or FMI ≥ 75th percentile. WC ≥ 90th percentile and WHtR with a 0.5 threshold were considered as criteria of abdominal obesity. Multilevel mixed-effect regressions were used to evaluate the association between flavonoids from cocoa-based products and adiposity parameters. Municipalities and schools were considered random effects.

Results

Participants with a higher flavonoid intake from cocoa-based products had lower WC z-score [B = -0.04, 95% CI (-0.07; -0.01), *P-for trend* = 0.045] and WHtR [B = -0.01, 95% CI (-0.02; -0.01), *P- for trend* < 0.001]. They also had lower probability of having abdominal obesity [OR 0.66, 95% CI (0.52; 0.85), *P- for trend* = 0.001]. Inverse associations were observed between flavonoids from cocoa powder and BMI z-score [B = -0.08, 95% CI (-0.12; -0.05), *P* < 0.001], WC z-score [B = -0.06, 95% CI (-0.11; -0.02), *P* = 0.003], WHtR [B = -0.01, 95% CI (-0.01; -0.00), *P* < 0.001], %FM [B = -1.11, 95% CI (-1.48; -0.75), *P* < 0.001], and FMI z-score [B = -0.18, 95% CI (-0.20; -0.17), *P* < 0.001]. Regarding dark chocolate, an inverse association only with WC z-score [B = -0.06, 95% CI (-0.08; -0.05), *P* < 0.001] was found. However, no association was observed between flavonoids from milk chocolate intake and anthropometric parameters.

Conclusion

In summary, a higher intake of flavonoids from cocoa-based products was associated with lower adiposity parameters and a lower probability of presenting abdominal obesity.

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Flavonoid Intake From Cocoa-Based Products and Adiposity Parameters in Adolescents in Spain

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Background: Cocoa-based products are a good source of flavonoids, which may have beneficial effects on metabolic health.

Objective: The aim of this study is to assess the relationship between flavonoids from cocoa-based products and adiposity parameters in adolescents.

Methods: A cross-sectional study was conducted involving 944 adolescents aged 11–14 years enrolled in the SI! Program for Secondary Schools trial in Spain with available baseline data from food frequency questionnaires and anthropometric measurements [weight, height, waist circumference (WC), and fat mass percentage (% FM) by bioimpedance analysis]. Fat mass index (FMI) and waist-to-height ratio (WHtR) were obtained by dividing fat mass by height and WC by height, respectively. Body mass index (BMI), WC, and FMI for age and gender z-score were calculated. Overweight/obesity was defined as BMI ≥ 85th percentile and excess adiposity as %FM or FMI ≥ 75th percentile. WC ≥ 90th percentile and WHtR with a 0.5 threshold were considered as criteria of abdominal obesity. Multilevel mixed-effect regressions were used to evaluate the association between flavonoids from cocoa-based products and adiposity parameters. Municipalities and schools were considered random effects.

Results: Participants with a higher flavonoid intake from cocoa-based products had lower WC z-score [B = −0.04, 95% CI (−0.07; −0.01), P-for trend = 0.045] and WHtR [B = −0.01, 95% CI (−0.02; −0.01), P- for trend < 0.001]. They also had lower probability of having abdominal obesity [OR 0.66, 95% CI (0.52; 0.85), P- for trend = 0.001]. Inverse associations were observed between flavonoids from cocoa powder and BMI z-score [B = −0.08, 95% CI (−0.12; −0.05), P < 0.001], WC z-score [B = −0.06, 95% CI (−0.11; −0.02), P = 0.003], WHtR [B = −0.01, 95% CI (−0.01; −0.00), P < 0.001],

%FM [B = −1.11, 95% CI (−1.48; −0.75), $P < 0.001$], and FMI z-score [B = −0.18, 95% CI (−0.20; −0.17), $P < 0.001$]. Regarding dark chocolate, an inverse association only with WC z-score [B = −0.06, 95% CI (−0.08; −0.05), $P < 0.001$] was found. However, no association was observed between flavonoids from milk chocolate intake and anthropometric parameters.

Conclusions: A higher intake of flavonoids from cocoa-based products was associated with lower adiposity parameters and a lower probability of presenting abdominal obesity.

Keywords: (poly)phenols, catechin, epicatechin, proanthocyanidins, cardiometabolic, obesity

INTRODUCTION

Obesity, which is characterized by abnormal or excessive body fat accumulation, is a serious public health problem worldwide (1, 2). Excessive adiposity in children and adolescents leads to metabolic disorders such as vascular dysfunction and subclinical indicators of atherosclerosis, increasing the risk of cardiovascular disease and mortality in adulthood (2, 3).

Marked by physiological and emotional changes, adolescence is a critical period for managing obesity. Behavioral modifications, particularly fomenting physical activity and healthy dietary patterns are one of the best strategies used in primary health care settings to reduce obesity among adolescents (2, 4). A diet based on polyphenol-rich foods is of interest because of the antioxidant and anti-inflammatory effect and their influence on physiological and molecular pathways related to body weight maintenance (5–8). The positive impact of cocoa-based products on obesity has been attributed to their content of flavonoids (a large class of phenolic compounds), specifically flavanols (catechins and procyanidins) (9, 10). Systematic reviews and meta-analyses support the beneficial effect of cocoa flavonoids on cardiovascular risk factors since they are reported to favorably improve blood pressure, lipid profile, inflammation, and adiposity parameters (11–13). However, as most of the research in this field has been performed in adults, there is a need for studies on adolescents to establish dietary recommendations for the consumption of cocoa-based products in this target population, always within the framework of a healthy lifestyle. Therefore, this study aimed to investigate the association between flavonoid intake from cocoa-based products and adiposity parameters in a large sample of adolescents in Spain.

MATERIALS AND METHODS

Study Population

The SI! (*Salud Integral-Comprehensive Health*) Program for Secondary Schools trial (NCT03504059) is a cluster-randomized controlled intervention trial conducted in adolescents from 24 secondary schools in Spain and conducted from 2017 to 2021. The main objective of this trial was to evaluate the effectiveness of an educational intervention to promote cardiovascular health at schools. A detailed description of the original study design and recruitment procedures has been previously published (14). Parents or caregivers provided assent and written informed consent before entering the study.

The present cross-sectional study derived from the SI! Program for Secondary Schools trial was carried out using baseline data (2017) collected from 944 participants with available information on food consumption frequency, and whose total energy intake ranged from 803 to 4,013 kcal/day in boys and 502 to 3,511 kcal/day in girls (15) (**Figure 1**).

Assessment of Flavonoids From Cocoa-Based Products

Dietary intake was assessed by a validated semi-quantitative food frequency questionnaire (15, 16). Cocoa-based product intake was expressed in grams and included cocoa powder (25% of pure cocoa), dark (more than 70% pure cocoa), and milk chocolate (about 30% pure cocoa). Cookies, pastries, and beverages made of cocoa-based products were not considered due to the lack of information on their content of flavonoids. Flavonoids from cocoa-based products were estimated using the Phenol-Explorer database (http://www.phenol-explorer.eu), which included flavanols, such as catechin, epicatechin, cinnamtannins, and proanthocyanidins (dimers, trimers, 4–6 mers, 7–10 mers, polymers, and monomers), and flavonol-like quercetin (17, 18). In brief, flavonoids from cocoa-based products were estimated (mg/100 g fresh food weight) for each food and then multiplied by intake of the respective foods (g/day). Total flavonoids from cocoa-based products were estimated as the sum of intakes of the individual flavonoids (catechin, epicatechin, cinnamtannins, proanthocyanidins, and quercetin). Energy-adjusted flavonoid intake was calculated by the residual method established by Willet et al. (19).

Assessment of Adiposity Parameters

All participants were evaluated by trained staff, who performed the anthropometric measurements of weight, height, and waist circumference (WC) according to standard procedures (14). Weight was obtained to the nearest 0.1 kg using a digital scale (OMRON BF511) and height to the nearest 0.1 cm with a portable SECA 213 stadiometer. WC was measured to the nearest 0.1 cm. To minimize measurement errors, WC was measured three times and a mean value was calculated. The percentage of fat

Abbreviations: BMI, body mass index; FDR, false discovery rate; FM, fat mass; FMI, fat mass index; SI, *Salud Integral;* WC, waist circumference; WHtR, waist-to-height ratio.

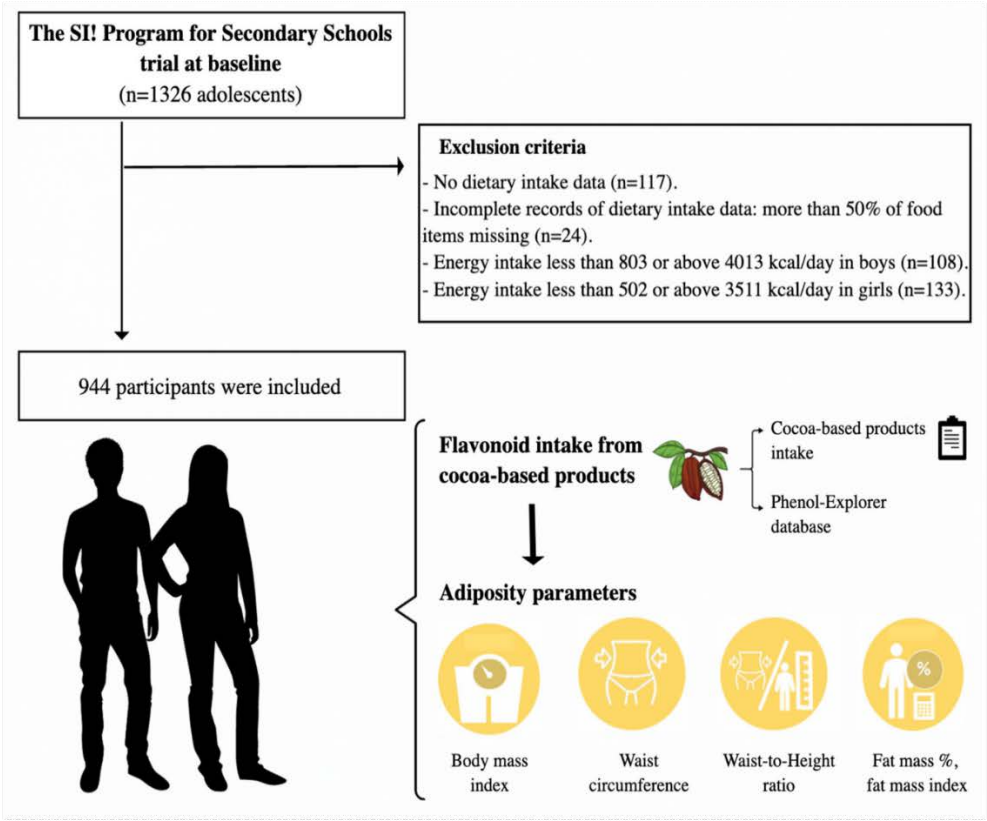

FIGURE 1 | Study design of the study.

mass (%FM) was estimated by bioelectrical impedance using a tetrapolar OMRON BF511 and fat mass weight was calculated as the product of fat percentage and body weight.

Body mass index (BMI) was calculated as body weight in kilograms divided by height squared in meters (kg/m^2). The fat mass index (FMI) and waist-to-height ratio (WHtR) were obtained by dividing fat weight (kg) by height squared (m^2) and WC (cm) by height (cm), respectively. Age- and gender-specific BMI, WC z-scores, and FMI z-scores (standard deviation score) were calculated according to the Center for Disease Control growth references and the National Health and Nutrition Examination Survey data (20–23).

Overweight was defined as BMI at or above the 85th percentile to less than the 95th percentile, and obesity as equal to or greater than the BMI 95th percentile (21, 22). Participants with a BMI percentile equal to or above the 85th percentile were classified as overweight/obese (21, 22). Abdominal obesity was defined by WC at or above the 90th percentile and/or WHtR equal to or above the 0.5 threshold (20, 24, 25). Finally, participants with a %FM and/or FMI greater than or equal to the age- and gender-specific 75th percentile were classified as excess adiposity, according to published reference data for %FM and FMI (23, 26).

Assessment of Covariates

Intake of energy, foods, and nutrients was determined from the semi-quantitative food frequency questionnaire that was previously described, with the use of values from Spanish food composition tables (27, 28).

Information on physical activity was obtained with the use of accelerometers and a standardized questionnaire. Physical activity was measured using accelerometers (Actigraph wGT3X-BT, ActiGraph, Pensacola, USA) worn on the non-dominant wrist for 7 days, except during water-based activities (14). Moderate-to-vigorous physical activity was estimated according to the cut points of Chandler et al. and is presented as the average minutes of moderate-to-vigorous physical activity per day (29). In participants with missing accelerometer data (n = 48), the information from the QAPACE survey (*Quantification*

TABLE 1 | Characteristics of participants according to quintiles of flavonoid intake from cocoa-based products (mg/day).

	Overall (*n* = 944)	Q1 (<12.1) (*n* = 189)	Q2 (12.1–32.0) (*n* = 189)	Q3 (32.1–53.2) (*n* = 189)	Q4 (53.3–83.8) (*n* = 189)	Q5 (>83.8) (*n* = 188)	*P-for trend*
Girls, *n* (%)	455 (48)	76 (40)	101 (53)	89 (47)	90 (48)	99 (53)	0.098
Age, years	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	0.740
Anthropometric measurements							
BMI, kg/m^2	20.2 (3.7)	20.2 (3.6)	20.4 (3.9)	20.5 (4.1)	20.4 (3.7)	19.7 (3.3)	0.286
WC, cm	71.9 (10.1)	72.2 (10.1)	71.7 (10.1)	72.7 (11.6)	72.5 (9.9)	70.5 (8.7)	0.232
WHtR	0.4 (0.1)	0.5 (0.1)	0.4 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.082
%FM	23.3 (8.3)	22.7 (8.2)	23.5 (8.1)	23.7 (9.0)	23.9 (8.2)	22.7 (8.0)	0.980
FMI, kg/m^2	5.0 (2.7)	4.9 (2.6)	5.1 (2.8)	5.2 (2.9)	5.1 (2.6)	4.7 (2.4)	0.656
Adiposity parameters, *n* (%)							
BMI ≥ 85th to <95th percentile	172 (18)	39 (21)	33 (18)	32 (17)	41 (22)	27 (14)	0.334
BMI ≥ 95th percentile	89 (9)	14 (7)	18 (10)	24 (13)	19 (10)	14 (7)	0.943
WC ≥ 90th percentile	153 (16)	34 (18)	25 (13)	36 (19)	33 (17)	25 (13)	0.546
WHtR ≥ 0.5	213 (23)	48 (25)	39 (21)	44 (23)	50 (26)	32 (17)	0.258
%FM ≥ 75th percentile	79 (8)	14 (7)	14 (7)	19 (10)	17 (9)	15 (8)	0.646
FMI ≥ 75th percentile	146 (16)	28 (15)	26 (14)	37 (20)	31 (16)	24 (13)	0.888
Physical activity, *n* (%)							
≥60 min/day MVPA	310 (33)	71 (38)	64 (34)	57 (30)	61 (32)	57 (30)	0.137
Parental education, *n* (%)							
University level	240 (26)	58 (31)	44 (24)	49 (27)	46 (25)	43 (24)	0.165
Municipality, *n* (%)							**0.021**
Barcelona	644 (68)	138 (73)	141 (75)	121 (64)	119 (63)	125 (66)	
Madrid	300 (32)	51 (27)	48 (25)	68 (36)	70 (37)	63 (34)	

Data are expressed as mean (SD) or frequency (percentage).

Q, quintiles of flavonoids from cocoa-based products; n, number; SD, standard deviation; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, body fat percentage; FMI, fat mass index; MVPA, moderate-to-vigorous physical activity.

Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables. P-for trend were obtained using orthogonal contrasts test. P < 0.05 are considered statistically significant.

Significant differences are bolded.

de L'Activité Physique en Altitude chez les Enfants) was used to estimate moderate-to-vigorous physical activity according to the frequency and duration of recreational physical activity and competitive sports performed inside or outside schools, on school days, and at weekends (14, 30). A conversion factor was used to calculate moderate-to-vigorous physical activity in terms of minutes per day. For the analysis, physical activity was categorized as below or equal to/above 60 min/day of moderate-to-vigorous physical activity based on physical activity recommendations for adolescents by the World Health Organization (31).

Information about parental education was obtained from a general questionnaire answered by the parents (14). A high level of parental education corresponded to university studies according to the International Standard Classification of Education (32). Puberty development was categorized according to the Tanner maturation stages using pictograms (33).

Statistical Analysis

A descriptive analysis of the population was carried out using mean (SD) and frequency distribution. Participants were categorized into quintiles of energy-adjusted flavonoids from cocoa-based products (Q1: <12.1, Q2: 12.1–32.0, Q3: 32.1–53.2, Q4: 53.3–83.8, and Q5 >83.8 mg/day). One-way analysis of variance, including Bonferroni *post-hoc* test, and chi-square analysis were performed to assess the differences in means and frequencies across quintiles of flavonoids from cocoa-based products, respectively.

Multilevel mixed-effects linear regression models with robust error variance were used to evaluate the association between quintiles of flavonoids from cocoa-based products with the anthropometric measurements (BMI *z*-score, WC *z*-score, WHtR, %FM, and FMI *z*-score) as continuous variables. Multilevel generalized logistic regression was performed to study the association between quintiles of flavonoids from cocoa-based products and adiposity parameters (BMI ≥ 80th to <95th percentile, BMI ≥ 95th percentile, WC ≥ 90th percentile, and WHtR ≥ 0.5). The fixed effects were gender (girls/boys), age (continuous, year), Tanner maturation stage (from I to V), physical activity (≥60 min/<60 min moderate-to-vigorous physical activity), parental education (university studies/lower than university studies), intake of energy (continuous, Kcal/day), sweetened products like breakfast cereals (continuous, g/day), pastries (continuous, g/day), sugar-sweetened beverages (continuous, g/day), meat, and processed meat (continuous,

TABLE 2 | Dietary food intake of participants according to quintiles of flavonoids from cocoa-based products (mg/day).

	Overall (n = 944)	Q1 (<12.1) (n = 189)	Q2 (12.1–32.0) (n = 189)	Q3 (32.1–53.2) (n = 189)	Q4 (53.3–83.8) (n = 189)	Q5 (>83.8) (n = 188)	*P-for trend*
Fish products, g/day	86.6 (55)	103.3 (67.2)^{a}	89.2 (52.6)^{a,b}	81.6 (47.0)^{b}	76.1 (49.6)^{b}	82.9 (53.1)^{b}	**<0.001**
Meat, g/day	172.6 (90.4)	207.4 (107.9)^{a}	161.2 (82.8)^{b}	172.5 (87.5)^{b}	155.8 (75.2)^{b}	166.0 (86.6)^{b}	**<0.001**
Processed meat, g/day	7.0 (6.9)	8.7 (8.5)^{a}	7.1 (7.1)^{a,b,c}	6.6 (6.3)^{b,c}	6.7 (6.3)^{a,b,c}	6.1 (5.4)^{c}	**0.001**
Dairy products, g/day	406.6 (253.9)	448.4 (275.8)^{a}	404.9 (254.3)^{a,b}	382.4 (231.5)^{a,b}	357.7 (193.6)^{b}	439.9 (293.3)^{a}	0.269
Refined grains, g/day	113.2 (69.4)	139.8 (85.8)^{a}	115.6 (70.1)^{b}	107.2 (59.1)^{b}	105.7 (62.9)^{b}	97.6 (58.7)^{b}	**<0.001**
Wholegrains, g/day	18.5 (32.2)	19.2 (33.6)	18.9 (32.0)	15.1 (25.9)	19.7 (34.1)	19.7 (34.8)	0.814
Breakfast cereals, g/day	14.6 (19.7)	17.8 (29.1)^{a}	15.3 (18.8)^{a}	13.4 (15.2)^{a}	14.6 (16.9)^{a}	12.1 (14.7)^{a}	**0.008**
Legumes, g/day	60.6 (44.2)	72.0 (64.7)^{a}	59.6 (37.8)^{a,b}	58.4 (36.2)^{b}	57.2 (37.3)^{b}	55.6 (36.4)^{b}	**0.001**
Vegetables, g/day	205.3 (150.6)	246.4 (199.8)^{a}	200.9 (137.9)^{b}	194.9 (134.7)^{b}	190.4 (129.7)^{b}	193.5 (133.1)^{b}	**0.001**
Fruits, g/day	334.7 (250.7)	430.6 (290.0)^{a}	338.6 (216.2)^{b}	310.3 (243.1)^{b}	292.4 (208.5)^{b}	301.1 (263.3)^{b}	**<0.001**
Nuts, g/day	11.3 (14.5)	16.1 (19.7)^{a}	10.8 (13.7)^{b}	9.4 (10.6)^{b}	9.7 (11.7)^{b}	10.6 (14.1)^{b}	**<0.001**
Olive oil, g/day	16.6 (14.5)	17.6 (13.6)	17.2 (17.2)	16.9 (13.6)	15.3 (12.6)	16.1 (15.0)	0.141
Cocoa-based products, g/day	7.4 (7.6)	2.6 (2.5)^{a}	3.9 (3.5)^{a}	5.7 (4.1)^{b}	8.0 (5.3)^{c}	16.6 (10.3)^{d}	**<0.001**
Sugar-sweetened beverages, g/day	54.8 (95.1)	73.2 (123.2)^{a}	52.4 (96.5)^{a,b}	50.6 (94.5)^{a,b}	55.3 (81.3)^{a,b}	42.3 (69.4)^{b}	**0.007**
Pastry products, g/day	69.1 (52.5)	74.2 (54.6)	67.2 (53.9)	68.4 (53.5)	66.5 (46.6)	73.0 (53.2)	0.561

Data are expressed as mean (SD).

Q, quintiles of flavonoids from cocoa-based products; n, number; SD, standard deviation.

Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables.

^{a,b,c,d}Data sharing the different letters are statistically different after Bonferroni post-hoc test. P-for trend were obtained using orthogonal contrasts test. $P < 0.05$ are considered statistically significant.

Significant differences are bolded.

g/day), and other polyphenol-rich food intakes like fruits (continuous, g/day), vegetables (continuous, g/day), legumes (continuous, g/day), nuts (continuous, g/day), and extra olive oil (continuous, g/day). Municipalities (Barcelona/Madrid) and schools were included as random effects. Gender interaction was considered to evaluate potential effect modification in the association between flavonoids from cocoa-based products and adiposity parameters. Orthogonal polynomial contrast was used to determine linear trends.

In addition, a multilevel mixed-effects linear regression analysis was conducted to explore associations between flavonoids of each cocoa-based product (cocoa powder, dark chocolate, and milk chocolate) and adiposity parameters, all of them as continuous variables. For this analysis, data from participants who reported daily intake of at least one cocoa-based product were considered (700 participants reported cocoa powder intake, 294 reported dark chocolate intake, and 644 reported milk chocolate intake). The model included the same fixed and random effects variables as described earlier. Moreover, Pearson correlation coefficients were used to explore the relationship between individual flavonoids from cocoa-based products and adiposity parameters. Finally, the false discovery rate (FDR) by the Benjamini-Hochberg procedure was applied to adjust p-values for multiple correlations (34). Before these analyses, values of flavonoids were normalized and scaled in 1-SD with the inverse normal transformation (35).

All statistical analyses were conducted using Stata statistical software package version 16.0 (StataCorp., College Station, TX, USA) and R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical tests were two-sided and statistical significance was set as 0.05.

RESULTS

General Characteristics of the Study Participants

The characteristics of the cohort stratified by quintiles of flavonoid intake from cocoa-based products are shown in **Table 1**. Based on BMI z-score, 18% of adolescents presented overweight and 9% obesity. Regarding the abdominal obesity parameters, 16% of participants had a WC greater than the 90th percentile, and 23% had a high WHtR (≥0.5 threshold). Finally, regarding the excess of adiposity, 8 and 16% of adolescents had %FM and FMI equal to or greater than the 75th percentile, respectively. Compared to the lowest quintile, participants in the highest quintile tended to have slightly lower BMI, WC, and WC z-score, although the differences were not significant in the univariate analysis.

The mean cocoa-based product intake was 7.4 (7.6) g/d, equivalent to one tablespoon of cocoa powder or one square piece of a chocolate bar. More than 90% of the participants reported daily intake of at least one cocoa-based product, from them, 75% (N = 700) reported intake of cocoa powder, 31% (N = 294) dark chocolate, and 68% (N = 644) milk chocolate. The mean flavonoid intake from cocoa-based products was 57.4 (74.5) mg/day, where 26.6 (35.3) mg/day were from cocoa powder, 24.0 (62.9 mg/day) from dark chocolate, and 6.7 (11.3) mg/day from milk chocolate (Data not shown). Participants with a

TABLE 3 | Nutrients and (poly)phenols intake of participants according to quintiles of flavonoids from cocoa-based products (mg/day).

	Overall (n = 944)	Q1 (<12.1) (n = 189)	Q2 (12.1–32.0) (n = 189)	Q3 (32.1–53.2) (n = 189)	Q4 (53.3–83.8) (n = 189)	Q5 (>83.8) (n = 188)	*P-for trend*
Nutrients intake							
Energy, Kcal/day	2,539.2 (601.8)	3,013.4 (435.9)^{a}	2,510.9 (490.9)^{b}	2,402.6 (562.7)^{b,c}	2,307.3 (614.7)^{c}	2,461.3 (622.6)^{b,c}	**<0.001**
Carbohydrates, g/day	256.5 (72.2)	303.2 (65.8)^{a}	255.8 (65.7)^{b}	240.6 (64.3)^{b}	236.3 (71.3)^{b}	246.7 (73.3)^{b}	**<0.001**
Fiber, g/day	29.4 (10.7)	35.5 (11.4)^{a}	29.1 (8.5)^{b}	27.6 (9.9)^{b}	27.2 (10.3)^{b}	27.6 (10.7)^{b}	**<0.001**
Proteins, g/day	120.9 (33.3)	144.2 (30.1)^{a}	118.9 (27.8)^{b}	115.9 (30.8)^{b,c}	108.3 (30.3)^{c}	117.5 (35.5)^{b}	**<0.001**
SFA, g/day	36.8 (11.5)	44.2 (11.3)^{a}	35.8 (9.5)^{b}	34.6 (10.6)^{b,c}	32.7 (10.8)^{c}	36.8 (11.7)^{b}	**<0.001**
MUFA, g/day	48.5 (16.2)	57.0 (14.2)^{a}	48.1 (15.9)^{b}	46.4 (15.3)^{b}	43.8 (15.8)^{b}	47.2 (16.5)^{b}	**<0.001**
PUFA, g/day	19.7 (6.8)	23.7 (6.4)^{a}	19.4 (5.7)^{b}	18.7 (6.2)^{b}	18.2 (7.3)^{b}	18.5 (6.9)^{b}	**<0.001**
Calcium, mg/day	1,012.9 (391.0)	1,198.1 (403.5)^{a}	1,001.6 (354.7)^{b,c}	959.7 (362.2)^{b,c}	896.9 (321.2)^{b}	1,008.1 (440.3)^{c}	**<0.001**
Vitamin A, μg/day	1,476.0 (1,465.2)	1,849.9 (1,719.5)^{a}	1,542.8 (1,759.5)^{a,b}	1,342.6 (1,107.7)^{b}	1,177.2 (754.9)^{b}	1,467.6 (1,637.2)^{a,b}	**0.001**
Vitamin D, μg/day	5.1 (2.6)	6.1 (3.0)^{a}	5.2 (2.4)^{b}	4.8 (2.4)^{b}	4.6 (2.5)^{b}	4.7 (2.4)^{b}	**<0.001**
(Polyp)phenols intake							
Flavonoids, mg/day	530.1 (331.3)	482.8 (314.0)^{a}	440.1 (283.9)^{a}	460.6 (263.3)^{a}	490.4 (241.1)^{a}	777.8 (406.1)^{b}	**<0.001**
Phenolic acids, mg/day	97.8 (64.5)	117.2 (70.8)^{a}	103.5 (74.4)^{b}	89.6 (53.8)^{b}	89.4 (60.4)^{b}	89.3 (56.5)^{b}	**<0.001**
Stilbenes, mg/day	0.2 (0.3)	0.2 (0.3)	0.2 (0.3)	0.2 (0.3)	0.2 (0.3)	0.2 (0.4)	0.677
Lignans, mg/day	3.8 (5.1)	5.4 (6.1)^{a}	3.2 (3.7)^{b}	3.5 (5.3)^{b}	3.7 (5.7)^{b}	3.4 (4.1)^{b}	**0.002**
Other, mg/day	51.3 (34.4)	59.5 (44.0)^{a}	55.1 (34.7)^{a,b}	46.5 (29.7)^{b}	48.9 (30.8)^{b}	46.5 (28.6)^{b}	**<0.001**

Data are expressed as mean (SD).

Q, quintiles of flavonoids from cocoa-based products; n, number; SD, standard deviation; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables.

^{a,b,c}Data sharing the different letters are statistically different after Bonferroni post-hoc test. P-for trend were obtained using orthogonal contrasts test. P < 0.05 are considered statistically significant.

Significant differences are bolded.

TABLE 4 | Association between flavonoid intake from cocoa-based products (mg/day) and anthropometric measurements.

Anthropometric variables	Q1 (<12.1)	Q2 (12.1–32.0) (ß, 95% CI)	Q3 (32.1–53.2) (ß, 95% CI)	Q4 (53.3–83.8) (ß, 95% CI)	Q5 (>83.8) (ß, 95% CI)	*P-for trend*
BMI *z*-score	Reference	0.25 (0.10; 0.40)	0.29 (0.17; 0.41)	0.06 (−0.04; 0.15)	−0.07 (−0.25; 0.10)	**<0.001**
WC *z*-score	Reference	0.09 (−0.01; 0.19)	0.23 (0.17; 0.29)	0.06 (−0.13; 0.24)	−0.04 (−0.07; −0.01)	**0.045**
WHtR	Reference	−0.00 (−0.01; 0.01)	0.01 (0.01; 0.01)	−0.00 (−0.00; −0.00)	−0.01 (−0.02; −0.01)	**<0.001**
%FM	Reference	0.72 (0.51; 0.94)	2.17 (1.95; 2.38)	0.09 (−0.14; 0.32)	−1.15 (−3.36; 1.05)	0.160
FMI *z*-score	Reference	0.41 (0.18; 0.64)	0.52 (0.39; 0.66)	0.21 (0.02; 0.40)	0.02 (−0.53; 0.56)	0.242

Q, quintiles of flavonoids from cocoa-based products (mg/day); ß, (beta) regression coefficient; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, percentage of fat mass; FMI, fat mass index.

Statistical analyses were conducted using multilevel mixed-effect linear regression analysis. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. P-for trend were obtained using orthogonal contrasts test across quintiles. P < 0.05 are considered statistically significant.

Significant differences are bolded.

higher intake of flavonoids from cocoa-based products tended to consume lower fish, meat, processed meat, refined grains, (poly)phenol-rich foods (legumes, vegetables, fruits, and nuts), and sugar-sweetened beverages (**Table 2**). In addition, compared to the lowest quintile, participants in the highest quintile of flavonoids from cocoa-based products had a lower energy intake and macro and micronutrients (**Table 3**), except for vitamin A. A higher intake of total dietary flavonoids was observed in the highest quintile, but lower values of phenolic acids, lignans, and other (poly)phenol intake were observed in the same group (**Table 3**).

Association of Dietary Flavonoids From Cocoa-Based Products With Adiposity Parameters

The results from the multivariate-adjusted linear regression analyses showed that a higher intake of flavonoids from cocoa-based products was associated with lower values of BMI z-score (*P-for trend* < 0.001); however, no significant difference was observed between the highest and lowest quintiles. Moreover, participants with highest intake of flavonoids from cocoa-based products had lower values of WC z-score [B = −0.04, 95% CI (−0.07; −0.01), *P-for trend* = 0.045] and WHtR [B = −0.01,

TABLE 5 | Association between flavonoids from cocoa powder, dark chocolate, and milk chocolate (mg/day) and anthropometric measurements.

Anthropometric measurements	Cocoa powder *N* = 700 (ß, 95% CI)	*P*	Dark chocolate *N* = 294 (ß, 95% CI)	*P*	Milk chocolate *N* = 644 (ß, 95% CI)	*P*
BMI *z*-score	−0.08 (−0.12; −0.05)	**<0.001**	−0.11 (−0.23; 0.12)	0.076	−0.02 (−0.11; 0.06)	0.593
WC *z*-score	−0.06 (−0.11; −0.02)	**0.003**	−0.06 (−0.08; −0.05)	**<0.001**	−0.01 (−0.02; 0.01)	0.403
WHtR	−0.01 (−0.01; −0.00)	**<0.001**	−0.003 (−0.01; 0.00)	0.110	−0.001 (−0.00; 0.00)	0.646
%FM	−1.11 (−1.48; −0.75)	**<0.001**	−0.42 (−1.63; 0.79)	0.494	0.112 (−0.49; 0.72)	0.718
FMI *z*-score	−0.18 (−0.20; −0.17)	**<0.001**	−0.16 (−0.36; 0.06)	0.147	−0.01 (−0.13; 0.11)	0.822

N, number of participants who reported cocoa powder, dark chocolate, or milk chocolate intake; ß, regression coefficient; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, body fat percentage; FMI, fat mass index.
Statistical analyses were conducted using multilevel mixed-effect linear regression analysis. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. Data from flavonoids were normalized with the inverse normal distribution before this analysis. P < 0.05 are statistically significant.
Significant differences are bolded.

TABLE 6 | Association between flavonoids from cocoa-based products (mg/day) and adiposity parameters.

Adiposity parameters	Q1 (<12.1)	Q2 (12.1–32.0) (OR, 95% CI)	Q3 (32.1–53.2) (OR, 95% CI)	Q4 (53.3–83.8) (OR, 95% CI)	Q5 (>83.8) (OR, 95% CI)	*P-for trend*
BMI ≥ 85th to <95th percentile	1	1.17 (1.11; 1.22)	0.94 (0.86; 1.03)	0.93 (0.51; 1.71)	0.52 (0.20; 1.35)	**0.023**
BMI ≥ 95th percentile	1	1.11 (0.76; 1.61)	2.86 (2.40; 3.41)	1.41 (1.22; 1.62)	0.96 (0.63; 1.45)	0.908
WC ≥ 90th percentile	1	0.52 (0.34; 0.80)	1.48 (1.16; 1.87)	0.83 (0.61; 1.14)	0.79 (0.68; 0.93)	0.520
WHtR ≥ 0.5	1	0.97 (0.54; 1.74)	1.14 (0.91; 1.42)	0.96 (0.68; 1.36)	0.60 (0.33; 1.09)	**<0.001**
%FM ≥ 85th percentile	1	0.79 (0.69; 0.90)	1.95 (1.45; 2.62)	1.02 (0.53; 1.98)	1.02 (0.53; 1.98)	0.736
FMI ≥ 75th percentile	1	0.75 (0.49; 1.14)	1.59 (1.42; 1.77)	0.92 (0.74; 1.16)	0.56 (0.14; 2.22)	0.859

Q, quintiles of flavonoids from cocoa-based products; OR, odds ratio; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, body fat percentage; FMI, fat mass index.
Statistical analyses were conducted using multilevel mixed-effect logistic regression model. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. P-for trend were obtained using orthogonal contrasts test. P < 0.05 are considered statistically significant.
Significant differences are bolded.

95% CI (−0.02; −0.01), *P-for trend* < 0.001] (**Table 4**). However, quartiles 2 and 3 had higher values of BMI *z*-score, %FM, and FMI *z*-score compared to quartile 1. No interaction with gender was found in the regression analysis.

Table 5 shows the association of flavonoids from each cocoa-based product and anthropometric parameters. Inverse associations were observed between flavonoids from cocoa powder and BMI *z*-score [B = −0.08, 95% CI (−0.12; −0.05), $P < 0.001$], WC *z*-score [B = −0.06, 95% CI [−0.11; −0.02], $P = 0.003$], WHtR [B = −0.01, 95% CI (−0.01; −0.00), $P < 0.001$], %FM [B = −1.11, 95% CI (−1.48; −0.75), $P < 0.001$], and FMI *z*-score [B = −0.18, 95% CI (−0.20; −0.17), $P < 0.001$]. Regarding dark chocolate, an inverse association only with WC *z*-score [B = −0.06, 95% CI (−0.08; −0.05), $P < 0.001$] was found. However, no association was observed between flavonoids from milk chocolate intake and anthropometric parameters.

Multivariate-adjusted logistic regression analyses revealed a tendency of having less probability of having overweight (BMI at or above the 85th percentile to less than the 95th percentile) and high WHtR (≥0.5 thresholds) in participants with higher flavonoid intake from cocoa-based products (**Table 6**). In addition, participants in the highest quintile had less probability of having abdominal obesity [OR 0.66, 95% CI (0.52; 0.85), *P-for trend* = 0.001] compared to the lowest quintile (**Figure 2**). However, participants in quintiles 4 and 3 had a higher probability of obesity (BMI at or above 95th percentile) compared to quintile 1.

Finally, in the correlation analysis between individual flavonoids from cocoa-based products and adiposity parameters, weak inverse correlations between WHtR and catechins (R = −0.08, FDR value = 0.027), epicatechins (R = −0.09, FDR value = 0.014), and proanthocyanidins (R = −0.08, FDR value = 0.021) were observed (Data not shown).

DISCUSSION

In the present study, a higher intake of flavonoids from cocoa-based products was inversely associated with individual adiposity parameters and abdominal obesity in adolescents. To our knowledge, this is one of the first studies to explore these associations in this target population.

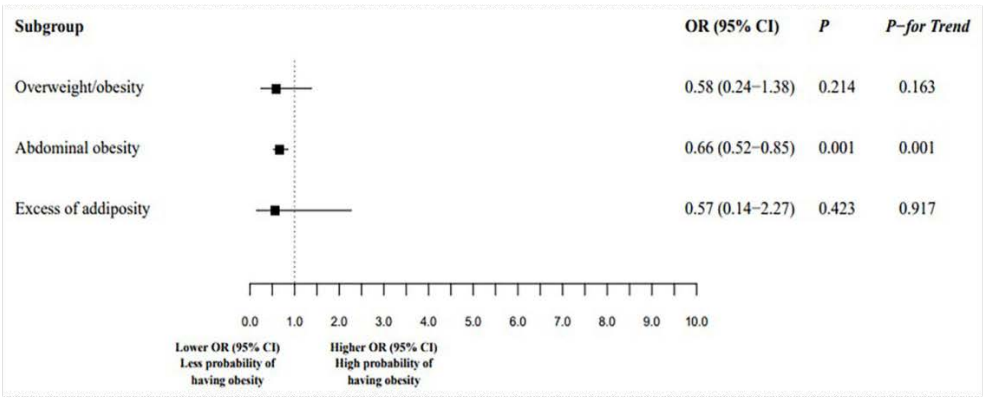

FIGURE 2 | Association between the highest and the lowest quintiles of flavonoids from cocoa-based products (mg/day) intake and obesity. Overweight/obesity was defined by body mass index percentile equal to or above the age- and gender-specific 85th percentile. Abdominal obesity was defined by WC at or above the 90th percentile and/or WHtR equal to or above the 0.5 threshold. Excess of adiposity was defined by %FM and or FMI greater than or equal to the age- and gender-specific 75th percentile. Q, quintiles of flavonoids from cocoa-based products; OR, odds ratio; CI, confidence interval. Statistical analyses were conducted using multilevel mixed-effect logistic regression model. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. *P*-values between Q5 vs. Q1 and *P- for trend* were obtained using orthogonal contrasts test. $P < 0.05$ are considered statistically significant.

Cocoa Flavonoid Intake in Adolescents

Cocoa-based products are an important dietary source of flavonoids. In our study, adolescents consumed a mean of 57.4 mg/day of flavonoids from cocoa-based products, representing 11% of the total dietary flavonoid intake (mean 530.1 mg/day). A lower intake of flavonoids from cocoa-based products was reported by Bawaked et al. in Spanish children aged 6 to 11 years, who consumed 10.9 mg/day of flavonoids from cocoa powder and chocolate, which provided 23.5% of the total flavonoid intake (mean 70.7 mg/day) (36). In the Healthy Lifestyle in Europe by Nutrition in Adolescence study, chocolate products were once the major source of dietary (poly)phenols and flavonoids in European adolescents (37).

Flavonoids From Cocoa-Based Products and Adiposity Parameters

The inverse association of flavonoids from cocoa-based products with adiposity parameters is in accordance with previous studies, although most of them were conducted in adults. The fact that the results remained consistent when using different adiposity parameters (BMI *z*-score, WC *z*-score, WHtR, %FM, and FMI *z*-score) further strengthens the study findings. In addition, in our cross-sectional, multivariate-adjusted model, clinical relevance was observed between extremes of quintiles of flavonoids from cocoa-based products (Q5 vs. Q1) and less probability of having abdominal obesity. These results were independent of physical activity, puberty development, parental education, intake of energy, sweetened products, meat, and processed meat, as well as other (poly)phenol-rich foods intake such as fruits, vegetables, legumes, nuts, and extra olive oil. Fruits and vegetables represent the main food source of flavonoids in the diet of adolescents and their consumption could influence the association of flavonoids from cocoa-based products with adiposity parameters (36, 38–40). According to the results of a cross-sectional study of European adolescents, consumption of energy-dense foods is associated with a higher probability of obesity (41). In our study, a tendency to consume less energy-dense and sugary foods was observed in participants with a higher intake of flavonoids from cocoa-based products.

In agreement with our findings, Cuenca-García et al. recently reported that higher chocolate consumption was associated with lower BMI, body fat, and WC in European adolescents (40). Similar results were obtained in a large cross-sectional analysis in non-diabetic US adults, where the BMI and WC of individuals who reported chocolate intake were lower by 0.92 Kg/m^2 and 2.07 cm, respectively, compared to the non-reporters, a difference that could be attributed to the intake of cocoa flavonoids (42). However, the authors did not define the type of chocolate being consumed (for example, dark or milk), and its flavonoid content was not calculated. In our study, inverse associations between flavonoids from cocoa powder and BMI *z*-score, WC *z*-score, WHtR, %FM, and FMI *z*-score were observed, but WC *z*-score was inversely associated only with dark chocolate. No association between anthropometric parameters and milk chocolate was found. These results could be attributed to the highest concentration of flavonoids in cocoa powder compared to dark or milk chocolate (17, 18, 43). In addition to this, it could be also explained by the fact that cocoa powder was consumed by most participants (75%), with fewer consuming milk (68%) and dark chocolates (31%).

Evidence between cocoa flavonoid consumption and adiposity in adults is conflicting. The results of a meta-analysis based on randomized clinical trials suggested that the consumption of at least 30 g/day of cocoa/dark chocolate for 4–8 weeks decreases BMI in adults (12). Similarly, weight reduction in overweight/obese adults (BMI $\geq$ 25 kg/m^2) was related to the consumption of flavanol-containing products such as tea, cocoa, and apple in a subgroup meta-analysis (13). In contrast, a meta-analysis of short-term trials did not find significant associations between flavonoid intake from cocoa-based products and BMI in adults, although this could have been due to the short-term nature of the studies (44). Longer-term randomized controlled clinical trials are needed to examine the magnitude of the effect of flavonoids from cocoa-based products on adiposity parameters. Instead, the limitations of BMI as an adiposity indicator are well-known, because it does not provide information on adiposity distribution, and therefore additional anthropometric measurements are required, such as WC, WHtR, %FM, and FMI (24, 45).

Cocoa-based products also contain other bioactive compounds like theobromine, a methylxanthine highly associated with body weight, lipid, and glucose metabolism (46). In our study, theobromine was not quantified so their possible association with adiposity parameters has been not determined.

The effect of cocoa-based product intake on body fat and obesity could be explained by an associated reduction in plasma adipokine (leptin and adiponectin) concentrations, although the mechanisms involved still need to be clarified (8, 47). Leptin and adiponectin are hormones mainly secreted by adipose tissue and delivered into the systemic circulation to modify glucose and lipid metabolism, insulin sensitivity, and cardiovascular function (48). Leptin promotes fatty acid oxidation and reduces lipogenesis by regulating peripheral metabolic pathways in skeletal muscle, adipose tissue, the liver, and the pancreas (49). Meanwhile, plasma adiponectin improves insulin sensitivity, activates muscle utilization of glucose, induces muscle and hepatic fatty acid oxidation, and reduces hepatic glucose production (48). In the present study, adipokine levels were not analyzed and their possible relationship with the intake of flavonoids from cocoa-based products was not determined.

The relationship between the consumption of cocoa-based products and adiposity parameters has been attributed to their flavanol (flavan-3-ol) content. Flavanols from cocoa include mainly monomers and polymers of catechin and epicatechin (7, 9, 10, 43). In our exploratory analysis, we observed a negative correlation between catechins, epicatechins, proanthocyanidins (polymers of flavanols), and WHtR. Catechins and epicatechins, both flavanols monomers, are rapidly absorbed from the upper portion of the small intestine and could influence metabolic pathways related to body weight (50, 51). Gutiérrez-Salmeán et al. suggested that epicatechin decreases the expression of proteins associated with mitochondrial function and increases the expression of protein-induced thermogenesis (51). Instead, although proanthocyanidins are the most abundant (poly)phenols in cocoa-based products, they are poorly absorbed in the small intestine due to their large number of hydrophilic hydroxyl groups (9, 50). Most proanthocyanidins reach the colon and are transformed by the gut microbiota into phenylvalerolactones and phenolic acids, such as hydroxyphenylpropionic acid, hydroxyphenylacetic acid, and benzoic acid (43, 50, 52, 53). These microbial metabolites might be responsible in part for health beneficial effects of proanthocyanidins and could be implicated in adipogenesis and lipogenesis mechanisms (6). Results from a cross-sectional study, based on 2,734 women twins aged 18–83 years, revealed that women with a higher dietary intake of proanthocyanidins-rich foods, which included apples and cocoa drinks, had lower fat mass and central fat mass, both measured by dual-energy-X-ray-absorptiometry (54). In another way, according to the results shown by Lee et al., 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone, a microbial flavanols metabolite, reduces lipid accumulation in 3T3-L1 mature adipocytes regulating free fatty acids metabolism through the suppression of the expression of lipogenic proteins (6). However, evidence for the effect of flavanols from cocoa-based products and their microbial metabolites on adipogenesis and lipogenesis metabolic pathways is yet inconclusive and further studies are needed to better understand the mechanisms of action implicated in weight maintenance.

Although cocoa-based products are an important source of flavonoids that might contribute to the improvement of adiposity parameters, their consumption should be promoted with caution, considering that most commercial formulations are high in calories, sugars, and fats (7). Thus, from a public health perspective, cocoa-based products low in fats and sugars might be recommended.

Limitations and Strengths

A limitation of the present study is its cross-sectional design, which precludes causal assumptions about flavonoid intake from cocoa-based products and differences in adiposity parameters. In addition, data derived from food frequency questionnaires are prone to bias because misreporting is common in dietary self-assessment in adolescents (55). Misreporting in adolescents is associated with several factors, specifically weight status, weight loss or weight maintenance, body image dissatisfaction, and skipping breakfast (56, 57). Adolescents with high values of BMI tend to report a lower consumption of food rich in energy, fats, and sugars, like cocoa-based products. Misreporting may reflect socially desirable answers where adolescents with self-image dissatisfaction are more likely to under-report the consumption of high fat/high sugar foods. Another plausible reason could be that under-eating is the result of a dietary regimen to lose or maintain weight, so there could be a control in the intake of cocoa-based products. Instead, adolescents with normal weight status could real over-eating to reflect higher intakes due to a growth spurt. Regarding the dietary flavonoids assessment, although our validated food frequency questionnaire specifies portion size, measurement error will be present with any assessment of the flavonoid content of cocoa-based commercial products because they depend on the manufacturing process like alkalinization treatment (58). Furthermore, a limitation of using a food frequency questionnaire is that there is no possible

way to determine the exact content of flavonoids from specific cocoa-based products since the percentage of cacao varies for each commercial product. In addition to this, flavonoid intake was estimated through a database, which may not reflect the true concentration of compounds reaching the target organs after digestion, absorption, and metabolism. Therefore, the association between flavonoids from cocoa-based products and adiposity parameters might be distorted by the dietary data bias, so these results should be interpreted with caution. Further longitudinal analyses will be necessary to clarify the true direction of these associations.

Strengths of the present study include the large sample size ($n = 944$) of well-characterized participants, the standardization of measures performed in the SI! Program for Secondary Schools trial, and the inclusion of a range of anthropometric variables, not only BMI, to evaluate adiposity.

In conclusion, a higher intake of flavonoids from cocoa-based products was associated with lower adiposity parameters and less probability of abdominal obesity. These findings are relevant for hypothesis generation regarding mechanisms underlying potential therapeutic effects of cocoa flavonoids against obesity and should stimulate further prospective studies and clinical trials to determine the health beneficial effects of cocoa flavonoids on adolescents.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because there are restrictions on the availability of the data for the SI! Program study, due to signed consent agreements around data sharing, which only allow access to external researcher for studies following project purposes. Requestor wishing to access the database used in this study can make a request to the Steering Committee (SC) chair. For the present study, the database was requested from the SC on 24 February 2022. Requests to access the datasets should be directed to gsantos@fundacionshe.org, rodrigo.fernandez@cnic.es, juanmiguel.fernandez@cnic.es, restruch@clinic.cat, lamuela@ub.edu, bibanez@cnic.es, and vfuster@cnic.es.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Instituto de Salud Carlos III in Madrid (CEI PI 35_2016), the Fundació Unió Catalana d'Hospitals (CEI 16/41), and the University of Barcelona (IRB00003099). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RL-R: conceptualization. EL-S, AT-R, and RL-R: methodology. EL-S and CA-R: formal analysis. EL-S, CA-R, and RL-R: investigation. AC-G: data curation. EL-S, CA-R, AT-R, and RL-R: writing—original draft preparation. AT-R, RF-J, JF-A, GS-B, MM, PB, AC-G, CR, AR-L, SC-B, RC, RE, and RL-R: writing—review and editing. EL-S: visualization. AT-R and RL-R: supervision. RF-J, JF-A, GS-B, MM, PB, AC-G, JM-G, AT-R, RE, and RL-R: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. *Nat Rev Endocrinol.* (2012) 9:13–27. doi: 10.1038/nrendo.2012.199
2. Cardel MI, Atkinson MA, Taveras EM, Holm J-C, Kelly AS. Obesity treatment among adolescents. *J Am Med Assoc Pediatr.* (2020) 174:609. doi: 10.1001/jamapediatrics.2020.0085
3. Kelly AS, Barlow SE, Rao G, Inge TH, Hayman LL, Steinberger J, et al. Severe obesity in children and adolescents: identification, associated health risks, and treatment approaches. *Circulation.* (2013) 128:1689–712. doi: 10.1161/CIR.0b013e3182a5cfb3
4. Fernandez-Jimenez R, Al-Kazaz M, Jaslow R, Carvajal I, Fuster V. Children present a window of opportunity for promoting health. *J Am Coll Cardiol.* (2018) 72:3310–9. doi: 10.1016/j.jacc.2018.10.031
5. Yasuda A, Natsume M, Sasaki K, Baba S, Nakamura Y, Kanegae M, et al. Cacao procyanidins reduce plasma cholesterol and increase fecal steroid

excretion in rats fed a high-cholesterol diet. *BioFactors.* (2008) 33:211–23. doi: 10.1002/biof.5520330307

6. Lee Y, Yang H, Hur G, Yu J, Park S, Kim JH, et al. 5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone, a metabolite of procyanidins in cacao, suppresses MDI-induced adipogenesis by regulating cell cycle progression through direct inhibition of CDK2/cyclin O. *Food Funct.* (2019) 10:2958–69. doi: 10.1039/C9FO00334G
7. Ali F, Ismail A, Kersten S. Molecular mechanisms underlying the potential antiobesity-related diseases effect of cocoa polyphenols. *Mol Nutr Food Res.* (2014) 58:33–48. doi: 10.1002/mnfr.201300277
8. Ángel García-Merino J, Moreno-Pérez D, de Lucas B, Montalvo-Lominchar MG, Muñoz E, Sánchez L, et al. Chronic flavanol-rich cocoa powder supplementation reduces body fat mass in endurance athletes by modifying the follistatin/myostatin ratio and leptin levels. *Food Funct.* (2020) 11:3441–50. doi: 10.1039/D0FO00246A
9. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr.* (2005) 81:230S−42S. doi: 10.1093/ajcn/81.1.230S
10. Perez M, Lopez-Yerena A, Vallverdú-Queralt A. Traceability, authenticity and sustainability of cocoa and chocolate products: a challenge for the chocolate industry. *Crit Rev Food Sci Nutr.* (2022) 62:475–89. doi: 10.1080/10408398.2020.1819769
11. Lin X, Zhang I, Li A, Manson JAE, Sesso HD, Wang L, et al. Cocoa flavanol intake and biomarkers for cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials. *J Nutr.* (2016) 146:2325–33. doi: 10.3945/jn.116.237644
12. Kord-Varkaneh H, Ghaedi E, Nazary-Vanani A, Mohammadi H, Shab-Bidar S. Does cocoa/dark chocolate supplementation have favorable effect on body weight, body mass index and waist circumference? A systematic review, meta-analysis and dose-response of randomized clinical trials. *Crit Rev Food Sci Nutr.* (2019) 59:2349–62. doi: 10.1080/10408398.2018.1451820
13. González-Sarrías A, Combet E, Pinto P, Mena P, Dall'Asta M, Garcia-Aloy M, et al. A systematic review and meta-analysis of the effects of flavanol-containing tea, cocoa and apple products on body composition and blood lipids: exploring the factors responsible for variability in their efficacy. *Nutrients.* (2017) 9:746. doi: 10.3390/nu9070746
14. Fernandez-Jimenez R, Santos-Beneit G, Tresserra-Rimbau A, Bodega P, de Miguel M, de Cos-Gandoy A, et al. Rationale and design of the school-based SI! Program to face obesity and promote health among Spanish adolescents: a cluster-randomized controlled trial. *Am Heart J.* (2019) 215:27–40. doi: 10.1016/j.ahj.2019.03.014
15. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br J Nutr.* (2010) 103:1808–16. doi: 10.1017/S0007114509993837
16. Juton C, Castro-Barquero S, Casas R, Freitas T, Ruiz-León AM, Crovetto F, et al. Reliability and concurrent and construct validity of a food frequency questionnaire for pregnant women at high risk to develop fetal growth restriction. *Nutrients.* (2021) 13:1629. doi: 10.3390/nu13051629
17. Neveu V, Perez-Jimenez J, Vos F, Crespy V, du Chaffaut L, Mennen L, et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database.* (2010) 2010:bap024. doi: 10.1093/database/bap024
18. Rothwell JA, Perez-Jimenez J, Neveu V, Medina-Remon A, M'Hiri N, Garcia-Lobato P, 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.* (2013) 2013:bat070. doi: 10.1093/database/bat070
19. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* (1997) 65:1220S−8S. doi: 10.1093/ajcn/65.4.1220S
20. Sharma AK, Metzger DL, Daymont C, Hadjiyannakis S, Rodd CJ. LMS tables for waist-circumference and waist-height ratio Z-scores in children aged 5–19 y in NHANES III: association with cardio-metabolic risks. *Pediatr Res.* (2015) 78:723–9. doi: 10.1038/pr.2015.160
21. Flegal KM, Cole TJ. Construction of LMS parameters for the Centers for Disease Control and Prevention 2000 growth charts. *Natl Health Stat Report.* (2013) 11:1–3.
22. Ogden CL, Kuczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, et al. Centers for disease control and prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics Version. *Pediatrics.* (2002) 109:45–60. doi: 10.1542/peds.109.1.45
23. Weber DR, Moore RH, Leonard MB, Zemel BS. Fat and lean BMI reference curves in children and adolescents and their utility in identifying excess adiposity compared with BMI and percentage body fat. *Am J Clin Nutr.* (2013) 98:49–56. doi: 10.3945/ajcn.112.053611
24. McCarthy HD, Ashwell M. A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message – 'keep your waist circumference to less than half your height.' *Int J Obes.* (2006) 30:988–92. doi: 10.1038/sj.ijo.0803226
25. Kromeyer-Hauschild K, Neuhauser H, Schaffrath Rosario A, Schienkiewitz A. Abdominal obesity in German adolescents defined by waist-to-height ratio and its association to elevated blood pressure: the KiGGS study. *Obes Facts.* (2013) 6:165–75. doi: 10.1159/000351066
26. Ogden CL, Li Y, Freedman DS, Borrud LG, Flegal KM. Smoothed percentage body fat percentiles for U.S. children and adolescents, 1999-2004. *Natl Health Stat Rep.* (2011) 43:1–7.
27. Moreiras O, Carvajal H, Cabrera L, Cuadrado C. *Tablas de composición de alimentos*. In: P Madrid, editor. Madrid: Ediciones Pirámide (2019). p. 1–494.
28. Mataix J, García L, Mañas M, Martínez E, Llopis J. *Tabla de composición de alimentos*. Granada: Universidad de Granada (2003).
29. Chandler JL, Brazendale K, Beets MW, Mealing BA. Classification of physical activity intensities using a wrist-worn accelerometer in 8-12-year-old children. *Pediatr Obes.* (2016) 11:120–7. doi: 10.1111/ijpo.12033
30. Barbosa N, Sanchez CE, Vera JA, Perez W, Thalabard J-C, Rieu M, et al. A physical activity questionnaire: reproducibility and validity. *J Sports Sci Med.* (2007) 6:505–18.
31. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med.* (2020) 54:1451–62. doi: 10.1136/bjsports-2020-102955
32. UNESCO Institute for Statistics. *International Standard Classification of Education (ISCED) 2011*. Montreal, QC: UNESCO Institute for Statistics (2012).
33. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child.* (1976) 51:170–9. doi: 10.1136/adc.51.3.170
34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Statist Soc.* (1995) 57:289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x
35. Blom G. *Statistical Estimates and Transformed Beta-Variables*. New York, NY: John Wiley & Sons A/S (1958).
36. Bawaked RA, Schröder H, Barba LR, Cárdenas G, Peña-Quintana L, Rodrigo CP, et al. Dietary flavonoids of Spanish youth: intakes, sources, and association with the mediterranean diet. *PeerJ.* (2017) 2017:e3304. doi: 10.7717/peerj.3304
37. Wisnuwardani RW, de Henauw S, Forsner M, Gottrand F, Huybrechts I, Knaze V, et al. Polyphenol intake and metabolic syndrome risk in European adolescents: the HELENA study. *Eur J Nutr.* (2020) 59:801–12. doi: 10.1007/s00394-019-01946-1
38. Johannot L, Somerset SM. Age-related variations in flavonoid intake and sources in the Australian population. *Public Health Nutr.* (2006) 9:1045–54. doi: 10.1017/PHN2006971
39. Rienks J, Penczynski KJ, Schmitting S, Buyken AE, Nöthlings U. Dietary flavonoids among children and adolescents in the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study: intake, food sources and trends from 1985 until 2016. *Br J Nutr.* (2020) 124:1198–206. doi: 10.1017/S000711452000183X
40. Cuenca-García M, Ruiz JR, Ortega FB, Castillo MJ. Association between chocolate consumption and fatness in European adolescents. *Nutrition.* (2014) 30:236–9. doi: 10.1016/j.nut.2013.07.011
41. Flieh SM, Miguel-Berges ML, González-Gil EM, Gottrand F, Censi L, Widhalm K, et al. The association between portion sizes from high-energy-dense foods and body composition in European adolescents: the HELENA study. *Nutrients.* (2021) 13:954. doi: 10.3390/nu13030954
42. Smith L, Grabovac I, Jackson SE, Veronese N, Shang C, López-Sánchez GF, et al. Chocolate consumption and indicators of adiposity in US adults. *Am J Med.* (2020) 133:1082–7. doi: 10.1016/j.amjmed.2020.01.020

43. Kim J, Kim J, Shim J, Lee CY, Lee KW, Lee HJ. Cocoa phytochemicals: recent advances in molecular mechanisms on health. *Crit Rev Food Sci Nutr.* (2014) 54:1458–72. doi: 10.1080/10408398.2011.641041
44. Shrime MG, Bauer SR, McDonald AC, Chowdhury NH, Coltart CEM, Ding EL. Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies. *J Nutr.* (2011) 141:1982–8. doi: 10.3945/jn.111.145482
45. Dias IBF, Panazzolo DG, Marques MF, Paredes BD, Souza MGC, Manhanini DP, et al. Relationships between emerging cardiovascular risk factors, z-BMI, waist circumference and body adiposity index (BAI) on adolescents. *Clin Endocrinol.* (2013) 79:12195. doi: 10.1111/cen.12195
46. Camps-Bossacoma M, Garcia-Aloy M, Saldana-Ruiz S, Cambras T, González-Domínguez R, Franch À, et al. Role of theobromine in cocoa's metabolic properties in healthy rats. *J Agric Food Chem.* (2019) 67:3605–14. doi: 10.1021/acs.jafc.8b07248
47. Eskandari M, Hooshmand Moghadam B, Bagheri R, Ashtary-Larky D, Eskandari E, Nordvall M, et al. Effects of interval jump rope exercise combined with dark chocolate supplementation on inflammatory adipokine, cytokine concentrations, and body composition in obese adolescent boys. *Nutrients.* (2020) 12:3011. doi: 10.3390/nu12103011
48. Lafontan M, Viguerie N. Role of adipokines in the control of energy metabolism: focus on adiponectin. *Curr Opin Pharmacol.* (2006) 6:580–5. doi: 10.1016/j.coph.2006.08.002
49. Ceddia RB. Direct metabolic regulation in skeletal muscle and fat tissue by leptin: implications for glucose and fatty acids homeostasis. *Int J Obes.* (2005) 29:1175–83. doi: 10.1038/sj.ijo.0803025
50. Ou K, Gu L. Absorption and metabolism of proanthocyanidins. *J Funct Foods.* (2014) 7:43–53. doi: 10.1016/j.jff.2013.08.004
51. Gutiérrez-Salmeán G, Ortiz-Vilchis P, Vacaseydel CM, Garduño-Siciliano L, Chamorro-Cevallos G, Meaney E, et al. Effects of (–)-epicatechin on a diet-induced rat model of cardiometabolic risk factors. *Eur J Pharmacol.* (2014) 728:24–30. doi: 10.1016/j.ejphar.2014.01.053
52. Urpi-Sarda M, Monagas M, Khan N, Llorach R, Lamuela-Raventós RM, Jáuregui O, et al. Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography–tandem mass spectrometry. *J Chromatogr A.* (2009) 1216:7258–67. doi: 10.1016/j.chroma.2009.07.058
53. Déprez S, Brezillon C, Rabot S, Philippe C, Mila I, Lapierre C, et al. Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J Nutr.* (2000) 130:2733–8. doi: 10.1093/jn/130.11.2733
54. Jennings A, MacGregor A, Spector T, Cassidy A. Higher dietary flavonoid intakes are associated with lower objectively measured body composition in women: evidence from discordant monozygotic twins. *Am J Clin Nutr.* (2017) 105:626–34. doi: 10.3945/ajcn.116.144394
55. Moreno LA, Kersting M, de Henauw S, González-Gross M, Sichert-Hellert W, Matthys C, et al. How to measure dietary intake and food habits in adolescence: the European perspective. *Int J Obes.* (2005) 29:S66–77. doi: 10.1038/sj.ijo.0803063
56. Bel-Serrat S, Julián-Almárcegui C, González-Gross M, Mouratidou T, Börnhorst C, Grammatikaki E, et al. Correlates of dietary energy misreporting among European adolescents: the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study. *Br J Nutr.* (2016) 115:1439–52. doi: 10.1017/S0007114516000283
57. Jones L, Ness A, Emmett P. Misreporting of energy intake from food records completed by adolescents: associations with sex, body image, nutrient, and food group intake. *Front Nutr.* (2021) 8:970. doi: 10.3389/fnut.2021.749007
58. Andres-Lacueva C, Monagas M, Khan N, Izquierdo-Pulido M, Urpi-Sarda M, Permanyer J, et al. Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. *J Agric Food Chem.* (2008) 56:3111–7. doi: 10.1021/jf0728754

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4.2. Total urinary (poly)phenols and cardiometabolic health in adolescents

4.2.1. Article 4: (Poly)phenols in Urine and Cardiovascular Risk Factors: A Cross-Sectional Analysis Reveals Gender Differences in Spanish Adolescents from the SI! Program

Emily P Laveriano-Santos, Isabella Parilli-Moser, Sonia L Ramírez-Garza, Anna Tresserra-Rimbau, Carolina E Storniolo, Ana María Ruiz-León, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Vanesa Carral, Gloria Santos-Beneit, Juan M Fernández-Alvira, Rodrigo Fernández-Jiménez, Valentín Fuster, Rosa M Lamuela-Raventós.

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Abstract

Introduction
Epidemiological studies have shown an inverse association between (poly)phenol intake and cardiovascular risk factors (CVRFs) in adults, but few have provided information about adolescents.

Objective
To evaluate the relationship between urinary total (poly)phenol excretion (TPE) and CVRFs in adolescents.

Methods
A cross-sectional study was performed in 1194 Spanish adolescents from the SI! (*Salud Integral*) program. TPE in urine samples was determined by the Folin-Ciocalteu method, after solid-phase extraction, and categorized into quartiles. The association between TPE and CVRFs was estimated using mixed-effect linear regression and a structural equation model (SEM).

Results
Linear regression showed negative associations among the highest quartile of TPE and body fat percentage (B = -1.75, *p*-value = <0.001), triglycerides (TG) (B = -17.68, *p*-value = <0.001), total cholesterol (TC) (B = -8.66, *p*-value = 0.002), and

low-density lipoprotein (LDL)-cholesterol (LDL-C) (B = -4.09, *p*-value = 0.008) in boys, after adjusting for all confounder variables. Negative associations between TPE quartiles and systolic blood pressure (SBP), diastolic blood pressure (DBP), and TC were also found in girls. Moreover, a structural equation model revealed that TPE was directly associated with body composition and blood glucose and indirectly associated with blood pressure, TG, LDL-C, and high-density lipoprotein-cholesterol (HDL-C) in boys.

Conclusion

In summary, higher concentrations of TPE were associated with a better profile of cardiovascular health, especially in boys, while in girls, the association was not as strong.

antioxidants

MDPI

Article

Polyphenols in Urine and Cardiovascular Risk Factors: A Cross-Sectional Analysis Reveals Gender Differences in Spanish Adolescents from the SI! Program

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Abstract: (1) Background: Epidemiological studies have shown an inverse association between polyphenol intake and cardiovascular risk factors (CVRFs) in adults, but few have provided information about adolescents. The aim of this study was to evaluate the relationship between urinary total polyphenol excretion (TPE) and CVRFs in adolescents. (2) Methods: A cross-sectional study was performed in 1194 Spanish adolescents from the SI! (*Salud Integral*) program. TPE in urine samples was determined by the Folin–Ciocalteu method, after solid-phase extraction, and categorized into quartiles. The association between TPE and CVRFs was estimated using mixed-effect linear regression and a structural equation model (SEM). (3) Results: Linear regression showed negative associations among the highest quartile of TPE and body fat percentage (B = −1.75, *p*-value = <0.001), triglycerides (TG) (B = −17.68, *p*-value = <0.001), total cholesterol (TC) (B = −8.66, *p*-value = 0.002), and low-density lipoprotein (LDL)-cholesterol (LDL-C) (B = −4.09, *p*-value = 0.008) in boys, after adjusting for all confounder variables. Negative associations between TPE quartiles and systolic blood pressure (SBP), diastolic blood pressure (DBP), and TC were also found in girls. Moreover, a structural equation model revealed that TPE was directly associated with body composition and blood glucose and indirectly associated with blood pressure, TG, LDL-C, and high-density lipoprotein-cholesterol (HDL-C) in boys.

(4) Conclusions: Higher concentrations of TPE were associated with a better profile of cardiovascular health, especially in boys, while in girls, the association was not as strong.

Keywords: antioxidants; pediatric; body composition; cardiovascular; lipid profile; Folin–Ciocalteu

1. Introduction

Although the clinical burden of cardiovascular disease (CVD) mainly occurs in adulthood, the process of developing CVD begins early in life and progresses throughout the lifespan. The principal cardiovascular risk factors (CVRFs) are obesity, diabetes mellitus, and hypertension, which are normally related to modifiable lifestyle factors, such as an unhealthy diet, physical inactivity, smoking, and excessive alcohol intake [1]. The high prevalence of obesity in the current adolescent population is associated with unhealthy habits, such as an inadequate diet and physical inactivity. This is of particular concern because the excess weight in childhood and adolescence is directly associated with hypertension, an adverse lipid profile, type II diabetes, and early atherosclerotic lesions, which can increase the risk of developing CVD during adulthood [2].

According to preliminary studies, the Mediterranean diet plays an important role in the prevention of CVRFs, such as diabetes, obesity, and hypertension [3–6]. Most evidence for the positive cardiovascular health effects of the Mediterranean diet indicates that bioactive compounds, including polyphenols, are, in part, responsible [3]. The intake of polyphenol-rich foods, such as vegetables, fruits, olive oil, and seeds, has been inversely associated with cardiovascular risk factors in elderly populations [6,7]. The beneficial health effects of polyphenols depend on intake and bioavailability, which varies from one molecule to another, and among individuals [8].

Most epidemiological studies have determined polyphenol intake using traditional dietary assessment tools, such as food frequency questionnaires or 24-h diet recall. A less biased and potentially more accurate approach is the determination of urinary polyphenols by the Folin–Ciocalteu assay, which can serve as a biomarker of total polyphenol intake and fruit and vegetable consumption [9,10].

The relationship of polyphenol biomarkers with CVD or CVRFs has been observed in several studies, suggesting that it is of great importance to maintain polyphenol biomarkers at high levels [11]. Lower all-cause and CVD mortality risks have been observed at higher total urinary polyphenol levels, especially enterolignans concentrations (enterolactone). In addition, urine excretion of enterolignans has been inversely associated with C-reactive protein (CRP) levels and metabolic syndrome components, such as type 2 diabetes (T2D) and obesity. Metabolites from flavanones (naringenin and hesperetin), flavonols (quercetin and isorhamnetin), phenolic acids (caffeic acid), and enterolignans (enterolactone) in spot urine samples have been significantly associated with a lower T2D risk [12]. Moreover, levels of urinary polyphenol biomarkers (caffeic acid, ferulic acid, 3-hydroxybenzoic acid), and especially gut microbial metabolites of polyphenols, have been inversely associated with overweight and obesity. This negative association has been more pronounced in the participants with higher CRP levels, a marker of chronic inflammation and a predictor of all-cause cardiovascular mortality [13].

The aim of the present study was to determine the association between total polyphenol excretion (TPE) in urine, as a biomarker of total polyphenol intake, and CVRFs in adolescents from the SI! (*Salud Integral*) program.

2. Materials and Methods

2.1. Study Design and Participants

The SI! (*Salud Integral*) program for secondary schools is a well-established cluster-randomized controlled intervention trial, registered at ClinicalTrials.gov (NCT03504059), aimed at evaluating the effectiveness of an educational intervention to promote cardiovascular health in 1326 adolescents

from 24 secondary public schools in Madrid and Barcelona, Spain. Schools were randomized 1:1:1 to receive a short-term (2-year) or a long-term (4-year) comprehensive educational program or the usual curriculum (control). The primary outcome was a change in obesity and other cardiovascular health parameters. The full details of the design of the SI! Program for Secondary Schools intervention have been published elsewhere [14].

The study protocol and procedures were approved by the Ethical Committee of *the Instituto de Salud Carlos III* in Madrid (CEI PI 35_2016), the *Fundació Unió Catalana d'Hospitals* (CEI 16/41), and the University of Barcelona (IRB00003099). Written informed consent was obtained from the parents or the legal guardians of all the participants.

The inclusion criteria were availability of spot urine samples, no diagnosis of diabetes or hypertension, and not having taken any drugs or supplements the day prior to the data collection at baseline. Based on these criteria, 1194 subjects were included in the analysis (Figure 1).

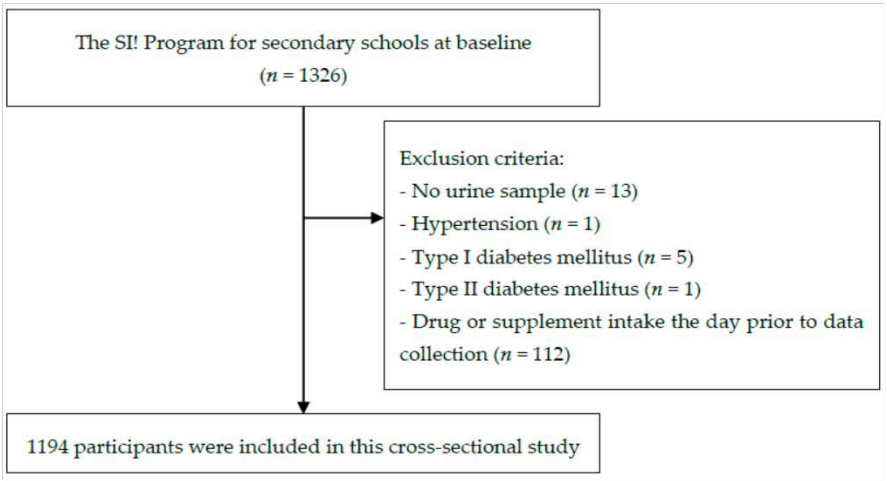

Figure 1. Flow chart of participant selection.

2.2. Anthropometry and Body Composition

Weight was measured to the nearest 0.1 kg using a digital scale (OMRON BF511, OMRON HEALTHCARE Co., Muko, Kyoto, Japan), and height to the nearest 0.1 cm with a portable stadiometer (SECA 213 of 0.1 cm precision), with participants wearing light clothes and no shoes. The body mass index (BMI) was calculated as weight in kilograms divided by square height in meters (kg/m^2). The body fat percentage was estimated by bioelectrical impedance using a tetrapolar OMRON BF511. Waist circumference (WC) was measured in triplicate to the nearest 0.1 cm using a non-stretchable Holtain tape [14]. The waist-to-height ratio (WtHR) was calculated as WC (cm) divided by height (cm). Measurements were taken early in the morning after overnight fasting. BMI and WC z-scores were calculated according to the age- and gender-specific median of the International Obesity Group (IOTF) and the National Health and Nutrition Examination Survey (NHANES), respectively [15,16].

2.3. Blood Pressure

Blood pressure (BP) was measured in the sitting position using a digital monitor OMRON M6 (OMRON HEALTHCARE Co., Muko, Kyoto, Japan). Duplicate measurements were taken at two- or three-minute intervals after the participants relaxed. If these differed by more than 10 mmHg for systolic blood pressure (SBP) and/or more than 5 mmHg for diastolic blood pressure (DBP), a third measurement was taken. Average values were calculated for the final SBP and DBP [14].

SBP and DBP z-scores were calculated according to the High Blood Pressure Working Group of the National Blood Pressure Education Program for children and adolescents [17].

2.4. Blood Analyte Measurements

Glucose and lipid profile (total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglycerides (TG)) levels were analyzed using a portable CardioCheck® Plus (PTS Diagnostic, Indianapolis, IN, USA) biochemical analyzer in finger-prick capillary whole blood samples (approximately 40 µL) taken early in the morning after overnight fasting. The coefficients of variation (CV) of TC, HDL-C, and glucose were 4.9%, 8.7%, and 4.0%, respectively [18].

2.5. TPE Determination in Urine

Fasting spot urine samples were collected in the morning and were immediately stored at −80 °C until analysis [14]. The validated Folin-Ciocalteu (F-C) spectrophotometric method used to determine TPE concentrations in urine has been previously described by Medina-Remón et al. This method includes a previous solid-phase extraction using 96-well plate hydrophilic-lipophilic-balanced cartridges water-wettable and reversed-phase solvent (Oasis® MAX 30 mg, Waters, Milford, MA, USA) to remove interferences with the F-C reagent (Sigma-Aldrich, St. Louis, MO, USA). Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) was used for curve calibration. The method has a percent relative standard deviation (precision) below 7.2% in each concentration from curve calibration. Recovery values were between 82.5 and 105.7%. The limit of detection (LOD) for gallic acid equivalent (GAE) was 0.07 mg/L [9]. Creatinine in urine samples was measured following the adapted Jaffé alkaline picrate method for thermo microtiter 96-well plates, according to Medina-Remón et al. [9]. In epidemiological studies and in the absence of disease, creatinine concentrations in urine can be used to determine urinary excretion of compounds in spot urine samples [9,19,20]. We considered values of mg of GAE and creatinine if the CV between measures were less than 15%. Finally, TPE was expressed as mg GAE/g creatinine.

2.6. Covariate Assessment

Information about dietary intake was assessed using an updated version of the validated 157-items semi-quantitative food frequency questionnaire (FFQ) [21]. From standard portions and frequencies of consumption, all items were calculated and reported in g per day (g/d) using Spanish food composition tables [22,23]. Dietary intake includes total energy, protein, carbohydrates, fiber, total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Food groups intake includes vegetables, fruits, legumes, cereals, dairy products, meat, olive oil, fish, nut, cookies, pastries, and sweets, chocolate, and soft drinks. Nutrient and food groups' intake was adjusted for total energy intake using the residual method [24].

A self-completed questionnaire for parents or legal guardians was used to assess sociodemographic parameters (place of birth, education level, and house income) [14]. The place of birth was categorized according to the area in which the participants were born, like Spain, the rest of Europe, Latin America, Africa, and others. The parental education level was categorized as low (families without studies or with primary studies), medium (secondary studies and professional training), and high (university studies), according to the International Standard Classification of Education (ISCED) [25]. As in the study by Bodega et al. [26], the highest parental education level was considered as a covariate in this study. House income was categorized into three levels (low, medium, and high) based on the annual survey of salary for the Spanish population [27].

Physical activity was measured with a triaxial Actigraph wGT3X-BT accelerometer (ActiGraph Corporation, Pensacola, FL, USA) on the non-dominant wrist for 7 consecutive days (except when bathing or swimming) [14]. The cut-off points of Chandler (2016) were applied to estimate total activity and minutes spent in moderate-to-vigorous physical activity [28].

Puberty development was assessed using pictograms, according to Tanner maturation stages [29].

2.7. Statistical Analysis

The baseline characteristics of participants were presented as means and standard deviations (SD) for continuous variables and frequencies and percentages for categorical variables. To assess the relation between TPE and CVRFs, participants were categorized into quartiles of TPE (mg GAE/g creatinine): Q1 (<71.8), Q2 (71.9–111.1), Q3 (111.2–161.2), and Q4 (>161.2). Continuous variables were used to compare the unadjusted sample means across TPE quartiles by one-way analysis of variance (ANOVA). Chi-square test analysis was used to assess qualitative variables across TPE quartiles.

Mixed-effect linear regression models were considered to evaluate associations between TPE (continuous and quartiles) and CVRFs (body composition, BP, glucose, TG, TC, HDL-C, and LDL-C), adjusted for factors previously related to TPE and CVRFs. The first model was unadjusted. In the second model, TPE was adjusted for gender (only for total participants), age (continuous), fasting (yes/no), moderate-to-vigorous physical activity (quartiles), Tanner stage (from I to V), a high parental education level (yes/no), place of birth (Spain, Rest of Europe, Latin America, Africa, others), and household income (low, medium, and high). The third model was additionally adjusted for energy intake (quartiles), fiber (quartiles of energy-adjusted intake), total fat (quartiles of energy-adjusted intake), MUFAs (quartiles of energy-adjusted intake), PUFAs (quartiles of energy-adjusted intake). The BMI and WtHR were also considered in the third model regression between TPE and blood analytes (glucose, TG, TC, HDL-C, and LDL-C). Municipalities (Barcelona/Madrid) were included as a random effect. To accommodate the use of some categorical variables, we estimated parameters using weighted least squares with robust standard errors. We evaluated potential effect modification in the association between quartiles of TPE and CVRFs by gender in an interaction analysis using the cross-product term between TPE and gender. This analysis was also stratified by gender to evaluate potential modification. Linear trends between TPE and mean of each CVRF were assessed using orthogonal polynomial contrasts.

Based on linear regression models, the data was re-analyzed using structural equation modeling (SEM) with robust maximum likelihood estimation to examine the relationship between TPE (continuous) and CVRFs (continuous). Our hypothesized model of study is shown in Figure 2. The main dependent variables were TG, TC, LDL-C as observed variables; body composition (included the WC z-score, the BMI z-score, and body fat percentage) and blood pressure (BP) (included the SBP z-score and the DBP z-score) as latent variables that are not measured directly. TPE was considered an independent variable. Other variables observed were considered as covariates: age, gender, physical activity, fasting, Tanner scale, energy intake, fat intake, MUFAs, PUFAs, fiber, high parental education, place of birth, and household income. The goodness-of-fit index model for SEM included the standardized root mean square residual (SRMR < 0.08) [30].

All statistical analyses were conducted using the Stata statistical software package version 16.0 (StataCorp, College Station, TX, USA). Statistical tests were two-sided, and p-values below 0.05 were considered significant.

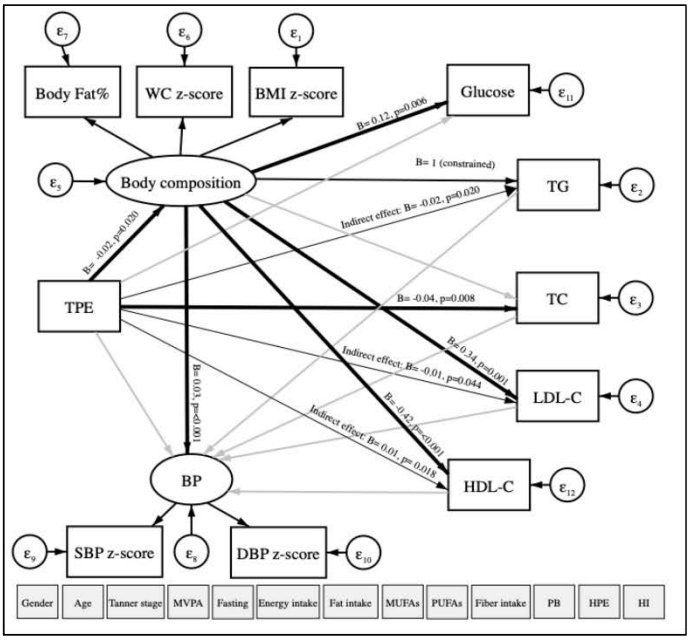

Figure 2. Path diagram of the association between TPE and CVRFs using structural equation modeling. TPE: Total polyphenol excretion in urine. CVRFs: Cardiovascular risk factors. WC: Waist circumference. BMI: Body mass index. BP: Blood pressure. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. TG: Triglycerides. TC: Total cholesterol. LDL-C: Low-density lipoprotein-cholesterol. HDL-C: High-density lipoprotein-cholesterol. MVPA: Moderate-to-vigorous physical activity. MUFAs: Monounsaturated fatty acids. PUFAs: Polyunsaturated fatty acids. PB: Place of birth. HPE: High parental education. HI: Household income. ε: error. Oval circles indicate a latent variable that is not measured directly. Age, gender, MVPA, fasting, Tanner scale, energy intake, fat intake, MUFAs, PUFAs, fiber intake, PB, HPE, and HI were considered as covariates. Significant paths ($p < 0.05$) are shown as black arrows, and non-significant paths are shown as grey arrows. Direct associations are presented with wider arrow widths than the indirect associations. Unstandardized regression coefficients are at the end of each arrow.

3. Results

3.1. General Characteristics

Table 1 summarizes the baseline gender-stratified characteristics of the 1194 participants (90% of the cohort) who were included in this cross-sectional study. Nearly half (48%) were girls with a mean age of 12.0 ± 0.5 years. The mean TPE was 125.7 ± 76.8 mg GAE/g creatinine, and the median 111.2 mg GAE/g creatinine with a CV of 61%, the minimum value was 5.1 mg GAE/g creatinine and the maximum 534.3 mg GAE/g creatinine. For the concentration of creatinine, the mean was 1.27 ± 0.49 g/L, with a CV of 39%. No significant differences were found in the TPE, according to gender. Regarding the CVRFs, significantly higher mean values of the BMI z-score, WC, the WC-z score, WtHR, SBP, and glucose were observed in boys compared to girls. Boys were also more sedentary, although they walked more than girls. On the other hand, significantly higher values of body fat percentage, DBP, the DBP-z score, TG, and TC were reported in girls. We observed gender-related differences in dietary food intake: boys showed a trend of having higher intakes of energy and less fiber intake.

Table 1. Baseline characteristics of the study population, according to gender (n = 1194).

Variable	Total	Girls n = 569 (48%)	Boys n = 625 (52%)	p-Value
Age (y), mean (SD)	12.04 (0.46)	11.99 (0.41)	12.08 (0.49)	**0.002**
Body composition, mean (SD)				
BMI (kg/m^2)	20.16 (3.78)	20.04 (3.71)	20.26 (3.83)	0.303
BMI z-score	0.65 (1.06)	0.54 (1.03)	0.74 (1.08)	**<0.001**
WC (cm)	71.87 (10.24)	70.47 (8.99)	73.14 (11.19)	**<0.001**
WC z-score	0.39 (0.86)	0.29 (0.71)	0.47 (0.91)	**<0.001**
WtHR	0.46 (0.06)	0.45 (0.06)	0.47 (0.07)	**<0.001**
Body fat (%)	22.93 (8.31)	24.45 (7.93)	21.52 (8.41)	**<0.001**
Blood pressure, mean (SD)				
SBP (mmHg)	104.59 (10.71)	103.47 (10.19)	105.60 (11.07)	**<0.001**
SBP z-score	−0.30 (0.95)	−0.35 (0.94)	−0.26 (0.96)	0.099
DBP (mmHg)	61.71 (8.74)	62.68 (8.58)	60.83 (8.81)	**<0.001**
DBP z-score	−0.12 (0.76)	−0.07 (0.78)	−0.17 (0.75)	**0.019**
Blood analytes, mean (SD)				
Glucose (mg/dL)	102.89 (11.76)	101.76 (11.70)	103.91 (11.72)	**0.002**
TG (mg/dL)	76.55 (41.07)	80.11 (37.79)	73.35 (43.59)	**0.005**
TC (mg/dL)	152.63 (34.46)	154.39 (32.54)	151.05 (36.04)	0.099
LDL-C (mg/dL)	77.96 (25.78)	78.41 (24.74)	77.47 (26.86)	0.585
HDL-C (mg/dL)	62.85 (15.89)	62.78 (14.26)	62.91 (17.24)	0.891
Physical activity, mean (SD)				
Sedentary (min/day)	605.89 (68.52)	596.78 (65.53)	614.51 (70.21)	**<0.001**
MVPA (min/day)	74.61 (23.41)	77.17 (22.89)	72.19 (23.66)	**<0.001**
Step counts per day	12,104.48 (2419.28)	11,711.49 (2175.84)	12,476.33 (2576.14)	**<0.001**
Tanner scale, mean (SD)	3.16 (0.85)	3.16 (0.84)	3.17 (0.85)	0.745
Dietary intake, mean (SD)				
Total energy (kcal/d)	2435.27 (546.59)	2343.71 (512.62)	2518.21 (563.47)	**<0.001**
Protein (g/d)	116.46 (16.65)	115.61 (15.98)	117.22 (17.22)	0.158
Carbohydrates (g/d)	243.83 (35.48)	244.13 (35.23)	243.55 (35.74)	0.812
Fiber (g/d)	27.91 (7.01)	28.62 (6.95)	27.27 (7.00)	**0.005**
Total fat (g/d)	110.31 (14.13)	110.52 (13.79)	110.11 (14.45)	0.672
SFAs (g/d)	35.89 (5.67)	35.98 (5.45)	35.80 (5.86)	0.642
MUFAs (g/d)	49.96 (8.98)	49.79 (9.66)	50.11 (8.33)	0.613
PUFAs (g/d)	18.70 (3.99)	18.66 (4.12)	18.74 (3.88)	0.780

Table 1. *Cont.*

Variable	Total	Girls $n = 569$ (48%)	Boys $n = 625$ (52%)	*p*-Value
Creatinine (g/L)	1.27 (0.49)	1.35 (0.51)	1.19 (0.45)	**<0.001**
TPE (mg GAE/g creatinine), mean (SD)	125.72 (76.84)	122.41 (74.59)	128.73 (78.78)	0.156
Place of birth, *n* (%)				0.800
Spain	1082 (90.62)	520 (91.39)	562 (89.92)	
Rest of Europe	15 (1.26)	7 (1.23)	8 (1.28)	
Latin America	58 (4.86)	26 (4.57)	32 (5.12)	
Africa	10 (0.84)	3 (0.53)	7 (1.12)	
Other	29 (2.43)	13 (2.28)	16 (2.56)	
Education of mother, *n* (%)				0.113
Low	282 (26.09)	152 (28.95)	130 (23.38)	
Medium	429 (39.69)	201 (38.29)	228 (41.01)	
High	370 (34.23)	172 (32.76)	198 (35.61)	
Education of father, *n* (%)				0.958
Low	248 (30.35)	115 (29.95)	133 (30.72)	
Medium	330 (40.39)	155 (40.36)	175 (40.42)	
High	239 (29.25)	114 (29.69)	125 (28.87)	
Household income, *n* (%)				0.748
Low	366 (33.09)	181 (34.09)	185 (32.17)	
Medium	348 (31.46)	167 (31.45)	181 (31.48)	
High	392 (35.44)	183 (34.46)	209 (36.35)	
Municipality, *n* (%)				0.351
Barcelona	845 (70.77)	410 (72.06)	435 (69.60)	
Madrid	349 (29.23)	159 (27.94)	190 (30.40)	

TPE: Total polyphenol excretion in urine. GAE: Gallic acid equivalent. BMI: Body mass index. WC: Waist circumference. WtHR: Waist-to-height ratio. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. TG: Triglycerides. TC: Total cholesterol. LDL-C: Low-density lipoprotein-cholesterol. HDL-C: High-density lipoprotein-cholesterol. MVPA: Moderate-to-vigorous physical activity. SFAs: Saturated fatty acids. MUFAs: Monounsaturated fatty acids. PUFAs: Polyunsaturated fatty acids. Statistical analyses were undertaken using the t-test for continuous variables and the chi-square ($\chi 2$) test for categorical variables. *p*-values refer to differences between girls and boy values < 0.05; values shown in bold are statistically significant.

The general characteristics of the participants, according to urinary TPE quartiles, are shown in Table 2. Significant differences in the mean BMI (*p*-trend = 0.017), the BMI z-score (*p*-trend = 0.022), body fat (*p*-trend = 0.003), glucose (*p*-trend = 0.009), TG (*p*-trend = 0.017), and TC (*p*-trend = 0.002) were observed among the quartiles. Differences in TPE stratified by gender are shown in Table A1. Lower values of body fat (*p*-trend = 0.039), glucose (*p*-trend = 0.006), TG (*p*-trend = 0.011), TC (*p*-trend = 0.012), and LDL-C (*p*-value = 0.045) were observed between quartiles of TPE in adolescent boys. The results indicated that boys with the highest TPE had a better CVRF profile, but this did not apply to girls. Other factors with significant differences among quartiles only in boys were the level of maternal education and place of birth. Significant differences were only found among quartiles regarding municipality in girls.

Table 2. Baseline characteristics of the study population, according to TPE quartiles (mg GAE in urine/g creatinine).

Variable	TPE (mg GAE in Urine/g Creatinine)						
	n	Q1 (<71.8)	Q2 (71.9–111.1)	Q3 (111.2–161.2)	Q4 (>161.2)	*p*-Value	*p*-Trend
Number of participants	1194	299	298	299	298		
Gender (Girls), *n* (%)	569	139 (46.49)	158 (53.02)	141 (47.16)	131 (43.96)	0.154	0.300
Age (y), mean (SD)	1194	12.03 (0.45)	12.09 (0.50)	12.01 (0.43)	12.02 (0.46)	0.123	0.341
Body composition, mean (SD)							
BMI (kg/m^2)	1193	20.44 (4.02)	20.47 (3.86)	19.85 (3.34)	19.86 (3.85)	0.057	**0.017**
BMI z-score	1193	0.72 (1.10)	0.72 (1.00)	0.59 (1.00)	0.55 (1.10)	0.115	**0.022**
WC (cm)	1194	72.49 (10.81)	72.59 (10.46)	70.80 (9.30)	71.60 (10.29)	0.110	0.093
WC z-score	1194	0.43 (0.87)	0.44 (0.84)	0.31 (0.86)	0.36 (0.87)	0.211	0.140
WtHR	1194	0.47 (0.07)	0.47 (0.07)	0.46 (0.06)	0.46 (0.06)	0.195	0.085
Body fat (%)	1182	23.79 (8.57)	23.57 (8.26)	22.25 (8.09)	22.10 (8.22)	**0.019**	**0.003**
Blood pressure, mean (SD)							
SBP (mmHg)	1191	105.35 (10.66)	104.35 (10.92)	104.24 (10.30)	104.40 (10.98)	0.559	0.289
SBP z-score	1191	−0.23 (0.95)	−0.34 (0.97)	−0.32 (0.92)	−0.32 (0.96)	0.436	0.274
DBP (mmHg)	1191	62.34 (9.19)	60.94 (8.56)	61.22 (8.54)	62.35 (8.61)	0.095	0.891
DBP z-score	1191	−0.06 (0.80)	−0.20 (0.75)	−0.16 (0.75)	−0.06 (0.75)	0.057	0.821
Blood analytes, mean (SD)							
Glucose (mg/dL)	1155	104.30 (11.84)	102.60 (11.70)	103.10 (12.45)	101.50 (10.87)	**0.033**	**0.009**
TG (mg/dL)	1154	77.90 (45.90)	80.58 (39.16)	77.21 (41.93)	70.43 (36.10)	**0.023**	**0.017**
TC (mg/dL)	1155	155.10 (33.63)	155.60 (34.06)	153.60 (36.76)	146.20 (32.59)	**0.003**	**0.002**
LDL-C (mg/dL)	893	78.57 (25.31)	79.11 (26.92)	79.26 (26.65)	74.60 (23.85)	0.196	0.131
HDL-C (mg/dL)	1153	63.35 (16.27)	63.18 (15.38)	63.45 (16.71)	61.41 (15.17)	0.367	0.185
Physical activity, mean (SD)							
Sedentary (min/day)	1121	608.49 (72.49)	604.05 (65.41)	609.12 (67.99)	601.94 (68.08)	0.539	0.426
MVPA (min/day)	1121	73.93 (23.88)	74.01 (22.81)	74.15 (23.17)	76.32 (23.80)	0.565	0.243
Step counts per day	1121	12,208.48 (2464.41)	11,977.63 (2375.30)	11,984.75 (2400.11)	12,243.93 (2436.11)	0.407	0.859
Tanner scale, mean (SD)	1188	3.11 (0.85)	3.28 (0.84)	3.17 (0.83)	3.08 (0.85)	**0.003**	0.365
Place of birth, *n* (%)						0.329	0.475
Spain	1082	268 (89.63)	268 (89.93)	275 (91.97)	271 (90.94)		
Rest of Europe	15	5 (1.67)	4 (1.34)	1 (0.33)	5 (1.68)		
Latin America	58	18 (6.02)	16 (5.37)	16 (5.35)	8 (2.68)		
Africa	10	1 (0.33)	4 (1.34)	1 (0.33)	4 (1.34)		
Other	29	7 (2.34)	6 (2.01)	6 (2.01)	10 (3.36)		

Table 2. *Cont.*

Variable	TPE (mg GAE in Urine/g Creatinine)						
	n	Q1 (<71.8)	Q2 (71.9–111.1)	Q3 (111.2–161.2)	Q4 (>161.2)	*p*-Value	*p*-Trend
Education of mother, *n* (%)						0.052	0.285
Low	282	73 (26.55)	78 (28.78)	77 (28.31)	54 (20.53)		
Medium	429	108 (39.27)	95 (35.06)	119 (43.75)	107 (40.68)		
High	370	94 (34.18)	98 (36.16)	76 (27.94)	102 (38.78)		
Education of father, *n* (%)						0.490	0.345
Low	248	58 (28.57)	65 (31.40)	66 (32.20)	59 (29.21)		
Medium	330	91 (44.83)	86 (41.55)	80 (39.02)	73 (36.14)		
High	239	54 (26.60)	56 (27.05)	59 (28.78)	70 (34.65)		
Household income, *n* (%)						0.450	0.057
Low	366	93 (33.82)	103 (36.52)	89 (32.25)	81 (29.67)		
Medium	348	91 (33.09)	87 (30.85)	89 (32.25)	81 (29.67)		
High	392	91 (33.09)	92 (32.62)	98 (35.51)	111 (40.66)		
Municipality, *n* (%)						0.050	**0.006**
Barcelona	845	226 (75.59)	217 (72.82)	205 (68.56)	197 (66.11)		
Madrid	349	73 (24.41)	81 (27.188)	94 (31.44)	101 (33.89)		

TPE: Total polyphenol excretion in urine. GAE: Gallic acid equivalent. Q: Quartile of TPE. BMI: Body mass index (calculated as weight in kilograms divided by height in square meters). WC: Waist circumference. WtHR: Waist-to-height ratio. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. TG: Triglycerides. TC: Total cholesterol. LDL: Low-density lipoprotein-cholesterol. HDL: High-density lipoprotein-cholesterol. MVPA: Moderate-to-vigorous physical activity. SFAs: Saturated fatty acids. MUFAs: Monounsaturated fatty acids. PUFAs: Polyunsaturated fatty acids. Statistical analyses were undertaken using one-way ANOVA for continuous variables and the chi-square ($\chi 2$) test for categorical variables. *p*-value refers to differences between quartiles of TPE. *p*-values < 0.05; values shown in bold are statistically significant.

We summarized dietary nutrients and the main food groups intake, according to quartiles of TPE, in Table 3. We did not observe significant differences between quartile groups except for cookies, pastries, sweets, and snacks, the intake of which tended to decrease inversely with quartiles, and legumes that presented higher values in the first and the third quartile, although no significant trend was appreciated.

Table 3. Mean dietary nutrients and food intake, according to TPE quartiles (mg GAE in urine/g creatinine).

		TPE (mg GAE in Urine/g Creatinine)					
	n	Q1 (<71.8)	Q2 (71.9–111.1)	Q3 (111.2–161.2)	Q4 (>161.2)	*p*-Value	*p*-Trend
Dietary intake, mean (SD)							
Total energy (Kcal/d)	850	2423.92 (546.44)	2400.96 (578.92)	2482.93 (531.79)	2435.43 (527.03)	0.471	0.488
Protein (g/d)	850	115.01 (15.49)	116.31 (16.02)	117.43 (18.18)	117.09 (16.85)	0.455	0.150
Carbohydrates (g/d)	850	248.07 (34.03)	242.18 (36.36)	243.48 (35.43)	241.69 (35.89)	0.235	0.102
Fiber (g/d)	850	28.50 (7.05)	27.06 (6.90)	28.38 (7.05)	27.76 (7.01)	0.127	0.669
Total fat (g/d)	850	109.10 (13.50)	111.05 (14.11)	110.08 (14.55)	110.96 (14.37)	0.450	0.290
SFAs (g/d)	850	35.74 (5.92)	35.90 (5.32)	35.66 (5.43)	36.24 (5.99)	0.730	0.474
MUFAs (g/d)	850	50.11 (9.19)	49.34 (8.94)	50.32 (9.15)	50.09 (8.69)	0.691	0.738
PUFAs (g/d)	850	18.49 (3.65)	18.98 (4.04)	18.50 (4.20)	18.81 (4.06)	0.501	0.694
Food intake, mean (SD)							
Vegetables (g/d)	850	248.48 (124.88)	258.17 (123.87)	241.57 (119.68)	254.44 (127.15)	0.536	0.973
Fruits (g/d)	850	295.26 (162.15)	293.99 (171.00)	299.10 (167.52)	289.67 (153.63)	0.949	0.817
Legumes (g/d)	850	61.38 (31.98)	53.70 (28.25)	62.41 (31.02)	55.96 (31.15)	**0.007**	0.422
Cereals (g/d)	850	123.01 (54.37)	121.05 (55.71)	124.95 (55.80)	117.06 (50.62)	0.484	0.403
Dairy (g/d)	850	390.33 (189.09)	389.29 (192.34)	378.07 (185.58)	392.64 (187.61)	0.863	0.941
Meat (g/d)	850	217.57 (81.89)	215.50 (75.33)	223.94 (75.99)	213.27 (75.49)	0.526	0.852
Olive oil (g/d)	850	15.58 (10.58)	15.86 (12.30)	17.04 (14.14)	16.01 (11.67)	0.638	0.509
Fish (g/d)	850	80.60 (40.17)	79.03 (39.25)	84.97 (45.68)	80.63 (40.66)	0.494	0.634
Nuts (g/d)	850	9.31 (8.99)	9.66 (8.45)	9.67 (8.52)	8.52 (8.62)	0.482	0.379
Cookies, pastries, sweets, and snacks (g/d)	850	83,17 (42.73)	83.60 (41.57)	78.58 (39.93)	76.01 (42.50)	0.174	**0.039**
Chocolate (g/d)	850	6.71 (8.33)	7.32 (6.94)	6.33 (6.43)	7.68 (7.35)	0.230	0.392
Soft drinks (g/d)	850	140.80 (123.35)	133.78 (120.13)	136.91 (142.04)	134.27 (124.75)	0.939	0.675

TPE: Total polyphenol excretion in urine. GAE: Gallic acid equivalent. Q: Quartile of TPE. SFAs: Saturated fatty acids. MUFAs: Monounsaturated fatty acids. PUFAs: Polyunsaturated fatty acids. Statistical analyses were undertaken using one-way ANOVA for continuous variables. *p*-value refers to differences between quartiles of TPE. *p*-values < 0.05; values shown in bold are statistically significant.

3.2. TPE, Body Composition, and BP

The associations among TPE quartiles with body composition and BP are shown in Table 4. After adjustment for age, gender (only for total participants), physical activity, fasting, Tanner scale,

energy intake, fat intake, MUFAs, PUFAs, fiber, high parental education, place of birth, and house income in the third model, the highest quartile of TPE was negatively associated with body fat percentage (B= −1.75, *p*-value < 0.001, 95% confidence interval (CI = −2.16; −1.36)), compared to the lowest quartile of TPE for boys. On the other hand, in girls, the highest quartile of TPE was negatively associated with the SBP z-score and the DBP z-score, compared to the lowest quartile of TPE.

3.3. TPE and Blood Analytes

Table 5 shows the relationship between urinary TPE and blood analysis estimated using linear regression. For all the participants, negative associations were found among the highest quartile of urinary polyphenols and TG (B= −9.31, *p*-value < 0.001, 95% CI= −12.69; −5.15), TC (B = −7.09, *p*-value < 0.001, 95% CI = −9.28; −4.98), and LDL-C (B= −1.98, *p*-value = 0.006, 95% CI = −4.09; −0.11), compared to the lowest quartile of TPE, after full adjustment for potential confounders. In the gender-stratified analysis, a negative association was found only in boys among the highest quartile of TPE and TG, and TC and LDL-C, compared to the lowest quartile in all regression models, with the exception of the second model in TG in which no significant result was found. No association was observed in boys between the highest and lower quartile of TPE and HDL-C in the fully adjusted model. In the case of the girls, a negative association was found between the highest quartile of TPE and TC, after adjusting for all confounder variables. Although the association between TPE quartiles and LDL-C and HDL-C was not significant in girls, an inverse trend was observed in the fully adjusted regression model.

3.4. TPE and CVRFs

Figure 2 illustrates our hypothesized relationship model between TPE and CVRFs, using SEM in a total of 566 participants. The model fit indicated a good fit in which the SRMR was 0.076, after adjustment for age, gender, physical activity, fasting, Tanner scale, energy, total fat, MUFAs, PUFAs, fiber intake, high parental education, place of birth, and house income. As expected, TPE was directly and negatively associated with body composition (B = −0.02, *p*-value = 0.020, 95% CI = −0.03; −0) and TC (B = −0.04, *p*-value = 0.008, 95% CI = −0.08; −0.01). Moreover, indirect and negative associations between TPE and TG (B = −0.02, *p*-value = 0.020, 95% CI = −0.03; −0) and LDL-C (B = −0.01, *p*-value = 0.044, 95% CI = −0.01; −0) were found. TPE was also indirectly and positively associated with HDL-C (B = 0.01, *p*-value = 0.018, 95% CI = 0; 0.01). No association was observed between TPE and BP (SBP z-score and DBP z-score). In addition, we observed that body composition was positively and directly associated with BP, blood glucose, and LDL-C and negatively associated with HDL-C.

In the boys model, a direct and negative relationship was observed among TPE with body composition (B = −0.03, *p*-value = 0.009, 95% CI = −0.05; −0.01) and blood glucose (B = −0.02, *p*-value = 0.022, 95% CI = −0.03; −0) using SEM. Indirect and negative associations were found among TPE with BP (B = −0.001, *p*-value = 0.003, 95% CI = −0; −0), TG (B = −0.03, *p*-value = 0.009, 95% CI = −0.05; −0.01), LDL-C ((B = −0.02, *p*-value = 0.013, 95% CI = −0.03; −0). TPE and HDL-C (B = 0.01, *p*-value = 0.008, 95% CI = 0; 0.02) were indirectly and positively associated. Although there was no association between TPE and CVRFs in girls, direct associations were found between body composition and TG with BP (Figure A1).

Table 4. Association between body composition and BP with quartiles of TPE (mg GAE/g creatinine), according to gender.

		Total (n = 1194)					Girls (n = 569)				Boys (n = 625)			
		n	Q1 vs. Q4	p-Value	p-Trend	p-for Interaction	n	Q1 vs. Q4	p-Value	p-Trend	n	Q1 vs. Q4	p-Value	p-Trend
BMI z-score	Margin Mean		0.69 vs. 0.53					0.59 vs. 0.40				0.78 vs. 0.63		
	B (CI)—Model 1	1193	−0.16 (−0.40; 0.09)	0.204	0.072	0.144	569	−0.19 (−0.93; 0.55)	0.615	0.499	624	−0.15 (−0.29; −0.01)	**0.047**	**0.023**
	B (CI)—Model 2	999	−0.19 (−0.56; 0.19)	0.326	0.331	0.788	483	−0.26 (−0.97; 0.44)	0.466	0.448	516	−0.15 (−0.15; −0.14)	**<0.001**	**<0.001**
	B (CI)—Model 3	736	−0.25 (−0.79; 0.30)	0.379	0.446	0.548	352	−0.33 (−0.99; 0.33)	0.330	0.347	384	−0.17 (−0.50; 0.17)	0.335	0.543
WC z-score	Margin Mean		0.32 vs. 0.30					0.23 vs. 0.24				0.42 vs. 0.34		
	B (CI)—Model 1	1194	−0.03 (−0.30; 0.24)	0.846	0.578	0.297	569	0.01 (−0.64; 0.65)	0.986	0.903	625	−0.07 (−0.08; −0.07)	**<0.001**	**<0.001**
	B (CI)—Model 2	1000	−0.02 (−0.39; 0.35)	0.897	0.854	0.832	483	−0.03 (−0.52; 0.46)	0.910	0.892	517	−0.04 (−0.21; 0.13)	0.655	0.595
	B (CI)—Model 3	737	−0.06 (−0.54; 0.43)	0.812	0.827	0.613	352	−0.07 (−0.46; 0.33)	0.734	0.753	385	−0.03 (−0.50; 0.44)	0.903	0.941
Body fat %	Margin Mean		23.52 vs. 21.92					25.08 vs. 23.58				22.32 vs. 20.67		
	B (CI)—Model 1	1182	−1.59 (−3.61; 0.42)	0.120	**0.045**	**0.001**	566	−1.49 (−6.53; 3.53)	0.559	0.381	616	−1.65 (−2.64; −0.68)	**0.001**	**<0.001**
	B (CI)—Model 2	989	−1.58 (−3.75; 0.59)	0.153	0.143	0.923	480	−1.92 (−6.91; 3.05)	0.448	0.336	509	−1.58 (−2.72; −0.44)	**0.007**	**<0.001**
	B (CI)—Model 3	728	−1.94 (−4.66; 0.78)	0.162	0.213	0.867	349	−2.29 (−6.43;1.84)	0.277	0.226	379	−1.75 (−2.16; −1.36)	**<0.001**	**0.022**
SBP z-score	Margin Mean		−0.12 vs. −0.25					−0.13 vs. −0.24				−0.11 vs. −0.28		
	B (CI)—Model 1	1191	−0.14 (−0.27; −0)	**0.046**	**0.008**	0.465	567	−0.11 (−0.31; 0.09)	0.283	0.078	624	−0.16 (−0.26; −0.07)	**<0.001**	**<0.001**
	B (CI)—Model 2	999	−0.13 (−0.46; 0.20)	0.432	0.431	0.727	482	−0.13 (−0.32; 0.05)	0.156	**0.047**	517	−0.13 (−0.55; 0.30)	0.553	0.647
	B (CI)—Model 3	737	−0.16 (−0.49; 0.16)	0.329	0.434	0.280	352	−0.28 (−0.29; −0.28)	**<0.001**	**<0.001**	385	−0.07 (−0.55; 0.42)	0.780	0.959
DBP z-score	Margin Mean		−0.11 vs. −0.09					−0.04 vs. −0.01				−0.14 vs. −0.15		
	B (CI)—Model 1	1191	0.02 (0; 0.03)	**0.009**	0.136	**<0.001**	567	0.04 (0.02; 0.05)	**<0.001**	0.102	624	−0.01 (−0.02; 0)	0.173	0.708
	B (CI)—Model 2	1004	0.04 (0.01; 0.07)	**0.020**	**0.021**	0.670	482	0.02 (−0.05; 0.08)	**0.585**	0.611	517	0.04 (−0.08; 0.16)	0.511	0.376
	B (CI)—Model 3	737	−0.02 (−0.11; 0.08)	0.836	0.673	0.207	352	−0.07 (−0.12; −0.02)	**0.003**	0.059	385	0.04 (−0.09; 0.18)	0.545	0.361

TPE: Total polyphenol excretion in urine. Q: Quartile of TPE. GAE: Gallic acid equivalent. BP: Blood pressure. BMI: Body mass index. WC: Waist circumference. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. B: Non-standardized coefficient. CI: Confidence interval. Model 1: unadjusted. Model 2: adjusted for gender (only for total participants), age, physical activity, fasting, Tanner stage, high parental education level, house income, and place of birth. Model 3: adjusted as in Model 2 plus energy intake, fiber, total fat, MUFAs, and PUFAs. p-value Q1 vs. Q4 of TPE, and p-trend < 0.05; values shown in bold are statistically significant.

Table 5. Association between blood analytes of cardiovascular health and quartiles of TPE (mg GAE/g creatinine), according to gender.

		Total (n = 1194)					Girls (n = 569)					Boys (n = 625)				
		n	Q1 vs. Q4	p-Value	p-Trend	p-for Interaction	n	Q1 vs. Q4	p-Value	p-Trend	n	Q1 vs. Q4	p-Value	p-Trend
Glucose (mg/dL)	Margin mean		103.05 vs. 100.66					101.76 vs. 99.55				105.09 vs. 101.54		
	B (CI)—Model 1	1155	−2.39 (−5.90; 1.12)	0.182	0.216	**<0.001**	546	−1.21 (−2.14; −0.27)	**0.012**	0.146	609	−2.57 (−9.74; 2.59)	0.256	0.233
	B (CI)—Model 2	1000	−2.66(−7.65, 2.33)	0.297	0.313	0.138	483	−1.54 (−4.31; 1.21)	0.272	0.482	517	−3.35 (−9.92; 3.23)	0.318	0.258
	B (CI)—Model 3	736	−1.76 (−6.24; 2.71)	0.440	0.491	**0.003**	352	2.40 (−0.71; 5.51)	0.130	**0.048**	384	−3.69 (−9.09; 1.71)	0.180	0.058
TG (mg/dL)	Margin mean		77.90 vs. 70.43					78.30 vs. 76.73				77.53 vs. 65.59		
	B (CI)—Model 1	1154	−7.47 (−9.11; −5.83)	**<0.001**	**<0.001**	**<0.001**	546	−1.57 (−5.53; 2.38)	0.435	0.260	608	−11.94 (−18.25; −5.62)	**<0.001**	**<0.001**
	B (CI)—Model 2	999	−7.41 (−16.11; 1.30)	0.096	**0.134**	0.354	483	−2.72 (−8.02; 2.59)	0.315	0.242	516	−10.18 (−23.27; 2.91)	0.127	0.182
	B (CI)—Model 3	735	−9.31 (−12.69; −5.15)	**<0.001**	**0.009**	**0.013**	352	4.40 (−4.81; 13.62)	0.349	0.577	383	−17.68 (−24.38; −10.99)	**<0.001**	**0.009**
TC (mg/dL)	Margin mean		149.29 vs. 142.42					149.05 vs. 145.99				149.61 vs. 139.87		
	B (CI)—Model 1	1155	−6.87 (−7.14; −6.60)	**<0.001**	**<0.001**	**0.048**	546	−3.07 (−3.91; −2.23)	**<0.001**	**0.001**	609	−9.74 (−10.78; −8.70)	**<0.001**	**<0.001**
	B (CI)—Model 2	1000	−6.54 (−8.06; −5.93)	**<0.001**	**<0.001**	**0.008**	483	−3.84 (−4.71; −2.97)	**<0.001**	**<0.001**	517	−8.55 (−9.01; −8.09)	**<0.001**	**<0.001**
	B (CI)—Model 3	736	−7.09 (−9.28; −4.90)	**<0.001**	**<0.001**	**0.017**	352	−3.11 (−4.19; −0.82)	0.060	**<0.001**	384	−8.66 (−14.23; −3.11)	**0.002**	**0.008**
LDL-C (mg/dL)	Margin mean		74.88 vs. 72.29					72.47 vs. 75.09				77.71 vs. 70.22		
	B (CI)—Model 1	893	−2.59 (−2.64; −2.55)	**<0.001**	**<0.001**	0.227	462	2.61 (0.98; 4.24)	**0.002**	**0.018**	431	−7.48 (−8.23; −6.75)	**<0.001**	**<0.001**
	B (CI)—Model 2	776	−2.83 (−5.81; 0.16)	0.063	0.057	**0.032**	408	1.82 (0.23; 3.42)	**0.025**	0.800	368	−7.53 (−11.35; −3.71)	**<0.001**	**0.007**
	B (CI)—Model 3	573	−1.98 (−4.09; −0.11)	**0.006**	**0.028**	0.177	300	−0.17 (−3.70; 4.05)	0.930	0.305	273	−4.09 (−9.75; 1.55)	**0.008**	**0.013**
HDL-C (mg/dL)	Margin mean		61.74 vs. 60.37					62.10 vs. 60.15				61.57 vs. 60.60		
	B (CI)—Model 1	1153	−1.37 (−1.82; −0.93)	**<0.001**	**<0.001**	0.183	546	−1.95 (−2.86; −1.04)	**<0.001**	**<0.001**	607	−0.96 (−1.12; −0.81)	**<0001**	**<0.001**
	B (CI)—Model 2	999	−1.39 (−3.25; 0.47)	0.142	0.249	0.563	483	−1.27 (−2.00; −0.53)	**0.001**	**<0.001**	516	−1.47 (−5.53; 2.59)	0.479	0.453
	B (CI)—Model 3	735	−1.88 (−4.92; 1.16)	0.227	0.272	0.685	352	−1.07 (−2.62; 0.47)	0.174	**<0.001**	383	−2.22 (−9.09; 4.64)	0.525	0.574

TPE: Total polyphenol excretion in urine. Q: Quartile of TPE. GAE: Gallic acid equivalent. TG: Triglycerides. TC: Total cholesterol. LDL-C: Low-density lipoprotein-cholesterol. HDL-C: High-density lipoprotein-cholesterol. B: Non-standardized coefficient. CI: Confidence interval. Model 1: unadjusted. Model 2: adjusted for gender (only for total participants), age, physical activity, fasting, Tanner stage, high parental education level, house income, and place of birth. Model 3: adjusted as in Model 2 plus BMI, WtHR, energy intake, fiber, total fat, MUFAs, and PUFAs. p-value Q1 vs. Q4 of TPE, and p-trend < 0.05; values shown in bold are statistically significant.

4. Discussion

To our knowledge, this is the first study to assess the association between total polyphenols in urine and CVRFs in adolescents. In this baseline cross-sectional study, we observed that a higher concentration of TPE was associated with a better profile of CVRFs, even though we observed different results according to gender.

The inverse association observed between TPE and body composition has been shown in a previous study by our research group in the PREDIMED (Prevención con Dieta Mediterránea) cohort, in which higher TPE in urine was associated with lower values of body weight, BMI, WC, and WtHR in elderly participants at high cardiovascular risk [31]. Although in this study, a direct and negative association between TPE and variables related to body composition (BMI z-score, WC z-score, and body fat percentage) was found in total participants using SEM, these results were gender-dependent, and stratified results showed significant associations only in boys. This result could be partly attributed to dietary intake. However, in the SEM and regression models, energy intake, fiber, total fat, MUFAs, and PUFAs intake were considered as covariates for removing the possible effects of the diet. This finding was consistent with a recent study, which reported that adolescents with a high total polyphenol intake presented lower BMI z-score values, even though it was based on an FFQ, in contrast with our work [32].

No relationship between urinary polyphenols and BP was observed in the total participants. Although, in linear regression models, we observed a negative association between TPE and SBP z-score and DBP z-score in girls, after SEM analysis, we found that body composition mediated this relationship. Besides, we observed indirect and a negative association between TPE and BP in boys after SEM analysis. The present finding was consistent with previous studies. A cross-sectional study found an inverse association between TPE and BP in subjects at high cardiovascular risk [33]. In the PREDIMED study, a diet rich in polyphenols was found to reduce SBP and DBP in adults with hypertension, possibly by stimulating the formation of vasoprotective factors, such as nitric oxide in plasma [6]. However, in a similar trial, Guo et al. only observed this correlation with SBP after five years of the intervention [34]. The difference between the results could be due to the different health conditions of the participants: in our case, young individuals were mainly normotensive, as opposed to a hypertensive elderly population.

A higher TPE in boys was associated with lower TG, TC, and LDL-C values but not HDL-C in the fully adjusted linear regression model. However, in the SEM analysis, we observed an indirect and positive association between TPE and HDL, mediated by body composition. In line with these findings, results from the PREDIMED cohort showed a similar inverse association with TG levels, but not with TC and LDL-C [34]. Previous studies reporting polyphenol intake in adults using questionnaires found that TG values improved as the polyphenol intake increased. A study of Polish adults indicated that TG values were significantly lower among individuals in the highest quartile of polyphenol intake [35,36]. The effects of polyphenols on lipids are not clear due to several factors: the little knowledge of active metabolites, the inter- and intra-individual variability of the intestinal microflora, and the number of subjects included in the studies. Nevertheless, possible mechanisms that could explain the favorable association between polyphenols and the lipid profile have been mentioned, such as a reduction in lipogenesis; an increase in lipolysis; stimulation of fatty acid β-oxidation; inhibition of adipocyte differentiation and growth; inhibition of expression and secretion of pro-inflammatory molecules; a decrease in oxidative stress, and an increase in antioxidant capacity in adipose tissue [37]. Polyphenols can decrease lipid digestion and absorption by reducing the activities of digestive enzymes and lipid emulsification. Green tea catechins, resveratrol, and curcumin have been considered to decrease fat accumulation in adipocytes by activating adenosine monophosphate-activated protein kinase (AMPK) and down-regulating the expression of lipogenic genes. These polyphenols have also been seen to increase lipolysis and stimulate fatty acid β-oxidation by upregulating hormone-sensitive lipase [38].

In boys, SEM analysis showed a direct and negative association between TPE and blood glucose. In a previous clinical trial, a high intake of polyphenol-rich foods for 8 weeks reduced glucose

concentrations in plasma and increased insulin secretion in adults at high cardiovascular risk [39]. Moreover, in the PREDIMED trial, a higher intake of total polyphenols, total flavonoids, stilbenes, and some flavonoid subclasses was inversely and linearly associated with the incidence of type 2 diabetes [40], and in the PREDIMED-Plus trial, high intakes of some polyphenols were inversely associated with the prevalence of type 2 diabetes in adults with metabolic syndrome, particularly in overweight subjects [41].

In this study, we observed gender-related differences between TPE and CVRFs. Although TPE concentrations are similar in boys and girls, inter-individual variability could be present. The heterogeneity in the cardiometabolic response to polyphenols may be influenced by gender, age, health status, and medication, variables that were considered in our regression models [42]. Sex-gender differences could have important implications in the bioavailability, distribution, metabolism, and excretion of phenolic compounds [43]. Sexual dimorphism in the absorption of polyphenols has been explained in animal models, where the expression of uridine 5′-diphospho (UDP)-glucuronosyltransferases (UGTs), the enzyme responsible for the absorption of polyphenols in the small intestine, was higher in males than females, affecting the glucuronidation of polyphenols [44]. In the human liver, some polyphenols, like caffeic acid, tyrosol, genistein, and daidzein, are metabolized by cytochrome P450 (CYPs) [45,46]. It is well known that CYP2B6, CYP2A6, and CYP3A activity is up-regulated by estrogen levels, indicating a higher activity in women than men [45,47]. Finally, gender-difference influences the excretion of polyphenols in the urine. In adults with hypercholesterolemia, men excreted more concentration of 3,5-diOH-benzoic acid, t-coutaric acid, naringenin, 4-hydroxybenzoic acid, and 4-hydroxyphenyl acetic acid than women [48]. Similar results were found in older adults, where men excreted more polyphenols than women [49]. Another important factor of this variability is the composition of gut microbiota; microbiota participates in the metabolism of polyphenols that cannot be absorbed in the small intestine. Microbial phenolic compounds might have a higher impact on human health than their parental polyphenols [50]. Although microbiota contributes to inter-individual variability, in this study, we did not evaluate microbial compounds.

In addition, there are gender differences in adiposity, metabolism, and predisposition for metabolic dysfunction that can be partially driven by sex hormones. Some studies have analyzed the effects of sex hormone levels on plasma lipid levels in children, and differences in HDL-C levels by sex during puberty have been related to the rise of testosterone levels in boys [51]. In addition, animal models have demonstrated that sex and sex hormones influence adipose tissue, gene expression profiles, regulating insulin resistance and lipolysis, as well as inflammatory tone and obesity [52]. Depending on sex, the level of specific sex hormones can improve or worsen metabolic dysfunction. Estrogen generally provides a protective effect in females, while adequate androgen levels in males are important in promoting appropriate adiposity and metabolic status, and an increase of testosterone decreases abdominal obesity and the metabolic risk profile [52]. Indeed, a study that included healthy schoolchildren ranging from 12 to 15 years old showed that sex hormone-binding globulin levels were related to a decrease in HDL-C and apolipoprotein A-I levels during puberty in boys and to a decrease in TG levels during puberty in both sexes [51]. Nevertheless, more mechanistic studies are needed to fully understand sex dimorphism.

A strong point of our study is the use of TPE as a biomarker to determine polyphenol intake, as this is a more reliable and accurate approach than food questionnaires. Moreover, the F-C method with microplates was used, which is a fast and cheap and reliable method to determine TPE [9].

The principal limitation of this study is the cross-sectional analysis design, and causality associations between exposure and outcomes cannot be established. Another limitation is that we did not measure specific phenolic metabolites but only total polyphenol excretion in urine. Moreover, we cannot rule out the possibility of residual confounding of the associations observed. In addition, the limited number of publications evaluating the relationship between polyphenols and cardiovascular health in adolescents did not allow comparison of the results with previous studies in similar populations.

5. Conclusions

In summary, the results of this study suggest an association between TPE and better CVRFs, mainly in male adolescents. However, direct associations between body composition and TG with BP were also found in females.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Baseline characteristics of the study population, according to gender and quartiles of TPE (n = 1194).

	Girls n = 569 (48%)							Boys n = 625 (52%)						
	Total	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**	**Total**	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**
TPE (mg GAE/g creatinine), mean (SD)	122.68 (74.98)	46.33 (16.46)	91.38 (11.15)	134.99 (15.00)	229.00 (68.00)			129.00 (79.00)	47.14 (15.94)	93.39 (11.71)	135.41 (14.09)	232.00 (67.00)		
Age (y), mean (SD)	11.99 (0.41)	11.96 (0.35)	12.03 (0.50)	12.00 (0.41)	11.97 (0.37)	0.475	0.923	12.08 (0.50)	12.09 (0.52)	12.16 (0.51)	12.01 (0.44)	12.06 (0.51)	0.087	0.193
Body composition, mean (SD)														
BMI (kg/m^2)	20.04 (3.71)	20.26 (3.96)	20.44 (3.87)	19.71 (3.07)	19.67 (3.86)	0.186	0.077	20.26 (3.84)	20.59 (4.08)	20.51 (3.86)	19.98 (3.56)	20.01 (3.84)	0.352	0.098
BMI z-score	0.54 (1.00)	0.59 (1.10)	0.65 (0.99)	0.49 (0.94)	0.40 (1.10)	0.195	0.065	0.74 (1.10)	0.83 (1.10)	0.80 (1.10)	0.69 (1.10)	0.67 (1.10)	0.451	0.117
WC (cm)	70.47 (8.99)	70.64 (9.31)	71.27 (9.02)	69.51 (8.12)	70.37 (9.49)	0.404	0.457	73.14 (11.12)	74.09 (11.76)	74.07 (11.73)	71.95 (10.13)	72.57 (10.80)	0.220	0.087
WC z-score	0.29 (0.79)	0.30 (0.83)	0.37 (0.74)	0.22 (0.79)	0.27 (0.81)	0.382	0.419	0.47 (0.91)	0.54 (0.89)	0.52 (0.94)	0.39 (0.92)	0.43 (0.91)	0.446	0.171
WtHR	0.45 (0.06)	0.46 (0.06)	0.46 (0.06)	0.45 (0.05)	0.45 (0.06)	0.305	0.401	0.47 (0.07)	0.48 (0.07)	0.47 (0.07)	0.47 (0.06)	0.47 (0.06)	0.357	0.089
Body fat (%)	24.45 (7.93)	25.08 (8.68)	25.16 (7.70)	23.85 (7.25)	23.58 (8.03)	0.212	0.057	21.53 (8.42)	22.65 (8.34)	21.77 (8.53)	20.81 (8.54)	20.92 (8.20)	0.178	**0.039**
Blood pressure, mean (SD)														
SBP (mmHg)	103.47 (10.19)	104.20 (10.19)	103.03 (10.22)	102.96 (9.86)	103.80 (10.44)	0.691	0.758	105.60 (11.07)	106.37 (10.89)	105.84 (11.50)	105.39 (10.57)	104.87 (11.39)	0.655	0.204
SBP z-score	−0.35 (0.94)	−0.28 (0.94)	−0.40 (0.95)	−0.40 (0.91)	−0.32 (0.94)	0.619	0.720	−0.26 (0.96)	−0.18 (0.96)	−0.29 (0.99)	−0.25 (0.92)	−0.33 (0.98)	0.566	0.236
DBP (mmHg)	62.68 (8.58)	63.15 (9.29)	62.03 (8.37)	62.20 (8.42)	63.20 (8.19)	0.398	0.711	60.83 (8.81)	61.63 (9.08)	59.73 (8.64)	60.35 (8.57)	61.46 (8.85)	0.186	0.975
DBP z-score	−0.07 (0.78)	−0.02 (0.84)	−0.13 (0.73)	−0.12 (0.77)	0.01 (0.74)	0.351	0.721	−0.17 (0.75)	−0.11 (0.77)	−0.29 (0.74)	−0.20 (0.73)	−0.12 (0.75)	0.132	0.915
Blood analytes, mean (SD)														
Glucose (mg/dL)	101.80 (11.70)	102.10 (11.60)	102.00 (11.81)	102.50 (12.30)	100.31 (11.03)	0.479	0.283	103.90 (11.72)	106.30 (11.74)	103.30 (11.58)	103.60 (12.59)	102.40 (10.69) [a]	**0.022**	**0.006**
TG (mg/dL)	80.11 (37.79)	78.31 (34.96)	83.34 (36.09)	83.34 (36.09)	76.73 (43.28)	0.464	0.649	73.35 (43.60)	77.54 (53.77)	77.41 (42.34)	73.71 (45.46)	65.60 (28.67)	0.051	**0.011**
TC (mg/dL)	154.40 (32.55)	155.90 (32.12)	157.80 (31.42)	152.90 (32.36)	149.93 (34.33)	0.202	0.073	151.10 (36.04)	154.30 (34.99)	153.00 (36.82)	154.20 (40.21)	143.30 (31.01) [a]	**0.015**	**0.012**
LDL-C (mg/dL)	78.41 (24.74)	76.54 (24.92)	81.02 (24.61)	77.73 (23.87)	77.57 (25.77)	0.489	0.987	77.47 (26.87)	80.58 (25.65)	76.40 (29.81)	80.89 (29.36)	72.05 (21.88)	**0.047**	0.062
HDL-C (mg/dL)	62.78 (14.27)	63.74 (14.88)	62.70 (13.97)	63.50 (14.96)	61.10 (13.17)	0.446	0.203	62.91 (17.24)	63.02 (17.44)	63.74 (16.90)	63.42 (18.10)	61.66 (16.56)	0.728	0.473

Table A1. *Cont.*

	Girls *n* = 569 (48%)							Boys *n* = 625 (52%)						
	Total	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**	**Total**	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**
Physical activity, mean (SD)														
Sedentary (min/day)	596.77 (65.53)	599.70 (72.69)	596.94 (59.57)	601.10 (62.28)	588.96 (67.80)	0.449	0.274	614.51 (70.21)	616.44 (71.62)	612.51 (71.06)	616.54 (72.31)	612.44 (66.69)	0.926	0.757
MVPA (min/day)	77.17 (22.89)	76.71 (23.76)	76.28 (22.25)	74.38 (22.59)	79.52 (23.13)	0.602	0.340	72.19 (23.66)	71.41 (23.79)	71.31 (23.25)	72.08 (23.58)	73.72 (24.09)	0.803	0.374
Step counts per day	11,711.49 (2175.84)	11,834.03 (2248.25)	11,567.89 (2102.69)	11,699.43 (2257.79)	11,766.61 (2111.09)	0.762	0.934	12,476.33 (2576.14)	12,546.90 (2606.32)	12,464.81 (2588.38)	12,248.44 (2503.41)	12,630.05 (2613.59)	0.615	0.972
Tanner scale, mean (SD)	3.16 (0.83)	3.11 (0.87)	3.23 (0.86)	3.19 (0.84)	3.08 (0.77)	0.364	0.652	3.17 (0.85)	3.13 (0.84)	3.33 (0.82)	3.16 (0.82)	3.10 (0.91)	0.083	0.386
Dietary intake, mean (SD)														
Energy (kcal/day)	2343.71 (512.62)	2392.14 (518.72)	2272.15 (538.42)	2390.47 (480.02)	2340.30 (500.28)	0.259	0.874	2518.21 (563.47)	2450.68 (569.62)	2562.96 (589.97)	2559.84 (561.85)	2507.17 (537.26)	0.406	0.476
Protein (g/d)	115.61 (15.98)	115.75 (14.96)	114.98 (15.92)	116.29 (17.10)	115.59 (16.15)	0.947	0.909	117.22 (17.22)	114.38 (15.95)	117.98 (16.08)	118.37 (19.06)	118.22 (17.35)	0.240	0.095
Carbohydrates (g/d)	244.13 (35.23)	246.67 (35.41)	242.87 (36.40)	241.17 (33.74)	246.20 (35.21)	0.652	0.847	243.55 (35.74)	249.24 (32.94)	241.32 (36.48)	245.41 (36.82)	238.29 (36.17)	0.101	0.052
Fiber (g/d)	28.62 (6.95)	29.52 (6.88)	27.69 (6.92)	29.11 (7.41)	28.41 (6.52)	0.227	0.545	27.27 (7.01)	27.64 (7.10)	26.27 (6.82)	27.76 (6.71)	27.27 (7.34)	0.417	0.898
Lipids (g/d)	110.52 (13.79)	109.37 (13.76)	111.32 (13.81)	111.62 (14.40)	109.56 (13.22)	0.549	0.892	110.11 (14.45)	108.87 (13.34)	110.72 (14.55)	108.80 (14.60)	112.01 (14.60)	0.255	0.210
Saturated fatty acids (g/d)	35.98 (5.45)	35.80 (5.78)	35.72 (5.13)	36.30 (5.17)	36.20 (5.85)	0.838	0.478	35.69 (6.06)	35.69 (5.56)	36.14 (6.91)	35.12 (5.61)	36.26 (6.11)	0.452	0.767
MUFAs (g/d)	49.01 (10.60)	48.89 (9.75)	49.88 (9.83)	50.00 (8.94)	50.40 (10.12)	0.738	0.293	50.11 (8.33)	51.13 (8.61)	48.66 (7.66)	50.59 (9.34)	49.85 (7.47)	0.163	0.577
PUFAs(g/d)	18.66 (4.12)	18.40 (3.68)	19.07 (4.01)	18.55 (4.66)	18.50 (4.15)	0.613	0.898	18.74 (3.88)	18.57 (3.63)	18.86 (4.09)	18.45 (3.81)	19.05 (4.00)	0.630	0.515
Food intake, mean (SD)														
Vegetables (g/d)	250.26 (120.73)	246.59 (118.87)	258.09 (126.01)	250.72 (121.79)	243.25 (115.65)	0.823	0.754	251.28 (126.83)	250.07 (130.22)	258.28 (121.77)	233.96 (117.90)	262.88 (135.03)	0.330	0.788
Fruits (g/d)	295.37 (161.74)	271.86 (156.85)	298.59 (163.70)	313.45 (165.07)	297.19 (160.48)	0.354	0.221	293.63 (165.20)	314.96 (164.59 9	288.20 (180.46)	287.17 (169.33)	284.00 (148.67)	0.460	0.171
Legumes (g/d)	56.79 (29.11)	56.80 (28.24)	54.55 (28.27)	61.87 (30.869	54.55 (29.09)	0.2481	0.966	59.65 (32.17)	65.23 (34.46)	52.64 (28.34)	62.86 (31.29)	57.02 (32.70)	**0.018**	0.276
Cereals (g/d)	122.50 54.70	126.78 (53.62)	123.09 (58.71)	124.12 (54.48)	115.60 (50.60)	0.544	0.196	120.55 (53.68)	119.83 (55.03)	118.47 (51.88)	125.64 (57.10)	118.16 (50.82)	0.701	0.923

Table A1. *Cont.*

	Girls n = 569 (48%)							Boys n = 625 (52%)						
	Total	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**	**Total**	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**
Dairy (g/d)	384.82 (184.41)	409.15 (198.92)	367.40 (177.41)	370.29 (171.69)	397.36 (189.49)	0.288	0.701	390.23 192.24	374.48 (179.75)	416.82 (207.27)	384.54 (196.91)	389.09 (186.89)	0.437	0.884
Meat (g/d)	213.92 (76.92)	206.00 (78.89)	212.15 (78.43)	226.14 (73.97)	212.04 (75.53)	0.321	0.363	220.76 (77.33)	227.31 (83.43)	219.72 (71.42)	222.12 (77.91)	214.21 (75.76)	0.628	0.628
Olive oil (g/d)	16.23 (12.41)	16.11 (10.97)	16.59 (13.03)	17.23 (14.34)	14.85 (10.84)	0.601	0.579	16.01 (12.06)	15.12 (10.27)	14.94 (11.31)	16.88 (14.03)	16.89 (12.24)	0.457	0.149
Fish (g/d)	83.55 (39.78)	88.20 (40.18)	80.89 (37.81)	81.36 (44.27)	84.48 (37.14)	0.535	0.558	79.20 (42.86)	74.20 (39.20)	76.68 (41.05)	87.98 (46.81)	77.74 (43.06)	0.079	0.216
Nuts (g/d)	9.72 8.85	9.50 (10.02)	10.15 (9.03)	9.62 (8.36)	9.49 (7.86)	0.937	0.887	7.80 (9.11)	9.14 (8.08)	9.04 (7.66)	9.70 (8.68)	7.80 (9.11)	0.361	0.337
Cookies, pastries, and sweets (g/d)	77.59 (39.80)	78.90 (39.23)	78.90 (39.23)	79.07 (41.43)	72.94 (35.76)	0.655	0.331	82.88 (43.34)	86.75 (42.58)	89.52 (43.82)	78.18 (38.82)	78.33 (46.96)	0.118	**0.041**
Chocolate (g/d)	7.21 (6.66)	6.78 (6.42)	7.75 (7.00)	6.02 (5.58)	8.15 (7.32)	0.109	0.436	6.85 (7.83)	6.65 (9.68)	6.78 (6.86)	6.60 (7.08)	7.32 (7.37)	0.889	0.573
Soft drinks (g/d)	130.19 (114.19)	141.97 (128.81)	129.12 (119.55)	120.00 (92.55)	129.70 (111.38)	0.620	0.381	142.03 (138.37)	139.82 (119.12)	139.64 (121.23)	150.98 (171.92)	137.72 (134.30)	0.885	0.930
Place of birth, n (%)						0.276	0.731						**0.039**	0.208
Spain	520(91.39)	129 (92.81)	144 (91.14)	126 (89.36)	121 (92.37)			562 (89.92)	139 (86.88)	124 (88.57)	149 (94.30)	150 (89.82)		
Rest of Europe	7 (1.23)	2 (1.44)	3 (1.90)	1 (0.71)	1(0.76)			8 (1.28)	3 (1.88)	1 (0.71)	0 (0)	4 (2.40)		
Latin America	26 (4.57)	6 (4.32)	4 (2.53)	11 (7.80)	5 (3.82)			32 (5.12)	12 (7.50)	12 (8.57)	5 (3.16)	3 (1.80)		
Africa	3 (0.53)	0 (0)	3 (1.90)	0 (0)	0 (0)			7(1.12)	1 (0.62)	1 (0.71)	1 (0.63)	4 (2.40)		
Other	13 (2.28)	2 (1.44)	4 (2.53)	3 (2.13)	4 (3.05)			16 (2.56)	5 (3.12)	2 (1.43)	3 (1.90)	6 (3.59)		
Education of mother, n (%)						0.610	0.422						**0.006**	**0.033**
Low	152 (28.95)	32 (24.43)	41 (28.41)	46 (34.59)	33 (28.21)			130 (23.38)	41 (28.47)	37 (29.13)	31 (22.30)	21 (14.38)		
Medium	201 (39.29)	55 (41.98)	52 (36.11)	50 (37.59)	44 (37.61)			228 (41.01)	53 (36.81)	43 (33.86)	69 (49.64)	63 (43.15)		
High	172 (32.76)	44 (33.59)	51 (35.42)	37 (27.82)	40 (34.19)			198 (35.61)	50 (34.72)	47 (37.01)	39 (28.06)	62 (42.47)		
Education of father, n (%)						0.526	0.216						0.922	0.861
Low	115 (29.95)	27 (28.12)	35 (32.41)	32 (33.33)	21 (25.00)			133 (30.72)	31 (28.97)	30 (30.30)	34 (31.19)	38 (32.20)		
Medium	155 (40.36)	44 (45.83)	44 (40.74)	36 (37.50)	31 (36.90)			175 (40.42)	47 (43.93)	42 (42.42)	44 (40.37)	42 (35.59)		
High	114 (29.69)	25 (26.04)	29 (26.85)	28 (29.17)	32 (38.10)			125 (28.87)	29 (27.10)	27 (27.27)	31 (28.44)	38 (32.20)		
Household income, n (%)						0.998	0.965						0.147	**0.001**
Low	181 (34.09)	44 (33.33)	53 (35.33)	44 (32.84)	41 (34.75)			185 (32.17)	50 (34.25)	50 (37.88)	45 (31.69)	40 (25.81)		
Medium	167 (31.45)	40 (31.01)	48 (32.00)	43 (32.09)	36 (30.51)			181 (31.48)	51 (34.93)	39 (29.55)	46 (32.39)	45 (29.03)		

Table A1. *Cont.*

	Girls n = 569 (48%)							Boys n = 625 (52%)						
	Total	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**	**Total**	**Q1**	**Q2**	**Q3**	**Q4**	***p*-Value**	***p*-Trend**
High	183 (34.46)	46 (35.66)	49 (32.67)	47 (35.07)	41 (34.75)			209 (36.35)	45 (30.82)	43 (32.58)	51 (35.92)	70 (45.16)		
Municipality, *n* (%)						**0.016**	**0.003**						0.639	0.318
Barcelona	410 (72.06)	109 (78.42)	122 (77.22)	94 (66.67)	85 (64.89)			435 (69.60)	117 (73.12)	95 (67.86)	111 (70.25)	112 (67.07)		
Madrid	159 (27.94)	30 (21.58)	36 (22.78)	47 (33.33)	46 (35.11)			190 (30.40)	43 (26.88)	45 (32.14)	47 (29.75)	55 (32.93)		

TPE: Total polyphenol excretion in urine. Q: Quartile of TPE. BMI: Body mass index. WC: Waist circumference. WtHR: Waist-to-height ratio. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. TG: Triglycerides. TC: Total cholesterol. LDL-C: Low-density lipoprotein-cholesterol. HDL-C: High-density lipoprotein-cholesterol. MVPA: Moderate-to-vigorous physical activity. MUFAs: Monounsaturated fatty acids. PUFAs: Polyunsaturated fatty acids. Statistical analyses were undertaken using a one-way ANOVA test for continuous variables and the chi-square ($\chi 2$) test for categorical variables. p-value < 0.05; values shown in bold are statistically significant. [a] Significant difference between the highest and lowest quartile of TPE after Bonferroni correction.

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Appendix B

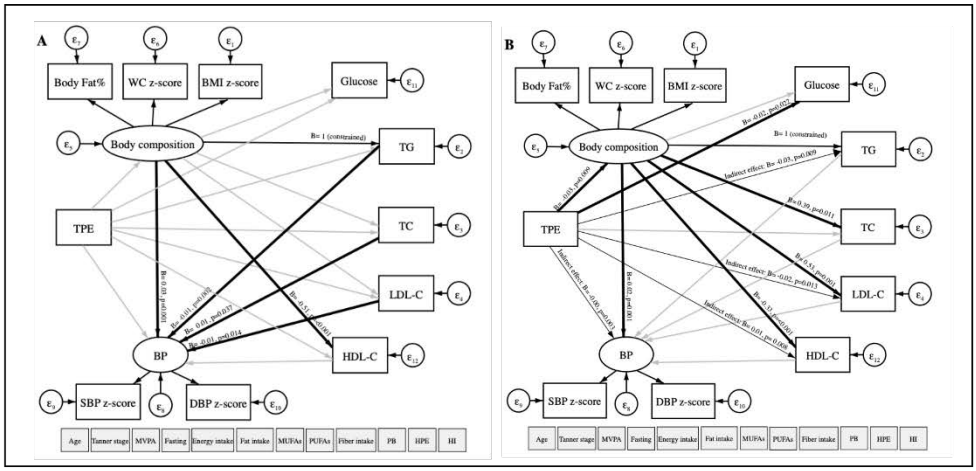

Figure A1. Path diagram of the association between TPE and CVRFs using structural equation modeling in girls (**A**) and boys (**B**). TPE: Total polyphenol excretion in urine. CVRFs: Cardiovascular risk factors. WC: Waist circumference. BMI: Body mass index. BP: Blood pressure. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. TG: Triglycerides. TC: Total cholesterol. LDL-C: Low-density lipoprotein-cholesterol. HDL-C: High-density lipoprotein-cholesterol. MVPA: Moderate-to-vigorous physical activity. MUFAs: Monounsaturated fatty acids. PUFAs: Polyunsaturated fatty acids. PB: Place of birth. HPE: High parental education. HI: Household income. ε: error. Oval circles indicate a latent variable that is not measured directly. Age, gender, MVPA, fasting, Tanner scale, energy intake, fat intake, MUFAs, PUFAs, fiber intake, PB, HPE, and HI were considered as covariates. Significant paths ($p < 0.05$) are shown as black arrows, and non-significant paths are shown as grey arrows. Direct associations are presented with wider arrow widths than indirect associations. Unstandardized regression coefficients are at the end of each arrow.

References

1. Piepoli, M.F.; Hoes, A.W.; Agewall, S.; Albus, C.; Brotons, C.; Catapano, A.L.; Cooney, M.-T.; Corrà, U.; Cosyns, B.; Deaton, C.; et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur. Heart J.* **2016**, *37*, 2315–2381.
2. Fernandez-Jimenez, R.; Al-Kazaz, M.; Jaslow, R.; Carvajal, I.; Fuster, V. Children Present a Window of Opportunity for Promoting Health JACC Review Topic of the Week. *J. Am. Coll. Cardiol.* **2018**, *72*, 3310–3319.
3. Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M.-I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *N. Engl. J. Med.* **2018**, *378*, e34. [CrossRef]
4. Franquesa, M.; Pujol-Busquets, G.; García-Fernández, E.; Rico, L.; Shamirian-Pulido, L.; Aguilar-Martínez, A.; Medina, F.; Serra-Majem, L.; Bach-Faig, A. Mediterranean Diet and Cardiodiabesity: A Systematic Review through Evidence-Based Answers to Key Clinical Questions. *Nutrients* **2019**, *11*, 655. [CrossRef]
5. Schröder, H.; Marrugat, J.; Vila, J.; Covas, M.I.; Elosua, R. Adherence to the Traditional Mediterranean Diet Is Inversely Associated with Body Mass Index and Obesity in a Spanish Population. *J. Nutr.* **2004**, *134*, 3355–3361. [CrossRef]

6. Medina-Remón, A.; Tresserra-Rimbau, A.; Pons, A.; Tur, J.A.; Martorell, M.; Ros, E.; Buil-Cosiales, P.; Sacanella, E.; Covas, M.I.; Corella, D.; et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. *Nutr. Metab. Cardiovasc. Dis.* **2015**, *25*, 60–67. [CrossRef] [PubMed]
7. Tresserra-Rimbau, A.; Rimm, E.B.; Medina-Remón, A.; Martínez-González, M.A.; de la Torre, R.; Corella, D.; Salas-Salvadó, J.; Gómez-Gracia, E.; Lapetra, J.; Arós, F.; et al. Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutr. Metab. Cardiovasc. Dis.* **2014**, *24*, 639–647. [CrossRef] [PubMed]
8. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxid. Redox Signal.* **2013**, *18*, 1818–1892. [CrossRef] [PubMed]
9. Medina-Remón, A.; Barrionuevo-González, A.; Zamora-Ros, R.; Andres-Lacueva, C.; Estruch, R.; Martínez-González, M.-Á.; Diez-Espino, J.; Lamuela-Raventos, R.M. Rapid Folin-Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Anal. Chim. Acta* **2009**, *634*, 54–60. [CrossRef] [PubMed]
10. Medina-Remón, A.; Tresserra-Rimbau, A.; Arranz, S.; Estruch, R.; Lamuela-Raventos, R.M. Polyphenols excreted in urine as biomarkers of total polyphenol intake. *Bioanalysis* **2012**, *4*, 2705–2713. [CrossRef] [PubMed]
11. Rienks, J.; Barbaresko, J.; Nöthlings, U. Association of Polyphenol Biomarkers with Cardiovascular Disease and Mortality Risk: A Systematic Review and Meta-Analysis of Observational Studies. *Nutrients* **2017**, *9*, 415.
12. Guasch-Ferré, M.; Merino, J.; Sun, Q.; Fitó, M.; Salas-Salvadó, J. Dietary Polyphenols, Mediterranean Diet, Prediabetes, and Type 2 Diabetes: A Narrative Review of the Evidence. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 1–16. [CrossRef] [PubMed]
13. Yang, R.; Xiu, H.; Zhou, Q.; Sun, L.; Mu, H.; Li, H.; Wang, S.; Li, X.; Chen, W.; Dong, J. Application of Urinary Polyphenol Biomarkers Measured by Liquid Chromatography Tandem Mass Spectrometry to Assess Polyphenol Intake and Their Association with Overweight and Obesity in Free-Living Healthy Subjects. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 1–11. [CrossRef]
14. Fernandez-Jimenez, R.; Santos-Beneit, G.; Tresserra-Rimbau, A.; Bodega, P.; de Miguel, M.; de Cos-Gandoy, A.; Rodríguez, C.; Carral, V.; Orrit, X.; Haro, D.; et al. Rationale and design of the school-based SI! Program to face obesity and promote health among Spanish adolescents: A cluster-randomized controlled trial. *Am. Heart J.* **2019**, *215*, 27–40. [CrossRef] [PubMed]
15. Cole, T.J. Establishing a standard definition for child overweight and obesity worldwide: International survey. *BMJ* **2000**, *320*, 1240. [CrossRef]
16. Sharma, A.K.; Metzger, D.L.; Daymont, C.; Hadjiyannakis, S.; Rodd, C.J. LMS tables for waist-circumference and waist-height ratio Z-scores in children aged 5–19 y in NHANES III: Association with cardio-metabolic risks. *Pediatr. Res.* **2015**, *78*, 723–729. [CrossRef]
17. Falkner, B.; Daniels, S.R. Summary of the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. *Hypertension* **2004**, *44*, 387–388. [CrossRef]
18. Whitehead, S.J.; Ford, C.; Gama, R. A combined laboratory and field evaluation of the Cholestech LDX and CardioChek PA point-of-care testing lipid and glucose analysers. *Ann. Clin. Biochem.* **2014**, *51*, 54–67. [CrossRef] [PubMed]
19. Zamora-Ros, R.; Rabassa, M.; Cherubini, A.; Urpi-Sarda, M.; Llorach, R.; Bandinelli, S.; Ferrucci, L.; Andres-Lacueva, C. Comparison of 24-h volume and creatinine-corrected total urinary polyphenol as a biomarker of total dietary polyphenols in the Invecchiare InCHIANTI study. *Anal. Chim. Acta* **2011**, *704*, 110–115. [CrossRef]
20. Wilson, T.; Garcia-Perez, I.; Posma, J.M.; Lloyd, A.J.; Chambers, E.S.; Tailliart, K.; Zubair, H.; Beckmann, M.; Mathers, J.C.; Holmes, E.; et al. Spot and Cumulative Urine Samples Are Suitable Replacements for 24-Hour Urine Collections for Objective Measures of Dietary Exposure in Adults Using Metabolite Biomarkers. *J. Nutr.* **2019**, *149*, 1692–1700. [CrossRef]
21. Fernández-Ballart, J.D.; Piñol, J.L.; Zazpe, I.; Corella, D.; Carrasco, P.; Toledo, E.; Perez-Bauer, M.; Martínez-González, M.Á.; Salas-Salvadó, J.; Martín-Moreno, J.M. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br. J. Nutr.* **2010**, *103*, 1808–1816. [CrossRef] [PubMed]

22. Mataix, J. *Tablas de Composición de Alimentos*, 4th ed.; Universidad de Granada: Granada, Spain, 2003.
23. Moreiras, O.; Carbajal, A.; Cabrera, L.; Hoyos, M.D.C.C. *Tablas de Composición de Alimentos: Guía de Prácticas*; Grupo Anaya, S., Ed.; Ediciones Pirámide: Madrid, Spain, 2018.
24. Willett, W.C.; Howe, G.R.; Kushi, L.H. Adjustment for total energy intake in epidemiologic studies. *Am. J. Clin. Nutr.* **1997**, *65*, 1220S–1228S. [CrossRef] [PubMed]
25. UNESCO International Standard Classification of Education. *ISCED 2011*; UNESCO Institute for Statistics: Montreal, QC, Canada, 2012; ISBN 9789291891238.
26. Bodega, P.; Fernández-Alvira, J.; Santos-Beneit, G.; de Cos-Gandoy, A.; Fernández-Jiménez, R.; Moreno, L.; de Miguel, M.; Carral, V.; Orrit, X.; Carvajal, I.; et al. Dietary Patterns and Cardiovascular Risk Factors in Spanish Adolescents: A Cross-Sectional Analysis of the SI! Program for Health Promotion in Secondary Schools. *Nutrients* **2019**, *11*, 2297. [CrossRef] [PubMed]
27. INE: Instituto Nacional de Estadística. Encuesta Anual de Estructura Salarial. Available online: https://www.ine.es/jaxiT3/Datos.htm?t=9948 (accessed on 10 June 2020).
28. Chandler, J.L.; Brazendale, K.; Beets, M.W.; Mealing, B.A. Classification of physical activity intensities using a wrist-worn accelerometer in 8–12-year-old children. *Pediatr. Obes.* **2016**, *11*, 120–127. [CrossRef] [PubMed]
29. Tanner, J.M.; Whitehouse, R.H. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch. Dis. Child.* **1976**, *51*, 170–179. [CrossRef]
30. Yu, C.Y. *Evaluation of Model Fit Indices for Latent Variable Models with Categorical and Continuous Outcomes*; American E: New Orleans, LA, USA, 2002.
31. Guo, X.; Tresserra-Rimbau, A.; Estruch, R.; Martínez-González, M.; Medina-Remón, A.; Fitó, M.; Corella, D.; Salas-Salvadó, J.; Portillo, M.; Moreno, J.; et al. Polyphenol Levels Are Inversely Correlated with Body Weight and Obesity in an Elderly Population after 5 Years of Follow up (The Randomised PREDIMED Study). *Nutrients* **2017**, *9*, 452. [CrossRef]
32. Wisnuwardani, R.W.; De Henauw, S.; Forsner, M.; Gottrand, F.; Huybrechts, I.; Knaze, V.; Kersting, M.; Donne, C.L.; Manios, Y.; Marcos, A.; et al. Polyphenol intake and metabolic syndrome risk in European adolescents: The HELENA study. *Eur. J. Nutr.* **2020**, *59*, 801–812. [CrossRef]
33. Medina-Remón, A.; Zamora-Ros, R.; Rotchés-Ribalta, M.; Andres-Lacueva, C.; Martínez-González, M.A.; Covas, M.I.; Corella, D.; Salas-Salvadó, J.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; et al. Total polyphenol excretion and blood pressure in subjects at high cardiovascular risk. *Nutr. Metab. Cardiovasc. Dis.* **2011**, *21*, 323–331. [CrossRef]
34. Guo, X.; Tresserra-Rimbau, A.; Estruch, R.; Martínez-González, M.A.; Medina-Remón, A.; Castañer, O.; Corella, D.; Salas-Salvadó, J.; Lamuela-Raventós, R.M. 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. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 1–11. [CrossRef]
35. Vitale, M.; Vaccaro, O.; Masulli, M.; Bonora, E.; del Prato, S.; Giorda, C.B.; Nicolucci, A.; Squatrito, S.; Auciello, S.; Babini, A.C.; et al. Polyphenol intake and cardiovascular risk factors in a population with type 2 diabetes: The TOSCA.IT study. *Clin. Nutr.* **2017**, *36*, 1686–1692. [CrossRef]
36. Grosso, G.; Stepaniak, U.; Micek, A.; Stefler, D.; Bobak, M.; Pająk, A. Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study. *Eur. J. Nutr.* **2017**, *56*, 1409–1420. [CrossRef] [PubMed]
37. Cicero, A.F.G.; Colletti, A. Polyphenols Effect on Circulating Lipids and Lipoproteins: From Biochemistry to Clinical Evidence. *Curr. Pharm. Des.* **2018**, *24*, 178–190. [CrossRef] [PubMed]
38. Wang, S.; Moustaid-Moussa, N.; Chen, L.; Mo, H.; Shastri, A.; Su, R. Novel insights of dietary polyphenols and obesity. *J. Nutr. Biochem.* **2014**, *25*, 1–18. [CrossRef] [PubMed]
39. Bozzetto, L.; Annuzzi, G.; Pacini, G.; Costabile, G.; Vetrani, C.; Vitale, M.; Griffo, E.; Giacco, A.; de Natale, C.; Cocozza, S.; et al. Polyphenol-rich diets improve glucose metabolism in people at high cardiometabolic risk: A controlled randomised intervention trial. *Diabetologia* **2015**, *58*, 1551–1560. [CrossRef] [PubMed]
40. Tresserra-Rimbau, A.; Guasch-Ferré, M.; Salas-Salvadó, J.; Toledo, E.; Corella, D.; Castañer, O.; Guo, X.; Gómez-Gracia, E.; Lapetra, J.; Arós, F.; et al. Intake of Total Polyphenols and Some Classes of Polyphenols Is Inversely Associated with Diabetes in Elderly People at High Cardiovascular Disease Risk. *J. Nutr.* **2015**, *146*, 767–777.

41. Tresserra-Rimbau, A.; Castro-Barquero, S.; Vitelli-Storelli, F.; Becerra-Tomas, N.; Vázquez-Ruiz, Z.; Díaz-López, A.; Corella, D.; Castañer, O.; Romaguera, D.; Vioque, J.; et al. Associations between Dietary Polyphenols and Type 2 Diabetes in a Cross-Sectional Analysis of the PREDIMED-Plus Trial: Role of Body Mass Index and Sex. *Antioxidants* **2019**, *8*, 537. [CrossRef]
42. Gibney, E.R.; Milenkovic, D.; Combet, E.; Ruskovska, T.; Greyling, A.; González-Sarrías, A.; de Roos, B.; Tomás-Barberán, F.; Morand, C.; Rodriguez-Mateos, A. Factors influencing the cardiometabolic response to (poly)phenols and phytosterols: A review of the COST Action POSITIVe activities. *Eur. J. Nutr.* **2019**, *58*, 37–47. [CrossRef]
43. Campesi, I.; Romani, A.; Franconi, F. The Sex-Gender Effects in the Road to Tailored Botanicals. *Nutrients* **2019**, *11*, 1637. [CrossRef]
44. Wu, B.; Kulkarni, K.; Basu, S.; Zhang, S.; Hu, M. First-Pass Metabolism via UDP-Glucuronosyltransferase: A Barrier to Oral Bioavailability of Phenolics. *J. Pharm. Sci.* **2011**, *100*, 3655–3681. [CrossRef]
45. Tanner, J.-A.; Tyndale, R. Variation in CYP2A6 Activity and Personalized Medicine. *J. Pers. Med.* **2017**, *7*, 18. [CrossRef]
46. Ronis, M.J.J. Effects of soy containing diet and isoflavones on cytochrome P450 enzyme expression and activity. *Drug Metab. Rev.* **2016**, *48*, 331–341. [CrossRef] [PubMed]
47. Waxman, D.J.; Holloway, M.G. Sex Differences in the Expression of Hepatic Drug Metabolizing Enzymes. *Mol. Pharmacol.* **2009**, *76*, 215–228. [CrossRef] [PubMed]
48. Conterno, L.; Martinelli, F.; Tamburini, M.; Fava, F.; Mancini, A.; Sordo, M.; Pindo, M.; Martens, S.; Masuero, D.; Vrhovsek, U.; et al. Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. *Eur. J. Nutr.* **2019**, *58*, 63–81. [CrossRef] [PubMed]
49. Zamora-Ros, R.; Achaintre, D.; Rothwell, J.A.; Rinaldi, S.; Assi, N.; Ferrari, P.; Leitzmann, M.; Boutron-Ruault, M.-C.; Fagherazzi, G.; Auffret, A.; et al. Urinary excretions of 34 dietary polyphenols and their associations with lifestyle factors in the EPIC cohort study. *Sci. Rep.* **2016**, *6*, 26905. [CrossRef] [PubMed]
50. Marhuenda-Muñoz, M.; Laveriano-Santos, E.P.; Tresserra-Rimbau, A.; Lamuela-Raventós, R.M.; Martínez-Huélamo, M.; Vallverdú-Queralt, A. Microbial Phenolic Metabolites: Which Molecules Actually Have an Effect on Human Health? *Nutrients* **2019**, *11*, 2725. [CrossRef] [PubMed]
51. Garcés, C.; de Oya, I.; Lasunción, M.A.; López-Simón, L.; Cano, B.; de Oya, M. Sex hormone-binding globulin and lipid profile in pubertal children. *Metabolism* **2010**, *59*, 166–171. [CrossRef] [PubMed]
52. Chang, E.; Varghese, M.; Singer, K. Gender and Sex Differences in Adipose Tissue. *Curr. Diab. Rep.* **2018**, *18*, 69. [CrossRef]

4.2.2. Article 5: Total urinary (poly)phenols and ideal cardiovascular health metrics in Spanish adolescents enrolled in the SI Program: a cross-sectional study

Emily P. Laveriano-Santos, Camila Arancibia-Riveros, Isabella Parilli-Moser, Sonia L. Ramírez-Garza, Anna Tresserra-Rimbau, Ana María Ruiz-León, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Vanesa Carral, Gloria Santos-Beneit, Juan M. Fernández-Alvira, Rodrigo Fernández-Jiménez, and Rosa M. Lamuela-Raventós

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Abstract

Introduction
Cardiovascular risk factor during the adolescence increases the risk of develop a cardiovascular event in adulthood. (Poly)phenols are associated with better cardiovascular health in adults, but it is still study in adolescents.

Objective
To study the relationship between urinary total (poly)phenol excretion (TPE) in adolescents and ideal cardiovascular (CVH) metrics.

Methods
1151 adolescents aged 12.04 (0.46) years participating in the SI! Program for Secondary Schools were selected based on the availability of urine samples and information required to assess CVH metrics. Data on health behaviours (smoking status, body mass index, physical activity, and healthy diet) and health factors (blood pressure, total cholesterol, and blood glucose) were used to calculate the CVH metrics. TPE in urine was analysed by a Folin-Ciocalteu method after solid-phase extraction. Associations between TPE (categorized into tertiles) and CVH metrics (total and separate scores) were assessed using multilevel mixed-effect regression models.

Results
Higher TPE levels were associated with higher (healthier) CVH scores and ideal smoking status (OR: 1.54, 95% CI:1.10; 1.87, p-value=0.007), physical activity (OR:

1.12, 95% CI:1.02; 1.23, p-value=0.022) and total cholesterol (OR: 1.78, 95% CI: 1.16; 2.73, p-value=0.009) after multivariate adjustment. An association between TPE and total CVH scores was observed only in boys. Girls with higher TPE had higher rates of ideal total cholesterol and blood pressure.

Conclusion

In summary, according to our findings, higher urinary TPE is related to better CVH scores, with relevant differences in this association by gender.

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Total urinary polyphenols and ideal cardiovascular health metrics in Spanish adolescents enrolled in the SI Program: a cross-sectional study

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To study the relationship between urinary total polyphenol excretion (TPE) in adolescents and ideal cardiovascular (CVH) metrics. 1151 adolescents aged 12.04 (0.46) years participating in the SI! Program for Secondary Schools were selected based on the availability of urine samples and information required to assess CVH metrics. Data on health behaviours (smoking status, body mass index, physical activity, and healthy diet) and health factors (blood pressure, total cholesterol, and blood glucose) were used to calculate the CVH metrics. TPE in urine was analysed by a Folin-Ciocalteu method after solid-phase extraction. Associations between TPE (categorized into tertiles) and CVH metrics (total and separate scores) were assessed using multilevel mixed-effect regression models. Higher TPE levels were associated with higher (healthier) CVH scores and ideal smoking status (OR 1.54, 95% CI 1.10; 1.87, *p* value = 0.007), physical activity (OR 1.12, 95% CI 1.02; 1.23, *p* value = 0.022) and total cholesterol (OR 1.78, 95% CI 1.16; 2.73, *p* value = 0.009) after multivariate adjustment. An association between TPE and total CVH scores was observed only in boys. Girls with higher TPE had higher rates of ideal total cholesterol and blood pressure. According to our findings, higher urinary TPE is related to better CVH scores, with relevant differences in this association by gender.

Abbreviations

BG	Blood glucose
BMI	Body mass index
BP	Blood pressure
CDC	Center for Disease Control
CEHQ	Children's Eating Habit Questionnaire
CVH	Cardiovascular health
CVD	Cardiovascular disease

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DBP	Diastolic blood pressure
FFQ	Food frequency questionnaire
GA	Gallic acid
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
MVPA	Moderate-to-vigorous physical activity
OR	Odds ratio
SBP	Systolic blood pressure
TC	Total cholesterol
TG	Triglycerides
TPE	Total polyphenol excretion

Although the clinical events of cardiovascular disease (CVD) generally occur in adulthood, cardiovascular risk factors develop in childhood and adolescence due to the accumulation of unhealthy behaviours[1]. In order to promote and monitor cardiovascular health (CVH), the American Heart Association defined the concept of ideal CVH based on seven metrics (smoking status, body mass index, physical activity, diet, blood pressure, total cholesterol, and blood glucose)[2].

Following a Mediterranean diet is known to help prevent CVD, partly because it includes polyphenol-rich foods[3,4]. Cardiovascular protection of polyphenols could be due to their anti-inflammatory and antioxidant properties, having an effect on endothelial function, oxidative stress, and the metabolism of glucose and lipids[5–7]. Besides, food frequency questionnaires, dietary history interviews, or 24-h diet recalls are the most common dietary assessment tools to estimate dietary polyphenol intake[8]. However, in recent years the measurement of urinary total polyphenols (TPE) has been considered a reliable biomarker of total polyphenol intake[9]. In a recent study on Spanish adolescents from the SI! Program, we found that higher polyphenol excretion in urine was associated with lower values of body fat percentage, triglycerides (TG), total cholesterol, and low-density lipoprotein cholesterol (LDL-c) in boys, and lower blood pressure in girls[10], all of which are well-known cardiovascular risk factors. Additionally, previous studies have reported a correlation between CVH metrics in adolescence with subclinical markers of CVD in adulthood[11,12]. Therefore, to shed light on the effect of polyphenol intake on CVH at an early stage of life, the present study investigated the relationship between urinary polyphenol levels and CVH metrics in adolescents.

Methods

Study design and participants. This is a sub-study carried out based on data from the SI! (*Salud Integral-Comprehensive Health*) Program for Secondary Schools is a cluster-randomized controlled intervention trial (NCT03504059) aiming to evaluate the effectiveness of an educational program to improve CVH in adolescents. It was conducted from 2017 to 2021 in 1326 participants from 24 Spanish secondary public schools. A detailed description of the study design and recruitment procedures is available elsewhere[13]. The study protocol was approved by the Joint Commission on Ethics of the Instituto de Salud Carlos III in Madrid (CEI PI 35_2016), the Fundació Unió Catalana d'Hospitals (CEI 16/41), and the University of Barcelona (IRB00003099) and carried out in accordance with the Helsinki Declaration. Parents or caregivers provided assent and written informed consent at the beginning of the study.

For this cross-sectional study, baseline data of 1151 adolescents (47% girls) enrolled in the SI! Program were used. Participants with unavailable urine samples (n = 13), diagnosed with diabetes (n = 6) or hypertension (n = 1), that had taken any drugs or supplements (n = 116) the day prior to the data collection, and with missing data for any of the CVH metrics (n = 39) were excluded.

Quantification of total polyphenol excretion (TPE) in urine samples. A validated Folin–Ciocalteu spectrophotometric method described by Medina-Remón et al. was used to determine TPE levels in spot urine samples[9]. A prior solid phase extraction was carried out using OASIS 30 mg MAX 96 well plates (Waters, Milford, MA) to remove potential interferences with the Folin–Ciocalteu reagent[9]. Gallic acid (GA) (Sigma-Aldrich, St. Louis, MO, USA) was used as a reference for TPE quantification, and its calibration curve ranged from 0.7 to 16 mg/L. Creatinine was measured using the Jaffé alkaline picrate method adapted for thermo microtiter 96-well plates by Medina-Remón et al.[9] A calibration curve for creatinine was prepared with a standard (Fluka, St. Louis, MO, USA) at values from 0.5 to 1 mg/L. The coefficient of variation between measures of GA and creatinine was less than 15%. Finally, TPE was normalized by creatinine, expressed as mg GA equivalent/g creatinine and categorized into tertiles.

Cardiovascular health assessment. Seven CVH metrics were calculated in the adolescents using the cut-off values stipulated by the American Health Association, as summarized in Table 1, comprising four health behaviours and three health factors[2].

Health Behaviours. Smoking status was evaluated by a confidential self-reported questionnaire[13] and was considered ideal when the participant had never smoked a whole cigarette.

Weight was measured using an electronic scale (OMRON BF511, OMRON HEALTHCARE Co., Muko, Kyoto, Japan) and height by a portable stadiometer (SECA 213, Hamburg, Germany) while participants wore light clothing and no shoes. Both measurements were conducted by a trained staff[13]. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). BMI z-scores and percentiles

CVH Component	Ideal metric	Non-ideal metric
Health behaviours		
Smoking status	Never smoked a whole cigarette	All other individuals
Body mass index	< 85th percentile	≥ 85th percentile
Physical activity	≥ 60 min/day MVPA every day	< 60 min/day MVPA or no physical activity every day
Healthy diet score	4 components[a]	0–3 components[a]
Health factors		
Total cholesterol	< 170 mg/dL	≥ 170 mg/dL
Blood glucose	< 100 mg/dL	≥ 100 mg/dL
Blood pressure	< 90th percentile	≥ 90th percentile

Table 1. Cardiovascular health metrics as defined by the American Health Association. CVH, cardiovascular health; MVPA, moderate-to-vigorous physical activity. [a]Diet score is based on the following dietary recommendations: fruits and vegetables ≥ 4.5 servings/day, fish ≥ two 3.5-oz servings/week, fibre-rich whole grains ≥ 3 servings/day, and sugar-sweetened beverages ≤ 450 kcal (36 oz)/week, all scaled to a diet of 2000 kcal/day.

were calculated based on the median values in adolescents by age and gender according to the Center for Disease Control (CDC)[14]. BMI was considered ideal when values were under the 85th percentile.

Moderate-to-vigorous physical activity (MVPA) was measured with an accelerometer (ACTIGRAPH WGT3X-BT, ActiGraph, Pensacola, USA) worn on the non-dominant wrist for seven consecutive days and applying the cut-points of Chandler et al.[15]. In participants with missing accelerometer data, we used information reported from a validated questionnaire[13,16], estimating the MVPA according to the frequency and duration of recreational physical activity and competitive sports done inside or outside schools, on schooldays and at weekends. A conversion factor was used to calculate MVPA in terms of minutes per day according to the questionnaire. Participants with ≥ 60 min/day of MVPA were considered to have an ideal level of physical activity.

Regarding diet, information about the intake of fruits, vegetables, fish, fibre-rich whole grains, and sugar-sweetened beverages was obtained using a validated 157-item semi-quantitative food frequency questionnaire (FFQ) filled out by the families[17,18]. A healthy diet score was based on fruits and vegetables ≥ 4.5 servings/day, fish ≥ 2 servings/week, fibre-rich whole grains ≥ 3 servings/day, and sugar-sweetened beverages ≤ 36 oz or 1065 mL/ week based on 2000 kcal of total daily energy intake. The validated non-quantitative self-reported Children's Eating Habits Questionnaire (CEHQ), was filled out by adolescents through the face-to-face interview method conducted by trained staff[19]. It was used to evaluate dietary intake in cases without available FFQ data. In the CEHQ, the frequency of food consumption was assessed as times per month, week, or day, and categorized in eight responses: 1 = never or less than once per month, 2 = once or twice per week, 3 = four or six times per week, 4 = once per day, 5 = two or three times per day, 6 = four or six times per day, 7 = more than six times per day, 8 = unknown. A conversion factor was used to transform questionnaire answers into weekly or daily consumption frequencies. Finally, subjects who had an ideal intake of all four diet components achieved an ideal healthy diet score.

Health factors. Total cholesterol (TC) and blood glucose (BG) levels were measured by trained staff and determined using a portable biochemical analyser (CardioChek Plus, Polymer Technology System Inc., Indianapolis, USA) in finger-prick capillary samples of whole blood (approximately 40 µL) taken early in the morning after overnight fasting[13]. In adolescents, ideal levels of TC have been defined as < 170 mg/dL and BG, < 100 mg/dL.

Blood pressure (BP) was measured when participants were in a sitting position using a digital monitor OMRON M6 (OMRON HEALTHCARE Co., Muko, Kyoto, Japan). Duplicate measurements were taken at two- or three-minute intervals after the participants relaxed[13]. Lowest BP values were used to calculate BP centiles according to gender-specific and age-specific z-scores from the High Blood Pressure Working Group of the National Blood Pressure Education Program for children and adolescents[20]. Systolic BP (SBP) and diastolic BP (DBP) were considered ideal when under the 90th percentile.

Cardiovascular health score. The overall CVH score was calculated by assigning one point for each ideal metric (health behaviour or factor), and zero points for each non-ideal metric, being categorized as poor (0–3 points), intermediate (4–5 points), and ideal (6–7 points), as previously described[21].

Sociodemographic characteristics. Parental education and household income were assessed based on a self-completed questionnaire for parents or legal guardians[13]. The highest parental education level corresponded to university studies according to the International Standard Classification of Education[22]. Household income was categorized as low, medium, or high, based on the reference salary for the Spanish population[23]. Puberty development was assessed according to Tanner maturation stages based on self-reports by the participants using pictograms[24].

Statistical analysis. Descriptive characteristics of participants were reported for the total population and by gender, using mean and standard deviations for continuous variables due to approximate normal distribution, and frequencies with percentages for categorical variables. T-test was carried out to analyze differences between gender. Participants were classified into tertiles of TPE (T1 < 85.8 mg GAE/g creatinine, T2 85.8–140.5 mg GAE/g creatinine, and T3 > 140.5 mg GAE/g creatinine). Pearson chi-square test (X^2) and one-way analysis of variance were used to assess the unadjusted difference in frequencies and mean across tertiles of TPE, respectively.

Multilevel mixed-effect linear regression models, with robust error variance, were used to evaluate the association between tertiles of TPE with the CVH score (continuous). The results of the regression models are expressed as unstandardized B coefficients and their 95% confidence interval (CI). In model 1, the fixed effect was gender (girls/boys); in model 2 were added age (continuous), fasting (yes/no), Tanner maturation stages (from I to V), and TG; finally, model 3 was additionally adjusted by highest parental education (yes/no), and household income (low, medium, and high). Akaike information criteria was applied to indicate the better regression model. To study the association between tertiles of TPE and each ideal CVH metric, multilevel mixed-effect logistic regression was performed using robust error variance, expressed as odds ratio (OR) and 95% CI and adjusted by the same variables considered in regression model 3. The associations of TPE with each CVH metric were analysed by comparing the highest with the lowest tertile of TPE. Municipalities (Barcelona/Madrid) and schools were included as a random effect. We evaluated the potential modifying effect of gender on the association between tertiles of TPE and CVH in an interaction analysis using cross-product terms between TPE and gender in the analysis. This analysis was also stratified by gender to evaluate potential modification. Linear trends were assessed using orthogonal polynomial contrasts. All statistical analyses were carried out using STATA statistical software package version 16.0 (StataCorp, College Station, TX, USA), and R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical tests were two-sided, and p values under 0.05 were considered significant.

Results

Characteristics of the study population. The characteristics of the study participants are summarized in Table 2. Over half of the adolescents were boys (53%), and the mean age was 12.04 (0.46) years. Boys had higher SBP and BG levels, whereas girls had higher DBP and triglyceride levels. A higher intake of fruits and vegetables, as well as a higher percentage of lower MVPA (< 60 min/day), was observed in girls. No significant differences in sociodemographic variables were observed by gender (Table 2).

Regarding the median overall CVH scores, 31% (n = 353) of participants had poor CVH, 64% (n = 735) intermediate, and 5% ideal (n = 63). Among the health behaviours, ideal smoking status was reported by more than 90% of boys and girls. No participant had an ideal healthy diet score. The BMI of approximately two-thirds of boys and three-quarters of girls was ideal. Only 23% of girls achieved ideal levels of physical activity compared with 43% of boys. Concerning the CVH factors, ideal TC and BP were reported in more than 60% and 90% of boys and girls, respectively. Less than 40% of all participants had ideal BG (Figure S1).

The mean urinary TPE of the participants was 125.29 (77.17) mg GAE/g creatinine. Overall, the adolescents in the highest tertile (T3) of TPE had lower values of body weight, BMI, BG, TC, LDL-C, and TG (Table S1). Boys and girls in T3 had lower values of TC, whereas only boys had lower values of BG (Table S2) (Table S3). All participants and girls had a higher daily intake of fruit and vegetables in the lowest tertile (T1) of TPE.

TPE and overall CVH score. Results for the association between CVH score and TPE are presented in Table 3. In all regression models, higher levels of TPE were positively associated with higher CVH scores in all participants, and significant gender interaction was found. The gender-stratified analysis showed that only in boys were TPE tertiles directly associated with CVH scores in all the regression models.

TPE and each CVH metric. The distribution of CVH score and individual CVH metrics according to tertiles of TPE is shown in Figure S2 and Table S4, respectively. Lower TPE levels were more frequent in adolescents with poor CVH (Figure S2). Regarding ideal CVH metrics, a major difference between tertiles of TPE was only found for TC (p value = 0.003, Table S4).

The results of logistic regressions between TPE and individual CVH metrics are shown in Table 4. A total of 1050 participants were included in the analysis after the adjustment of covariates. The adjusted analysis revealed that the highest TPE tertile was associated with increased odds of ideal smoking status, ideal physical activity, and ideal TC, whereas it was less associated with ideal BMI compared to the lowest tertile of TPE. All participants followed a non-ideal healthy diet and logistic regression analysis could not be applied.

Gender-specific analysis revealed that in boys, T3 was less associated with an ideal BMI compared to T1 and directly associated with TC, whereas in girls, the odds of having ideal TC (OR:1.22, 95% CI:1.22; 1.23, p value < 0.001) and ideal BP (OR:1.27, 95% CI:1.05; 1.55, p value = 0.016) were higher in T3 compared to T1 (Fig. 1). As all participants followed a non-ideal healthy diet, logistic regression analysis could not be applied.

Discussion

In this cross-sectional study, the relationship between urinary TPE and CVH was investigated in 1151 adolescents aged 11 to 14 years. The findings indicate that higher TPE in urine is positively associated with a higher (healthier) CVH score, mainly due to specific CVH metrics, namely ideal smoking status, physical activity and total cholesterol, with some gender differences observed.

Prevalence of ideal overall CVH and individual metrics. CVH metrics in adolescence have been associated with subclinical CVD in adulthood, indicating the importance of maintaining an ideal CVH from an

	N	Boys (n = 607)	Girls (n = 544)	*p* value
Age (years)	1151	12.08 (0.50)	11.99 (0.42)	0.002
Anthropometric measurements				
Weight (kg)	1151	49.21 (11.90)	48.66 (10.27)	0.403
Height (cm)	1151	155.13 (7.86)	155.41 (6.74)	0.514
BMI (kg/m^2)	1151	20.28 (3.84)	20.06 (3.64)	0.318
Blood pressure				
SBP (mmHg)	1151	105.67 (11.09)	103.50 (10.24)	0.001
DBP (mmHg)	1151	60.81 (8.85)	62.63 (8.54)	< 0.001
Biochemical analytes				
BG (mg/dL)	1151	103.93 (11.75)	101.82 (11.71)	0.002
TC (mg/dL)	1151	151.59 (35.69)	154.59 (32.19)	0.133
HDL-c (mg /dL)	1149	62.92 (17.12)	62.85 (14.28)	0.934
LDL-c (mg/dL)	1083	75.80 (26.36)	77.43 (24.77)	0.296
TG (mg/dL)	1150	75.01 (42.48)	81.06 (36.88)	0.010
Smoking status, n (%)				0.014
Never smoked	1057	546 (90)	511 (94)	
Physical activity, n (%)				
≥ 60 min/day MVPA	387	263 (57)	124 (23)	< 0.001
< 60 min/day MVPA	764	344 (43)	420 (77)	
Dietary intake				
Fruit and vegetables (servings /day)	1149	3.31 (1.96)	3.64 (2.27)	0.010
Whole grains (servings/day)	1149	0.31 (0.57)	0.33 (0.62)	0.604
Fish (servings/week)	1150	4.44 (4.63)	4.19 (2.82)	0.258
Sweet beverages (mL/week)	1150	694.77 (1844.77)	533.78 (1453.81)	0.099
Sociodemographicfactors				
Parental education, n (%)				
Low/medium	217	110 (19)	107 (20)	0.573
Medium	445	228 (40)	217 (42)	
High	431	234 (41)	197 (38)	
Household income, n (%)				
Low	353	181 (32)	172 (34)	0.734
Medium	333	172 (31)	161 (32)	
High	380	205 (37)	175 (34)	
Municipality, n (%)				
Barcelona	813	429 (69)	394 (72)	0.206
Madrid	338	188 (31)	150 (28)	

Table 2. Baseline characteristics of the SI! Program cohort at baseline by gender. Data are expressed as mean (SD) or frequency (percentage). N, number; SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; BG, blood glucose; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; MVPA, moderate-to-vigorous physical activity. Statistical analyses were carried out using the t-test for continuous variables and the chi-square test for categorical variables. *p* value s refer to differences between gender and are considered statistically significant when < 0.05.

early stage in life to prevent future ill health[11,12]. A low prevalence of ideal CVH (5%) was observed in the adolescent participants, due above all to low levels of physical activity and healthy diet, results similar to those reported by Fernandez-Jimenez et al. in a previous study of the same cohort, as well as in other studies in adolescents[25,26].

Polyphenols and ideal cardiovascular health. The mean urinary TPE of 125.3 (77.2) mg GAE/g creatinine was higher to the results of Hussein et al., who reported an average TPE of 89.5 (8.4) mg GAE/g creatinine in 49 Egyptian children aged 7–14 years, using the same Folin–Ciocalteu method[27]. Concerning the association between urinary TPE and the overall CVH score, the highest tertile of TPE was correlated with a higher CVH score in all regression models.

Behaviours such as smoking, insufficient physical activity, and an unhealthy diet during adolescence are reported to increase the risk of subsequently developing CVD[1,11]. Although health behaviours are independently related to increased cardiovascular risk factors, clustering analysis of multiple lifestyle factors reveals a synergetic effect. Santaliestra-Pasías et al. suggested that the joint influence of low fruit and vegetable intake, a highly

Overall CVH score	Models	n	TPE						
			T1	T2		T3		AIC	p-trend
				(B, 95% CI)	p value	(B, 95% CI)	p value		
All participants	Model 1	1151		0.21 (−0.02; 0.43)	0.070	0.25 (0.12; 0.38)	< 0.001	3336.26	0.031
	Model 2	1144	Reference	0.17 (−0.06; 0.40)	0.140	0.19 (0.14; 0.25)	< 0.001	3230.34	0.028
	Model 3	1050		0.11 (−0.12; 0.34)	0.329	0.13 (0.10; 0.15)	< 0.001	2953.36	0.003
Boys	Model 1	607		0.15 (−0.03; 0.32)	0.095	0.20 (0.12; 0.29)	< 0.001	1813.87	< 0.001
	Model 2	604	Reference	0.13 (−0.08; 0.34)	0.232	0.18 (0.17; 0.19)	< 0.001	1765.86	< 0.001
	Model 3	551		0.08 (−0.14; 0.31)	0.452	0.13 (0.12; 0.14)	< 0.001	1600.82	< 0.001
Girls	Model 1	544		0.09 (−0.17; 0.35)	0.506	0.12 (−0.08; 0.32)	0.235	1523.74	0.235
	Model 2	540	Reference	0.14 (−0.07; 0.35)	0.182	0.12 (−0.07; 0.30)	0.210	1461.21	0.210
	Model 3	499		0.12 (−0.04; 0.27)	0.134	0.09 (−0.07; 0.25)	0.261	1359.07	0.261

Table 3. Association between CVH score and tertiles of TPE. Multilevel mixed-effect linear regression models were used to evaluate the relationship between tertiles of TPE and overall CVH score (continuous). Model 1: adjusted by gender (also interaction). Model 2: adjusted as in model 1 plus age, Tanner stage, fasting, and triglycerides. Model 3: adjusted as in model 2 plus parental education and household income. Municipalities and schools were included as a random effect. *p* value T3 vs. T1 of TPE, and p-trend of tertiles of TPE < 0.05 are statistically significant. AIC, Akaike information criteria; B, non-standardized coefficient; CI, confidence interval; CVH, cardiovascular health; TPE, total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine; T1, first tertile of TPE (< 85.8 mg GAE/g creatinine); T2, second tertile of TPE (85.8–140.5 mg GAE/g creatinine); T3, third tertile of TPE (> 140.5 mg GAE/g creatinine).

CVH metrics	TPE All participants (n = 1050)					
	T1	T2		T3		p-trend
		(OR, 95% CI)	p value	(OR, 95% CI)	p value	
Health behaviours						
Ideal SS	Reference	1.16 (0.99; 1.35)	0.070	1.44 (1.10; 1.87)	0.007	0.231
Ideal BMI percentile	Reference	1.01 (0.92; 1.10)	0.872	0.87 (0.80; 0.93)	< 0.001	0.696
Ideal PA level	Reference	1.19 (0.57; 2.51)	0.642	1.12 (1.02; 1.23)	0.022	0.999
Health factors						
Ideal TC	Reference	1.21 (0.75; 1.94)	0.433	1.78 (1.16; 2.73)	0.009	0.009
Ideal BG	Reference	1.22 (0.82; 1.81)	0.321	1.10 (0.84, 1.46)	0.486	0.850
Ideal BP	Reference	1.25 (0.69; 2.27)	0.467	1.09 (0.68; 1.76)	0.709	0.419

Table 4. Association between CVH metrics and tertiles of TPE. Multilevel mixed-effect logistic regression was used to evaluate the relationship between tertiles of TPE and overall CVH metrics, adjusted by gender (also interaction), age, Tanner stage, fasting, triglycerides, parent education, and household income. OR, odds ratio; CI, confidence interval; CVH, cardiovascular health; TPE, total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine; T1, first tertile of TPE (< 85.8 mg GAE/g creatinine); T2, second tertile of TPE (85.8–140.5 mg GAE/g creatinine); T3, third tertile of TPE (> 140.5 mg GAE/g creatinine); SS, smoking status; BMI, body mass index; PA, physical activity; TC, total cholesterol; BP, blood pressure; BG, blood glucose.

sedentary lifestyle, and low physical activity levels are related to excess body fat in European children aged 2–9 years[28]. The Mediterranean diet, characterized by the daily consumption of polyphenol-rich foods, is directly associated with higher TPE[9], but in addition, adherence to the Mediterranean diet is linked with cultural behaviours that facilitate a healthy lifestyle[29]. In the present study, higher TPE was correlated with a greater likelihood of ideal smoking status and physical activity. Olmedo-Requena et al. reported that lower adherence to the MD was positively related with smoking habits and a sedentary lifestyle in young Spanish women[30].

Higher urinary TPE was associated with lower odds of having an ideal BMI, in contrast with a previous study with the same cohort, where higher TPE was related to a lower BMI z-score (continuous) in boys[10]. In the present study, the ideal BMI was analysed as a dichotomic variable, above or equal to the 85th percentile and under the 85th percentile, the latter therefore including participants below the 5th percentile of BMI (underweight, n = 31). However, when the analysis was carried out with and without this underweight group, the results were similar. Also, linear regression between the BMI z-score (continuous) and tertiles of TPE was analysed, and a negative association was found between the highest tertile and the BMI z-score (B = -0.17, *p* value < 0.001). Wisnuwardani et al. showed that a higher intake of polyphenols, mainly flavonoids, was related with a lower BMI z-score in European adolescents aged 12.5 to 17.5 years[31].

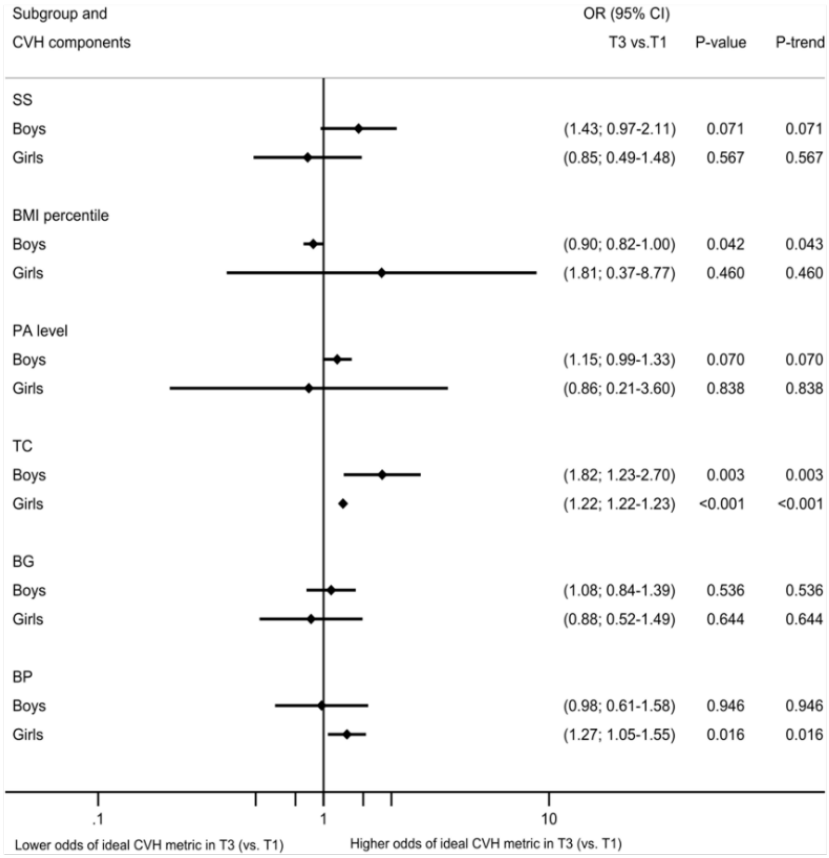

Figure 1. Association between ideal CVH metrics and tertiles of TPE by gender. OR, odds ratio; CI, confidence interval; CVH, cardiovascular health; TPE, total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine; T1, first tertile of TPE (< 85.8 mg GAE/g creatinine); T2, second tertile of TPE (85.8–140.5 mg GAE/g creatinine); T3, third tertile of TPE (> 140.5 mg GAE/g creatinine); SS, smoking status; BMI, body mass index; PA, physical activity; TC, total cholesterol; BP, blood pressure; BG, blood glucose. Multilevel mixed-effect logistic regression was used to evaluate the relationship between tertiles of TPE and each ideal CVH metric. All the analysis was adjusted by age, Tanner stage, fasting, TG, parent education, and household income. Municipalities and schools were included as a random effect. *p* value T3 vs. T1 of TPE, p-trend of tertiles of TPE, and p-interaction < 0.05 are statistically significant. All participants presented a non-ideal healthy diet and logistic regression analysis could not be applied.

Regarding health factors, higher TPE was associated with a greater probability of having ideal TC in both genders, and ideal BP in girls. In a meta-analysis of five prospective cohort studies, Godos et al. reported that a higher intake of anthocyanins was associated with a reduced hypertension risk[32]. A reduction of TC in overweight/obese subjects due to the intake of products rich in ellagitannins and anthocyanins has been observed in several clinical trials[33].

Gender differences in the relation of polyphenols with cardiovascular health. In this study gender differences were observed in the associations between TPE levels and overall CVH and individual metrics. The stimulatory effect of polyphenols on the growth of beneficial microbiota and inhibition of pathogenic strains can vary according to gender and BMI. Studies suggest that women harbour a higher portion of *Firmicutes/Bacteroidetes*, whereas men with a lower BMI have a lower *Firmicutes/Bacteroidetes* ratio and therefore less risk of dysbiosis[34]. Additionally, women have been observed to have a higher number of *lactobacilli,* which could also

generate gender-differential effects on BP[35]. The influence of polyphenols on the microbiota was not analysed here.

The changes in metabolic health status induced by increased sexual hormone secretion after puberty onset, including fat mass distribution, and levels of TC, leptin, and adiponectin, also differ between boys and girls[36,37]. Moreover, the secretion of androgens and oestrogens can be affected by phenolic phytoestrogens[38]. Accordingly, the Tanner stage was included as an adjustment variable in our study.

Gender differences have also been described in fatty acid metabolism, with women being more sensitive to the antilipolytic effects of insulin than men, resulting in a greater release of free fatty acids that contribute to the production of TG and TC[39]. This could explain the higher triglyceride levels found in girls, although TC levels did not differ from those of boys. Other gender-specific mechanisms related to CVH and TPE need to be identified in further research.

Possible mechanisms for the effect of polyphenols on cardiovascular health. Polyphenols are associated with a protective effect against CVD[40]. Clinical trials have demonstrated that these plant secondary metabolites have a therapeutic role in vascular disorders, inflammation processes, glucose metabolism, dyslipidemia, hypertension, and oxidative stress[5–7]. The mechanisms underlying their biological effects involve the initiation of cell signalling responses and their interaction with both extracellular and intracellular receptors[41].

By altering lipid metabolism and inhibiting the oxidation of LDL-C, polyphenols can reduce atherosclerotic lesions and platelet aggregation, as well as ameliorate endothelial function, resulting in lower BP[42]. Catechins are reported to activate nitric oxide synthase, thus modulating flow-mediated dilation and the vasodilation of endothelial cells, which reduces BP[7,43]. Flavonoids from tea, cocoa, and apples have been associated with lower levels of TC, LDL-C, and an increase in HDL-C[6]. The effects of polyphenols on metabolic pathways related to TC and BP, both CVH risk factors, could explain the results obtained in this study, in which polyphenol intake, measured by polyphenol excretion in urine, was seen to have a positive impact on CVH metrics. A higher consumption of polyphenol-rich foods was also associated with other aspects of a healthy lifestyle, as participants with higher TPE were more likely to have an ideal smoking status and physical activity level. Inter-individual variability, such as gender, also played a role.

Strengths and limitations. The main strength of this study is the large sample size (n = 1151), and the use of standardized procedures. Moreover, the stratified analysis according to boys and girls increases the generality of the results. To the best of our knowledge, no studies about the association between polyphenol intake and ideal CVH in adolescents have been published to date.

This study has limitations that should be considered when interpreting the results. The cross-sectional design does not allow the identification of causal relationships between exposure and outcomes. The use of self-reported questionnaires risks biased reporting of diets, physical activity, and smoking status. As participants did not use accelerometers in water sports or during sports championships, the physical activity levels could have been underestimated. Given that fasting can alter BG concentrations, it was included as an adjustment variable in the analysis, even though 3% (n = 29) of the participants were not fasting. Another limitation is the issue of residual confounding due to the use of categorical variables.

Conclusion

The results of the present study suggest that higher concentrations of polyphenols excreted in urine are associated with a more favourable CVH score in adolescents, mainly explained by the metrics of smoking status, physical activity, and TC. Gender differences were observed in the results; in boys, a higher TPE was associated with a better overall CVH score and ideal TC, and in girls with higher odds of having ideal TC and BP. The important finding of this study indicates the need to conduct similar studies in other European countries and worldwide. Additionally, longitudinal studies and randomized trials are needed to confirm the relationship of polyphenols with CVH and evaluate their effectiveness in preventing CVD.

Data availability

The datasets generated during and/or analysed during the current study are not publicly available due to there are restrictions on the availability of the data for the SI! Program study. Requestor wishing to access the database used in this study can make a request to the Steering Committee (SC) chair: gsantos@fundacionshe.org, rodrigo.fernandez@cnic.es, juanmiguel.fernandez@cnic.es, RESTRUCH@clinic.cat, lamuela@ub.edu, bibanez@cnic.es, vfuster@cnic.es. For the present study, the database was requested from the SC on 4th June 2021.

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References

1. Fernandez-Jimenez, R., Al-Kazaz, M., Jaslow, R., Carvajal, I. & Fuster, V. Children present a window of opportunity for promoting health. *J. Am. Coll. Cardiol.* **72**, 3310–3319 (2018).
2. Lloyd-Jones, D. M. *et al.* Defining and setting national goals for cardiovascular health promotion and disease reduction. *Circulation* **121**, 586–613 (2010).
3. Estruch, R. *et al.* Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. *N. Engl. J. Med.* **378**, e34 (2018).
4. Tresserra-Rimbau, A. *et al.* Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutr. Metab. Cardiovasc. Dis.* **24**, 639–647 (2014).

5. Hurtado-Barroso, S. *et al.* Changing to a low-polyphenol diet alters vascular biomarkers in healthy men after only two weeks. *Nutrients* **10**, 1766 (2018).
6. Koch, W. Dietary polyphenols—Important non-nutrients in the prevention of chronic noncommunicable diseases. A systematic review. *Nutrients* **11**, 1039 (2019).
7. Li, P.-G. *et al.* Quercetin induces rapid eNOS phosphorylation and vasodilation by an Akt-independent and PKA-dependent mechanism. *Pharmacology* **89**, 220–228 (2012).
8. Probst, Y., Guan, V. & Kent, K. A systematic review of food composition tools used for determining dietary polyphenol intake in estimated intake studies. *Food Chem.* **238**, 146–152 (2018).
9. Medina-Remón, A. *et al.* Rapid Folin–Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Anal. Chim. Acta* **634**, 54–60 (2009).
10. Laveriano-Santos, E. P. *et al.* Polyphenols in urine and cardiovascular risk factors: a cross-sectional analysis reveals gender differences in Spanish adolescents from the SI! Program. *Antioxidants* **9**, 910 (2020).
11. Pahkala, K. *et al.* Ideal cardiovascular health in adolescence. *Circulation* **127**, 2088–2096 (2013).
12. Laitinen, T. T. *et al.* Childhood lifestyle and clinical determinants of adult ideal cardiovascular health. *Int. J. Cardiol.* **169**, 126–132 (2013).
13. Fernandez-Jimenez, R. *et al.* Rationale and design of the school-based SI! Program to face obesity and promote health among Spanish adolescents: A cluster-randomized controlled trial. *Am. Heart J.* **215**, 27–40 (2019).
14. Fryar, C. D., Gu, Q. & Ogden, C. L. Anthropometric reference data for children and adults: United States, 2007–2010. *Vital Health Stat* **11**, 1–48 (2012).
15. Chandler, J. L., Brazendale, K., Beets, M. W. & Mealing, B. A. Classification of physical activity intensities using a wrist-worn accelerometer in 8–12-year-old children. *Pediatr. Obes.* **11**, 120–127 (2016).
16. Barbosa, N. *et al.* A physical activity questionnaire: reproducibility and validity. *J Sports Sci Med* **6**, 505–518 (2007).
17. Fernández-Ballart, J. D. *et al.* Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br. J. Nutr.* **103**, 1808–1816 (2010).
18. Juton, C. *et al.* Reliability and concurrent and construct validity of a food frequency questionnaire for pregnant women at high risk to develop fetal growth restriction. *Nutrients* **13**, 1629 (2021).
19. Bel-Serrat, S. *et al.* Relative validity of the Children's Eating Habits Questionnaire–food frequency section among young European children: the IDEFICS Study. *Public Health Nutr.* **17**, 266–276 (2014).
20. Falkner, B. & Daniels, S. R. Summary of the fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Hypertension* **44**, 387–388 (2004).
21. Fernandez-Jimenez, R. *et al.* Prevalence and correlates of cardiovascular health among early adolescents enrolled in the SI! Program in Spain: a cross-sectional analysis. *European Journal of Preventive Cardiology* (2020).
22. UNESCO. *International Standard Classification of Education, ISCED 2011* (2012).
23. INE: Instituto Nacional de Estadística. Encuesta anual de estructura salarial.
24. Tanner, J. M. & Whitehouse, R. H. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch. Dis. Child.* **51**, 170–179 (1976).
25. Fernandez-Jimenez, R. *et al.* Prevalence and correlates of cardiovascular health among early adolescents enrolled in the SI! Program in Spain: a cross-sectional analysis. *Eur. J. Prev. Cardiol.* **29**, e7–e10 (2022).
26. Pacor, J. M. *et al.* Prevalence of ideal cardiovascular health metrics in children & adolescents: A systematic review. *Prog. Pediatr. Cardiol.* **43**, 141–146 (2016).
27. Hussein, L., Medina, A., Barrionnevo, A., Lammuela-Raventos, R. M. & Andres-Lacueva, C. Normal distribution of urinary polyphenol excretion among Egyptian males 7–14 years old and changes following nutritional intervention with tomato juice (Lycopersicon esculentum). *Int. J. Food Sci. Nutr.* **60**, 302–311 (2009).
28. Santaliestra-Pasias, A. M. *et al.* Clustering of lifestyle behaviours and relation to body composition in European children. The IDEFICS study. *Eur. J. Clin. Nutr.* **69**(7), 811–816 (2015).
29. Bach-Faig, A. *et al.* Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr.* **14**, 2274–2284 (2011).
30. Olmedo-Requena, R. *et al.* Factors associated with a low adherence to a Mediterranean diet pattern in healthy Spanish women before pregnancy. *Public Health Nutr.* **17**, 648–656 (2014).
31. Wisnuwardani, R. W. *et al.* Polyphenol intake and metabolic syndrome risk in European adolescents: the HELENA study. *Eur. J. Nutr.* **59**(2), 801–812 (2019).
32. Godos, J. *et al.* Dietary polyphenol intake, blood pressure, and hypertension: A systematic review and meta-analysis of observational studies. *Antioxidants* **8**, 152 (2019).
33. García-Conesa, M. T. *et al.* Meta-analysis of the effects of foods and derived products containing ellagitannins and anthocyanins on cardiometabolic biomarkers: Analysis of factors influencing variability of the individual responses. *Int. J. Mol. Sci.* **19**, 694 (2018).
34. Razavi, A. C., Potts, K. S., Kelly, T. N. & Bazzano, L. A. Sex, gut microbiome, and cardiovascular disease risk. *Biol. Sex Differ.* **10**, 1–14 (2019).
35. Khalesi, S., Sun, J., Buys, N. & Jayasinghe, R. Effect of probiotics on blood pressure: A systematic review and meta-analysis of randomized, controlled trials. *Hypertension* **64**, 897–903 (2014).
36. Guzzetti, C. *et al.* Cardiovascular risk factors in children and adolescents with obesity: Sex-related differences and effect of puberty. *Front. Endocrinol.* **10**, 591 (2019).
37. Moran, A. *et al.* Changes in insulin resistance and cardiovascular risk during adolescence. *Circulation* **117**, 2361–2368 (2008).
38. Domínguez-López, I., Yago-Aragón, M., Salas-Huetos, A., Tresserra-Rimbau, A. & Hurtado-Barroso, S. Effects of dietary phytoestrogens on hormones throughout a human lifespan: A review. *Nutrients* **12**, 2456 (2020).
39. Hatch-Stein, J. A., Kelly, A., Gidding, S. S., Zemel, B. S. & Magge, S. N. Sex differences in the associations of visceral adiposity, homeostatic model assessment of insulin resistance, and body mass index with lipoprotein subclass analysis in obese adolescents. *J. Clin. Lipidol.* **10**, 757–766 (2016).
40. Marx, W. *et al.* The effect of polyphenol-rich interventions on cardiovascular risk factors in haemodialysis: A systematic review and meta-analysis. *Nutrients* **9**, 1345 (2017).
41. Kang, G. G. *et al.* Dietary polyphenols and gene expression in molecular pathways associated with type 2 diabetes mellitus: A review. *Int. J. Mol. Sci.* **21**, 140 (2019).
42. Baião, D. D. S. *et al.* Polyphenols from root, tubercles and grains cropped in Brazil: Chemical and nutritional characterization and their effects on human health and diseases. *Nutrients* **9**, 1044 (2017).
43. Tang, W.-J., Hu, C.-P., Chen, M.-F., Deng, P.-Y. & Li, Y.-J. Epigallocatechin gallate preserves endothelial function by reducing the endogenous nitric oxide synthase inhibitor level. *Can. J. Physiol. Pharmacol.* **84**, 163–171 (2006).

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Author contributions

Conceptualization, R.M.L.-R.; methodology, E.P.L.-S., A.T.-R., and R.M.L.-R.; formal analysis, E.P.L.-S. and C.A.-R.; investigation, E.P.L.-S., C.A.-R., and R.M.L.-R.; data curation, A.d.C.-G.; writing—original draft preparation, E.P.L.-S., C.A.-R., A.T.-R. and R.M.L.-R.; writing—review and editing, A.T.-R., R.F.-J., J.M.F.-A., G.S.-B., M.d.M., P.B., A.d.C.-G., V.C., A.M.R.-L., S. C.-B., R.E. and R.M.L.-R.; visualization, E.P.L.-S; supervision, A.T.-R. and R.M.L.-R.; funding acquisition; R.F.-J., J.M.F.-A., G.S.-B., M.d.M., P.B., A.d.C.-G., V.C., A.T.-R., R.E. and R.M.L.-R. All authors have read and agreed to the published version of the manuscript.

Competing interests

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Additional information

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Total urinary polyphenols and ideal cardiovascular health metrics in Spanish adolescents enrolled in the SI Program: a cross-sectional study

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Online Supplementary Material

Supplementary tables

Table S1. Baseline characteristics of the SI! Program cohort at baseline by tertiles of TPE.

	N	Missing N (%)	T1 (n=384)	T2 (n=384)	T3 (n=383)	*P-value*
Girls	544		187 (49)	187 (49)	170 (44)	0.386
Age (y)	1151	0 (0)	12.03 (0.48)	12.05 (0.45)	12.02 (0.47)	0.638
Anthropometric measurements						
Weight (kg)	1151	0 (0)	49.95 (12.00)	48.98 (10.27)	47.92 (11.07)	0.043
Height (cm)	1151	0 (0)	155.17 (7.34)	155.47 (7.12)	155.14 (7.61)	0.794
BMI (kg/m^2)	1151	0 (0)	20.61 (4.11)	20.15 (3.46)	19.78 (3.61)	0.008
Blood pressure						
SBP (mmHg)	1151	0 (0)	105.34 (10.66)	103.89 (10.86)	104.69 (10.70)	0.173
DBP (mmHg)	1151	0 (0)	61.89 (9.12)	60.92 (8.64)	62.20 (8.46)	0.108
Biochemical analyses						
BG (mg/dL)	1151	0 (0)	103.99 (11.93)	103.16 (12.19)	101.64 (11.09)	0.020
TC (mg/dL)	1151	0 (0)	155.69 (32.33)	156.61 (37.05)	146.70 (31.87)	<0.001
HDL-C (mg /dL)	1149	2 (0)	63.39 (15.71)	63.95 (17.04)	61.31 (14.56)	0.052
LDL-C (mg/dL)	1083	68 (6)	77.63 (24.52)	78.55 (27.79)	73.52 (24.18)	0.020
TG (mg/dL)	1150	1 (0)	79.91 (44.80)	80.00 (38.06)	73.68 (36.48)	0.043
Smoking status, n(%)						0.866
Never smoked	1057	94 (8)	352 (92)	351 (91)	354 (92)	
Physical activity, n(%)	1151	0 (0)				0.683
≥60 min/day MVPA	387		122 (32)	131 (34)	134 (35)	
<60 min/day MVPA	764		262 (68)	253 (66)	249 (65)	
Dietary intake						
Fruit and vegetables (servings /day)	1149	2 (0)	3.69 (2.37)	3.33 (2.06)	3.38 (1.87)	0.033
Whole grains (servings /day)	1149	2 (0)	0.34 (0.69)	0.30 (0.53)	0.32 (0.54)	0.641
Fish (servings /week)	1150	1 (0)	4.48 (4.07)	4.32 (3.69)	4.15 (3.87)	0.499
Sweet beverages (mL/week)	1150	1 (0)	680.48 (1765.85)	552.45 (1483.01)	653.17 (1752.67)	0.376
Sociodemographic factors	1093	58 (5)				
Parental education, n(%)						0.866
Low	217		77 (21)	75 (21)	65 (18)	
Medium	445		147 (40)	148 (41)	150 (41)	
High	431		142 (39)	142 (38)	147 (41)	
Household income, n(%)	1066	85 (7)				0.444
Low	353		124 (35)	125 (34)	104 (30)	
Medium	333		113 (32)	112 (31)	108 (31)	
High	380		116 (33)	128 (35)	136 (39)	
Municipality, n(%)	1151	0 (0)				
Barcelona	813		283 (74)	284 (74)	246 (64)	0.003
Madrid	338		101 (26)	100 (26)	137 (36)	

Data are expressed as the mean (SD) or as percentage. Abbreviations: N number; SD standard deviation, TPE total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine, T1 first tertile of TPE (< 85.8mg GAE/g creatinine), T2 second tertile of TPE (85.8-140.5 mg GAE/g creatinine), T3 third tertile of TPE (> 140.5 mg GAE/g creatinine), BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, BG blood glucose, TC total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, TG triglycerides, MVPA moderate-to-vigorous physical activity. Statistical analyses were undertaken using one-way ANOVA for continuous variables and the chi-square test for categorical variables. P-values refer to differences between tertiles of TPE and are considered statistically significant when < 0.05.

Table S2. Baseline characteristics of the SI! Program cohort at baseline by tertiles of TPE in boys

	N	T1 (n=197)	T2 (n=197)	T3 (n=213)	*P-value*
Age (y)	607	12.09 (0.54)	12.09 (0.44)	12.05 (0.52)	0.647
Anthropometric measurements					
Weight (kg)	607	50.27 (12.49)	48.86 (11.05)	48.55 (12.10)	0.301
Height (kg)	607	155.19 (7.81)	155.34 (7.52)	154.89 (8.25)	0.842
BMI (kg/m^2)	607	20.72 (4.13)	20.10 (3.57)	20.06 (3.80)	0.160
Blood pressure					
SBP (mmHg)	607	106.61 (11.07)	105.13 (11.22)	105.29 (11.00)	0.342
DBP (mmHg)	607	61.29 (9.08)	59.81 (8.81)	61.28 (8.64)	0.159
Biochemical analytes					
BG (mg/dL)	607	105.76 (11.85)	103.63 (12.36)	102.51 (10.90)	0.018
TC (mg/dL)	607	154.08 (33.43)	155.86 (41.11)	145.33 (31.37)	0.006
HDL-C (mg /dL)	605	62.42 (16.61)	65.28 (18.83)	61.22 (15.71)	0.050
LDL-C (mg/dL)	563	78.26 (25.32)	76.68 (30.18)	72.70 (23.13)	0.104
TG (mg/dL)	606	79.71 (53.56)	75.76 (39.13)	69.93 (32.24)	0.064
Smoking status, n(%)					
Never smoked	546	175 (89)	176 (89)	195 (92)	0.620
Physical activity, n(%)					0.426
≥60 min/day MVPA	263	78 (40)	90 (46)	95 (45)	
<60 min/day MVPA	344	119 (60)	107 (54)	118 (55)	
Dietary intake					
Fruit and vegetables (servings /day)	607	3.38 (1.97)	3.15 (1.96)	3.40 (1.93)	0.372
Whole grains (servings /day)	605	0.31 (0.55)	0.31 (0.54)	0.31 (0.61)	0.985
Fish (servings /week)	606	4.60 (5.05)	4.50 (4.01)	4.24 (4.76)	0.718
Sweet beverages (ml /week)	606	808.80 (1963.97)	532.53 (1536.93)	739.88 (1986.25)	0.302
Sociodemographic factors					
Parental education, n(%)					
Low	110	45 (24)	38 (21)	27 (13)	0.040
Medium	228	62 (33)	74 (40)	92 (46)	
High	234	79 (43)	73 (39)	82 (41)	
Household income, n(%)					
Low	181	63 (35)	63 (34)	55 (28)	0.287
Medium	172	59 (33)	56 (31)	57 (29)	
High	205	58 (32)	64 (35)	83 (43)	
Municipality, n(%)					
Barcelona	419	138 (70)	142 (72)	139 (65)	0.306
Madrid	188	59 (30)	55 (28)	74 (35)	

Data are expressed as the mean (SD) or as percentage. Abbreviations: N number, SD standard deviation, TPE total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, BG blood glucose, TC total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, TG triglycerides, MVPA moderate-to-vigorous physical activity. Statistical analyses were carried out using one-way ANOVA for continuous variables and the chi-square test for categorical variables. P-values refer to differences between tertiles of TPE and are considered statistically significant when < 0.05.

Table S3. Baseline characteristics of the SI! Program cohort at baseline by tertiles of TPE in girls

	N	T1 (n=187)	T2 (n=187)	T3 (n=170)	*P-value*
Age (y)	544	11.97 (0.41)	12.02 (0.45)	11.99 (0.39)	0.602
Anthropometric measurements					
Weight (kg)	544	49.60 (11.49)	49.10 (9.41)	47.15 (9.64)	0.060
Height (kg)	544	155.16 (6.84)	155.61 (6.69)	155.46 (6.73)	0.806
BMI (kg/m^2)	607	20.50 (4.09)	20.20 (3.35)	19.42 (3.33)	0.015
Blood pressure					
SBP (mmHg)	544	104.01 (10.07)	102.59 (10.34)	103.95 (10.30)	0.326
DBP (mmHg)	544	62.51 (9.14)	62.09 (8.31)	63.35 (8.10)	0.366
Biochemical analytes					
BG (mg/dL)	544	102.13 (11.76)	102.66 (12.02)	100.56 (11.26)	0.218
TC (mg/dL)	544	157.40 (31.13)	157.40 (32.32)	148.42 (32.51)	0.010
HDL-C (mg /dL)	544	64.41 (14.69)	62.57 (14.86)	61.44 (13.04)	0.138
LDL-C (mg/dL)	520	77.00 (23.75)	80.50 (24.99)	74.53 (25.46)	0.084
TG (mg/dL)	544	80.11 (33.33)	84.47 (36.47)	78.35 (40.78)	0.267
Smoking status, n(%)					
Never smoked	511	177 (95)	175 (94)	159 (94)	0.879
Physical activity, n(%)					
≥60 min/day MVPA	124	44 (26)	41 (22)	39 (23)	0.932
<60 min/day MVPA	420	143 (74)	146 (78)	131 (77)	
Dietary intake					
Fruit and vegetables (servings /day)	542	4.02 (2.69)	3.51 (2.15)	3.36 (1.80)	0.014
Whole grains (servings /day)	544	0.37 (0.81)	0.29 (0.52)	0.33 (0.44)	0.467
Fish (servings /week)	544	4.36 (2.68)	4.14 (3.33)	4.05 (2.32)	0.548
Sweet beverages (mL /week)	544	545.97(1526 .07)	511.83 (1428.06)	544.53 (1404.67)	0.968
Sociodemographic factors					
Parental education, n(%)					
Low	107	32 (18)	37 (21)	38 (24)	0.325
Medium	217	85 (47)	74 (41)	58 (36)	
High	197	63 (35)	69 (38)	65 (40)	
Household income, n(%)					
Low	172	61 (35)	62 (34)	49 (32)	0.972
Medium	161	54 (31)	56 (31)	51 (33)	
High	175	58 (33)	64 (35)	53 (35)	
Municipality, n(%)					
Barcelona	394	145 (78)	142 (76)	107 (63)	0.004
Madrid	150	42 (22)	45 (24)	63 (37)	

Data are expressed as the mean (SD) or as percentage. Abbreviations: N number; SD standard deviation, TPE total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, BG blood glucose, TC total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, TG triglycerides, MVPA moderate-to-vigorous physical activity. Statistical analyses were carried out using one-way ANOVA for continuous variables and the chi-square test for categorical variables. P-values refer to differences between tertiles of TPE and are considered statistically significant when < 0.05.

Table S4. CVH metrics by tertiles of TPE

	Total (n=1151)	T1 (n=384))	T2 (n=384)	T3 (n=383)	*P-value*
Overall CVH, n (%)					
Ideal	63 (5)	21 (6)	15 (4)	27 (7)	<0.001
Intermediate	735 (64)	216 (56)	262 (68)	257 (67)	
Poor	353 (31)	147 (38)	107 (28)	99 (26)	
SS, n (%)					
Ideal	1057 (92)	352 (92)	351 (91)	354 (92)	0.866
Non-ideal	94 (8)	32 (8)	33 (9)	29 (8)	
BMI percentile, n (%)					
Ideal	841 (73)	267 (70)	289 (75)	285 (74)	0.155
Non-ideal	310 (27)	117 (30)	95 (25)	98 (26)	
PA level, n (%)					
Ideal	387 (34)	122 (32)	131 (34)	134 (35)	0.622
Non-ideal	764 (66)	262 (68)	253 (66)	249 (65)	
HDS, n (%)					
Non-ideal	1151 (100)	384 (100)	384 (100)	383 (100)	
TC, n (%)					
Ideal	809 (70)	253 (66)	263 (68)	293 (76)	0.004
Non-ideal	342 (30)	131 (34)	121 (32)	90 (24)	
BG, n (%)					
Ideal	429 (37)	133 (35)	146 (38)	150 (39)	0.402
Non-ideal	722 (63)	251 (65)	238 (62)	233 (61)	
BP, n (%)					
Ideal	1044 (91)	342 (89)	351 (91)	351 (92)	0.396
Non-ideal	107 (9)	42 (11)	33 (9)	32 (8)	

Data are expressed as percentages. Abbreviations: N number; CVH cardiovascular health, TPE total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine, T1 first tertile of TPE (< 85.8mg GAE/g creatinine), T2 second tertile of TPE (85.8-140.5 mg GAE/g creatinine), T3 third tertile of TPE (> 140.5 mg GAE/g creatinine), SS smoking status, BMI body mass index, PA physical activity, HDS healthy diet score, TC total cholesterol, BP blood pressure, BG blood glucose. Statistical analyses were undertaken using the chi-square test. P-values refer to differences between tertiles of TPE and are considered statistically significant when < 0.05.

Figures

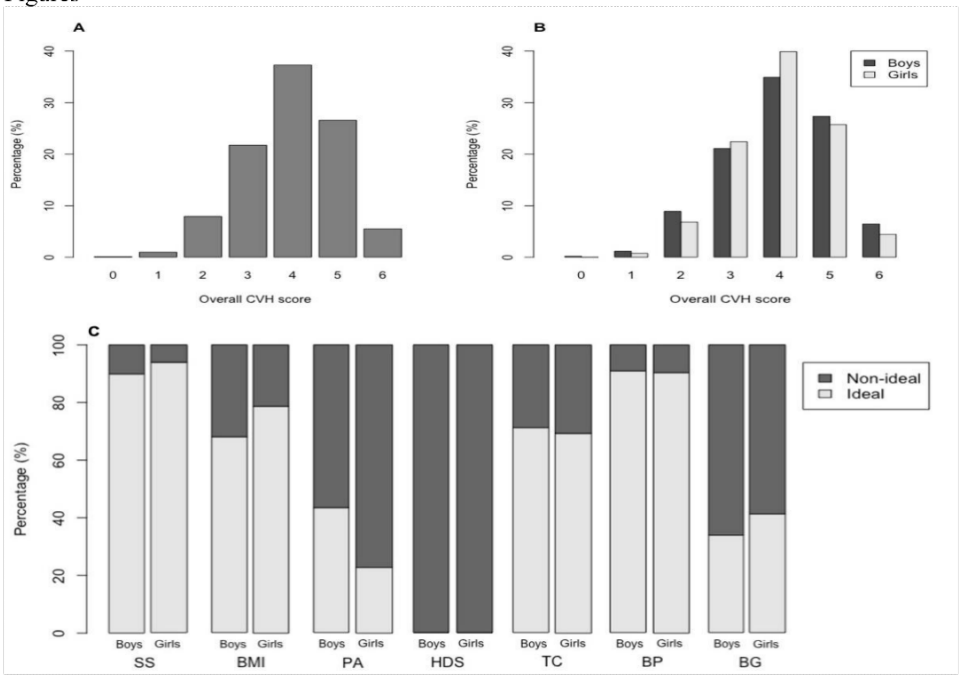

Figure S1. Prevalence of Cardiovascular Health (CVH) score, CVH behaviors and factors.

A: Ideal CVH score in all participants. B: Ideal CVH score by gender. C: Ideal CVH behaviors and factors. SS smoking status, BMI body mass index, PA physical activity, HDS healthy diet score, TC total cholesterol, BP blood pressure, BG blood glucose.

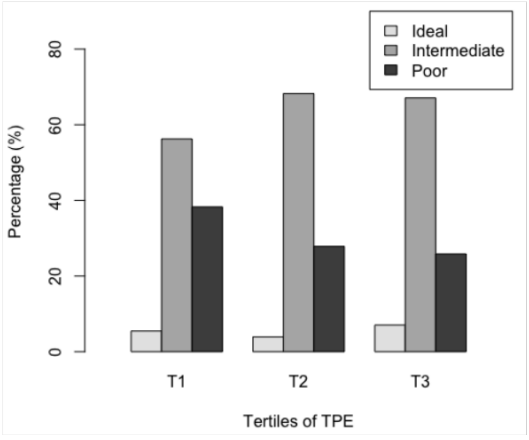

Figure S2. Cardiovascular Health (CVH) categories by tertiles of TPE.

TPE total polyphenol excretion expressed as mg gallic acid equivalent (GAE)/g creatinine, T1 first tertile of TPE (< 85.8mg GAE/g creatinine), T2 second tertile of TPE (85.8-140.5 mg GAE/g creatinine), T3: third tertile of TPE (> 140.5 mg GAE/g creatinine).

4.3. Validation of an analytical method to identify and quantify urinary phenolic metabolites

4.3.1. Article 6: Identification and quantification of urinary microbial phenolic metabolites by HPLC-ESI-LTQ-Orbitrap-HRMS and their relationship with dietary (poly)phenols in adolescents

Emily P Laveriano-Santos, María Marhuenda-Muñoz, Anna Vallverdú-Queralt, Miriam Martínez-Huélamo, Anna Tresserra-Rimbau, Elefterios Miliarakis, Camila Arancibia-Riveros, Olga Jáuregui, Ana María Ruiz-León, Sara Castro-Baquero, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Jesús Martínez-Gómez, Gloria Santos-Beneit, Juan M Fernández-Alvira, Rodrigo Fernández-Jiménez, Rosa M Lamuela-Raventós.

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Abstract

Introduction
High resolution mass spectrometry using an Orbitrap mass analyzer has been used to identification of phenolic metabolites, however, there are scare studies that used this approach to quantified microbial phenolic metabolites in urine in a large cohort in young population.

Objective
To develop and validate a liquid chromatography/electrospray ionization-linear ion trap quadrupole-Orbitrap-high-resolution mass spectrometry (HPLC/ESI-LTQ-Orbitrap-HRMS) method to identify and quantify urinary microbial phenolic metabolites (MPM), as well as to explore the relationship between MPM and dietary (poly)phenols in Spanish adolescents.

Methods
A total of 601 spot urine samples of adolescents aged 12.02 ± 0.41 years were analyzed. The quantitative method was validated for linearity, limit of detection, limit of quantification, recovery, intra- and inter-day accuracy and precision, as well as postpreparative stability according to the criteria established by the Association of Official Agricultural Chemists International.

Results

A total of 17 aglycones and 37 phase II MPM were identified and quantified in 601 spot urine samples. Phenolic acids were the most abundant urinary MPM, whereas stilbenes, hydroxytyrosol, and enterodiol were the least abundant. Urinary hydroxycoumarin acids (urolithins) were positively correlated with flavonoid and total (poly)phenol intake.

Conclusion

In summary, an HPLC-ESI-LTQ-Orbitrap-HRMS method was developed and fully validated to quantify MPM. The new method was performed accurately and is suitable for MPM quantification in large epidemiological studies. Urinary lignans and urolithins are proposed as potential biomarkers of grain and nut intake in an adolescent population.

antioxidants

MDPI

Article

Identification and Quantification of Urinary Microbial Phenolic Metabolites by HPLC-ESI-LTQ-Orbitrap-HRMS and Their Relationship with Dietary Polyphenols in Adolescents

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Abstract: This study aimed to develop and validate a liquid chromatography/electrospray ionization-linear ion trap quadrupole-Orbitrap-high-resolution mass spectrometry (HPLC/ESI-LTQ-Orbitrap-HRMS) method to identify and quantify urinary microbial phenolic metabolites (MPM), as well as to explore the relationship between MPM and dietary (poly)phenols in Spanish adolescents. A total of 601 spot urine samples of adolescents aged 12.02 ± 0.41 years were analyzed. The quantitative method was validated for linearity, limit of detection, limit of quantification, recovery, intra- and inter-day accuracy and precision, as well as postpreparative stability according to the criteria established by the Association of Official Agricultural Chemists International. A total of 17 aglycones and 37 phase II MPM were identified and quantified in 601 spot urine samples. Phenolic acids were the most abundant urinary MPM, whereas stilbenes, hydroxytyrosol, and enterodiol were the least abundant. Urinary hydroxycoumarin acids (urolithins) were positively correlated with flavonoid and total (poly)phenol intake. An HPLC-ESI-LTQ-Orbitrap-HRMS method was developed and fully validated to quantify MPM. The new method was performed accurately and is suitable for MPM quantification in large epidemiological studies. Urinary lignans and urolithins are proposed as potential biomarkers of grain and nut intake in an adolescent population.

Keywords: polyphenol; phytochemical; biomarker; microbiota; dietary antioxidants

1. Introduction

The beneficial health effects of dietary (poly)phenols have been reported in several epidemiological and clinical trials [1–3], although their biological activities are not all attributed to their native form. After ingestion, modification by phase I and II metabolic enzymes reduces the concentrations of native (poly)phenols in the systemic circulation [4,5]. More than 80% of dietary (poly)phenols are not absorbed in the small intestine and reach the colon, where they undergo conjugation and are metabolized by gut microbiota through a range of enzymatic reactions (deglycosylation, dehydroxylation, demethylation, deconjugation, epimerization, ring fission, hydrolysis, and chain-shortening) [5–7]. The microbial phenolic metabolites (MPM) may be more bioactive than the parental (poly)phenol when they reach the target cells or tissues [8–11]. Fewer studies have reported MPM in young populations, such as adolescents.

High-resolution mass spectrometry (HRMS) using an Orbitrap mass analyzer is a well-established method for rapid targeted and untargeted identification of (poly)phenols in nutrimetabolomics studies [12]. This equipment provides exact mass information, two-stage mass analysis (MS/MS), and multi-stage mass analysis (MS^n), which facilitates the structural elucidation of known and unknown compounds [12–15]. Therefore, HRMS constitutes a versatile and robust system for quantitative analysis [16–19]. However, to date, few methods are available to quantify MPM in human biological samples using high performance liquid chromatography (HPLC)/Orbitrap-HRMS.

The aim of this study was to develop and validate a high-performance liquid chromatography/electrospray ionization-linear ion trap quadrupole-Orbitrap-high-resolution mass spectrometry (HPLC/ESI-LTQ-Orbitrap-HRMS) method to identify and quantify urinary MPM in adolescents, and to explore the relationship of MPM with dietary (poly)phenols.

2. Materials and Methods

2.1. Study Design and Sample Selection

This work was carried out as a cross-sectional analysis within the SI! (Salud Integral) Program for Secondary Schools trial in Spain, a cluster-randomized controlled intervention trial (NCT03504059) aiming to evaluate the impact of a lifestyle educational program on cardiometabolic health in adolescents. A total 1326 participants were recruited in the baseline of the trial. Details of the study design, recruitment procedures, and Commission on Ethics are available elsewhere [20]. Informed consent was obtained for all the parents or caregivers.

For the current study, baseline data of 601 randomly chosen participants (53% girls) with available baseline urine samples were included, equivalent to 45% of the original cohort.

2.2. Chemicals and Urine Samples

The provenance of chemicals and standards is listed in the Supplemental data. Urine samples were collected in 2017 and stored at −80 °C until analysis.

2.3. Sample Preparation and Extraction of (Poly)Phenols

All the spot urine samples were analyzed in a room with filtered light and kept on ice to avoid phenolic oxidation, following the procedure proposed by Martínez-Huelamo et. al., with some modifications [21]. Firstly, 1 mL of urine was acidified with 2 µL of formic acid and centrifuged at 15,000× *g* at 4 °C for 4 min. After centrifugation, the urine underwent a solid-phase extraction (SPE) and clean-up procedure using Waters Oasis HLB 96-well plates 30 µm (30 mg) (Waters Oasis, Milford, MA, USA). Plates were activated by consecutively adding 1 mL of methanol (MeOH) and 1 mL of 1.5 M formic acid. After loading 1 mL of sample, clean-up was performed with 0.5 mL of 1.5 M formic acid and 0.5% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid.

The eluted fraction was evaporated to dryness under a stream of nitrogen gas in a sample concentrator (Techne, Duxford, Cambridge, UK) at room temperature, and reconstituted with 100 µL of 0.05% formic acid in water. The 96-well plate was then vortexed for 20 min and filtered through 0.22 µm polytetrafluoroethylene 96-well plate filters (Millipore, Burlington, MA, USA). To prepare calibration curves, synthetic urine was spiked with increasing concentrations of a mixture of 18 phenolic standards (3-hydroxybenzoic acid, 3-hydroxytyrosol, 3′-hydroxytyrosol-3′-glucuronide, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, enterodiol, enterolactone, urolithin-B, gallic acid, dihydroresveratrol, urolithin-A, 3,4-dihydroxyphenylpropionic acid, 3′-hydroxyphenylacetic acid, *o*-coumaric acid, *m*-coumaric acid, and *p*-coumaric acid) before being processed and subjected to the same extraction procedure exactly as the samples. Abscisic acid d6 was used as an internal standard.

Synthetic urine was used as a blank, composed of calcium chloride (0.65 g/L), magnesium chloride (0.65 g/L), sodium chloride (4.6 g/L), sodium sulfate (2.3 g/L), sodium citrate (0.65 g/L), dihydrogen phosphate (2.8 g/L), potassium chloride (1.6 g/L), ammonium chloride (1.0 g/L), urea (25 g/L), and creatinine (1.1 g/L) [22].

2.4. HPLC/ESI-LTQ-Orbitrap-HRMS Instrumentation

2.4.1. Chromatographic Conditions

Analysis was performed using an Accela chromatograph (Thermo Scientific, Hemel Hempstead, UK) equipped with a quaternary pump and a thermostated autosampler set at 4 °C, all operated by Chromeleon Xpress software. Chromatographic separation was accomplished with a reverse phase chromatographic column Kinetex F5 (50 × 4.6 mm i.d., 2.6 µm) (Phenomenex, Torrance, CA, USA) kept at 40 °C. Gradient elution was carried out with (A) water (0.05% formic acid) and (B) acetonitrile (0.05% formic acid) at a constant flow rate of 0.5 mL/min. The injection volume was 5 µL. A non-linear gradient was applied: 0 min, 2% B; 1 min, 2% B; 2.5 min, 8% B; 7 min, 20% B; 9 min, 30% B; 11 min, 50% B; 12 min, 70% B, 15 min, 100% B; 16 min, 100% B; 16.5 min, 2% B; 21.5 min, 2% B. The total run time was 21.5 min.

2.4.2. Mass Spectrometry Parameters

Accurate mass measurements were performed on an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) equipped with an ESI source working in negative mode. Mass spectra were acquired in profile mode with a setting of 30,000 resolution at m/z 400, and the mass range was from m/z 100 to 2000. Operation parameters were as follows: source voltage, 5 kV; sheath gas, 50 units; auxiliary gas, 20 units; sweep gas, 2 units, and capillary temperature, 375 °C.

2.5. Validation of the HPLC/ESI-LTQ-Orbitrap-HRMS Method

The method was validated following the criteria of the Association of Official Agricultural Chemists (AOAC) International in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, intra- and inter-day accuracy and precision, and postpreparative stability [23]. All parameters were examined based on three concentrations (low, medium, and high) of each phenolic compound standard, as shown in Table 1.

Table 1. Concentration levels of each phenolic standards for HPLC/ESI-LTQ-Orbitrap-HRMS method validation.

Phenolic Standards	Concentration Level (µg/L)		
	Low	Medium	High
Enterodiol	5	200	766
3′-Hydroxytyrosol-3′-glucuronide	5	200	766
3-Hydroxybenzoic acid	5	200	766

Table 1. *Cont.*

Phenolic Standards	Concentration Level (µg/L)		
	Low	Medium	High
3-Hydroxytyrosol	5	200	766
4-Hydroxybenzoic acid	5	200	766
Enterolactone	5	200	766
m-coumaric acid	5	200	766
p-coumaric acid	5	200	766
Protocatechuic acid	5	200	766
o-coumaric acid	5	200	766
Syringic acid	5	200	766
Urolithin-B	5	200	766
Vanillic acid	5	200	766
Dihydroresveratrol	12.5	500	1915
Gallic acid	12.5	500	1915
Urolithin-A	12.5	500	1915
3,4-Dihydroxyphenylpropionic acid	25	100	3830
3′-Hydroxyphenylacetic acid	50	2000	7660

HPLC high performance liquid chromatography, ESI electrospray ionization, LTQ linear ion trap quadrupol, Orbitrap-HRMS.

2.5.1. Linearity and Sensitivity

Calibration curves were prepared by spiking synthetic urine in triplicate using nine different concentrations of standard mixtures ranging from 1 to 1000 µg/L for 3-hydroxybenzoic acid, 3-hydroxytyrosol, 3′-hydroxytyrosol-3′-glucuronide, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, *m*-coumaric acid, *p*-coumaric acid, *o*-coumaric acid, enterodiol, enterolactone, and urolithin-B; 2.5 to 2500 µg/L for gallic acid, dihydroresveratrol, and urolithin-A; 5 to 5000 µg/L for 3,4-dihydroxyphenylpropionic acid; and 10 to 10,000 µg/L for 3′-hydroxyphenylacetic acid, with the internal standard (IS) (+)cis, trans-abscisic acid d6 (500 µg/L). Calibration curves were created by quadratic regression analysis with residual plots lower than 15%. The adequacy of the model and linearity were assessed by coefficient of determination (R^2).

LOD and LOQ were estimated for a signal-to-noise (S/N) ratio of 3 and 10, respectively.

2.5.2. Accuracy and Precision

Accuracy was determined by analyzing five replicates of spiked synthetic urine with three known concentrations (Table 1) to evaluate the closeness of agreement between the calculated amount and the nominal amount of analyte. The results were expressed as the percentage of the ratio of the mean concentration observed and the known spiked concentration in the biological matrices. Precision was calculated using relative standard deviation (RSD) between the five spiked urine samples at three different levels on three different days. Intra- and inter-day precision was assessed using five determinations per three concentration levels (Table 1) in a single analytical run or on three different days, respectively.

2.5.3. Recovery and Matrix Effect

Recovery and matrix effects (ME) were evaluated following the procedure described by Matuszewski et al., and Pereira-Caro et al. [19,24], analyzing three synthetic urines spiked at the three standard concentration levels (Table 1). Recoveries were calculated as the ratio between the area responses of standard concentration levels dissolved in pre-extracted samples and the analyte area responses of post-extracted urine spiked at the same concentrations. The results were expressed as recovery rate.

MEs were determined with the same concentration levels by comparing area responses of the spiked pre-extracted samples with the analyte area responses with neat standards

dissolved in the mobile phase. The results were expressed as percentages. ME values above 100% are considered to indicate ion enhancement, and below 100% ion suppression.

2.5.4. Stability

Postpreparative stability and freeze and thaw stability were assessed in this method. Postpreparative stability of the sample extraction process and during the time inside the autosampler at 4 °C were evaluated by injecting the post-extracted synthetic urine spiked with two standard concentrations (low and high) (Table 1) into the HPLC-ESI-LTQ-Orbitrap-HRMS system at 0 and 24 h. Freeze and thaw stability were assessed by injecting the post-extracted synthetic urine spiked at the same concentration levels into the HPLC-ESI-LTQ-Orbitrap-HRMS system after three freeze (−80 °C) and thaw (room temperature) cycles.

2.5.5. Selectivity

The selectivity of the method was assessed by comparing chromatograms of blank human urine from three individuals and urine spiked with analytes at a known low concentration to discriminate between analytes and other endogenous components in urine.

2.6. Analysis of Urinary MPM by HPLC/ESI-LTQ-Orbitrap-HRMS in Adolescent Samples

2.6.1. Targeted Identification of MPM

MPM were identified by comparing retention times with those of available standards. A semi-targeted screening method was established to identify phase II metabolites (glucuronides and sulfates) when reference standards were not available. The molecular formula of each compound was generated with an accurate mass and error of 5 ppm using the Xcalibur software v2.0.7 (Thermo Fisher Scientific, San Jose, CA, USA). Data acquisition techniques, including Fourier transform mass spectrometry (FTMS) mode (scan range from m/z 100–1000) in combination with product ion scan experiments (MS2) (Orbitrap resolution from 15,000 to 30,000 FWHM), were performed to obtain information about the m/z of precursor and fragment ions, retention time, and isotope pattern. Finally, analytes were confirmed by comparing MS/MS spectra with fragments found in the literature and The Human Metabolome Database 4.0 [25].

2.6.2. Quantification of MPM

Calibration curves were constructed with available standards in synthetic urine and subjected to the same procedure as described above. To quantify phase II metabolites (glucuronides and sulfates), calibration curves of the aglycon form were used. Samples with concentrations that exceeded the highest point of the calibration curve were diluted and reinjected into the HPLC-FTMS system. Quantitative data processing was performed using Trace Finder software (LC version 4.1, Thermo Fisher Scientific, San Jose, CA, USA).

MPM concentration was normalized by urinary creatinine concentrations, which were determined using the Jaffé alkaline picrate method adapted to microtiter 96-well plates [26] and expressed as μg MPM/g creatinine.

2.7. Dietary (Poly)Phenols

Dietary intake was estimated using a semiquantitative food frequency questionnaire [27]. Dietary (poly)phenol intake was assessed by matching data from the Phenol-Explorer database v.3.6. [28]. Flavonoids, phenolic acids, stilbenes, lignans, phenolic acids, tyrosols, and other minor (poly)phenols, such as alkylphenols and alkylmethoxyphenols, were included in this analysis. Total (poly)phenol intake was estimated as the sum of individual (poly)phenol intakes and categorized into tertiles. Energy-adjusted (poly)phenol intake was calculated by the residual method established by Willet et al. [29].

2.8. Data Analysis

General characteristics of the studied population are presented as means (standard deviation (SD)) or median (interquartile range (IQR)) for quantitative variables and percentages (number) for categorical variables. MPM concentrations are presented as the mean, standard error of the mean (SEM). Student's *t*-test was used to compare mean values of general characteristics between girls and boys, but also to compare MPM and postpreparative stability.

For the statistical analysis, MPM levels below the LOQ were set to values corresponding to half the LOQ. Pearson correlation was used to assess the relationship between urinary MPM and dietary (poly)phenols, as well as polyphenol-rich food sources. The false discovery rate (FDR) method was applied to adjust p-values for multiple correlations [30]. Data were normalized with the inverse normal distribution before the analysis.

The overall urinary MPM pattern and tertiles of total phenolic intake were assessed using principal component analysis (PCA) and presented as biplots in which eigenvectors were plotted as lines and the scores of individual samples as points. Beforehand, MPM data were standardized to unit variance.

Statistical analyses were conducted using the Stata statistical software package version 16.0 (StataCorp, College Station, TX, USA) and R v.4.1.1 (https://www.r-project.org, accessed on 1 April 2022). Statistical tests were two-sided, and p-values below 0.05 were considered significant.

3. Results and Discussion

3.1. Optimization of the HPLC/ESI-LTQ-Orbitrap-HRMS Method

Several SPEs solutions, as well as two SPE cartridges (Table S1 and Figure S1), were tested in order to obtain optimum recoveries. Two reverse-phase chromatographic columns were tested: Kinetex F5 (50 × 4.6 mm i.d., 2.6 µm) (Phenomenex, Torrance, CA, USA) and Atlantis T3 C18 (100 × 2.1 mm i.d., 3 µm) (Waters, Milford, MA, USA), obtaining better recoveries with Kinetex F5 and SPE 2 procedure (Figure S1). Different percentages of formic acid (from 0.05% to 0.1%) in mobile phases were tested to achieve desirable peak shapes and compound separation. The best results were obtained with 0.05% formic acid (data not shown). Two injection volumes (5 and 10 µL) were also tested to ensure optimum separation and detection of the analytes, and 5 µL sample injection gave the best results (data not shown). Details of the analytical conditions tested are available in the Supplemental Data.

3.2. Method Validation

3.2.1. Linearity, LOD, and LOQ

The HPLC/ESI-LTQ-Orbitrap-HRMS method provided quadratic responses with coefficients of determination (R^2) above 0.995 for all standards (Table S2). Weighted factors (1/x statistical weight) were used to obtain the most reliable calibration curves.

The sensitivity of the method was evaluated by determining the LOD and LOQ of a synthetic urine sample spiked with standards. The LOD ranged from 0.02 to 3.29 µg/L, and LOQ from 0.06 to 10.96 µg/L.

3.2.2. Precision and Accuracy

Intra- and inter-day precision varied in the ranges of 0−15% and 1−16%, respectively, in accordance with the values proposed by the AOAC (RDS < 15%) [23]. However, inter-day precision values for the lowest concentration of gallic acid, 3-hydroxytyrosol, and 3,4-dihydroxyphenylpropionic acid were 58, 31, and 26%, respectively (Table S3), possibly due to early elution, which leads to a lower resolution peak when the concentration is low. Pereira et al. reported an intra-day precision of less than 15% (0% to 10%) for flavan-3-ols and their metabolites in a study using ultra high-performance liquid chromatography (UHPLC)-HRMS [19].

The accuracy was within the accepted limits of the AOAC guidelines [23] at all tested concentration levels for 89% of the metabolites analyzed, ranging from 80 to 120%. However, at the lowest concentrations the inter-day accuracy of gallic acid, 3,4-dihydroxyphenylpropionic acid, *m*-coumaric acid, and urolithin-A fell outside this range (Table S3).

3.2.3. Matrix Effect and Recovery

The average ME was 83%, with ranges from 53% to 126%, except those of urolithins-A and -B, which were below 35%. Minor ion suppression was also reported by Ordoñez et al. and Pereira-Caro et al. [18,19]. Ion enhancement was observed for 4-hydroxybenzoic acid and 3-hydroxyphenyacetic acid (Table S3).

The average recovery of the three concentration levels was 89%, ranging between 70% and 99%. The lowest recovery was for gallic acid and 3-hydroxytyrosol, which was 70% at the lowest concentration (Table S3). Similarly, Ordoñez et al. reported a mean recovery of 73% of urinary (poly)phenols extracted by an HLB cartridge and using an HPLC-HRMS method, obtaining a good recovery rate of 79% to 104% for free phenolic and glucuronide derivatives [18]. Better recoveries were reported by Pereira-Caro et al., with values ranging from 95% to 102% for 34 flavan-3-ol and its metabolites in rat urine samples analyzed by UHPLC-HRMS [19].

3.2.4. Stability

The postpreparative stability assay showed no significant variation of analyte concentration in the urine matrix 24 h post-extraction at both low and high concentrations, except for 3-hydroxyphenylacetic acid, which was the analyte with the highest reduction (13%) (Figure S2). The freeze and thaw stability assay showed a signal decline of 14% for most analytes after the third freeze-thaw cycle. Likewise, Martínez-Huélamo et al., described a 12.9% reduction in signal for 3-hydroxyphenylacetic acid [21].

3.2.5. Selectivity

Selectivity was confirmed by the absence of endogenous peaks in chromatograms at the same retention time as the analytes in three human urine samples. The method was, therefore, found to be selective for analytes at low concentrations and was able to discriminate between analytes and other components in urine.

3.3. Microbial Phenolic Metabolites Measured in Urine Samples

3.3.1. General Characteristics of the Study Population

Out of the 601 randomized participants selected in this cross-sectional analysis, 546 had available information of food intake. The general characteristics of participants are presented in Table 2. The average age and body mass index (BMI) were 12.0 (0.4) years and 20.9 (4.2) kg/m^2, respectively. The mean energy-adjusted (poly)phenol intake was 683.5 (335.3) mg/day. No differences were observed between boys and girls in terms of BMI and total (poly)phenol intake (Figure S3). Higher mean intakes of energy (p-value = 0.002), carbohydrates (p-value = 0.001), total fat (p-value = 0.010), and proteins (p-value < 0.001) were observed in boys (Figure S3).

Table 2. General characteristics of the participants.

	N	Mean (SD)	Median (IQR)
Age, years	601	12.0 (0.4)	12.0 (0.0)
Body mass, kg	601	50.8 (12.2)	48.5 (14.8)
Height, cm	601	155.2 (6.9)	155.2 (9.2)
BMI, kg/m^2	601	20.9 (4.2)	20.1 (5.0)
BMI z-score	601	0.6 (1.0)	0.6 (1.4)

Table 2. *Cont.*

	N	Mean (SD)	Median (IQR)
Energy and nutrients intake			
Energy, kcal/day	546	2498.8 (579.6)	2474.9 (828.6)
Carbohydrates, g/day	546	132.1 (47.3)	124.3 (63.0)
Fiber, g/day	546	29.6 (10.6)	28.1 (13.5)
Fat, g/da	546	112.0 (32.8)	109.4 (41.6)
Protein, g/day	546	119.5 (32.3)	117.8 (42.1)
Energy-adjusted (poly)phenol intake			
Total (poly)phenol intake, mg/day	546	683.5 (335.3)	639.8 (354.9)
Flavonoids, mg/day	546	533.9 (310.3)	480.8 (298.8)
Stilbenes, mg/day	546	0.2 (0.3)	0.1 (0.2)
Tyrosols, mg/day	546	21.3 (13.7)	17.8 (12.6)
Lignans, mg/day	546	3.7 (4.1)	2.5 (2.5)
Phenolic acids, mg/day	546	94.9 (50.4)	89.2 (51.7)

BMI: body mass index, IQR: interquartile range, SD standard deviation Values are given as means (SD) and medians (IQR).

3.3.2. Identification and Quantification of Urinary MPM

Identification of MPM according to classes of (poly)phenols (lignans, hydroxybenzoic acids, hydroxycinnamic acids, hydroxyphenylacetic acids, hydroxyphenylpropanoic acids, stilbenes, hydroxycoumarins, and tyrosols) are presented in Table S4. A total of 54 MPM were identified in urine. Enterolactone and urolithin diglucuronides were determined only in one sample.

Concentrations of MPM are summarized in Table 3. Excretion of urinary MPM varied highly between participants, and the majority of MPM were detected in the form of glucuronides and sulfates. Consistent with our results, Ordónez et al. reported that an HPLC-HRMS method was suitable for the analysis of phase II metabolites [18]. The most abundant MPM in the urine of all participants were phenolic acids, namely 3-hydroxyphenylacetic acid, hydroxyphenylacetic sulfate and glucuronide, protocatechuic acid sulfate-I, 3,4-dihydroxyphenylpropionic acid sulfate, hydroxybenzoic acid sulfate, and vanillic acid sulfate. These results are in agreement with Zamora-Ros et al., who detected phenolic acids as the most abundant urinary MPM in adult participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) study [31]. Similarly, Hurtado-Barroso et al. found phenylacetic acids to be among the most abundant urinary MPM in young adults [32].

Table 3. Quantification of urinary MPM by HPLC-ESI-LTQ-Orbitrap-HRMS.

Urinary MPM, µg/g Creatinine	<LOQ (*n*)	Mean *	SEM *	CV *
Lignans				
Enterodiol [a]	136	4.5	0.9	1.0
Enterodiol glucuronide I (ED)	4	740.7	151.1	4.8
Enterodiol glucuronide II (ED)	3	209.2	67.8	5.1
Enterodiol sulfate (ED)	18	158.0	34.1	4.9
Enterolactone [a]	179	30.6	3.2	1.7
Enterolactone glucuronide (EL)	3	6984.5	419.2	1.5
Enterolactone sulfate (EL)	19	639.3	168.8	6.3
Phenolic acids—Hydroxybenzoic acids				
Gallic acid [a]	223	9.1	1.1	1.4
Gallic acid glucuronide (GA)	80	4.6	1.2	1.4
Gallic acid sulfate (GA)	87	22.8	1.5	1.3
3-Hydroxybenzoic acid [a]	206	113.4	41.1	5.6
4-Hydroxybenzoic acid [a]	1	824.5	157.8	4.6
Hydroxybenzoic acid glucuronide I (HBA)	229	33.4	4.2	1.3

Table 3. *Cont.*

Urinary MPM, µg/g Creatinine	<LOQ (*n*)	Mean *	SEM *	CV *
Hydroxybenzoic acid glucuronide II (HBA)	13	69.5	6.0	1.8
Hydroxybenzoic acid sulfate (HBA)	0	25,034.4	1607.6	1.6
Protocatechuic acid [a]	1	173.8	31.7	4.2
Protocatechuic acid glucuronide (PCA)	57	30.2	2.1	1.5
Protocatechuic acid sulfate I (PCA)	3	33,703.3	5368.2	3.8
Protocatechuic acid sulfate II (PCA)	0	228.0	41.8	3.6
Syringic acid [a]	4	99.6	6.7	1.3
Syringic acid glucuronide I (SA)	0	297.6	26.8	2.0
Syringic acid glucuronide II (SA)	2	181.0	53.5	3.4
Syringic acid sulfate (SA)	32	249.9	26.9	1.8
Vanillic acid [a]	0	1027.5	198.9	3.5
Vanillic acid glucuronide I (VA)	16	6847.5	857.4	2.5
Vanillic acid glucuronide II (VA)	2	3795.8	1038.3	4.7
Vanillic acid sulfate (VA)	1	17,227.2	1610.6	2.2
Phenolic acids—Hydroxycinnamic acids				
m-Coumaric acid [a]	38	69.9	11.8	2.8
o-Coumaric acid [a]	42	15.8	2.4	1.6
p-Coumaric acid [a]	16	23.4	2.3	1.6
Coumaric acid glucuronide I	18	36.5	2.8	1.6
Coumaric acid glucuronide II	162	20.4	1.7	1.2
Coumaric acid glucuronide III	11	72.4	8.8	2.7
Coumaric acid sulfate I	39	46.8	9.2	2.6
Coumaric acid sulfate II	13	240.4	75.8	6.1
Coumaric acid sulfate III	5	788.7	208.6	5.3
Phenolic acids—Hydroxyphenylacetic acids				
3-Hydroxyphenylacetic acid [a]	13	40,797.6	3248.4	1.8
Hydroxyphenylacetic acid glucuronide (3-HPAA)	122	13,860.5	4363.6	5.3
Hydroxyphenylacetic acid sulfate (3-HPAA)	22	45,815.5	6160.0	2.4
Phenolic acids—Hydroxyphenylpropanoic acids				
3,4-dihydroxyphenylpropionic acid [a]	25	132.8	17.1	2.0
Dihydroxyphenylpropionic acid sulfate (3,4-DHPPA)	1	30,942.7	2700.1	2.0
Stilbenes				
Dihydroresveratrol [a]	78	3.3	0.5	0.5
Dihydroresveratrol sulfate I (DHR)	4	753.5	57.8	1.8
Dihydroresveratrol sulfate II (DHR)	47	991.6	379.0	5.2
Other polyphenols—Hydroxycoumarins				
Urolithin A [a]	57	1338.1	270.3	2.4
Urolithin A glucuronide (Uro A)	41	3030.2	482.1	2.7
Urolithin A sulfate (Uro A)	26	801.0	399.9	3.5
Urolithin B [a]	86	1334.4	1067.1	4.4
Urolithin B glucuronide (Uro B)	63	3062.8	1565.1	6.1
Other polyphenols-Tyrosols				
3-Hydroxytyrosol [a]	143	9.1	0.9	0.6
3′hydroxytyrosol-3′-glucuronide [a]	71	62.4	28.9	7.5
Hydroxytyrosol sulfate (3-HT)	5	398.0	88.8	5.0

3,4-DHPPA 3,4-dihydroxyphenylpropionic acid, 3-HPAA 3-hydroxyphenylacetic acid, 3-HBA 3-hydroxybenzoic acid, 3-HT 3-hydroxytyrosol, 3-HT-G 3-hydroxytyrosol glucuronide, 4-HBA 4-hydroxybenzoic acid, DHRSV dihydroresveratrol, ED enterodiol, EL enterolactone, GA gallic acid, PCA protocatechuic acid, SA syringic acid, Uro A urolithin A, Uro B urilithin B, VA vanillic acid, LOQ limit of quantification, SEM mean standard error, CV coefficient of variance. When standards were not available, aglycone was used for quantification. The molecule used for the quantification is shown in brackets. [a] Commercial standards. * Data obtained from samples with microbial phenolic metabolites quantified by HPLC-ESI-LTQ-Orbitrap-HRMS. This table does not include data below the LOQ or non-detected compounds.

Urinary concentrations of stilbenes (dihydroresveratrol), tyrosols (3-hydroxytyrosol), and lignans (enterodiol) were low, with mean values below 10 µg/g of creatinine. Those reported by Zamora-Ros et al. in adults from the EPIC study were also low, being less than 5 µg/24 h [31]. These levels could be explained by a low dietary intake of stilbenes, tyrosols, and lignans, as reported in the food frequency questionnaire.

A high percentage of participants had a urinary MPM concentration below the LOQ for hydroxybenzoic acid glucuronide-I (38%), gallic acid (37%), 3-hydroxybenzoic acid (34%), enterolactone (30%), coumaric acid glucuronide-II (27%), 3-hydroxytyrosol (24%), and enterodiol (23%).

Interindividual variations in MPM could be explained by the gut microbiota profile, which is affected by age, gender, hormonal status, dietary habits, and other lifestyle

variables [33]. In this study, the gut microbiota profile was not analyzed and thus the influence of the microbial family on MPM production was not determined.

Differences in urinary MPM between boys and girls are shown in Figure 1. Boys had higher values of 3,4-dihydroxyphenylpropionic, dihydroxyphenylpropionic sulfate, gallic acid, gallic acid sulfate, p-coumaric acid, vanillic acid glucuronide and sulfate, hydroxybenzoic acid glucuronide-I and sulfate-I, protocatechuic acid sulfate, 3-hydroxyphenylacetic acid and hydroxyphenylacetic acid glucuronide and sulfate than girls. Our findings are in line with those of Zamora et al., who observed that the median urinary concentrations of tyrosol, vanillic acid, and 4-hydroxyphenylacetic acid were at least 1.4-fold higher in men than women [31]. Similarly, Mumford et al., found higher values of enterodiol and enterolactone in females than males [34]. As sex hormones may be responsible for these differences [8,35], a limitation of the current study is that the follicular phase of the menstrual cycle was not considered during the urine collection to minimize bias related to the hormonal status of the participants.

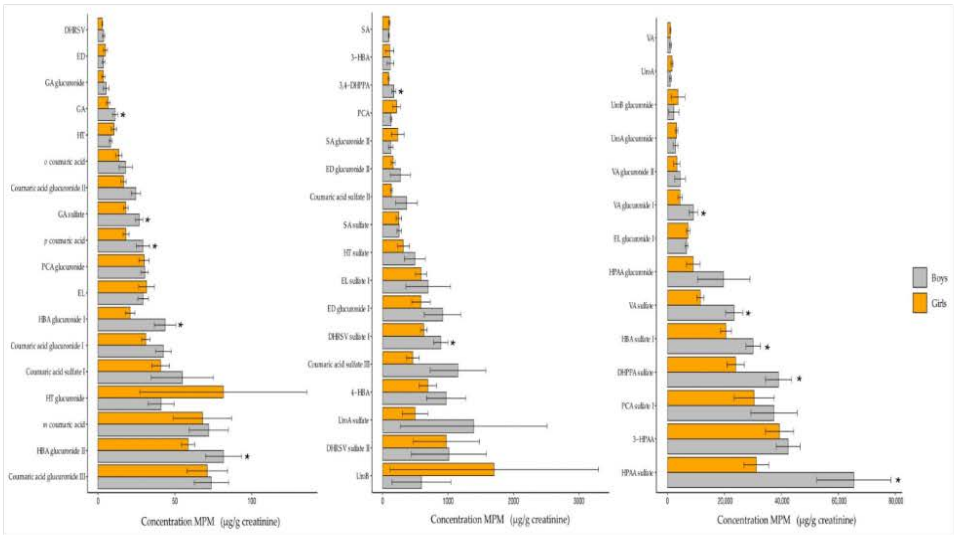

Figure 1. Urinary MPM of adolescents by gender. 3,4-DHPPA 3,4-dihydroxyphenylpropionic acid, 3-HPAA 3-hydroxyphenylacetic acid, 3-HBA 3-hydroxybenzoic acid, 3-HT 3-hydroxytyrosol, 3-HT-G 3-hydroxytyrosol glucuronide, 4-HBA 4-hydroxybenzoic acid, DHRSV dihydroresveratrol, ED enterodiol, EL enterolactone, GA gallic acid, PCA protocatechuic acid, SA syringic acid, Uro-A urolithin A, uro-B urilithin B, VA vanillic acid. Bar graphs are plotted as the mean (SEM). * p-values < 0.05 from t-test analysis.

3.3.3. Urinary MPM and Dietary (Poly)Phenols

No differences were found between classes of urinary MPM and tertiles of total phenolic intake in the PCA (Figure S4). However, positive correlations were observed between urinary hydroxycoumarins (urolithins) and flavonoid intake and TPI (Figure 2). Additionally, positive correlations were observed between urinary lignans and intake of whole grains (R = 0.13, FDR-adjusted p = 0.007) and green-leaf vegetables (R = 0.13, FDR-adjusted p = 0.008). Urinary hydroxycinnamic acids also correlated with whole grains (R = 0.11, FDR-adjusted p = 0.015), green-leaf vegetables (R = 0.15, FDR-adjusted p = 0.002), and tomato or tomato-based products (R = 0.12, FDR-adjusted p = 0.011) (Figure S5). Urolithins are produced by gut microbiota through the metabolism of ellagitannins [11,36], whose main food sources are red fruits, nuts, and seeds [36], but in our study, urolithins were only positively correlated with nuts and seeds (R = 0.13, FDR-adjusted p = 0.014).

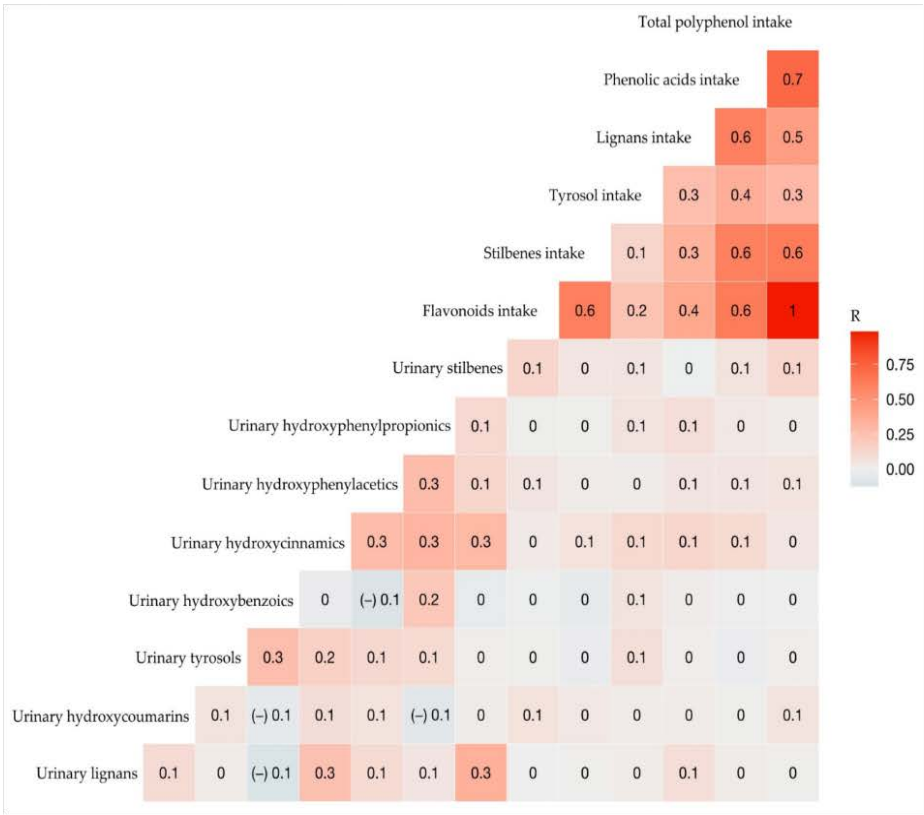

Figure 2. Heatmap of the Pearson correlation between subclasses of urinary MPM and energy-adjusted (poly)phenol intake in adolescents.

4. Conclusions

In conclusion, an HPLC-ESI-LTQ-Orbitrap-HRMS method was developed and fully validated to quantify urinary MPM in terms of linearity, sensitivity, recovery, accuracy, and precision. To our knowledge, this is the first time that several MPM have been identified and quantified in urine samples of an adolescent population using an HPLC-ESI-LTQ-Orbitrap-HRMS method on a large scale. Variations in MPM were observed between participants, which were associated with variability in dietary (poly)phenol intake and sex. Finally, some MPM were found to be potential dietary biomarkers of specific food groups, namely lignans for whole grains and urolithins for nuts. Further investigations are needed to explore the relationship between MPM and dietary sources of (poly)phenols.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11061167/s1. Standards and chemicals. Analytical condition testing before validation HPLC/ESI-LTQ-Orbitrap-HRMS method. Figure S1: Recovery obtained according to different solid phase extraction and reverse-phase chromatographic columns (Kinetex F5 (50 × 4.6 mm i.d., 2.6 µm) and Atlantis T3 C18 (100 × 2.1 mm i.d., 3 µm). Figure S2: Postpreparative stability. Mean concentrations (µg/L) of phenolic compounds recovered at the start (t = 0) and at 24 h with two standard concentrations prepared in synthetic urine. Figure S3: General characteristics of participants according to gender. Figure S4: Principal component (PC) biplot of subclass of microbial phenolic metabolites (MPM) according to tertiles of total polyphenol intake (n = 546). Figure S5: Heatmap of the Pearson correlation between subclass of microbial phenolic metabolites and polyphenol-rich food intake in adolescents. Table S1: Recovery obtained in Oasis HLB and

PRiMe HLB. Table S2: Validation data: Linearity ranges, coefficient of determination, and low limits of detection and quantification of microbial phenolic metabolite. Table S3: Intra- and inter-day precision and accuracy, matrix effect and recovery results for three concentration levels (high, medium, and low); RSD (%) was calculated for the recovery values for three replicates. Table S4: Identification of microbial phenolic metabolites in urine by HPLC/ESI-LTQ-Orbitrap-HRMS. Table S5: Pearson correlation coefficients between microbial phenolic metabolites and dietary polyphenols in adolescents. References [37,38] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, A.V.-Q., M.M.-H., E.P.L.-S., M.M.-M., O.J. and R.M.L.-R.; methodology, A.V.-Q., M.M.-H., E.P.L.-S., M.M.-M. and O.J.; software, E.P.L.-S. and M.M.-M.; validation, E.P.L.-S., M.M.-M., A.V.-Q., M.M.-H. and R.M.L.-R.; formal analysis E.P.L.-S., M.M.-M. and A.V.-Q.; investigation, E.P.L.-S., M.M.-M., A.V.-Q. and R.M.L.-R.; sources, R.M.L.-R.; data curation, E.P.L.-S., M.M.-M., A.V.-Q., O.J. and E.M.; writing-original draft preparation, E.P.L.-S., M.M.-M., A.V.-Q., A.T.-R. and R.M.L.-R.; writing-review and editing, E.P.L.-S., M.M.-M., A.V.-Q., M.M.-H., C.A.-R., A.T.-R., R.M.L.-R., O.J., S.C.-B., A.M.R.-L., R.E., R.F.-J., J.M.F.-A., G.S.-B., M.d.M., P.B., A.d.C.-G. and J.M.-G.; visualization, E.P.L.-S., M.M.-M. and A.V.-Q.; supervision, A.V.-Q., M.M.-H. and R.M.L.-R. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all parents or caregivers of the participants involved in the SI! (Salud Integral) Program for Secondary Schools trial.

Data Availability Statement: There are restrictions on the availability of the data for the SI! Program study due to signed consent agreements around data sharing, which only allow access to external researcher for studies following project purposes. Requesters wishing to access the database used in this study can make a request to the Steering Committee (SC) chair: gsantos@fundacionshe.org, rodrigo.fernandez@cnic.es, juanmiguel.fernandez@cnic.es, RESTRUCH@clinic.cat, lamuela@ub.edu, bibanez@cnic.es, vfuster@cnic.es. For the present study, the database was requested from the SC on 24 February 2022.

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Abbreviations

AOAC	Association of Official Agricultural Chemists
BMI	body mass index
ESI	electrospray ionization
FDR	false discovery rate
FTMS	Fourier transform mass spectrometry
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
IQR	interquartile range
LOD	limit of detection
LOQ	limit of quantification
LTQ	linear ion trap quadrupole
MeOH	methanol
ME	matrix effect
MPM	microbial phenolic metabolites
MS/MS	two-stage mass analysis
MS^n	multi-stage mass analysis
PCA	principal component analysis
R^2	coefficient of determination
RSD	relative standard deviation
SD	standard deviation
SEM	standard error of mean
S/N	signal-to-noise
SPE	solid-phase extraction
TPI	total (poly)phenol intake
UHPLC	ultra-high performance liquid chromatography

References

1. Tresserra-Rimbau, A.; Guasch-Ferré, M.; Salas-Salvadó, J.; Toledo, E.; Corella, D.; Castañer, O.; Guo, X.; Gómez-Gracia, E.; Lapetra, J.; Arós, F.; et al. Intake of Total Polyphenols and Some Classes of Polyphenols Is Inversely Associated with Diabetes in Elderly People at High Cardiovascular Disease Risk. *J. Nutr.* **2015**, *146*, 767–777. [CrossRef]
2. Sun, Q.; Wedick, N.M.; Tworoger, S.S.; Pan, A.; Townsend, M.K.; Cassidy, A.; Franke, A.A.; Rimm, E.B.; Hu, F.B.; van Dam, R.M. 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. *J. Nutr.* **2015**, *145*, 1280–1288. [CrossRef]
3. Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M.-I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *N. Engl. J. Med.* **2018**, *378*, e34. [CrossRef]
4. Marhuenda-Muñoz, M.; Laveriano-Santos, E.P.; Tresserra-Rimbau, A.; Lamuela-Raventós, R.M.; Martínez-Huélamo, M.; Vallverdú-Queralt, A. Microbial Phenolic Metabolites: Which Molecules Actually Have an Effect on Human Health? *Nutrients* **2019**, *11*, 2725. [CrossRef]
5. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food Sources and Bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747. [CrossRef]
6. Duarte, L.; Gasaly, N.; Poblete-Aro, C.; Uribe, D.; Echeverria, F.; Gotteland, M.; Garcia-Diaz, D.F. Polyphenols and Their Anti-Obesity Role Mediated by the Gut Microbiota: A Comprehensive Review. *Rev. Endocr. Metab. Disord.* **2021**, *22*, 367–388. [CrossRef]
7. Bohn, T. Dietary Factors Affecting Polyphenol Bioavailability. *Nutr. Rev.* **2014**, *72*, 429–452. [CrossRef]
8. Fan, Y.; Qian, H.; Wu, Z.; Li, Z.; Li, X.; Zhang, Y.; Xu, Q.; Lu, C.; Wang, X. Exploratory Analysis of the Associations between Urinary Phytoestrogens and Thyroid Hormones among Adolescents and Adults in the United States: National Health and Nutrition Examination Survey 2007–2010. *Environ. Sci. Pollut. Res.* **2022**, *29*, 2974–2984. [CrossRef]

9. Gaya, P.; Medina, M.; Sánchez-Jiménez, A.; Landete, J. Phytoestrogen Metabolism by Adult Human Gut Microbiota. *Molecules* **2016**, *21*, 1034. [CrossRef]
10. Perez-Cornago, A.; Appleby, P.N.; Boeing, H.; Gil, L.; Kyrø, C.; Ricceri, F.; Murphy, N.; Trichopoulou, A.; Tsilidis, K.K.; Khaw, K.-T.; et al. Circulating Isoflavone and Lignan Concentrations and Prostate Cancer Risk: A Meta-Analysis of Individual Participant Data from Seven Prospective Studies Including 2,828 Cases and 5,593 Controls. *Int. J. Cancer* **2018**, *143*, 2677–2686. [CrossRef]
11. Selma, M.V.; González-Sarrías, A.; Salas-Salvadó, J.; Andrés-Lacueva, C.; Alasalvar, C.; Örem, A.; Tomás-Barberán, F.A.; Espín, J.C. 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. *Clin. Nutr.* **2018**, *37*, 897–905. [CrossRef]
12. López-Yerena, A.; Domínguez-López, I.; Vallverdú-Queralt, A.; Pérez, M.; Jáuregui, O.; Escribano-Ferrer, E.; Lamuela-Raventós, R.M. Metabolomics Technologies for the Identification and Quantification of Dietary Phenolic Compound Metabolites: An Overview. *Antioxidants* **2021**, *10*, 846. [CrossRef]
13. Eliuk, S.; Makarov, A. Evolution of Orbitrap Mass Spectrometry Instrumentation. *Annu. Rev. Anal. Chem.* **2015**, *8*, 61–80. [CrossRef]
14. Vallverdú-Queralt, A.; Jáuregui, O.; Medina-Remón, A.; Andrés-Lacueva, C.; Lamuela-Raventós, R.M. Improved Characterization of Tomato Polyphenols Using Liquid Chromatography/Electrospray Ionization Linear Ion Trap Quadrupole Orbitrap Mass Spectrometry and Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2986–2992. [CrossRef]
15. Sasot, G.; Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Mercader-Martí, M.; Estruch, R.; Lamuela-Raventós, R.M. Identification of Phenolic Metabolites in Human Urine after the Intake of a Functional Food Made from Grape Extract by a High Resolution LTQ-Orbitrap-MS Approach. *Food Res. Int.* **2017**, *100*, 435–444. [CrossRef]
16. Grund, B.; Marvin, L.; Rochat, B. Quantitative Performance of a Quadrupole-Orbitrap-MS in Targeted LC–MS Determinations of Small Molecules. *J. Pharm. Biomed. Anal.* **2016**, *124*, 48–56. [CrossRef]
17. Henry, H.; Sobhi, H.R.; Scheibner, O.; Bromirski, M.; Nimkar, S.B.; Rochat, B. Comparison between a High-Resolution Single-Stage Orbitrap and a Triple Quadrupole Mass Spectrometer for Quantitative Analyses of Drugs. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 499–509. [CrossRef]
18. Ordóñez, J.L.; Pereira-Caro, G.; Ludwig, I.; Muñoz-Redondo, J.M.; Ruiz-Moreno, M.J.; Crozier, A.; Moreno-Rojas, J.M. A Critical Evaluation of the Use of Gas Chromatography- and High Performance Liquid Chromatography-Mass Spectrometry Techniques for the Analysis of Microbial Metabolites in Human Urine after Consumption of Orange Juice. *J. Chromatogr. A* **2018**, *1575*, 100–112. [CrossRef]
19. Pereira-Caro, G.; Ordóñez, J.L.; Ludwig, I.; Gaillet, S.; Mena, P.; del Rio, D.; Rouanet, J.-M.; Bindon, K.A.; Moreno-Rojas, J.M.; Crozier, A. Development and Validation of an UHPLC-HRMS Protocol for the Analysis of Flavan-3-Ol Metabolites and Catabolites in Urine, Plasma and Feces of Rats Fed a Red Wine Proanthocyanidin Extract. *Food Chem.* **2018**, *252*, 49–60. [CrossRef]
20. Fernandez-Jimenez, R.; Santos-Beneit, G.; Tresserra-Rimbau, A.; Bodega, P.; de Miguel, M.; de Cos-Gandoy, A.; Rodríguez, C.; Carral, V.; Orrit, X.; Haro, D.; et al. Rationale and Design of the School-Based SI! Program to Face Obesity and Promote Health among Spanish Adolescents: A Cluster-Randomized Controlled Trial. *Am. Heart J.* **2019**, *215*, 27–40. [CrossRef]
21. Martínez-Huélamo, M.; Tulipani, S.; Jáuregui, O.; Valderas-Martinez, P.; Vallverdú-Queralt, A.; Estruch, R.; Torrado, X.; Lamuela-Raventós, R.M. Sensitive and Rapid UHPLC-MS/MS for the Analysis of Tomato Phenolics in Human Biological Samples. *Molecules* **2015**, *20*, 20409–20425. [CrossRef]
22. Roura, E.; Andrés-Lacueva, C.; Estruch, R.; Lamuela-Raventós, R.M. Total Polyphenol Intake Estimated by a Modified Folin–Ciocalteu Assay of Urine. *Clin. Chem.* **2006**, *52*, 749–752. [CrossRef]
23. Lynch, J.; Horwitz, W.; Latimer, G.W. *Appendix E: Laboratory Quality Assurance*; Official Methods of Analysis of AOAC International: Washington, DC, USA, 2005; Available online: http://www.eoma.aoac.org/app_e.pdf (accessed on 1 January 2018).
24. Matuszewski, B.K.; Constanzer, M.L.; Chavez-Eng, C.M. Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC−MS/MS. *Anal. Chem.* **2003**, *75*, 3019–3030. [CrossRef]
25. Wishart, D.S.; Feunang, Y.D.; Marcu, A.; Guo, A.C.; Liang, K.; Vázquez-Fresno, R.; Sajed, T.; Johnson, D.; Li, C.; Karu, N.; et al. HMDB 4.0: The Human Metabolome Database for 2018. *Nucleic Acids Res.* **2018**, *46*, D608–D617. [CrossRef]
26. Medina-Remón, A.; Barrionuevo-González, A.; Zamora-Ros, R.; Andres-Lacueva, C.; Estruch, R.; Martínez-González, M.-Á.; Diez-Espino, J.; Lamuela-Raventos, R.M. Rapid Folin–Ciocalteu Method Using Microtiter 96-Well Plate Cartridges for Solid Phase Extraction to Assess Urinary Total Phenolic Compounds, as a Biomarker of Total Polyphenols Intake. *Anal. Chim. Acta* **2009**, *634*, 54–60. [CrossRef]
27. Juton, C.; Castro-barquero, S.; Casas, R.; Freitas, T.; Ruiz-león, A.M.; Crovetto, F.; Domenech, M.; Crispi, F.; Vieta, E.; Gratacós, E.; et al. Reliability and Concurrent and Construct Validity of a Food Frequency Questionnaire for Pregnant Women at High Risk to Develop Fetal Growth Restriction. *Nutrients* **2021**, *13*, 1629. [CrossRef]
28. Neveu, V.; Perez-Jiménez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D.; et al. Phenol-Explorer: An Online Comprehensive Database on Polyphenol Contents in Foods. *Database* **2010**, *2010*, bap024. [CrossRef]
29. Willett, W.C.; Howe, G.R.; Kushi, L.H. Adjustment for Total Energy Intake in Epidemiologic Studies. *Am. J. Clin. Nutr.* **1997**, *65*, 1220S–1228S; discussion 1229S–1231S. [CrossRef]

30. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B* **1995**, *57*, 289–300. [CrossRef]
31. Zamora-Ros, R.; Achaintre, D.; Rothwell, J.A.; Rinaldi, S.; Assi, N.; Ferrari, P.; Leitzmann, M.; Boutron-Ruault, M.-C.; Fagherazzi, G.; Auffret, A.; et al. Urinary Excretions of 34 Dietary Polyphenols and Their Associations with Lifestyle Factors in the EPIC Cohort Study. *Sci. Rep.* **2016**, *6*, 26905. [CrossRef]
32. Hurtado-Barroso, S.; Quifer-Rada, P.; Marhuenda-Muñoz, M.; Rinaldi de Alvarenga, J.F.; Tresserra-Rimbau, A.; Lamuela-Raventós, R.M. Increase of 4-Hydroxybenzoic, a Bioactive Phenolic Compound, after an Organic Intervention Diet. *Antioxidants* **2019**, *8*, 340. [CrossRef]
33. Liu, C.; Vervoort, J.; Beekmann, K.; Baccaro, M.; Kamelia, L.; Wesseling, S.; Rietjens, I.M.C.M. Interindividual Differences in Human Intestinal Microbial Conversion of (-)-Epicatechin to Bioactive Phenolic Compounds. *J. Agric. Food Chem.* **2020**, *68*, 14168–14181. [CrossRef]
34. Mumford, S.L.; Sundaram, R.; Schisterman, E.F.; Sweeney, A.M.; Barr, D.B.; Rybak, M.E.; Maisog, J.M.; Parker, D.L.; Pfeiffer, C.M.; Buck Louis, G.M. Higher Urinary Lignan Concentrations in Women but Not Men Are Positively Associated with Shorter Time to Pregnancy. *J. Nutr.* **2014**, *144*, 352–358. [CrossRef]
35. Campesi, I.; Marino, M.; Cipolletti, M.; Romani, A.; Franconi, F. Put "Gender Glasses" on the Effects of Phenolic Compounds on Cardiovascular Function and Diseases. *Eur. J. Nutr.* **2018**, *57*, 2677–2691. [CrossRef]
36. Espín, J.C.; Larrosa, M.; García-Conesa, M.T.; Tomás-Barberán, F. Biological Significance of Urolithins, the Gut Microbial Ellagic Acid-Derived Metabolites: The Evidence So Far. *Evid.-Based Complement. Altern. Med.* **2013**, *2013*, 270418. [CrossRef]
37. Martínez-Huélamo, M.; Tulipani, S.; Torrado, X.; Estruch, R.; Lamuela-Raventós, R.M. Validation of a New LC-MS/MS Method for the Detection and Quantification of Phenolic Metabolites from Tomato Sauce in Biological Samples. *J. Agric. Food Chem.* **2012**, *60*, 4542–4549. [CrossRef]
38. Quifer-Rada, P.; Martínez-Huélamo, M.; Jáuregui, O.; Chiva-Blanch, G.; Estruch, R.; Lamuela-Raventós, R.M. Analytical Condition Setting a Crucial Step in the Quantification of Unstable Polyphenols in Acidic Conditions: Analyzing Prenylflavanoids in Biological Samples by Liquid Chromatography-Electrospray Ionization Triple Quadruple Mass Spectrometry. *Anal. Chem.* **2013**, *85*, 5547–5554. [CrossRef]

Identification and quantification of urinary microbial phenolic metabolites by HPLC-ESI-LTQ-Orbitrap-HRMS and their relationship with dietary polyphenols in adolescents.

Supplementary Data

Table of contents

Standards and chemicals

Gallic acid, 3-hydroxytyrosol, protocatechuic acid, 4-hydroxybenzoic acid, 3,4-dihydroxyphenylpropionic acid, 3′-hydroxyphenylacetic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, enterodiol, urolithin-A, and urolithin-B were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3′-hydroxytyrosol-3′-glucuronide, dihydroresveratrol, and (+)*cis, trans*-abscisic acid D_6 were obtained from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA). 3-hydroxybenzoic acid, vanillic acid, syringic acid, enterolactone, and creatinine were purchased from Fluka (St. Louis, MO, USA). Standards were stored in powder form and protected from light. Methanol (MeOH) of LC-MS and acetonitrile (MeCN) of HPLC grade were obtained from Sigma-Aldrich (St. Louis, MO, USA), formic acid (≥ 98 %) from Panreac Química S.A. (Barcelona, Spain), and ultrapure water (Milli-Q) from a Millipore system (Bedford, USA).

Analytical condition testing before validation HPLC/ESI-LTQ-Orbitrap-HRMS method

- **Solid phase extraction (SPE) cartridge selection**

  Two different chemical cartridges, Waters Oasis HBL (hydrophilic-lipophilic-balance cartridge) 96-well plates 30 µm (30 mg) and Oasis PRiME HLB 96-well plates 3 mg, for the extraction of 3-hydroxybenzoic acid, 3-hydroxytyrosol, 3'-hydroxytyrosol-3'-glucuronide, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, enterodiol, enterolactone, urolithin-B, gallic acid, dihydroresveratrol, urolithin-A, 3,4-dihydroxyphenylpropionic acid, 3'-hydroxyphenylacetic acid, *o*-coumaric acid, *m*-coumaric acid, and *p*-coumaric acid. Synthetic urine was spiked with 1000 µg/L of phenolic standards, and 500 µg/L of internal standard. Oasis HLB procedure was performed using the method previously describe by Martínez-Huélamo et al. for the analysis of polyphenols and their metabolites in urine samples, with some modifications [1]. Briefly, cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 0.1% formic acid. After loading 1 mL of acidified sample, clean-up was performed with 1 mL of 0.1% formic acid and 0.5% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.1% formic acid in water. To assess extraction recovery, blank synthetic urine extract spiked after SPE were also prepared at the same concentration (Table S1).

- **Optimization of SPE using different solutions**

  After the selection of Waters Oasis HLB 96-well plates 30 µm (30 mg) as cartridge for the SPE, four different SPE procedures were tested to obtain higher extraction of 3-hydroxybenzoic acid, 3-hydroxytyrosol, 3'-hydroxytyrosol-3'-glucuronide, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, enterodiol, enterolactone, urolithin-B, gallic acid, dihydroresveratrol, urolithin-A, 3,4-dihydroxyphenylpropionic acid, 3'-hydroxyphenylacetic acid, *o*-coumaric acid, *m*-coumaric acid, and *p*-coumaric acid.

  Synthetic urine was spiked with 400 µg/L of phenolic standards and internal standard. These procedures were performed using the methods previously described by Martínez-Huélamo et al. and Quifer-Rada [1–3], with some modifications, following the next extractions:

  - *SPE 1:* cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 0.1% formic acid. After loading 1 mL of acidified sample, clean-up was performed with 1 mL of 0.1% formic acid and 0.5% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.
  - *SPE 2:* cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 1.5 M formic acid. After loading 1 mL of sample, clean-up was performed with 0.5 mL of 1.5 M formic acid and 0.5% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.
  - *SPE 3:* cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 1.5 M formic acid. After loading 1 mL of sample, clean-up was performed with 0.5 mL of 1.5 M formic acid and 0.5 mL of 5% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.
  - *SPE 4:* cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 1% formic acid. After loading 1 mL of acidified sample, clean-up was performed with 1 mL of 1% formic acid and 2% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.

- **Chromatographic column selection**

  Two different reverse-phase chromatographic columns were tested: Kinetex F5 (50 x 4.6 mm i.d., 2.6 µm) (Phenomenex, Torrance, CA, USA) and Atlantis T3 C18 (100 x 2.1 mm i.d., 3 µm) (Waters, Milford, MA, USA) (Figure S1).

Figures

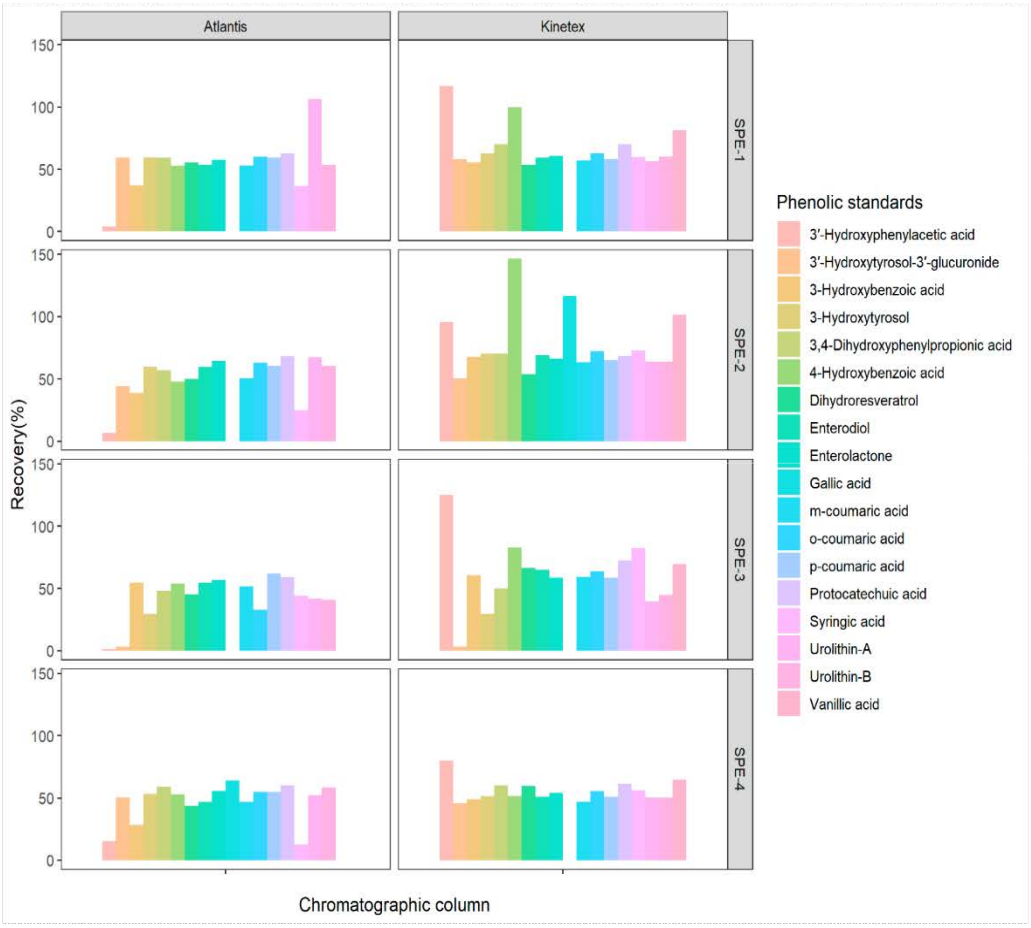

Figure S1. Recovery obtained according to different solid phase extraction and reverse-phase chromatographic columns (Kinetex F5 (50 x 4.6 mm i.d., 2.6 µm) and Atlantis T3 C18 (100 x 2.1 mm i.d., 3 µm).

SPE-1: cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 0.1% formic acid. After loading 1 mL of acidified sample, clean-up was performed with 1 mL of 0.1% formic acid and 0.5% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.

SPE-2: cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 1.5 M formic acid. After loading 1 mL of sample, clean-up was performed with 0.5 mL of 1.5 M formic acid and 0.5% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.

SPE-3: cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 1.5 M formic acid. After loading 1 mL of sample, clean-up was performed with 0.5 mL of 1.5 M formic acid and 0.5 mL of 5% MeOH, and the elution with 1 mL MeOH acidified with

0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.

SPE-4: cartridges were activated with 1 mL of methanol (MeOH) and 1 mL of 1% formic acid. After loading 1 mL of acidified sample, clean-up was performed with 1 mL of 1% formic acid and 2% MeOH, and the elution with 1 mL MeOH acidified with 0.1% of formic acid. The eluted fraction was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of 0.05% formic acid in water.

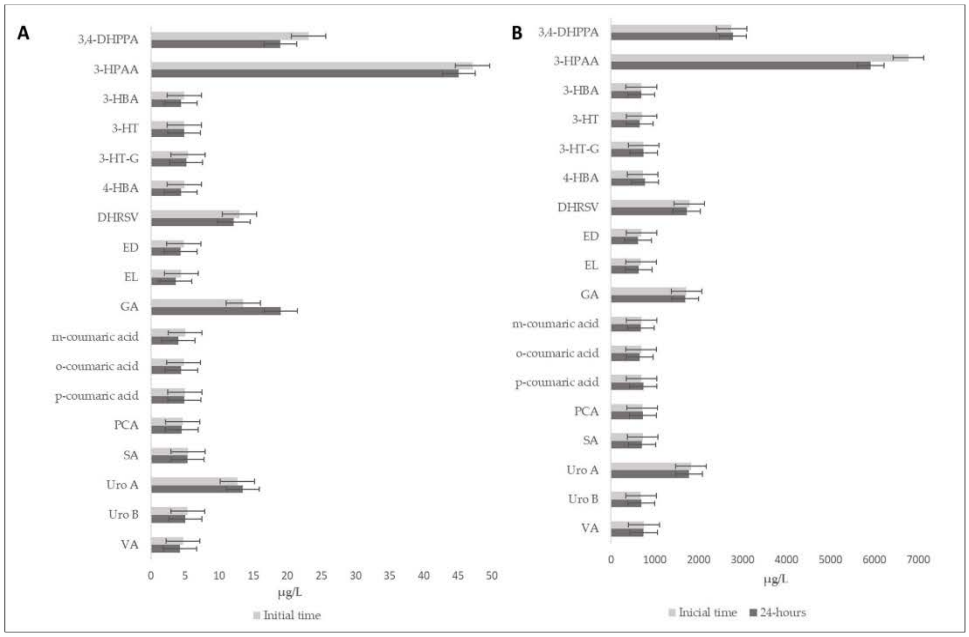

Figure S2. Postpreparative stability. Mean concentrations (µg/L) and SEM of phenolic compounds recovered at 0 h (t = 0) and at 24 h with two standard concentrations prepared in synthetic urine. **A**: stability of the low concentration *(5 µg L⁻ for 3-HBA, 3-HT, 3-HT-G, PCA, 4-HBA, VA, SA, p,m,o-coumaric acids, ED, EL, Uro B; 12.5µg L⁻¹ for GA, DHRSV, and Uro A; 25 µg L⁻¹ for 3,4-DHPPA, 50 µg L⁻¹ for 3-HPAA).* **B**: stability of the high concentration *(766 µg L⁻ for 3-HBA, 3-HT, 3-HT-G, PCA, 4-HBA, VA, SA, p,m,o-coumaric acids, ED, EL, Uro B; 1915µg L⁻¹ for GA, DHRSV, and Uro A; 3830 µg L⁻¹ for 3,4-DHPPA, 7660 µg L⁻¹ for 3-HPAA).* 3,4-DHPPA 3,4-dihydroxyphenylpropionic acid, 3-HPAA 3-hydroxyphenylacetic acid, 3-HBA 3-hydroxybenzoic acid, 3-HT 3-hydroxytyrosol, 3-HT-G 3-hydroxytyrosol glucuronide, 4-HBA 4-hydroxybenzoic acid, DHRSV dihydroresveratrol, ED enterodiol, EL enterolactone, GA gallic acid, PCA protocatechuic acid, SA syringic acid, Uro A urolithin A, Uro B urilithin B, VA vanillic acid.

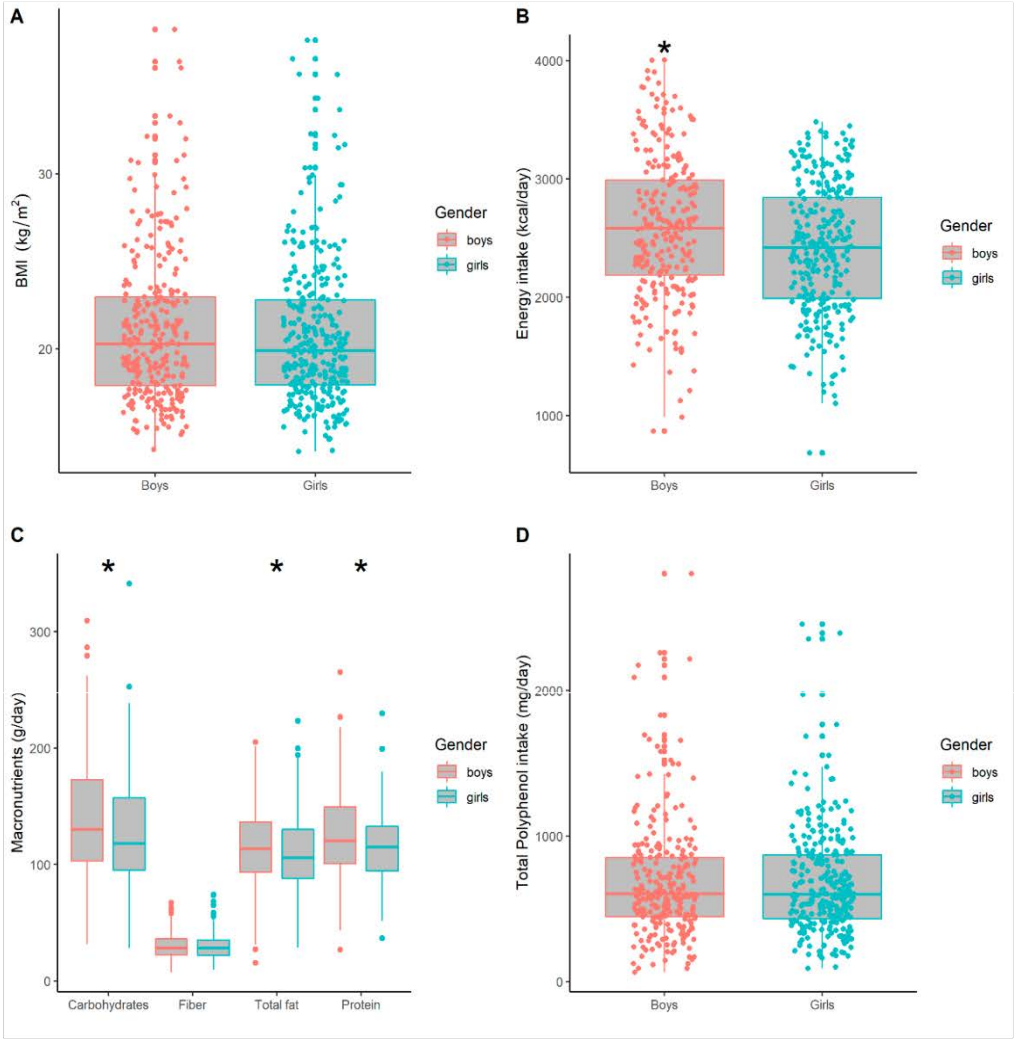

Figure S3. Boxplot of general characteristics of participants according to gender.

BMI body mass index.**A**: BMI (kg/m²), **B**: Total Energy Intake (kcal/day), **C**: Macronutrients (g/day), **D**: Total polyphenol intake (mg/day).* *p*-values < 0.05 from T-test analysis.

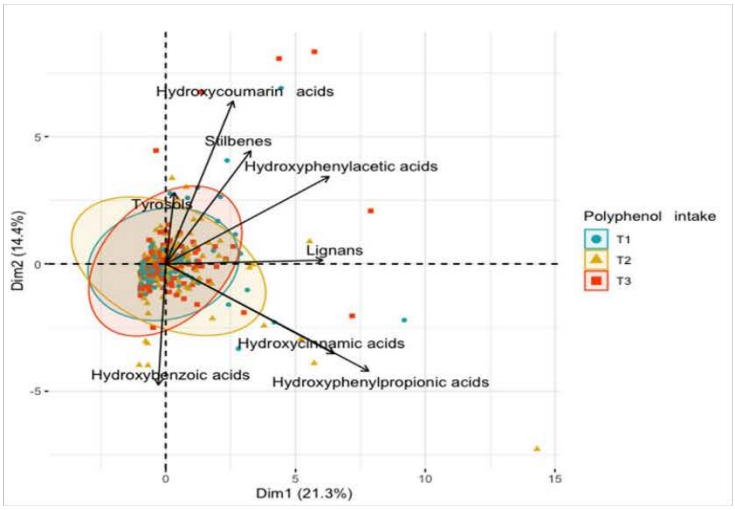

Figure S4. Principal Component (PC) biplot of subclass of microbial phenolic metabolites (MPM) according to tertiles of total polyphenol intake (*n*=546).

PC1, accounting for 21.33% of the total variance, included lignans; and PC2, accounting for 14.40%, included hydroxycoumarin acids, hydroxybenzoic acids, tyrosols, and stilbenes.

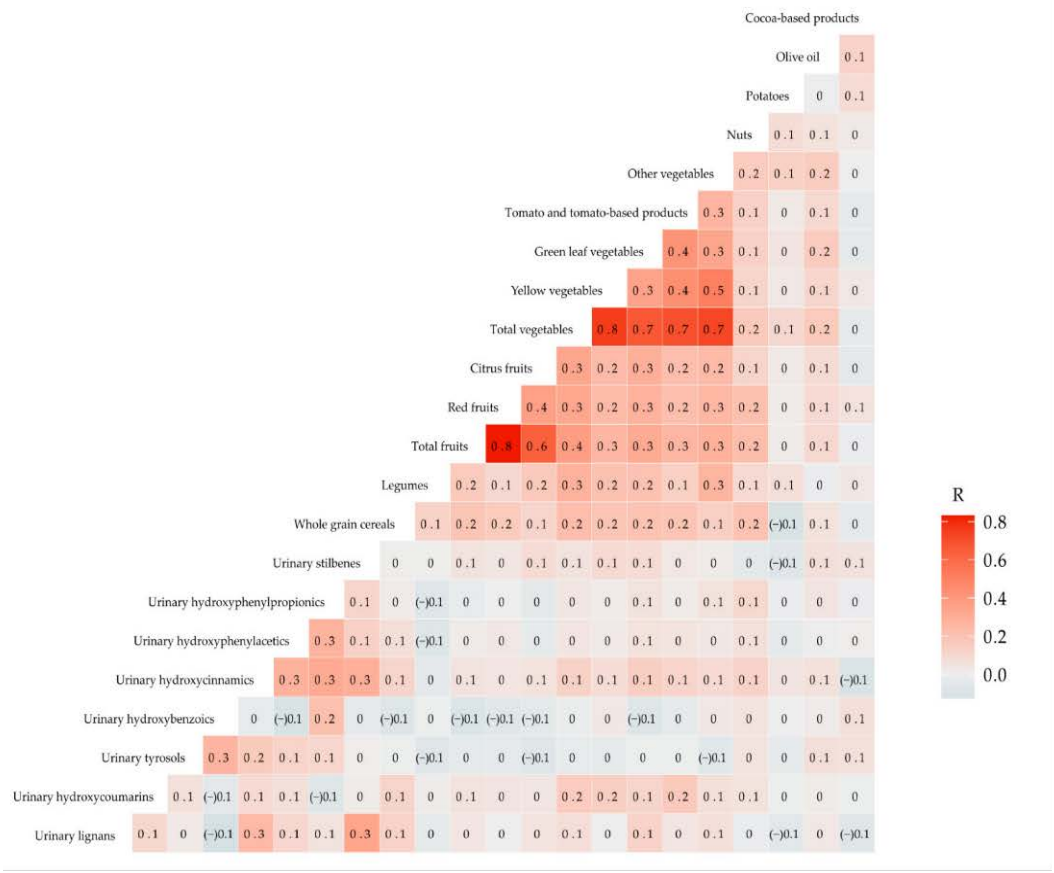

Figure S5. Heatmap of Pearson correlation between subclass of microbial phenolic metabolites and polyphenol-rich food group intake in adolescents.

Table S1. Recovery obtained in Oasis HLB and PRiME HLB.

Phenolic standard	Recovery (%) HLB	Recovery (%) PRiME HLB
Dihydroresveratrol	75%	71%
3,4-Dihydroxyphenylpropionic acid	62%	55%
Enterodiol	69%	76%
Enterolactone	69%	81%
Gallic acid	94%	84%
3-Hydroxybenzoic acid	89%	85%
4-Hydroxybenzoic acid	83%	75%
3′-Hydroxyphenylacetic acid	79%	77%
3-Hydroxytyrosol	86%	78%
3′-Hydroxytyrosol-3′-glucuronide	83%	75%
m-coumaric acid	85%	73%
o-coumaric acid	106%	101%
p-coumaric acid	89%	88%
Protocatechuic acid	98%	88%
Syringic acid	105%	87%
Urolithin-A	96%	58%
Urolithin-B	68%	40%
Vanillic acid	82%	68%

HBL (hydrophilic-lipophilic-balance cartridge). After the selection of Waters Oasis HLB 96-well plates 30 µm (30 mg) as cartridge for the SPE due its higher recovery, four different SPE procedures were tested to obtain higher extraction of phenolic standards. More details are available in "Analytical condition testing before validation HPLC/ESI-LTQ-Orbitrap-HRMS method" from this supplementary data.

Table S2. Characteristics of the validation of the HPLC/ESI-LTQ-Orbitrap-HRMS method: Linearity ranges, coefficient of determination, and low limits of detection and quantification of microbial phenolic metabolites.

Compounds	Rt (min)	Linearity Range (μg/L)	Calibration Curve Equation	R^2	LOD (μg/L)	LOQ (μg/L)
Gallic acid	1.69	2.5–2500	$Y=1875.73x-0.381799x^2+14499.5$	0.9988	0.35	1.16
3-Hydroxytyrosol	2.11	1–1000	$Y = 1582.99x-0.627282x^2+3434.06$	0.9984	0.13	0.43
3′-Hydroxytyrosol-3′-glucuronide	2.11	1–1000	$Y=2284.9x-1.07371x^2+3850.89$	0.9982	0.19	0.64
Protocatechuic acid	2.25	1–1000	$Y = 2456.96x-1.16492x^2+5769.12$	0.9979	0.04	0.13
4-Hydroxybenzoic acid	3.15	1–1000	$Y = 1603.96x-0.383033x^2+5829.04$	0.9992	0.06	0.20
3,4-dihydroxyphenylpropionic acid	3.84	5–5000	$Y = 1584.35x-0.185698x^2+26132.5$	0.9975	0.33	1.11
3-Hydroxybenzoic acid	4.02	1–1000	$Y = 1390.37x-0.660198x^2+937.023$	0.9972	0.21	0.71
3′-Hydroxyphenylacetic acid	4.09	10–100000	$Y = 126.989x-0.00834377x^2-169.942$	0.9979	3.29	10.96
Vanillic acid	4.74	1–1000	$Y = 453.431x-0.023629x^2+304.713$	0.9977	0.18	0.61
Syringic acid	5.63	1–1000	$Y = 1000.29x-0.180888x^2-981.368$	0.9984	0.16	0.53
p-Coumaric acid	5.96	1–1000	$Y = 3148.49x-1.46337x^2+1029.2$	0.9989	0.11	0.36
m-Coumaric acid	6.54	1–1000	$Y = 2498.93x-1.00467x^2+10900.8$	0.9990	0.10	0.32
o-Coumaric acid	7.20	1–1000	$Y = 2340.75x-1.03397x^2-432.812$	0.9988	0.23	0.77
Dihydroresveratrol	8.73	2.50-2500	$Y = 678.148x-0.0970142x^2-1417.62$	0.9992	0.56	1.85
Enterodiol	9.17	1–1000	$Y = 3931.4x-1.75738x^2+5384.13$	0.9979	0.05	0.18
Urolithin A	9.56	2.50–2500	$Y = 484.123x-0.0277372x^2-3593.79$	0.9994	0.61	2.03
Enterolactone	10.80	1–1000	$Y = 4229.75x-1.81222x^2+16770$	0.9956	0.02	0.06
Urolithin B	11.12	1–1000	$Y = 1661.47x-0.424359x^2-3176.34$	0.9990	0.18	0.58

Rt retention time, R^2 coefficient of determination, LOD limit of detection, LOQ limit of quantification.

Table S3. Intra- and inter-day precision and accuracy, matrix effect and recovery results for three concentration levels (high, medium, and low); RSD (%) was calculated for the recovery values for three replicates.

Compound	Concentration (µg/L)	Intra-day assay		Inter-day assay		Matrix effect average (%) (CV)	Recovery Average (%) (CV)
		Precision (RSD%)	Accuracy (%)	Precision (RSD%)	Accuracy (%)		
Gallic acid	**12.5**	**7.4**	113.4	58.1	30.8	65.8 (4.6)	70.1 (4.8)
	500	8.8	116.6	13.7	102.6	84.2 (0.7)	88.1 (2.3)
	1915	11.7	87.3	13.3	86.3	88.9 (0.4)	94.0 (1.1)
3-Hydroxytyrosol	5	3.9	99.8	31.1	81.8	116.5 (0.2)	70.1 (1.5)
	200	6.6	101.3	10.1	91.3	88.5 (0.1)	78.1 (6.8)
	766	11.0	90.8	5.2	90.9	94.3 (0.8)	89.6 (4.2)
3′-Hydroxytyrosol-3′-glucuronide	5	4.3	107.8	15.5	92.4	80.4 (1.8)	98.7 (1.7)
	200	6.1	102.9	0.6	102.2	101.8 (1.7)	82.5 (11.2)
	766	8.1	90.4	5.6	90.9	95.1 (0.2)	87.4 (4.6)
Protocatechuic acid	5	5.0	90.2	11.9	86.5	81.5 (0.3)	84.1 (0.6)
	200	3.9	101.4	8.6	106.0	90.5 (1.6)	88.3 (2.7)
	766	7.0	90.9	9.2	96.2	91.8 (0.2)	93.9 (4.2)
4-Hydroxybenzoic acid	5	0.7	99.1	2.6	101.8	125.8 (0.0)	78.1 (0.7)
	200	4.0	96.6	5.2	100.5	101.5 (1.5)	86.7 (3.5)
	766	6.3	97.4	4.9	101.8	99.1 (0.7)	90.5 (1.8)
3,4-dihydroxyphenylpropionic acid	25	8.1	95.1	26.6	13.6	100.9 (3.6)	71.7 (2.7)
	1000	7.2	117.9	7.2	113.5	98.4 (0.9)	92.9 (0.4)
	3830	10.7	81.3	2.2	83.2	95.8 (1.3)	96.4 (2.2)
3-Hydroxybenzoic acid	5	5.3	97.9	4.6	93.1	88.9 (1.4)	104.0 (1.8)
	200	5.7	103.4	3.6	104.4	89.1 (1.4)	95.5 (8.6)
	766	4.3	91.5	3.1	88.5	93.6 (0.1)	96.2 (2.9)
3-Hydroxyphenylacetic acid	50	2.8	94.8	2.0	92.6	91.3 (3.3)	80.2 (1.8)
	2000	3.3	101.1	9.0	111.5	114.0 (0.2)	83.5 (1.3)
	7660	1.7	88.3	8.4	80.9	101.6 (1.7)	94.2 (0.0)
Vanillic acid	5	0.4	94.4	14.3	86.7	110.8 (0.5)	96.0 (4.2)
	200	4.4	94.4	4.4	93.9	91.5 (1.2)	91.1 (6.9)
	766	6.2	97.8	2.3	96.1	96.1 (2.2)	91.9 (10.4)
Syringic acid	5	2.6	110.5	6.5	106.8	77.5 (3.6)	104.5 (0.4)
	200	4.2	99.1	5.2	97.5	93.1 (0.0)	88.8 (4.2)

	766	7.1	97.4	3.5	94.8	91.3 (0.3)	97.6 (2.0)
p-Coumaric acid	5	2.3	99.2	8.6	94.6	83.2 (0.2)	101.4 (0.8)
	200	3.2	101.7	6.9	105.8	93.5 (0.8)	90.8 (3.0)
	766	5.7	89.6	6.0	93.2	101.8 (0.3)	95.5 (1.1)
m-Coumaric acid	5	4.3	98.3	4.3	63.9	110.8 (0.5)	73.8 (0.2)
	200	0.9	100.0	4.7	100.8	94.7 (0.7)	87.4 (1.4)
	766	7.8	93.1	1.2	94.0	94.2 (0.2)	97.6 (3.2)
o-Coumaric acid	5	0.7	95.5	10.5	89.2	75.5 (2.6)	95.0 (0.8)
	200	2.1	101.5	4.6	104.0	87.5 (1.3)	85.8 (0.1)
	766	5.5	92.7	2.2	93.1	89.5 (0.1)	96.2 (0.4)
Dihydroresveratrol	12.5	1.4	105.0	10.2	106.5	60.7 (5.1)	79.1 (1.3)
	500	2.9	100.8	11.7	101.4	66.6 (5.8)	83.0 (4.5)
	1915	8.7	98.3	9.9	100.3	79.0 (1.7)	91.4 (2.8)
Enterodiol	5	5.6	96.9	10.3	87.0	74.1 (6.3)	97.4 (6.9)
	200	3.6	103.2	8.8	98.2	91.6 (0.2)	86.8 (5.3)
	766	10.6	88.0	3.0	85.2	94.2 (0.9)	95.9 (2.1)
Urolithin A	12.5	14.5	112.1	15.4	136.2	7.8 (2.7)	79.9 (3.4)
	500	14.4	92.4	14.6	105.7	17.7 (5.3)	96.4 (3.8)
	1915	8.8	98.8	5.7	101.0	27.7 (8.9)	106.5 (8.6)
Enterolactone	5	4.9	89.8	12.3	100.9	53.9 (2.1)	101.0 (1.1)
	200	4.7	105.6	6.8	110.2	72.5 (0.2)	95.9 (0.2)
	766	9.2	86.2	10.9	90.8	76.2 (3.1)	93.4 (3.6)
Urolithin B	5	5.2	113.1	14.6	117.8	23.7 (9.3)	85.7 (9.0)
	200	4.8	98.3	10.9	108.8	34.0 (4.7)	85.7 (4.3)
	766	10.8	95.3	13.8	105.5	47.2 (5.9)	79.7 (13.2)

RSD relative standard deviation, CV coefficient of variation.

Table S4. Identification of microbial phenolic metabolites in urine by HPLC/ESI-LTQ-Orbitrap-HRMS.

Compound	Neutral Molecular Formula	R_t (min)	Ion mass [M-H]-		mDa error	MS^2 fragment ions [M-H]-
			Theoretical	Experimental		
Lignans - Lignans						
Enterodiol [a]	C18H22O4	9.17	301.1434	301.1434	0.00	271.1334
Enterodiol glucuronide I (ED)	C24H30O10	7.48	477.1755	477.1751	0.40	459.1652, 301.1442, 175.0244
Enterodiol glucuronide II (ED)	C24H30O10	7.61	477.1755	477.1748	0.70	459.1644, 348.1826, 301.1436, 175.0242
Enterodiol sulfate (ED)	C18H20O7S	7.50	381.0996	381.1005	−0.90	382.1031, 301.1442
Enterolactone [a]	C18H18O4	10.80	297.1121	297.1114	0.70	253.12275, 217.0500, 107.0499
Enterolactone glucuronide (EL)	C24H26O10	8.77	473.1442	473.1434	0.80	455.1339, 343.0944, 297.1128, 175.0243
Enterolactone diglucuronide (EL)	C30H34O16	7.24	649.1763	649.1783	−2.00	473.1469, 297.1135
Enterolactone sulfate (EL)	C18H18O7S	8.57	377.0683	377.0690	−0.70	297.1131
Phenolic acids-Hydroxybenzoic acids						
Gallic acid [a]	C7H6O5	1.69	169.0131	169.0141	−1.00	125.0243
Gallic acid glucuronide (GA)	C13H14O11	1.85	345.0452	345.0447	0.50	169.0141, 125.0242
Gallic acid sulfate (GA)	C7H6O8S	1.72	248.9693	248.9700	−0.70	230.0651,204.9810, 169.0141
3- hydroxybenzoic acid [a]	C7H6O3	4.02	137.0233	137.0244	−1.10	93.0342
4-hydroxybenzoic acid [a]	C7H6O3	3.15	137.0233	137.0247	−1.40	93.0342
Hydroxybenzoic acid glucuronide I (HBA)	C13H14O9	2.50	313.0554	313.0548	0.60	175.0244, 137.0241, 93.0342
Hydroxybenzoic acid glucuronide II (HBA)	C13H14O9	2.00	313.0554	313.0551	0.30	295.0821,175.0245, 137.0242, 93.0136
Hydroxybenzoic acid sulfate (HBA)	C7H6O6S	1.89	216.9795	216.9809	−1.40	137.0241, 172.9908, 93.0341
Protocatechuic acid [a]	C7H6O4	2.25	153.0182	153.0197	−1.50	109.029
Protocatechuic acid glucuronide (PCA)	C13H14O10	1.67	329.0503	329.0498	0.50	153.0189, 175.0189, 134.0469
Protocatechuic acid sulfate I (PCA)	C7H6O7S	1.75	232.9744	232.9753	−0.90	153.0191, 188.9856, 109.0290, 96.9596
Protocatechuic acid sulfate II (PCA)	C7H6O7S	1.94	232.9744	232.9754	−1.00	233.9755, 214.9653, 153.0034
Syringic acid [a]	C9H10O5	5.63	197.0444	197.0459	−1.50	153.0557, 121.0295
Syringic acid glucuronide I (SA)	C15H18O11	4.68	373.0765	373.0760	0.50	355.1039, 329.1321, 197.0456, 175.0249
Syringic acid glucuronide II (SA)	C15H18O11	4.88	373.0765	373.0755	1.00	354.0912, 329.1267, 197.0817, 175.0246
Syringic acid sulfate (SA)	C9H10O8S	4.24	277.0006	277.0008	−0.20	197.0455, 182.0219, 167.0715
Vanillic acid [a]	C8H8O4	4.74	167.0339	167.0354	−1.50	123.0450
Vanillic acid glucuronide I (VA)	C14H16O10	2.76	343.0660	343.0651	0.90	175.0245, 167.0347, 123.0448
Vanillic acid glucuronide II (VA)	C14H16O10	2.31	343.0660	343.0650	1.00	175.0245, 167.0348, 131.9496

Vanillic acid sulfate (VA)	C8H8O7S	2.03	246.9910	246.9910	0.00	203.0017, 167.0344, 123.0450
Phenolic acids - Hydroxycinnamic acids						
m-coumaric acid [a]	C9H8O3	6.54	163.0390	163.0405	−1.50	119.0500
o-coumaric acid [a]	C9H8O3	7.20	163.0390	163.0403	−1.30	119.0500
p-coumaric acid [a]	C9H8O3	5.96	163.0390	163.0405	−1.30	119.0500
Coumaric acid glucuronide I	C15H16O9	4.77	339.0711	339.0710	0.10	321.0609, 175.0245, 163.0368
Coumaric acid glucuronide II	C15H16O9	5.09	339.0711	339.0707	0.40	295.0391, 175.0247, 163.0400, 119.0500
Coumaric acid glucuronide III	C15H16O9	5.82	339.0711	339.0709	0.20	321.0612, 295.1294, 175.0250, 163.0403, 119.0502
Coumaric acid sulfate I	C9H8O6S	3.53	242.9952	242.9953	−0.10	199.0068, 163.0403, 119.0501, 96.9599
Coumaric acid sulfate II	C9H8O6S	4.27	242.9952	242.9962	−1.00	199.0034, 163.0398, 119.0493
Coumaric acid sulfate III	C9H8O6S	4.74	242.9952	242.9965	−1.30	199.0062, 163.0399, 119.0497
Phenolic acids - Hydroxyphenylacetic acids						
3-hydroxyphenylacetic acid [a]	C8H8O3	4.09	151.0390	151.0401	−1.10	107.0208
Hydroxyphenylacetic acid glucuronide (3-HPAA)	C14H16O9	4.8	327.0711	327.0709	0.20	309.0609, 175.0243, 151.0397
Hydroxyphenylacetic acid sulfate (3-HPAA)	C8H8O6S	2.24	230.9952	230.9963	−1.10	151.0399, 108.0208
Phenolic acids - Hydroxyphenylpropanoic acids						
3,4-dihydroxyphenylpropionic acid [a]	C9H10O4	3.84	181.0495	181.0508	−1.30	137.0603
Dihydroxyphenylpropionic acid sulfate (3,4-DHPPA)	C9H10O7S	2.52	261.0057	261.0066	−0.90	217.0160, 181.0504, 137.0603
Stilbenes						
Dihydroresveratrol [a]	C14H14O3	8.73	229.0865	229.0874	−0.90	185.0819
Dihydroresveratrol sulfate I (DHR)	C14H14O6S	7.07	309.0427	309.0430	−0.30	245.0817, 229.0871, 193.0504, 123.0449
Dihydroresveratrol sulfate II (DHR)	C14H14O6S	7.40	309.0427	309.0430	−0.30	245.0810, 229.0861,175.0235
Other polyphenols - Hydroxycoumarins						
Urolithin A [a]	C13H8O4	9.56	227.0339	227.0353	−1.40	183.0047
Urolithin A glucuronide (Uro A)	C19H16O10	6.98	403.0659	403.0666	−0.70	385.0551, 227.0344, 175.0243
Urolithin A diglucuronide (Uro A)	C25H24O16	6.88	579.0981	579.0988	−0.70	403.0688, 227.0352
Urolithin A sulfate (Uro A)	C13H8O7S	7.49	306.9901	306.9906	−0.50	227.0347
Urolithin B [a]	C13H8O3	11.12	211.0390	211.0404	−1.40	167.0499
Urolithin B glucuronide (Uro B)	C19H16O9	8.51	387.0711	387.0713	−0.20	369.1538, 211.0393, 175.0242
Other polyphenols -Tyrosols						
3-Hydroxytyrosol [a]	C8H10O3	2.11	153.0546	153.0561	−1.50	123.0452
3'hydroxytyrosol-3'-glucuronide [a]	C14H18O9	2.11	329.0873	329.0878	−0.50	153.056, 123.0452
Hydroxytyrosol sulfate (3-HT)	C8H10O6S	1.82	233.0108	233.0113	−0.50	153.0557, 188.9862, 96.9600

[a]commercial standards, Rt retention time, mDa millidalton of error between the mass found and the accurate mass of each (poly)phenol (absolute value). 3,4-DHPPA 3,4-dihydroxyphenylpropionic acid, 3-HPAA 3-hydroxyphenylacetic acid, 3-HBA 3-hydroxybenzoic acid, 3-HT 3-hydroxytyrosol, 3-HT-G 3-Hydroxytyrosol glucuronide, 4-HBA 4-hydroxybenzoic acid, DHRSV Dihydroresveratrol, ED enterodiol, EL enterolactone, GA gallic acid, PCA protocatechuic acid, SA syringic acid, Uro A urolithin A, Uro B urilithin B, VA vanillic acid. When standards were not available, the aglycone was used for quantification (shown in brackets).

Table S5. Pearson correlation coefficients between microbial phenolic metabolites and dietary polyphenols in adolescents.

Parameter1	Parameter2	R	95% CI		*P*-value
			Low	High	
Urinary lignans	Urinary hydroxycoumarins acids	0.09	0.01	0.17	0.058
Urinary lignans	Urinary tyrosols	0.03	−0.05	0.11	0.654
Urinary lignans	Urinary hydroxybenzoic acids	−0.12	−0.20	−0.04	**0.011**
Urinary lignans	Urinary hydroxycinnamic acids	0.25	0.18	0.33	**<0.001**
Urinary lignans	Urinary hydroxyphenylacetic acids	0.11	0.03	0.19	**0.021**
Urinary lignans	Urinary hydroxyphenylpropionic acids	0.05	−0.03	0.13	0.389
Urinary lignans	Urinary stilbenes	0.34	0.27	0.41	**<0.001**
Urinary lignans	Flavonoid intake	0.02	−0.06	0.11	0.712
Urinary lignans	Phenolic acid intake	0.05	−0.04	0.13	0.468
Urinary lignans	Stilbene intake	0.02	−0.07	0.10	0.797
Urinary lignans	Lignan intake	0.09	0.01	0.18	0.073
Urinary lignans	Tyrosol intake	0.06	−0.02	0.15	0.278
Urinary lignans	Total polyphenol intake	0.04	−0.05	0.12	0.560
Urinary hydroxycoumarin acids	Urinary tyrosols	0.06	−0.02	0.14	0.286
Urinary hydroxycoumarin acids	Urinary hydroxybenzoic acids	−0.04	−0.12	0.04	0.468
Urinary hydroxycoumarin acids	Urinary hydroxycinnamic acids	0.11	0.03	0.19	**0.021**
Urinary hydroxycoumarin acids	Urinary hydroxyphenylacetic acids	0.08	0.00	0.16	0.123
Urinary hydroxycoumarin acids	Urinary hydroxyphenylpropionic acids	−0.04	−0.12	0.04	0.515
Urinary hydroxycoumarin acids	Urinary stilbenes	0.04	−0.04	0.12	0.515
Urinary hydroxycoumarin acids	Flavonoid intake	0.13	0.05	0.21	**0.008**
Urinary hydroxycoumarin acids	Phenolic acids intake	0.08	0.00	0.17	0.123
Urinary hydroxycoumarin acids	Stilbens intake	0.08	−0.01	0.16	0.156
Urinary hydroxycoumarin acids	Lignan intake	0.04	−0.04	0.13	0.497
Urinary hydroxycoumarin acids	Tyrosol intake	0.03	−0.05	0.11	0.608
Urinary hydroxycoumarin acids	Total polyphenol intake	0.13	0.05	0.21	**0.008**
Urinary tyrosols	Urinary hydroxybenzoic acids	0.26	0.19	0.34	**<0.001**
Urinary tyrosols	Urinary hydroxycinnamic acids	0.17	0.09	0.25	**<0.001**
Urinary tyrosols	Urinary hydroxyphenylacetic acids	0.12	0.04	0.19	**0.015**
Urinary tyrosols	Urinary hydroxyphenylpropionic acids	0.13	0.05	0.20	**0.008**
Urinary tyrosols	Urinary stilbenes	0.00	−0.08	0.08	0.952
Urinary tyrosols	Flavonoid intake	−0.03	−0.11	0.06	0.654
Urinary tyrosols	Phenolic acids intake	−0.05	−0.14	0.03	0.389
Urinary tyrosols	Stilbene intake	−0.05	−0.14	0.03	0.389
Urinary tyrosols	Lignan intake	−0.01	−0.09	0.07	0.857
Urinary tyrosols	Tyrosol intake	0.08	−0.01	0.16	0.153
Urinary tyrosols	Total polyphenol intake	−0.02	−0.11	0.06	0.675
Urinary hydroxybenzoic acids	Urinary hydroxycinnamic acids	−0.03	−0.11	0.05	0.585
Urinary hydroxybenzoic acids	Urinary hydroxyphenylacetic acids	−0.11	−0.19	−0.03	**0.016**
Urinary hydroxybenzoic acids	Urinary hydroxyphenylpropionic acids	0.23	0.15	0.30	**<0.001**
Urinary hydroxybenzoic acids	Urinary stilbenes	−0.03	−0.11	0.05	0.585
Urinary hydroxybenzoic acids	Flavonoid intake	−0.01	−0.10	0.07	0.847
Urinary hydroxybenzoic acids	Phenolic acid intake	−0.02	−0.11	0.06	0.712
Urinary hydroxybenzoic acids	Stilbene intake	−0.05	−0.13	0.04	0.468
Urinary hydroxybenzoic acids	Lignans intake	0.02	−0.06	0.10	0.722
Urinary hydroxybenzoic acids	Tyrosol intake	0.05	−0.03	0.14	0.389
Urinary hydroxybenzoic acids	Total polyphenol intake	−0.01	−0.09	0.08	0.927

Urinary hydroxycinnamic acids	Urinary hydroxyphenylacetic acids	0.29	0.21	0.36	**<0.001**
Urinary hydroxycinnamic acids	Urinary hydroxyphenylpropionic acids	0.33	0.25	0.40	**<0.001**
Urinary hydroxycinnamic acids	Urinary stilbenes	0.30	0.22	0.37	**<0.001**
Urinary hydroxycinnamic acids	Flavonoid intake	−0.04	−0.12	0.05	0.560
Urinary hydroxycinnamic acids	Phenolic acid intake	0.05	−0.03	0.13	0.441
Urinary hydroxycinnamic acids	Stilbene intake	0.01	−0.07	0.10	0.843
Urinary hydroxycinnamic acids	Lignan intake	0.09	0.00	0.17	0.092
Urinary hydroxycinnamic acids	Tyrosol intake	0.09	0.01	0.18	0.077
Urinary hydroxycinnamic acids	Total polyphenol intake	−0.02	−0.11	0.06	0.684
Urinary hydroxyphenylacetic acids	Urinary hydroxyphenylpropionic acids	0.27	0.19	0.34	**<0.001**
Urinary hydroxyphenylacetic acids	Urinary stilbenes	0.13	0.05	0.21	**0.005**
Urinary hydroxyphenylacetic acids	Flavonoid intake	0.03	−0.05	0.11	0.608
Urinary hydroxyphenylacetic acids	Phenolic acids intake	0.04	−0.04	0.12	0.515
Urinary hydroxyphenylacetic acids	Stilbene intake	−0.01	−0.09	0.08	0.928
Urinary hydroxyphenylacetic acids	Lignan intake	0.04	−0.04	0.12	0.505
Urinary hydroxyphenylacetic acids	Tyrosol intake	0.00	−0.08	0.09	0.946
Urinary hydroxyphenylacetic acids	Total polyphenol intake	0.03	−0.05	0.12	0.585
Urinary hydroxyphenylpropionic acids	Urinary stilbenes	0.12	0.04	0.20	**0.011**
Urinary hydroxyphenylpropionic acids	Flavonoid intake	−0.05	−0.13	0.04	0.441
Urinary hydroxyphenylpropionic acids	Phenolic acid intake	−0.03	−0.11	0.06	0.663
Urinary hydroxyphenylpropionic acids	Stilbene intake	−0.03	−0.12	0.05	0.585
Urinary hydroxyphenylpropionic acids	Lignan intake	0.04	−0.04	0.13	0.505
Urinary hydroxyphenylpropionic acids	Tyrosol intake	0.02	−0.07	0.10	0.788
Urinary hydroxyphenylpropionic acids	Total polyphenol intake	−0.05	−0.13	0.04	0.441
Urinary stilbenes	Flavonoid intake	0.09	0.01	0.17	0.081
Urinary stilbenes	Phenolic acid intake	0.00	−0.08	0.08	0.991
Urinary stilbenes	Stilbene intake	0.03	−0.05	0.11	0.608
Urinary stilbenes	Lignan intake	−0.07	−0.15	0.02	0.234
Urinary stilbenes	Tyrosol intake	0.01	−0.08	0.09	0.927
Urinary stilbenes	Total polyphenol intake	0.09	0.01	0.18	0.074
Flavonoid intake	Phenolic acid intake	0.51	0.45	0.57	**<0.001**
Flavonoid intake	Stilbene intake	0.60	0.54	0.65	**<0.001**
Flavonoid intake	Lignan intake	0.23	0.15	0.31	**<0.001**
Flavonoid intake	Tyrosol intake	0.05	−0.04	0.13	0.468
Flavonoid intake	Total polyphenol intake	0.98	0.98	0.98	**<0.001**
Phenolic acid intake	Stilbene intake	0.60	0.54	0.65	**<0.001**
Phenolic acid intake	Lignan intake	0.49	0.42	0.55	**<0.001**

Phenolic acid intake	Tyrosol intake	0.16	0.08	0.24	**0.001**
Phenolic acid intake	Total polyphenol intake	0.62	0.56	0.67	**<0.001**
Stilbene intake	Lignan intake	0.28	0.20	0.36	**<0.001**
Stilbene intake	Tyrosol intake	0.07	−0.01	0.15	0.219
Stilbene intake	Total polyphenol intake	0.63	0.57	0.67	**<0.001**
Lignan intake	Tyrosol intake	0.13	0.04	0.21	**0.011**
Lignan intake	Total polyphenol intake	0.29	0.21	0.37	**<0.001**
Tyrosol intake	Total polyphenol intake	0.09	0.01	0.17	0.081

CI confidence interval, R correlation coefficient. False discovery rate method (Benjamini & Hochberg, 1995) was used to adjust *p*-values. *p*-values < 0.5 are in bold.

References

1. Martínez-Huélamo, M.; Tulipani, S.; Jáuregui, O.; Valderas-Martinez, P.; Vallverdú-Queralt, A.; Estruch, R.; Torrado, X.; Lamuela-Raventós, R. Sensitive and Rapid UHPLC-MS/MS for the Analysis of Tomato Phenolics in Human Biological Samples. *Molecules* **2015**, *20*, 20409–20425, doi:10.3390/molecules201119702.
2. Martínez-Huélamo, M.; Tulipani, S.; Torrado, X.; Estruch, R.; Lamuela-Raventós, R.M. Validation of a New LC-MS/MS Method for the Detection and Quantification of Phenolic Metabolites from Tomato Sauce in Biological Samples. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 4542–4549, doi:10.1021/jf205266h.
3. Quifer-Rada, P.; Martínez-Huélamo, M.; Jáuregui, O.; Chiva-Blanch, G.; Estruch, R.; Lamuela-Raventós, R.M. Analytical Condition Setting a Crucial Step in the Quantification of Unstable Polyphenols in Acidic Conditions: Analyzing Prenylflavanoids in Biological Samples by Liquid Chromatography-Electrospray Ionization Triple Quadruple Mass Spectrometry. *Analytical Chemistry* **2013**, *85*, 5547–5554, doi:10.1021/AC4007733

4.4. Urinary phenolic metabolites and cardiometabolic health parameters in adolescents

4.4.1. Article 7: Microbial Phenolic Metabolites in Urine Are Inversely Linked to Certain Features of Metabolic Syndrome in Spanish Adolescents

Emily P. Laveriano-Santos, Paola Quifer-Rada, María Marhuenda-Muñoz, Camila Arancibia-Riveros, Anna Vallverdú-Queralt, Anna Tresserra-Rimbau, Ana María Ruiz-León, Rosa Casas, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Jesús Martínez-Gómez, Gloria Santos-Beneit, Juan M. Fernández-Alvira, Rodrigo Fernández-Jiménez, and Rosa M. Lamuela-Raventós.

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Abstract

Introduction
Several studies have documented the effect of phenolic metabolites on cardiometabolic outcomes, especially those derived from gut microbiota. However, scare evidence is available in young population such as adolescents.

Objective
To explore the relationship between urinary phenolics and metabolic syndrome (MetS) and its components in adolescents.

Methods
A cross-sectional study was conducted on 560 adolescents aged 12.02 ± 0.41 years, enrolled at baseline in the SI! Program for Secondary Schools trial. The following MPM, hydroxycinnamic acids (m-, *o*-, *p*- coumaric acids), dihydroxy-phenylpropionic acid, dihydroresveratrol, enterolignans, gallic acid, hydroxybenzoic acids, hydroxyphenylacetic acids, hydroxytyrosol, protocatechuic acid, syringic acid, urolithins (A, B), and vanillic acid, were analyzed by HPLC-LTQ-Orbitrap-HRMS. MetS and its clinical features were defined in accordance with the International Diabetes Federation.

Results
Out of all MPM, urolithin A was inversely associated with the diastolic blood pressure z-score. Urolithin B was inversely associated with the MetS score and waist circumference z-score. Additionally, higher levels of gallic acid were associated with lower odds of presenting MetS (OR=0.85, 95% CI: 0.77; 0.93) and abdominal obesity (OR=0.93, 95% CI:

(0.89; 0.98). Higher urolithin B levels were inversely associated with abdominal obesity (OR= 0.94, 95% CI: 0.89; 0.98) and high blood glucose (OR= 0.92, 95% CI:0.88; 0.96)

Conclusion

Gallic acid, urolithins A and B were associated with lower odds of developing MetS and/or some of its clinical features. These findings are relevant to formulating new hypotheses and elucidating the effect of these compounds on metabolic syndrome in adolescents.

antioxidants

MDPI

Article

Microbial Phenolic Metabolites in Urine Are Inversely Linked to Certain Features of Metabolic Syndrome in Spanish Adolescents

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Abstract: (1) Background: To explore the association between microbial phenolic metabolites (MPM) and metabolic syndrome (MetS) and its clinical features in adolescents aged 12.02 ± 0.41 years. (2) Methods: a cross-sectional study was conducted in 560 participants at baseline in the SI! Program for Secondary Schools trial. The following MPM, coumaric acids (*m*-, *o*-, *p*-coumaric acids), dihydroxyphenylpropionic acid, dihydroresveratrol, enterolignans, gallic acid, hydroxybenzoic acids, hydroxyphenylacetic acid, hydroxytyrosol, protocatechuic acid, syringic acid, urolithins (A, B), and vanillic acid, were analyzed by HPLC-LTQ-Orbitrap-HRMS. MetS and its clinical features were defined in accordance with the International Diabetes Federation. (3) Results: Out of all MPM, urolithin A was inversely associated with the diastolic blood pressure z-score. Urolithin B was inversely associated with the MetS score and waist circumference z-score. Additionally, higher levels of gallic acid were associated with lower odds of presenting MetS (OR = 0.85, 95% CI: 0.77; 0.93) and abdominal obesity (OR = 0.93, 95% CI: 0.89; 0.98). Higher urolithin B levels were inversely associated with abdominal obesity (OR = 0.94, 95% CI: 0.89; 0.98) and high blood glucose (OR = 0.92, 95% CI:0.88; 0.96); (4) Conclusions: gallic acid, urolithin A and B were associated with lower odds of presenting MetS or some of its clinical features in adolescents. This is the first study that evaluates several MPM with MetS in adolescents, highlighting the importance of MPM on cardiometabolic health at early life stages.

Keywords: microbiota; phytochemical; antioxidant compound; cardiovascular

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1. Introduction

Metabolic syndrome (MetS) refers to a cluster of physiological, clinical features, biochemical, and metabolic conditions, including abdominal obesity, elevated blood pressure, dyslipidemia, and hyperglycemia, which are cardiovascular risk factors and are associated with insulin resistance [1,2]. MetS in childhood and adolescence is strongly associated with a high risk of maintaining MetS in adulthood and developing atherosclerosis and type 2 diabetes mellitus later in life [3]. In 2020, it was estimated that about 5% of adolescents worldwide had MetS; its prevalence was found to be unrelated to the wealth of individual countries [4] but linked to an increase in the rate of obesity stemming from an unhealthy diet and sedentary lifestyle [5]. Moreover, exposition to endocrine-disrupting chemicals, such as bisphenol A, parabens, and phthalates at early life stages contributes to obesity and other MetS features in adolescents [6,7].

As mentioned, diet is one of the modifiable factors strongly associated with obesity and MetS. The Mediterranean diet, characterized by foods rich in phenolic compounds, is known to reduce cardiometabolic risk factors [8–10]. The impact of dietary (poly)phenols on cardiometabolic health can be partly explained by their potential antioxidant action in preventing reactive oxygen species (ROS) production and cellular oxidative stress. The particular chemical structure of (poly)phenols makes them good electron or hydrogen atom donors, neutralizing ROS [11]. Prebiotic properties and the ability to modulate gut microbiota activity also have been attributable to phenolic compounds [12]. After ingestion, 85–90% of dietary (poly)phenols reach the large intestine, where they are metabolized by the gut microbiota into new compounds (metabolites) with potentially beneficial health effects [13,14]. The health effects of dietary (poly)phenols depend on the quantity consumed and their absorption, distribution, metabolism, and elimination in biological fluids [13]. The rate and extent of their absorption depend on the phenolic structure; whereas aglycones and some glycosides can be absorbed in the small intestine, esters and polymers are partly metabolized by gut microbiota and transformed into lower-molecular-weight compounds. These can be absorbed in the large intestine and reach the liver, where they may undergo further phase II metabolism. Once in the systematic circulation, phenolic metabolites are distributed to different target tissues and excreted through biological fluids, including urine, and can be identified through metabolomic approaches [13,15,16]. As dietary (poly)phenols undergo extensive modification, the forms that appear in human urine (mainly glucuronides and sulfates) are usually different from the parent compounds ingested in foods [13,14]. Several studies have documented the effect of phenolic metabolites on cardiometabolic outcomes, especially those derived from gut microbiota such as urolithins, phenolic acids (hydroxycinnamic acids, hydroxybenzoic acids, hydroxyphenylpropionic acids, hydroxyphenylacetic acids), enterolignans, and stilbenes (dihydroresveratrol) [13,17,18]. However, most of them have performed in adult populations.

Despite the plausible role of microbial phenolic metabolites (MPM) in cardiometabolic health and MetS [17,18] and the importance of investigating their role in young population, the aim of the present study was to evaluate the association between MPM and MetS and their clinical features in young adolescents in Spain using a targeted metabolomic approach.

2. Materials and Methods

2.1. Study Population

This is a sub-study nested within the SI! (Salud Integral-Comprehensive Health) Program for Secondary Schools trial (NCT03504059), a cluster-randomized controlled intervention trial that evaluated the effectiveness of different educational intervention strategies on cardiovascular health parameters in 1326 adolescents from 24 public Secondary Schools in Spain. A detailed description of the original study design and recruitment procedures has been published by Fernandez-Jiménez et al. [19].

The 601 subjects initially included in the present cross-sectional study had available baseline data on MPM and were selected by simple random sampling [20].

2.2. Assessment of Cardiometabolic Parameters

Waist circumference (WC) was measured using non-elastic tape; to minimize errors, the measurement was repeated three times, and the results were averaged [19]. Age- and sex-specific WC z-scores were calculated according to the National Health and Nutrition Examination III Survey data [21].

Systolic and diastolic blood pressure (SBP, DBP) (mm Hg) readings were taken with the subject in a sitting position using a digital device (Omron M6, OMROM Healthcare Co., Kyoto, Japan) according to standardized procedures [19]. Two readings were taken at intervals of 3–5 min, and if they differed by at least 5 mm Hg for DBP and/or 10 mm Hg for SBP, a third reading was taken. For this analysis, the lowest SBP and DBP values were considered. The mean arterial pressure (MAP) was obtained using the lowest values of SBP and DBP and calculated using the following formula: by the [(SBP − DPB)/3] + DBP. DBP and SBP z-scores were estimated following the cut-offs specified by the High Blood Pressure Working Group of the National Blood Pressure Education Program for children and adolescents [22].

The levels of blood glucose (BG), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were determined using a portable whole-blood analyzer in capillary blood samples collected early in the morning and fasting [19]. All of the measurements were performed by trained staff under standardized conditions.

Adolescents (aged 10 to <16 years) were considered to have MetS if they had abdominal obesity (WC ≥ 90th percentile) and at least two other clinical features, as defined by the International Diabetes Foundation [1]: (1) SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg, (2) TG ≥ 150 mg/dL, (3) HDL-c ≤ 40 mg/dL, or (4) BG ≥ 110 mg/dL. Additionally, a continuous MetS score was calculated following the methodology of Shafiee et al. [23] as the sum of standardized residuals for WC, MAP, HDL-c, TG, and BG, regressed for age and sex [23]. The HDL-c was multiplied by −1 because a low value of this parameter is an unfavorable factor of cardiometabolic risk. Thus, lower continuous MetS scores indicate a better cardiometabolic profile [23].

2.3. Determination of Urinary Phenolic Metabolites

2.3.1. Reagents and Standards

Gallic acid (3,4,5-trihydroxybenzoic acid), 3-hydroxytyrosol (4-(2-hydroxyethyl)benzene-1,2-diol), protocatechuic acid (3,4-dihydroxybenzoic acid), 4-hydroxybenzoic acid, 3,4-dihydroxyphenylpropionic acid, 3′-hydroxyphenylacetic acid (2-(3-hydroxyphenyl)acetic acid), *o*-coumaric acid ((*E*)-2-(3-hydroxyphenyl)acetic acid), *m*-coumaric acid ((*E*)-3-(3-hydroxyphenyl)prop-2-enoic acid), *p*-coumaric acid ((*E*)-3-(4-hydroxyphenyl)prop-2-enoic acid), enterodiol ((2*R*,3*R*)-2,3-bis[(3-hydroxyphenyl)methyl]butane-1,4-diol), urolithin-A (3,8-dihydroxybenzo[c]chromen-6-one), and urolithin-B (3-hydroxybenzo[c]chromen-6-one) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 3′-hydroxytyrosol-3′-glucuronide ((2*S*,3*S*,4*S*,5*R*,6*S*)-3,4,5-trihydroxy-6-[2-hydroxy-5-(2-hydroxyethyl)phenoxy]oxane-2-carboxylic acid), dihydroresveratrol (5-[2-(4-hydroxyphenyl)ethyl]benzene-1,3-diol), and (+)-cis,trans-abscisic acid D6 ((2*Z*,4*E*)-3-methyl-5-[(1*S*)-3,5,5-trideuterio-1-hydroxy-6,6-dimethyl-4-oxo-2-(trideuteriomethyl)cyclohex-2-en-1-yl]penta-2,4-dienoic acid) were obtained from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA, USA). 3-hydroxybenzoic acid, vanillic acid (4-hydroxy-3-methoxybenzoic acid), syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid), enterolactone ((3*R*,4*R*)-3,4-bis[(3-hydroxyphenyl)methyl]oxolan-2-one), and creatinine were obtained from Fluka (St. Louis, MO, USA). The standards were stored, protected from light, and in powder form. Methanol and acetonitrile grade were obtained from Sigma-Aldrich

Chemical Co. (St. Louis, MO, USA), formic acid (≥98%) from Panreac Química S.A. (Barcelona, Spain), and ultrapure water (Milli-Q) from a Milli-Q system (Bedford, MA, USA).

2.3.2. Urine Sample Collection and Treatment for MPM Analysis

Baseline spot urine samples were collected in the morning and fasting in 1 mL polyethylene containers and stored at −80 °C prior to analysis. The extraction of phenolic compounds was carried out using a method developed by our research group [20]. For the sample preparation, urine (1 mL) was acidified with 2 µL of formic acid and centrifuged (4 min, 15,000× *g*, 4°C) one time. The supernatant was then loaded onto Waters Oasis 96-well reversed-phase phase extraction plates (30 µm) (MA, USA) prior to plate activation with 1 mL of methanol and 1 mL of 1.5 M formic acid. Sample clean-up was performed with 500 µL of 1.5 M formic acid and 0.5% methanol, and elution was achieved using 1 mL of methanol (acidified with 0.1% formic acid). Then, the eluted fraction was evaporated to dryness under a nitrogen stream at room temperature and reconstituted with Milli-Q water (0.05% formic acid) up to 100 µL. The extract was filtered with a 0.22 µm polytetrafluoroethylene 96-well plate filter from Millipore (Burlington, MA, USA). (+)-cis,trans-abscisic acid d6 (500 µg/L) was used as the internal standard.

The calibration curves were prepared following the same procedure by spiking synthetic urine at nine different concentrations of standard mixtures according to our previous method [20].

2.3.3. Chromatographic Conditions

MPM were identified and quantified by reverse-phase high-performance liquid chromatography coupled to linear trap quadrupole Orbitrap high-resolution mass spectrometry (HPLC -LTQ-Orbitrap-HRMS), as previously described [20]. The liquid chromatography system was an Accela chromatograph (Thermo Scientific, Hemel Hempstead, UK) equipped with a quaternary pump and a thermostated autosampler set at 4 °C. The HPLC column employed was a Kinetex F5 100 Å (50 × 4.6 mm i.d., 2.6 µm particle size from Phenomenex (Torrance, CA, USA) at 40 °C. The mobile phases consisted of water/0.05% formic acid (A) and acetonitrile/0.05% formic acid (B), with a flow rate of 0.5 mL min^{-1}. The non-linear gradient program started with 2% of solvent B, reaching 8% solvent B at 2.5 min, 20% solvent B at 7 min, 30% solvent B at 9 min, 50% solvent B at 11 min, 70% solvent B at 12 min, and 100% of solvent B at 15 min. The initial conditions were re-established at 16.5 min and maintained up to 21.5 min.

For accurate mass measurements, the HPLC system was coupled with an LTQ-Orbitrap-HRMS (Thermo Scientific, Hemel Hempstead, UK) equipped with an electrospray ionization (ESI) source on the negative mode, with the following parameters: source voltage, 5 kV; sheath gas, 50 units; auxiliary gas, 20 units; sweep gas, 2 units; and capillary temperature, 375 °C. Mass spectra (MS) were acquired in profile mode with a setting of 30,000 resolution at m/z 400. The mass range in Fourier transform mass spectrometry mode was from m/z 100 to 2000, in combination with product ion scan experiments (resolution range 15,000–30,000 full-width at half maximum) [20].

MPM identification, described in detail by Laveriano-Santos et al., was based on their accurate mass measurements with an error of 5 ppm and isotopic patterns, as well as the existing literature [20]. Xcalibur 3.0 and Trace Finder version 4.1 (Thermo Fisher Scientific, San Jose, CA, USA) were applied for the instrument control and chromatographic data analysis.

In this study, 54 MPM were identified and quantified (aglycones and phase II metabolites in glucuronide and sulfate form) (Table S1). MPM values below the limit of quantitation (LOQ) were replaced by the mean LOQ/2.

2.3.4. Creatinine Analysis

Urinary creatinine was measured using a Jaffé alkaline picrate method adapted for microtiter 96-well plates described by Medina-Remón et al. [24]. Briefly, 3 µL of a spot urine sample was mixed with 60 µL of aqueous picric acid solution (1%) and 5 µL of sodium hydroxide (10%) and maintained in darkness for 15 min. After adding 232 µL of Milli-Q water, the absorbance was measured at 500 nm by a UV–vis spectrophotometer; all of the experiments were performed in triplicate. The calibration curve was prepared with a creatinine standard to quantify the creatinine concentrations in the spot urine samples. MPM concentrations were normalized by creatinine and expressed as µg MPM/g creatinine.

2.4. Assessment of Covariates

The energy intake (kcal/day) was estimated from a semi-quantitative food frequency questionnaire [25] using Spanish food composition tables [26,27].

Physical activity was measured by accelerometry (Actigraph wGT3X-BT, ActiGraph, Pensacola, FL, USA). The participants wore accelerometers on the non-dominant wrist for 7 consecutive days [19]. Moderate-to-vigorous physical activity (min/day) was estimated according to Chandler et al. (2016) [28]. Physical activity self-reported information obtained by questionnaires was used if accelerometer data were missing (n = 13; 2%) [19,29]. Finally, for this study, physical activity was categorized based on the recommendation of the World Health Organization as (1) below or (2) at least 60 min/day of moderate-to-vigorous physical activity [30]. Sexual maturity status was established using pictograms according to the Tanner & Whitehouse method [31].

Information about household income was obtained from a validated questionnaire answered by the parents [19]. Household incomes were categorized as low, medium, or high based on salaries in Spain [32].

2.5. Statistical Analysis

Stata software version 16.0 (StataCorp., College Station, TX, USA) and R 4.2.1 (R Studio, 250 Northern Ave, Boston, MA,, USA) were used to perform the data analyses. The data are expressed as mean ± SD and frequency (percentage).

MPM outlier values were identified using a robust mean absolute deviation method (MAD) [33], and 41 participants (7% of the selected sample) with MPM values of more than 3MAD were excluded from the analysis, leaving a total of 560 participants (Figure S1). The MPM were divided into 14 groups according to their chemical structure, and each group included all the aglycone, glucuronide, and sulfate forms. Thus, statistical analysis was carried out with the following MPM groups: coumaric acids (including *p*-, *o*-, and *m*-coumaric acid), dihydroxyphenylacetic acid, dihydroresveratrol, enterodiol, enterolactone, gallic acid, hydroxybenzoic acids (including 3- and 4-hydroxybenzoic acids), hydroxyphenylacetic acid, hydroxytyrosol, protocatechuic acid, syringic acid, urolithin A, urolithin B, and vanillic acid. As the MPM levels had a skewed distribution, the analysis was carried out with their natural log-transformed concentrations.

Association between the log-transformed MPM (continuous) and cardiometabolic health parameters (WC, DBP, SBP, BG, TG, HDL-c, and MetS scores, all continuous) was determined using mixed-effects linear regression analyses. The fixed effect included two different models: model 1 was adjusted for sex (females/males) and age (continuous, years), whereas model 2 was model 1 plus Tanner maturation stage (from I to V), physical activity (≥60 min/<60 min moderate-to-vigorous physical activity), household income (low/medium/high), and energy intake (continuous, kcal/day). Mixed-effects logistic regression was used to assess the associations between the log-transformed MPM (continuous) and MetS presence (yes/no), as well as its clinical features, ((1) WC ≥ 90th percentile, (2) SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg, (3) TG ≥ 150 mg/dL, (4) HDL-c ≤ 40 mg/dL, (5) BG ≥ 110 mg/dL), adjusting for the same covariates as mentioned above. Municipality and

schools were considered as random effects in all adjusted models. The results are expressed as unstandardized β coefficients or odds ratios (OR) and their 95% confidence intervals (CI). To compensate for multiple testing, the Benjamin–Hochberg procedure was applied, considering a false discovery rate (FDR) < 0.05 as significant [34], although all *p* values below 0.05 are provided. To summarize the regression analyses, Forest plots were applied using the "forester" package for R software. An analysis flowchart was designed to visualize all the statistical analyses (Figure S2).

3. Results

3.1. Participant Characteristics

Out of a total sample of 601 adolescents, 560 (54% female) aged 12.0 ± 0.4 years were included in the study (Figure S1). The following mean measurements were obtained for this sub-study: WC 73.6 cm, SBP 105.2 mm Hg, DBP 62.5 mm Hg, BG 102.9 mg/dL, TG 87.9 mg/dL, and HDL-c 62.4 mg/dL. Overall, 72% of the participants reported at least 60 min/day of moderate-to-vigorous physical activity. Regarding sociodemographic factors, more than a third reported a high household income (Table 1).

Table 1. General characteristics of the participants (*n* = 560).

Characteristics	Total
Female, *n* (%)	304 (54)
Age, years	12.0 ± 0.4
WC, cm	73.6 ± 11.4
SBP, mm Hg	105.3 ± 11.2
DBP, mm Hg	62.5 ± 9.3
BG, mg/dL	102.9 ± 14.2
TG, mg/dL	87.9 ± 47.2
HDL-c, mg/dL	62.4 ± 16.5
MetS score	0.01 ± 3.04
MetS, *n* (%)	16 (3)
Clinical features	
Abdominal obesity, *n* (%)	122 (22)
High blood pressure, *n* (%)	2 (0.4)
Low HDL-c, *n* (%)	32 (6)
High TG, *n* (%)	43 (8)
High BG, *n* (%)	159 (28)
Total energy intake, kcal/day	2492.1 ± 586.4
Moderate-to-vigorous physical activity, *n* (%)	
<60 min/day	156 (28)
≥60 min/day	404 (72)
Household income, *n* (%)	
Low	173 (32)
Medium	144 (27)
High	216 (41)

Values are expressed as mean ± SD for continuous variables or as a frequency (percentage) for categorical variables. BG blood glucose, DBP diastolic blood pressure, HDL-c high-density lipoprotein-cholesterol, SBP systolic blood pressure, MetS metabolic syndrome, TG triglycerides, WC waist circumference.

Sixteen participants were diagnosed with MetS, and the overall crude prevalence of MetS was 3%. 240 (43%) participants had one or two components of MetS, while 304 (54%) had none. The mean MetS score was 0.01 ± 3.04. Regarding MetS clinical features, 122 (22%)

participants presented abdominal obesity (WC ≥ 90th), 2 (less than 1%) had high blood pressure (≥130 mm Hg SBP or ≥85 mm Hg DBP), 43 (8%) had high TG (≥150 mg/dL), 32 (6%) had low HDL-c (≤40 mg/dL), and 159 (28%) had high BG (≥110 mg/dL) (Table 1).

3.2. Association between MPM and Cardiometabolic Health Parameters

Mixed-effects linear regression models were generated to evaluate the association between the log-transformed groups of MPM and the cardiometabolic health parameters. In the fully adjusted multivariable model, adjusted for sex, age, Tanner maturation stage, physical activity, household income, and energy intake, after multitesting adjustment for FDR, the results showed that higher urolithin B levels (aglycone, glucuronide, and sulfate forms) were associated with lower MetS score (β= −0.08, 95% CI: −0.12; −0.04) (Figure 1 and Table S2), but also with lower WC z-scores (β= −0.03, 95% CI: −0.04; −0.01) (Figure 2 and Table S2). Higher concentrations of urolithin A (aglycone, glucuronide, and sulfate forms) were associated with lower DBP z-scores (β= −0.02, 95% CI: −0.03; −0.01) (Figure 2 and Table S2).

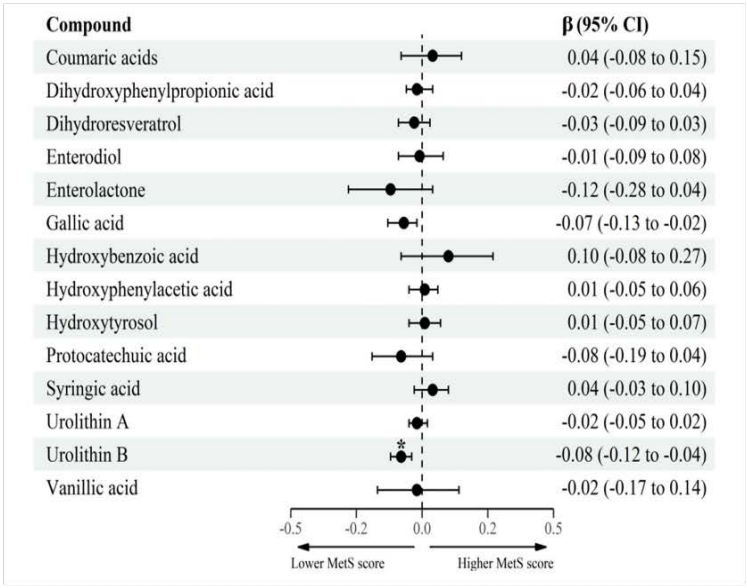

Figure 1. Association between log-transformed groups of MPM and MetS score in adolescents. β coefficient, CI confidence interval, MetS metabolic syndrome, MPM microbial phenolic metabolites. Mixed-effects linear regression between log-transformed MPM and MetS score. Fixed effect: sex (female/male), age (continuous, years), Tanner maturation stage (score from I to V), physical activity (≥60 min/<60 min moderate-to-vigorous physical activity), household income (low/medium/high), and energy intake (continuous, kcal/day). Municipality and schools were considered as random effects). * *p*-adjusted for multiple-testing using the Benjamin–Hochberg procedure considering a false discovery rate < 0.05 as significant.

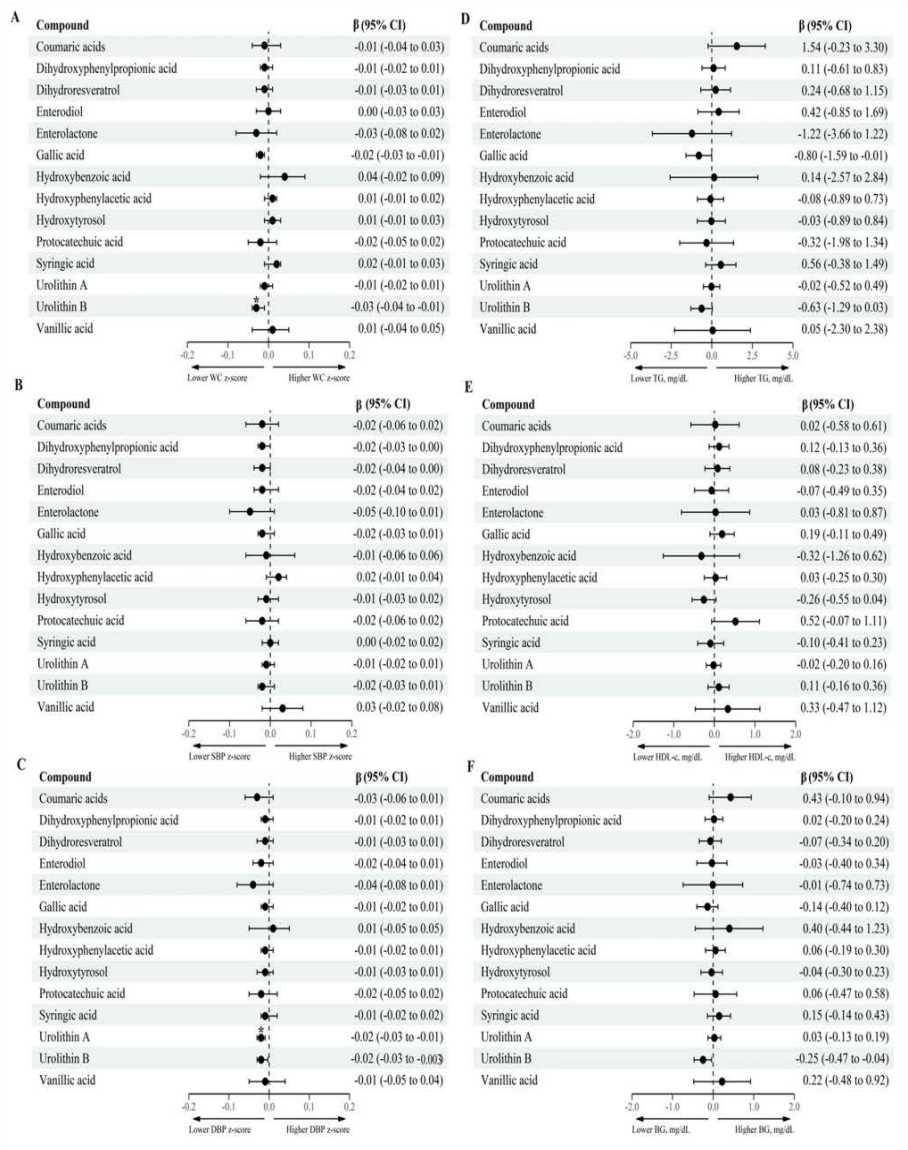

Figure 2. Association between log-transformed groups of MPM and cardiometabolic health parameters in adolescents. (**A**): WC z-score, (**B**): SBP- z-score, (**C**): DBP z-score, (**D**): TG, mg/dL, (**E**): HDL-c, mg/dL, (**F**): BG, mg/dL. β coefficient, BG blood glucose, CI confidence interval, DBP diastolic blood pressure, HDL-c high-density lipoprotein-cholesterol, MPM microbial phenolic metabolites, SBP systolic blood pressure, TG triglycerides, WC waist circumference. Mixed-effects linear regression between log-transformed MPM and cardiometabolic health parameters. Fixed effect: sex (female/male), age (continuous, year), Tanner maturation stage (score from I to V), physical activity (≥60 min/<60 min moderate-to-vigorous physical activity), household income (low/medium/high), and energy intake (continuous, kcal/day). Municipality and schools were considered random effects. * *p*-adjusted for multiple-testing using the Benjamin–Hochberg procedure, considering a false discovery rate < 0.05 as significant.

3.3. Association between MPM and MetS and Its Clinical Features

The association between MPM and MetS and its clinical components was assessed through mixed-effects logistic regression analyses (Figures 3 and 4 and Table S3). After adjustment for an FDR, higher gallic acid levels (aglycone, glucuronide, and sulfate forms) were associated with lower odds of presenting MetS (OR = 0.85, 95% CI: 0.77; 0.93) and abdominal obesity (OR = 0.93, 95% CI: 0.89; 0.98) defined as WC ≥ 90th. Similarly, participants with higher urolithin B levels (aglycone, glucuronide, and sulfate forms) had lower odds of having abdominal obesity (OR = 0.94, 95% CI: 0.89; 0.98) and high BG (OR = 0.92, 95% CI:0.88; 0.96). High blood pressure was not considered in the statistical analysis as only 2 participants (less than 1%) presented this condition (SBP ≥ 130 mm Hg or SBP ≥ 85 mm Hg), and therefore the analysis did not converge.

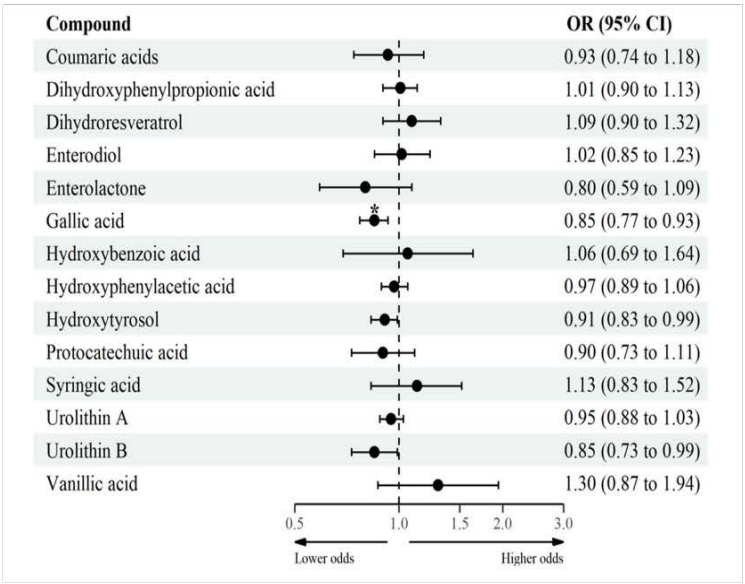

Figure 3. Log-transformed groups of MPM with MetS in adolescents. CI confidence interval, MetS metabolic syndrome; MPM microbial phenolic metabolites, OR odds ratio. Mixed effect logistic regression between groups of MPM (log-transformed, continuous) and MetS (dichotomous). Fixed effect: sex (female/male), age (continuous, years), Tanner maturation stage (score from I to V), physical activity (≥60 min/<60 min moderate-to-vigorous physical activity), household income (low/medium/high), and energy intake (continuous, kcal/day). Municipality and schools were considered as random effects. * *p*-adjusted for multiple-testing using the Benjamin–Hochberg procedure, considering a false discovery rate < 0.05 as significant.

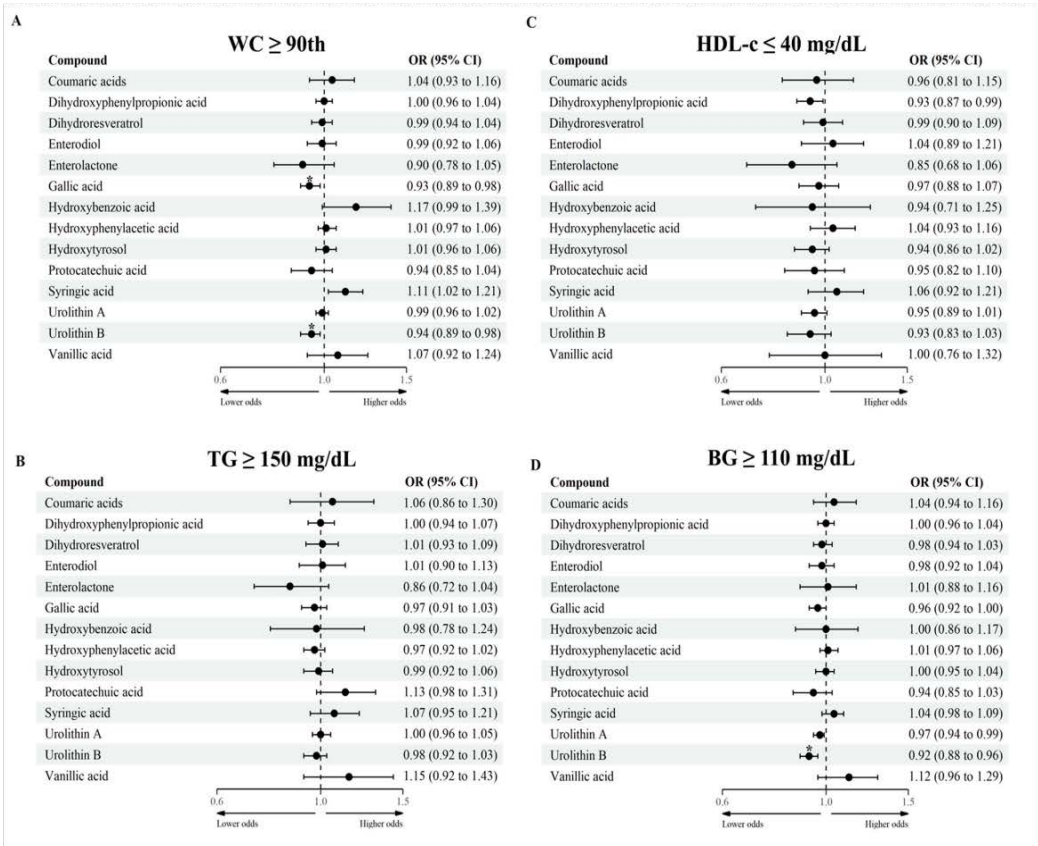

Figure 4. Log-transformed groups of MPM with MetS clinical features in adolescents. (**A**): WC ≥ 90th, (**B**): TG ≥ 150 mg/dL, (**C**): HDL-c ≤ 40 mg/dL, (**D**): BG ≥ 110 mg/dL. BG blood glucose, CI confidence interval, HDL-c high-density lipoprotein-cholesterol, MPM microbial phenolic metabolites, TG triglycerides, WC waist circumference. Mixed-effects logistic regression between groups of MPM (log-transformed, continuous) and MetS clinical features (dichotomous). Fixed effect: sex (female/male), age (continuous, years), Tanner maturation stage (score from I to V), physical activity (≥60 min/<60 min moderate-to-vigorous physical activity), household income (low/medium/high), and energy intake (continuous, kcal/day). Municipality and schools were considered random effects. High blood pressure was not considered in the statistical analysis as only 2 participants had this condition (systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg), and therefore analysis did not converge. * p-adjusted for multiple-testing using the Benjamin–Hochberg procedure, considering a false discovery rate < 0.05 as significant.

4. Discussion

The results of the present cross-sectional study of 560 adolescents show that higher urinary excretion of phenolic metabolites (gallic acid, and urolithin A and B) was associated with a lower WC z-score, DBP z-score, and MetS score and reduced odds of presenting MetS or some of its clinical features (abdominal obesity and high BG), after adjustment for sex, age, Tanner maturation stage, physical activity, household income, and energy intake.

The potential role of MPM on MetS and its clinical features have been documented in several studies; however, most of them have been conducted in adult, in vitro, or animal models.

We observed that higher levels of urinary gallic acid (aglycone, glucuronide, and sulfate forms) were associated with lower odds of having MetS and abdominal obesity. Gallic acid is a small phenolic acid, widespread in fruits and vegetables as a free molecule in aglycone or esterified form [14,35]. Moreover, gallic acid is one of the main microbial metabolites arising from hydrolysable tannins (gallotannins), anthocyanins, and galloylated catechin (epigallocatechin gallate and epicatechin gallate) [14]. According to Esteban-Torres et al., gut microbiota phyla such as *Firmicutes*, *Proteobacteria*, and *Actinobacteria* contain gallate decarboxylase and/or tannase enzymes responsible for the degradation of dietary gallates and tannins, transforming into simpler compounds, such as gallic acid and pyrogallol [36]. The therapeutic properties of gallic acid related to its strong antioxidant activities have been demonstrated in several studies [35], but its effects have been scarcely studied in adolescents. Gallic acid promotes mitochondrial biogenesis, a vital process for maintaining cellular homeostasis in terms of energy production and heat generation, which are closely associated with obesity, diabetes, and other metabolic disorders [35]. In animal models, gallic acid and epicatechin gallate induce thermogenesis and mitochondrial biogenesis in brown adipocytes [37]. Gallic acid and its ester derivatives improve the function of several mitochondrial enzymes involved in cellular energy homeostasis regulating body weight and lipid and glucose metabolism [35,38]. The thermogenic effect of galloylated catechins, especially epigallocatechin gallate, on weight loss has been studied in human adults. The results from a meta-analysis based on five randomized, double-blind, placebo-controlled clinical trials involving 112 participants showed that a moderate daily intake of epigallocatechin gallate (300–600 mg dose) for a period of 2 days to 12 weeks increases energy expenditure in 158 kJ/day or 38 kcal/day compared to placebo [39]. In the same study, epigallocatechin gallate supplementation reduced WC and body fat mass but not body mass index [39]. Another clinical trial showed that daily supplementation of green tea extract capsules, with a high concentration of galloylated catechins (approximately 1000 mg) for twelve weeks, reduced body weight and increased energy expenditure and fat oxidation in sixty subjects aged 40–60 years and with overweight [40].

Concerning glucose metabolism, which is closely linked to MetS, gallic acid can reduce BG levels by suppressing glucose absorption in the intestinal cell via the inhibition of sodium-dependent transporters [41]. Additionally, gallic acid stimulates glucose transporter 4 translocation and increases glucose uptake activity in adipocytes, thus demonstrating its antihyperglycemic properties [13,42–44]. A study of streptozotocin-induced diabetic rats reported that supplementation of 10 and 20 mg/kg body weight of gallic acid for 21 days decreased body weight, glycosylated hemoglobin, glucose-6-phosphatase activity, pancreatic lipid peroxidation, and pancreatic damage [45]. In a randomized clinical trial conducted in thirty-seven individuals aged 16–65 years with MetS, the daily consumption of a beverage (650 mL) with high gallic acid content for twelve weeks did not show changes in BG in the placebo and intervention group [46]. However, a significant decrease in inflammatory biomarkers (interleukin 6 and 8-isoprostane), closely associated with MetS, was observed in the intervention group [46]. These finding showed in the literature agree with our results and provide strong support for the possible effect of gallic acid on MetS and obesity.

In the present study, higher levels of urinary urolithin A and B (aglycone, glucuronide, and sulfate forms) were associated with better cardiometabolic health parameters (lower MetS score, WC z-score, and DBP z-score) and reduced odds of presenting abdominal obesity and high BG (the latter only with urolithin B). Urolithins are the final metabolites of ellagitannins, the main group of hydrolysable tannins [13,14,47], which are found in nuts and red fruits, including grapes, pomegranates, and some berries. Ellagitannins are broken down into ellagic acids, which are transformed into urolithins by the action of the gut microbiota [14,47,48]. Urolithins are metabolized in enterocytes and hepatocytes into glucuronide, sulfate, and methylated phase II metabolites and are excreted in breast milk and urine [13,49]. Several studies in vitro and in animal models and some human clinical trials have revealed a protective effect of urolithins (ellagitannin

metabolites) against obesity, type 2 diabetes mellitus, cardiovascular diseases, and some cancers [17,18,47]. Regarding blood pressure, we found that a higher excretion of urolithin A was related to a lower DBP score, in agreement with Istas G. et al., who reported a beneficial effect of plasma urolithins on endothelial function in healthy individuals aged 18–35 years after the consumption of 200 and 400 g of raspberries. Additionally, a reduction in blood pressure (central and peripheral) was observed after raspberry intake [50]. The results from a meta-analysis conducted in thirty randomized clinical trials showed that pomegranate and nut (sources of ellagitannins) supplementation did not have a significant reduction in SBP and DBP levels in adults. However, in a separate analysis, differences in the regulation of blood pressure were observed; meanwhile, pomegranate intake significantly reduced DBP levels, and nut intake increased DBP [51]. The association between urolithin A and blood pressure found in our study could be explained by the antioxidant and anti-inflammatory properties of Urolithin A by decreasing inflammation markers (prostaglandins E2, cyclo-oxigenase-2, and microsomal prostaglandins E synthase-1) and inhibiting the activation of mitogen-activated protein kinase and nuclear factor kappa B [52]. Moreover, urolithin A mediates vascular function by inhibiting the adhesion of monocytes and endothelial cell migration, as well as activating endothelial nitric oxide synthase and releasing nitric oxide [53,54]. We also observed that higher urinary levels of urolithin B were associated with lower odds of presenting abdominal obesity, which could be attributed to its enhancing effects on thermogenesis, lipolysis, fatty oxidation, and inhibition of lipogenesis and adipogenesis [47]. Significant interindividual variation has been observed in urolithin production and excretion, with an effect on cardiometabolic health [14,17,18,48]. Based on urinary excretion, three urolithin metabotypes (UMs) have been identified: UM-A individuals produce only urolithin A, those with UM-B produce urolithin B and isourolithin A, and those with UM-0 are nonproducers [14,17,18]. UMs could be used as biomarkers of gut microbiota [55]. In a Spanish cohort of 415 children and adolescents aged 5–17 years, who consumed 25 g of walnuts (peeled raw) or 250 mL of pomegranate juice daily for three days, UM-B was associated with higher odds of overweight-obesity defined by body mass index cut-offs compared to UM-A. In this study, urolithin B was not evaluated separately; therefore, our results cannot be comparable. In our study, we did not evaluate urolithin metabotypes, and therefore their possible associations with cardiometabolic parameters cannot be determined [55]. Additionally, we found that urolithin B was associated with lower odds of presenting high BG. By contrast, Selma et al. did not find a relationship between urolithin B + isourolithin A in urine and BG in fifty MetS subjects aged 42–61 years [17]. Our findings could be explained by the protective effect of urolithins on the exocrine pancreas through the inhibition of inflammatory signaling pathways, autophagy activation, and maintenance of mitochondrial function in pancreatic cells [48]. Hyperglycemia plays a pivotal role in the pathogenesis of cardiometabolic diseases through the stimulation of cellular oxidative stress, mitochondrial dysfunction, and inflammation [56]. According to the results from an in vitro study, 1 μM urolithin B and B-glucuronide prevents inflammatory responses in rat cardiac myocytes exposed to high glucose concentrations. Thus, the authors highlighted the importance of a regular intake of ellagitannin-rich foods in the modulation of pro-inflammatory mediators in hyperglycemic conditions [11,57]

In our cross-sectional study, coumaric acids (including *p*-, *o*-, and *m*- coumaric acid), dihydroxyphenylacetic acid, dihydroresveratrol, enterodiol, enterolactone, hydroxybenzoic acids (including 3- and 4-hydroxybenzoic acids), hydroxyphenylacetic acid, hydroxytyrosol, protocatechuic acid, syringic acid, and vanillic acid; were not associated with MetS and its clinical features in adolescents after the full-adjustment for confounder variables, in contrast with the literature reported in studies conducted in human adults, in vitro, and in animal models [13,43,58,59].

To our knowledge, this is the first observational study to investigate the associations of several MPM with cardiometabolic parameters and MetS and its clinical components in young adolescents. Another strong point of the study is the precise extraction of

phenolic metabolites from urine samples and the use of a novel targeted metabolomic approach for their highly accurate identification and quantification. However, several limitations also need to be acknowledged. First, as this is a cross-sectional study, causal associations cannot be assumed, and the possibility of reverse causation cannot be excluded. Second, the scope of the study did not include the elucidation of molecular mechanisms underlying these associations. Third, inter-individual variation could affect the bioavailability of MPM such as food matrix and processing, gut microbiota profile, genetic polymorphisms, enzymatic capacity (especially phase II enzymes) of the host [42,60], biological rhythms [61], and environmental exposure (e.g., phthalates and phenols as endocrine disruptors related to obesity indicators) [6,7], all of them were not determined in our study. Fourth, as the participants did not use accelerometers in water sports or during competitions, the physical activity levels may have been underestimated. Finally, as some participants were probably evaluated in non-fasting conditions, the BG might be overestimated: only 1/560 participants reported non-fasting, but BG levels seem too high for a healthy adolescent population.

5. Conclusions

In summary, a higher concentration of MPM (gallic acid and urolithin A and B) was associated with better cardiometabolic health (lower MetS scores) in a sub-sample of adolescents enrolled at baseline in the SI! Program for Secondary Schools trial. Additionally, MPM were associated with lower odds of presenting MetS or some of its clinical features, such as abdominal obesity and high BG. These findings highlight the importance of (poly)phenols from gut microbiota metabolism on cardiometabolic health at early life stages. Further prospective analysis and clinical trials are strongly warranted to investigate the effect of MPM on cardiometabolic health in this young population.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11112191/s1, Figure S1: Flow-chart of study participants; Figure S2: Analysis flow-chart; Table S1: Phenolic metabolites identified and quantified in urine by HPLC-LTQ-Orbitrap-HRMS; Table S2: Log-transformed group of MPM and cardiometabolic health parameters; Table S2: Log-transformed group of MPM and MetS and its components.

Author Contributions: Conceptualization, R.M.L.-R., A.T.-R. and E.P.L.-S.; methodology, E.P.L.-S. and P.Q.-R.; software, E.P.L.-S.; validation, R.M.L.-R. and A.T.-R.; formal analysis, E.P.L.-S. and P.Q.-R.; investigation, R.M.L.-R., A.T.-R. and E.P.L.-S.; data curation, E.P.L.-S., M.M.-M., C.A.-R., A.V.-Q., A.d.C.-G.; writing—original draft preparation, E.P.L.-S., A.T.-R. and R.M.L.-R.; writing—review and editing, R.F.-J., J.M.F.-A., J.M.-G., G.S.-B., M.d.M., P.B., A.d.C.-G., E.P.L.-S., P.Q.-R., M.M.-M., C.A.-R., A.V.-Q., A.T.-R., A.M.R.-L., R.C., R.E. and R.M.L.-R.; visualization, E.P.L.-S.; supervision, R.M.L.-R. and A.T.-R.; funding acquisition, R.E., R.F.-J., J.M.F.-A., G.S.-B. and R.M.L.-R. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The SI! Program trial was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of ISCIII in Madrid (CEI PI 35_2016), the Fundació Unió Catalana d'Hospitals (CEI 16/41), and the University of Barcelona (IRB00003099).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. All participants were informed in advance about the study methodology and assessments, and parents or caregivers provided assent and written informed consent.

Data Availability Statement: The database analyzed during this cross-sectional study is not publicly available. Requestors wishing to access the database used in this study can make a request to the Steering Committee chair of the SI! Program trial: gsantos@fundacionshe.org, rodrigo.fernandez@cnic.es, juanmiguel.fernandez@cnic.es, RESTRUCH@clinic.cat, lamuela@ub.edu, bibanez@cnic.es, vfuster@cnic.es.

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Conflicts of Interest: R.M.L-R reports receiving lecture fees from Cerveceros de España and receiving lecture fees and travel support from Adventia and Idilia Foods SL. RE reports grants from Fundación Dieta Mediterránea (Spain), and Cerveza y Salud (Spain). Additionally, personal fees for given lectures from Brewers of Europe (Belgium), Fundación Cerveza y Salud (Spain), Pernaud-Ricard (Mexico), Instituto Cervantes, Lilly Laboratories (Spain), and Wine and Culinary International Forum (Spain), and non-financial support to organize a National Congress on Nutrition. Additionally, feeding trials with products from Grand Fountain and Uriach Laboratories (Spain). These funders had no role in the design, data collection and analyses, results, and the writing of the present cross-sectional study. The other authors declare no conflict of interest.

Abbreviations

BG	blood glucose
CI	confidence interval
DBP	diastolic blood pressure
FDR	false discovery rate
HDL-c	high-density lipoprotein cholesterol
HPLC-LTQ-Orbitrap-HRMS	High-performance liquid chromatography coupled to a linear trap quadrupole Orbitrap high-resolution mass spectrometer
LOQ	limit of quantitation
MAP	mean arterial pressure
MAD	mean absolute deviation method
MetS	metabolic syndrome
MS	mass spectra
MPM	
microbial phenolic metabolites	
OR	odds ratio
SBP	systolic blood pressure
TG	triglycerides
UM	Urinary metabotypes

WC	waist circumference

References

1. Zimmet, P.; Alberti, G.; Kaufman, F.; Tajima, N.; Silink, M.; Arslanian, S.; Wong, G.; Bennett, P.; Shaw, J.; Caprio, S. The Metabolic Syndrome in Children and Adolescents. *Lancet* **2007**, *369*, 2059–2061. https://doi.org/10.1016/S0140-6736(07)60958-1.
2. Magliano, D.J.; Zimmet, P.; Shaw, J.E. Classification of Diabetes Mellitus and Other Categories of Glucose Intolerance. In *International Textbook of Diabetes Mellitus*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2015; pp. 1–16. https://doi.org/10.1002/9781118387658.CH1.
3. Magnussen, C.G.; Koskinen, J.; Chen, W.; Thomson, R.; Schmidt, M.D.; Srinivasan, S.R.; Kivimäki, M.; Mattsson, N.; Kähönen, M.; Laitinen, T.; et al. Pediatric Metabolic Syndrome Predicts Adulthood Metabolic Syndrome, Subclinical Atherosclerosis, and Type 2 Diabetes Mellitus but Is No Better Than Body Mass Index Alone. *Circulation* **2010**, *122*, 1604–1611. https://doi.org/10.1161/CIRCULATIONAHA.110.940809.
4. Noubiap, J.J.; Nansseu, J.R.; Lontchi-Yimagou, E.; Nkeck, J.R.; Nyaga, U.F.; Ngouo, A.T.; Tounouga, D.N.; Tianyi, F.L.; Foka, A.J.; Ndoadoumgue, A.L.; et al. Global, Regional, and Country Estimates of Metabolic Syndrome Burden in Children and Adolescents in 2020: A Systematic Review and Modelling Analysis. *Lancet Child Adolesc. Health* **2022**, *6*, 158–170. https://doi.org/10.1016/S2352-4642(21)00374-6.
5. Després, J.-P.; Lemieux, I.; Bergeron, J.; Pibarot, P.; Mathieu, P.; Larose, E.; Rodés-Cabau, J.; Bertrand, O.F.; Poirier, P. Abdominal Obesity and the Metabolic Syndrome: Contribution to Global Cardiometabolic Risk. *Arter. Thromb. Vasc. Biol.* **2008**, *28*, 1039–1049. https://doi.org/10.1161/ATVBAHA.107.159228.
6. Casas, L.; Fernández, M.F.; Llop, S.; Guxens, M.; Ballester, F.; Olea, N.; Irurzun, M.B.; Rodríguez, L.S.M.; Riaño, I.; Tardón, A.; et al. Urinary Concentrations of Phthalates and Phenols in a Population of Spanish Pregnant Women and Children. *Environ. Int.* **2011**, *37*, 858–866. https://doi.org/10.1016/J.ENVINT.2011.02.012.
7. Güil-Oumrait, N.; Cano-Sancho, G.; Montazeri, P.; Stratakis, N.; Warembourg, C.; Lopez-Espinosa, M.J.; Vioque, J.; Santa-Marina, L.; Jimeno-Romero, A.; Ventura, R.; et al. Prenatal Exposure to Mixtures of Phthalates and Phenols and Body Mass Index and Blood Pressure in Spanish Preadolescents. *Environ. Int.* **2022**, *169*, 107527. https://doi.org/10.1016/J.ENVINT.2022.107527.
8. Martínez-González, M.A.; Gea, A.; Ruiz-Canela, M. The Mediterranean Diet and Cardiovascular Health. *Circ. Res.* **2019**, *124*, 779–798. https://doi.org/10.1161/CIRCRESAHA.118.313348.
9. Hurtado-Barroso, S.; Quifer-Rada, P.; de Alvarenga, J.F.R.; Pérez-Fernández, S.; Tresserra-Rimbau, A.; Lamuela-Raventos, R.M. Changing to a Low-Polyphenol Diet Alters Vascular Biomarkers in Healthy Men after Only Two Weeks. *Nutrients* **2018**, *10*, 1766. https://doi.org/10.3390/NU10111766.
10. Tresserra-Rimbau, A.; Rimm, E.B.; Medina-Remón, A.; Martínez-González, M.A.; de la Torre, R.; Corella, D.; Salas-Salvadó, J.; Gómez-Gracia, E.; Lapetra, J.; Arós, F.; et al. Inverse Association between Habitual Polyphenol Intake and Incidence of Cardiovascular Events in the PREDIMED Study. *Nutr. Metab. Cardiovasc. Dis.* **2014**, *24*, 639–647. https://doi.org/10.1016/j.numecd.2013.12.014.
11. Zhang, H.; Tsao, R. Dietary Polyphenols, Oxidative Stress and Antioxidant and Anti-Inflammatory Effects. *Curr. Opin. Food Sci.* **2016**, *8*, 33–42. https://doi.org/10.1016/J.COFS.2016.02.002.
12. Loo, Y.T.; Howell, K.; Chan, M.; Zhang, P.; Ng, K. Modulation of the Human Gut Microbiota by Phenolics and Phenolic Fiber-Rich Foods. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 1268–1298. https://doi.org/10.1111/1541-4337.12563.
13. Marhuenda-Muñoz, M.; Laveriano-Santos, E.P.; Tresserra-Rimbau, A.; Lamuela-Raventós, R.M.; Martínez-Huélamo, M.; Vallverdú-Queralt, A. Microbial Phenolic Metabolites: Which Molecules Actually Have an Effect on Human Health? *Nutrients* **2019**, *11*, 2725. https://doi.org/10.3390/NU11112725.
14. Mosele, J.I.; Macià, A.; Motilva, M.J. Metabolic and Microbial Modulation of the Large Intestine Ecosystem by Non-Absorbed Diet Phenolic Compounds: A Review. *Molecules* **2015**, *20*, 17429. https://doi.org/10.3390/MOLECULES200917429.
15. Teng, H.; Chen, L. Polyphenols and Bioavailability: An Update. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 2040–2051. https://doi.org/10.1080/10408398.2018.1437023.
16. López-Yerena, A.; Domínguez-López, I.; Vallverdú-Queralt, A.; Pérez, M.; Jáuregui, O.; Escribano-Ferrer, E.; Lamuela-Raventós, R.M. Metabolomics Technologies for the Identification and Quantification of Dietary Phenolic Compound Metabolites: An Overview. *Antioxidants* **2021**, *10*, 846. https://doi.org/10.3390/ANTIOX10060846.
17. Selma, M. v.; González-Sarrías, A.; Salas-Salvadó, J.; Andrés-Lacueva, C.; Alasalvar, C.; Örem, A.; Tomás-Barberán, F.A.; Espín, J.C. 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. *Clin. Nutr.* **2018**, *37*, 897–905. https://doi.org/10.1016/J.CLNU.2017.03.012.
18. Romo-Vaquero, M.; Cortés-Martín, A.; Loria-Kohen, V.; Ramírez-de-Molina, A.; García-Mantrana, I.; Collado, M.C.; Espín, J.C.; Selma, M.V. Deciphering the Human Gut Microbiome of Urolithin Metabotypes: Association with Enterotypes and Potential Cardiometabolic Health Implications. *Mol. Nutr. Food Res.* **2019**, *63*, 1800958. https://doi.org/10.1002/MNFR.201800958.
19. Fernandez-Jimenez, R.; Santos-Beneit, G.; Tresserra-Rimbau, A.; Bodega, P.; de Miguel, M.; de Cos-Gandoy, A.; Rodríguez, C.; Carral, V.; Orrit, X.; Haro, D.; et al. Rationale and Design of the School-Based SI! Program to Face Obesity and Promote Health

among Spanish Adolescents: A Cluster-Randomized Controlled Trial. *Am. Heart J.* **2019**, *215*, 27–40. https://doi.org/10.1016/j.ahj.2019.03.014.

20. Laveriano-Santos, E.P.; Marhuenda-Muñoz, M.; Vallverdú-Queralt, A.; Martínez-Huélamo, M.; Tresserra-Rimbau, A.; Miliarakis, E.; Arancibia-Riveros, C.; Jáuregui, O.; Ruiz-León, A.M.; Castro-Baquero, S.; et al. Identification and Quantification of Urinary Microbial Phenolic Metabolites by HPLC-ESI-LTQ-Orbitrap-HRMS and Their Relationship with Dietary Polyphenols in Adolescents. *Antioxidants* **2022**, *11*, 1167. https://doi.org/10.3390/antiox11061167.
21. Sharma, A.K.; Metzger, D.L.; Daymont, C.; Hadjiyannakis, S.; Rodd, C.J. LMS Tables for Waist-Circumference and Waist-Height Ratio Z-Scores in Children Aged 5–19 y in NHANES III: Association with Cardio-Metabolic Risks. *Pediatr. Res.* **2015**, *78*, 723–729. https://doi.org/10.1038/pr.2015.160.
22. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. *Pediatrics* **2004**, *114*, 555–576.
23. Shafiee, G.; Kelishadi, R.; Heshmat, R.; Qorbani, M.; Motlagh, M.E.; Aminaee, T.; Ardalan, G.; Taslimi, M.; Poursafa, P.; Larijani, B. First Report on the Validity of a Continuous Metabolic Syndrome Score as an Indicator for Metabolic Syndrome in a National Sample of Paediatric Population—The CASPIAN-III Study. *Endokrynol. Pol.* **2013**, *64*, 278–284. https://doi.org/10.5603/EP.2013.0006.
24. Medina-Remón, A.; Barrionuevo-González, A.; Zamora-Ros, R.; Andres-Lacueva, C.; Estruch, R.; Martínez-González, M.-Á.; Diez-Espino, J.; Lamuela-Raventos, R.M. Rapid Folin–Ciocalteu Method Using Microtiter 96-Well Plate Cartridges for Solid Phase Extraction to Assess Urinary Total Phenolic Compounds, as a Biomarker of Total Polyphenols Intake. *Anal. Chim. Acta* **2009**, *634*, 54–60. https://doi.org/10.1016/j.aca.2008.12.012.
25. Juton, C.; Castro-Barquero, S.; Casas, R.; Freitas, T.; Ruiz-León, A.M.; Crovetto, F.; Domenech, M.; Crispi, F.; Vieta, E.; Gratacós, E.; et al. Reliability and Concurrent and Construct Validity of a Food Frequency Questionnaire for Pregnant Women at High Risk to Develop Fetal Growth Restriction. *Nutrients* **2021**, *13*, 1629. https://doi.org/10.3390/nu13051629.
26. Moreiras, O.; Carvajal, H.; Cabrera, L.; Cuadrado, C. *Tablas de Composición de Alimentos*; Guía de Prácticas 19th ed.; Editorial Pirámide; Madrid, Spain, 2018; ISBN 978-84-368-3947-0.
27. Farrán A., Zamora R.C.P. Tablas de Composición de Alimentos del CESNID. McGraw-Hill Interamericana, Edicions Universitat de Barcelona; Barcelona, Spain: 2003.
28. Chandler, J.L.; Brazendale, K.; Beets, M.W.; Mealing, B.A. Classification of Physical Activity Intensities Using a Wrist-Worn Accelerometer in 8-12-Year-Old Children. *Pediatr. Obes.* **2016**, *11*, 120–127. https://doi.org/10.1111/ijpo.12033.
29. Barbosa, N.; Sanchez, C.E.; Vera, J.A.; Perez, W.; Thalabard, J.-C.; Rieu, M. A Physical Activity Questionnaire: Reproducibility and Validity. *J. Sport. Sci. Med.* **2007**, *6*, 505–518.
30. Bull, F.C.; Al-Ansari, S.S.; Biddle, S.; Borodulin, K.; Buman, M.P.; Cardon, G.; Carty, C.; Chaput, J.-P.; Chastin, S.; Chou, R.; et al. World Health Organization 2020 Guidelines on Physical Activity and Sedentary Behaviour. *Br. J. Sport. Med.* **2020**, *54*, 1451–1462. https://doi.org/10.1136/bjsports-2020-102955.
31. Tanner, J.M.; Whitehouse, R.H. Clinical Longitudinal Standards for Height, Weight, Height Velocity, Weight Velocity, and Stages of Puberty. *Arch. Dis. Child* **1976**, *51*, 170–179. https://doi.org/10.1136/adc.51.3.170.
32. INE: Instituto Nacional de Estadística Encuesta Anual de Estructura Salarial. Available online: https://www.ine.es/jaxiT3/Datos.htm?t=9948 (accessed on 19 June 2022).
33. Rusiman, M.S.; Nor, S.N.M.; Suparman, S.; Razali, S.N.A.M. Robust Method in Multiple Linear Regression Model on Diabetes Patients. *Math. Stat.* **2020**, *8*, 36–39. https://doi.org/10.13189/ms.2020.081306.
34. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B (Methodol.)* **1995**, *57*, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.
35. Kosuru, R.Y.; Roy, A.; Das, S.K.; Bera, S. Gallic Acid and Gallates in Human Health and Disease: Do Mitochondria Hold the Key to Success? *Mol. Nutr. Food Res.* **2018**, *62*, 1700699. https://doi.org/10.1002/MNFR.201700699.
36. Esteban-Torres, M.; Santamaría, L.; Cabrera-Rubio, R.; Plaza-Vinuesa, L.; Crispie, F.; de Las Rivas, B.; Cotter, P.; Muñoz, R. A Diverse Range of Human Gut Bacteria Have the Potential To Metabolize the Dietary Component Gallic Acid. *Appl. Environ. Microbiol.* **2018**, *84*, e01558-18. https://doi.org/10.1128/AEM.01558-18.
37. Kim, H.S.; Moon, J.H.; Kim, Y.M.; Huh, J.Y. Epigallocatechin Exerts Anti-Obesity Effect in Brown Adipose Tissue. *Chem. Biodivers.* **2019**, *16*, e1900347. https://doi.org/10.1002/CBDV.201900347.
38. Yang, C.S.; Zhang, J.; Zhang, L.; Huang, J.; Wang, Y. Mechanisms of Body Weight Reduction and Metabolic Syndrome Alleviation by Tea. *Mol. Nutr. Food Res.* **2016**, *60*, 160–174. https://doi.org/10.1002/MNFR.201500428.
39. Kapoor, M.P.; Sugita, M.; Fukuzawa, Y.; Okubo, T. Physiological Effects of Epigallocatechin-3-Gallate (EGCG) on Energy Expenditure for Prospective Fat Oxidation in Humans: A Systematic Review and Meta-Analysis. *J. Nutr. Biochem.* **2017**, *43*, 1–10. https://doi.org/10.1016/J.JNUTBIO.2016.10.013.
40. Auvichayapat, P.; Prapochanung, M.; Tunkamnerdthai, O.; Sripanidkulchai, B. orn; Auvichayapat, N.; Thinkhamrop, B.; Kunhasura, S.; Wongpratoom, S.; Sinawat, S.; Hongprapas, P. Effectiveness of Green Tea on Weight Reduction in Obese Thais: A Randomized, Controlled Trial. *Physiol. Behav.* **2008**, *93*, 486–491. https://doi.org/10.1016/J.PHYSBEH.2007.10.009.

41. Eid, H.M.; Wright, M.L.; Anil Kumar, N. v.; Qawasmeh, A.; Hassan, S.T.S.; Mocan, A.; Nabavi, S.M.; Rastrelli, L.; Atanasov, A.G.; Haddad, P.S. Significance of Microbiota in Obesity and Metabolic Diseases and the Modulatory Potential by Medicinal Plant and Food Ingredients. *Front. Pharm.* **2017**, *8*, 387. https://doi.org/10.3389/fphar.2017.00387.
42. Bento-Silva, A.; Koistinen, V.M.; Mena, P.; Bronze, M.R.; Hanhineva, K.; Sahlstrøm, S.; Kitrytė, V.; Moco, S.; Aura, A.M. Factors Affecting Intake, Metabolism and Health Benefits of Phenolic Acids: Do We Understand Individual Variability? *Eur. J. Nutr.* **2019**, *59*, 1275–1293. https://doi.org/10.1007/S00394-019-01987-6.
43. Kasprzak-Drozd, K.; Oniszczuk, T.; Stasiak, M.; Oniszczuk, A. Beneficial Effects of Phenolic Compounds on Gut Microbiota and Metabolic Syndrome. *Int. J. Mol. Sci.* **2021**, *22*, 3715. https://doi.org/10.3390/IJMS22073715.
44. Vishnu Prasad, C.N.; Anjana, T.; Banerji, A.; Gopalakrishnapillai, A. Gallic Acid Induces GLUT4 Translocation and Glucose Uptake Activity in 3T3-L1 Cells. *FEBS Lett.* **2010**, *584*, 531–536. https://doi.org/10.1016/J.FEBSLET.2009.11.092.
45. Punithavathi, V.R.; Prince, P.S.M.; Kumar, R.; Selvakumari, J. Antihyperglycaemic, Antilipid Peroxidative and Antioxidant Effects of Gallic Acid on Streptozotocin Induced Diabetic Wistar Rats. *Eur. J. Pharm.* **2011**, *650*, 465–471. https://doi.org/10.1016/J.EJPHAR.2010.08.059.
46. Kim, H.; Simbo, S.Y.; Fang, C.; McAlister, L.; Roque, A.; Banerjee, N.; Talcott, S.T.; Zhao, H.; Kreider, R.B.; Mertens-Talcott, S.U. Açaí (Euterpe Oleracea Mart.) Beverage Consumption Improves Biomarkers for Inflammation but Not Glucose- or Lipid-Metabolism in Individuals with Metabolic Syndrome in a Randomized, Double-Blinded, Placebo-Controlled Clinical Trial. *Food Funct.* **2018**, *9*, 3097–3103. https://doi.org/10.1039/C8FO00595H.
47. Hasheminezhad, S.H.; Boozari, M.; Iranshahi, M.; Yazarlu, O.; Sahebkar, A.; Hasanpour, M.; Iranshahy, M. A Mechanistic Insight into the Biological Activities of Urolithins as Gut Microbial Metabolites of Ellagitannins. *Phytother. Res.* **2021**, *36*, 112–146. https://doi.org/10.1002/ptr.7290.
48. Li, K.; Xiao, Y.; Bian, J.; Han, L.; He, C.; El-Omar, E.; Gong, L.; Wang, M. Ameliorative Effects of Gut Microbial Metabolite Urolithin A on Pancreatic Diseases. *Nutrients* **2022**, *14*, 2549. https://doi.org/10.3390/nu14122549.
49. García-Muñoz, C.; Hernández, L.; Pérez, A.; Vaillant, F. Diversity of Urinary Excretion Patterns of Main Ellagitannins' Colonic Metabolites after Ingestion of Tropical Highland Blackberry (*Rubus adenotrichus*) Juice. *Food Res. Int.* **2014**, *55*, 161–169. https://doi.org/10.1016/J.FOODRES.2013.10.049.
50. Istas, G.; Feliciano, R.P.; Weber, T.; Garcia-Villalba, R.; Tomas-Barberan, F.; Heiss, C.; Rodriguez-Mateos, A. Plasma Urolithin Metabolites Correlate with Improvements in Endothelial Function after Red Raspberry Consumption: A Double-Blind Randomized Controlled Trial. *Arch. Biochem. Biophys.* **2018**, *651*, 43–51. https://doi.org/10.1016/J.ABB.2018.05.016.
51. García-Conesa, M.T.; Chambers, K.; Combet, E.; Pinto, P.; Garcia-Aloy, M.; Andrés-Lacueva, C.; Pascual-Teresa, S. de; Mena, P.; Ristic, A.K.; Hollands, W.J.; et al. Meta-Analysis of the Effects of Foods and Derived Products Containing Ellagitannins and Anthocyanins on Cardiometabolic Biomarkers: Analysis of Factors Influencing Variability of the Individual Responses. *Int. J. Mol. Sci.* **2018**, *19*, 694. https://doi.org/10.3390/IJMS19030694.
52. González-Sarrías, A.; Larrosa, M.; Tomás-Barberán, F.A.; Dolara, P.; Espín, J.C. NF-KappaB-Dependent Anti-Inflammatory Activity of Urolithins, Gut Microbiota Ellagic Acid-Derived Metabolites, in Human Colonic Fibroblasts. *Br. J. Nutr.* **2010**, *104*, 503–512. https://doi.org/10.1017/S0007114510000826.
53. Giménez-Bastida, J.A.; González-Sarrías, A.; Larrosa, M.; Tomás-Barberán, F.; Espín, J.C.; García-Conesa, M.T. Ellagitannin Metabolites, Urolithin A Glucuronide and Its Aglycone Urolithin A, Ameliorate TNF-α-Induced Inflammation and Associated Molecular Markers in Human Aortic Endothelial Cells. *Mol. Nutr. Food Res.* **2012**, *56*, 784–796. https://doi.org/10.1002/MNFR.201100677.
54. Spigoni, V.; Mena, P.; Cito, M.; Fantuzzi, F.; Bonadonna, R.C.; Brighenti, F.; Cas, A.D.; Rio, D. del Effects on Nitric Oxide Production of Urolithins, Gut-Derived Ellagitannin Metabolites, in Human Aortic Endothelial Cells. *Molecules* **2016**, *21*, 1009. https://doi.org/10.3390/MOLECULES21081009.
55. Cortés-Martín, A.; Colmenarejo, G.; Selma, M.V.; Espín, J.C. Genetic Polymorphisms, Mediterranean Diet and Microbiota-Associated Urolithin Metabotypes Can Predict Obesity in Childhood-Adolescence. *Sci. Rep.* **2020**, *10*, 7850. https://doi.org/10.1038/s41598-020-64833-4.
56. Bugger, H.; Abel, E.D. Molecular Mechanisms of Diabetic Cardiomyopathy. *Diabetologia* **2014**, *57*, 660. https://doi.org/10.1007/S00125-014-3171-6.
57. Sala, R.; Mena, P.; Savi, M.; Brighenti, F.; Crozier, A.; Miragoli, M.; Stilli, D.; del Rio, D. Urolithins at Physiological Concentrations Affect the Levels of Pro-Inflammatory Cytokines and Growth Factor in Cultured Cardiac Cells in Hyperglucidic Conditions. *J. Funct. Foods* **2015**, *15*, 97–105. https://doi.org/10.1016/J.JFF.2015.03.019.
58. Williamson, G.; Kay, C.D.; Crozier, A. The Bioavailability, Transport, and Bioactivity of Dietary Flavonoids: A Review from a Historical Perspective. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 1054–1112. https://doi.org/10.1111/1541-4337.12351.
59. Frankenfeld, C.L. Cardiometabolic Risk Factors Are Associated with High Urinary Enterolactone Concentration, Independent of Urinary Enterodiol Concentration and Dietary Fiber Intake in Adults. *J. Nutr.* **2014**, *144*, 1445–1453. https://doi.org/10.3945/JN.114.190512.
60. Gibney, E.R.; Milenkovic, D.; Combet, E.; Ruskovska, T.; Greyling, A.; González-Sarrías, A.; de Roos, B.; Tomás-Barberán, F.; Morand, C.; Rodriguez-Mateos, A. Factors Influencing the Cardiometabolic Response to (Poly)Phenols and Phytosterols: A Review of the COST Action POSITIVe Activities. *Eur. J. Nutr.* **2019**, *58*, 37–47. https://doi.org/10.1007/s00394-019-02066-6.

61. Ávila-Román, J.; Soliz-Rueda, J.R.; Bravo, F.I.; Aragonès, G.; Suárez, M.; Arola-Arnal, A.; Mulero, M.; Salvadó, M.J.; Arola, L.; Torres-Fuentes, C.; et al. Phenolic Compounds and Biological Rhythms: Who Takes the Lead? *Trends Food Sci. Technol.* **2021**, *113*, 77–85. https://doi.org/10.1016/J.TIFS.2021.04.050.

Supplementary data

Microbial Phenolic Metabolites in Urine are Inversely Linked to Certain Features of Metabolic Syndrome in Spanish Adolescents

Content:

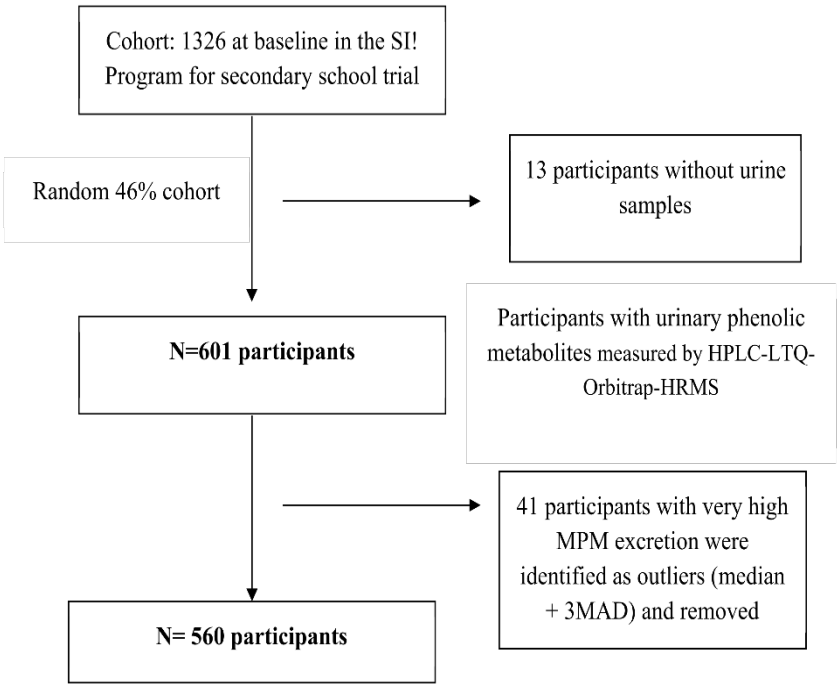


Figure S1. Flow-chart of study participants.

HPLC-LTQ-Orbitrap-HRMS High-performance liquid chromatography coupled to linear trap quadrupole Orbitrap high-resolution mass spectrometer, MAD median absolute deviation, MPM microbial phenolic metabolites.

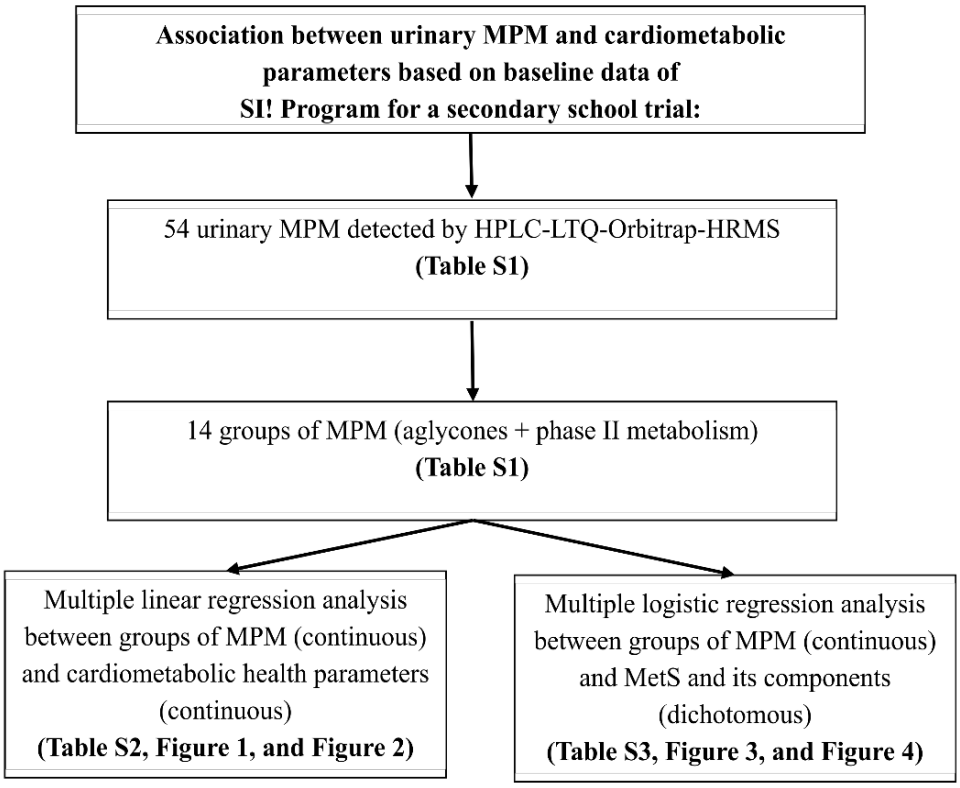


Figure S2. Analysis flow-chart.

HPLC-LTQ-Orbitrap-HRMS High-performance liquid chromatography coupled to linear trap quadrupole Orbitrap high-resolution mass spectrometer, MetS metabolic syndrome, MPM microbial phenolic metabolites.

Table S1. Phenolic metabolites identified and quantified in urine by HPLC-LTQ-Orbitrap-HRMS.

Compound	Neutral Molecular Formula	R_t (min)
Coumaric acids		
m-coumaric acid [a]	$C_9H_8O_3$	6.54
o-coumaric acid [a]	$C_9H_8O_3$	7.20
p-coumaric acid [a]	$C_9H_8O_3$	5.96
Coumaric acid glucuronide I (CA)	$C_{15}H_{16}O_9$	4.77
Coumaric acid glucuronide II (CA)	$C_{15}H_{16}O_9$	5.09
Coumaric acid glucuronide III (CA)	$C_{15}H_{16}O_9$	5.82
Coumaric acid sulfate I (CA)	$C_9H_8O_6S$	3.53
Coumaric acid sulfate II (CA)	$C_9H_8O_6S$	4.27
Coumaric acid sulfate III (CA)	$C_9H_8O_6S$	4.74
Dihydroxyphenylpropionic acid		
3,4-dihydroxyphenylpropionic acid [a]	$C_9H_{10}O_4$	3.84
Dihydroxyphenylpropionic acid sulfate (3,4-DHPPA)	$C_9H_{10}O_7S$	2.52
Dihydroresveratrol		
Dihydroresveratrol [a]	$C_{14}H_{14}O_3$	8.73
Dihydroresveratrol sulfate I (DHRSV)	$C_{14}H_{14}O_6S$	7.07
Dihydroresveratrol sulfate II (DHRSV)	$C_{14}H_{14}O_6S$	7.40
Enterodiol		
Enterodiol [a]	$C_{18}H_{22}O_4$	9.17
Enterodiol glucuronide I (ED)	$C_{24}H_{30}O_{10}$	7.48
Enterodiol glucuronide II (ED)	$C_{24}H_{30}O_{10}$	7.61
Enterodiol sulfate (ED)	$C_{18}H_{20}O_7S$	7.50
Enterolactone		
Enterolactone [a]	$C_{18}H_{18}O_4$	10.80
Enterolactone glucuronide (EL)	$C_{24}H_{26}O_{10}$	8.77
Enterolactone diglucuronide (EL)	$C_{30}H_{34}O_{16}$	7.24
Enterolactone sulfate (EL)	$C_{18}H_{18}O_7S$	8.57
Gallic acid		
Gallic acid [a]	$C_7H_6O_5$	1.69
Gallic acid glucuronide (GA)	$C_{13}H_{14}O_{11}$	1.85
Gallic acid sulfate (GA)	$C_7H_6O_8S$	1.72
Hydroxybenzoic acid		
3- hydroxybenzoic acid [a]	$C_7H_6O_3$	4.02
4-hydroxybenzoic acid [a]	$C_7H_6O_3$	3.15
Hydroxybenzoic acid glucuronide I (HBA)	$C_{13}H_{14}O_9$	2.00
Hydroxybenzoic acid glucuronide II (HBA)	$C_{13}H_{14}O_9$	2.50
Hydroxybenzoic acid sulfate (HBA)	$C_7H_6O_6S$	1.89

Hydroxyphenylacetic acid		
3-hydroxyphenylacetic acid [a]	$C_8H_8O_3$	4.09
Hydroxyphenylacetic acid glucuronide (3-HPAA)	$C_{14}H_{16}O_9$	4.8
Hydroxyphenylacetic acid sulfate (3-HPAA)	$C_8H_8O_6S$	2.24
Hydroxytyrosol		
3-hydroxytyrosol [a]	$C_8H_{10}O_3$	2.11
3'hydroxytyrosol-3'-glucuronide [a]	$C_{14}H_{18}O_9$	2.11
Hydroxytyrosol sulfate (3-HT)	$C_8H_{10}O_6S$	1.82
Protocatechuic acid		
Protocatechuic acid [a]	$C_7H_6O_4$	2.25
Protocatechuic acid glucuronide (PCA)	$C_{13}H_{14}O_{10}$	1.67
Protocatechuic acid sulfate I (PCA)	$C_7H_6O_7S$	1.75
Protocatechuic acid sulfate II (PCA)	$C_7H_6O_7S$	1.94
Syringic acid		
Syringic acid [a]	$C_9H_{10}O_5$	5.63
Syringic acid glucuronide I (SA)	$C_{15}H_{18}O_{11}$	4.68
Syringic acid glucuronide II (SA)	$C_{15}H_{18}O_{11}$	4.88
Syringic acid sulfate (SA)	$C_9H_{10}O_8S$	4.24
Urolithin A		
Urolithin A [a]	$C_{13}H_8O_4$	9.56
Urolithin A glucuronide (Uro A)	$C_{19}H_{16}O_{10}$	6.98
Urolithin A diglucuronide (Uro A)	$C_{25}H_{24}O_{16}$	6.88
Urolithin A sulfate (Uro A)	$C_{13}H_8O_7S$	7.49
Urolithin B		
Urolithin B [a]	$C_{13}H_8O_3$	11.12
Urolithin B glucuronide (Uro B)	$C_{19}H_{16}O_9$	8.51
Vanillic acid		
Vanillic acid [a]	$C_8H_8O_4$	4.74
Vanillic acid glucuronide I (VA)	$C_{14}H_{16}O_{10}$	2.31
Vanillic acid glucuronide II (VA)	$C_{14}H_{16}O_{10}$	2.76
Vanillic acid sulfate (VA)	$C_8H_8O_7S$	2.03

[a] Commercial standards, Rt retention time, CA coumaric acid, 3,4-DHPPA 3,4-dihydroxyphenylpropionic acid, 3-HPAA 3-hydroxyphenylacetic acid, 3-HBA 3-hydroxybenzoic acid, 3-HT 3-hydroxytyrosol, 3-HT-G 3-Hydroxytyrosol glucuronide, 4-HBA 4-hydroxybenzoic acid, DHRSV Dihydroresveratrol, ED enterodiol, EL enterolactone, GA gallic acid, PCA protocatechuic acid, SA syringic acid, Uro A urolithin A, Uro B urolithin B, VA vanillic acid. When standards were not available, the aglycone was used for quantification (shown in brackets).

Table S2. Log-transformed group of MPM and cardiometabolic health parameters.

	WC z-score			SBP z-score			DBP z-score			TG			HDL-c			BG			MetS score		
	β (95 %CI)	*p*-value	*p*-adj*	β (95% CI)	*p*-value	*p*-adj*	β (95% CI)	*p*-value	*p*-adj*	β (95% CI)	*p*-value	*p*-adj*	β (95% CI)	*p*-value	*p*-adj*	β (95% CI)	*p*-value	*p*-adj*	β (95% CI)	*p*-value	*p*-adj*
Coumaric acids																					
Model 1	0.01 (-0.02;0.04)	0.523	0.666	0.00 (-0.04;0.04)	0.976	0.976	-0.01 (-0.04;0.03)	0.705	0.868	1.75 (0.18;3.33)	**0.030**	0.330	-0.2 (-0.73;0.35)	0.484	0.847	0.27 (-0.19;0.72)	0.254	0.889	0.08 (-0.03;0.18)	0.144	0.271
Model 2	-0.01 (-0.04;0.03)	0.646	0.822	-0.02 (-0.06;0.02)	0.343	0.437	-0.03 (-0.06;0.01)	0.142	0.387	1.54 (-0.23;3.3)	0.088	0.411	0.02 (-0.58;0.61)	0.956	0.956	0.43 (-0.1;0.94)	0.108	0.756	0.04 (-0.08;0.15)	0.513	0.798
Dihydroxyphenylpropionic acid																					
Model 1	-0.01 (-0.02;0.01)	0.608	0.709	-0.01 (-0.03;0.01)	0.180	0.360	-0.01 (-0.02;0.01)	0.444	0.777	0.09 (-0.58;0.75)	0.799	0.974	0.20 (-0.03;0.43)	0.086	0.569	0.02 (-0.18;0.21)	0.853	0.919	-0.02 (-0.07;0.03)	0.394	0.549
Model 2	-0.01 (-0.02;0.01)	0.735	0.858	-0.02 (-0.03;0.00)	**0.048**	0.231	-0.01 (-0.02;0.01)	0.249	0.387	0.11 (-0.61;0.83)	0.766	0.972	0.12 (-0.13;0.36)	0.351	0.956	0.02 (-0.2;0.24)	0.870	0.948	-0.02 (-0.06;0.04)	0.572	0.801
Dihydroresveratrol																					
Model 1	-0.01 (-0.02;0.01)	0.475	0.666	-0.02 (-0.04;0.01)	0.093	0.301	-0.01 (-0.02;0.01)	0.313	0.626	0.07 (-0.75;0.89)	0.870	0.974	0.22 (-0.06;0.5)	0.122	0.569	-0.09 (-0.32;0.15)	0.468	0.919	-0.04 (-0.09;0.02)	0.155	0.271
Model 2	-0.01 (-0.03;0.01)	0.505	0.786	-0.02 (-0.04;0.00)	**0.048**	0.231	-0.01 (-0.03;0.01)	0.204	0.387	0.24 (-0.68;1.15)	0.612	0.972	0.08 (-0.23;0.38)	0.623	0.956	-0.07 (-0.34;0.2)	0.611	0.948	-0.03 (-0.09;0.03)	0.345	0.634
Enterodiol																					
Model 1	-0.01 (-0.03;0.02)	0.441	0.666	-0.02 (-0.05;0.01)	0.126	0.301	-0.02 (-0.04;0.01)	0.101	0.445	0.51 (-0.67;1.67)	0.399	0.931	-0.05 (-0.45;0.35)	0.803	0.950	-0.07 (-0.4;0.27)	0.704	0.919	-0.03 (-0.11;0.05)	0.431	0.549
Model 2	0.00 (-0.03;0.03)	0.981	0.981	-0.02 (-0.04;0.02)	0.275	0.385	-0.02 (-0.04;0.01)	0.219	0.387	0.42 (-0.85;1.69)	0.516	0.972	-0.07 (-0.49;0.35)	0.741	0.956	-0.03 (-0.4;0.34)	0.880	0.948	-0.01 (-0.09;0.08)	0.851	0.866
Enterolactone																					
Model 1	-0.04 (-0.08;0.01)	0.115	0.350	-0.05 (-0.1;0.01)	0.058	0.301	-0.03 (-0.07;0.02)	0.159	0.445	-1.89 (-4.19;0.41)	0.107	0.330	0.42 (-0.39;1.23)	0.306	0.774	-0.15 (-0.82;0.54)	0.682	0.919	-0.17 (-0.32;-0.02)	**0.033**	0.231
Model 2	-0.03 (-0.08;0.02)	0.178	0.498	-0.05 (-0.1;0.01)	0.053	0.231	-0.04 (-0.08;0.01)	0.129	0.387	-1.22 (-3.66;1.22)	0.326	0.913	0.03 (-0.81;0.87)	0.945	0.956	-0.01 (-0.74;0.73)	0.988	0.988	-0.12 (-0.28;0.04)	0.129	0.602
Gallic acid																					
Model 1	-0.01 (-0.03;0.01)	0.239	0.478	-0.01 (-0.03;0.02)	0.498	0.697	-0.01 (-0.02;0.02)	0.926	0.955	-0.67 (-1.42;0.09)	0.082	0.330	0.08 (-0.21;0.37)	0.581	0.904	-0.08 (-0.32;0.17)	0.558	0.919	-0.05 (-0.1;0.01)	0.100	0.271
Model 2	-0.02 (-0.03;-0.01)	**0.041**	0.287	-0.02 (-0.03;0.01)	0.189	0.378	-0.01 (-0.02;0.01)	0.542	0.747	-0.8 (-1.59;-0.01)	**0.048**	0.411	0.19 (-0.11;0.49)	0.200	0.933	-0.14 (-0.4;0.12)	0.291	0.948	-0.07 (-0.13;-0.02)	**0.009**	0.063
Hydroxybenzoic acid																					

Model 1	0.04 (-0.01;0.09)	0.097	0.350	0.01 (-0.05;0.06)	0.796	0.976	0.01 (-0.04;0.05)	0.744	0.868	0.16 (-2.33;2.64)	0.904	0.974	-0.03 (-0.91;0.85)	0.950	0.950	0.45 (-0.3;1.2)	0.235	0.889	0.10 (-0.07;0.27)	0.232	0.361
Model 2	0.04 (-0.02;0.09)	0.137	0.480	-0.01 (-0.06;0.06)	0.931	0.983	0.01 (-0.05;0.05)	0.942	0.949	0.14 (-2.57;2.84)	0.923	0.972	-0.32 (-1.26;0.62)	0.505	0.956	0.4 (-0.44;1.23)	0.348	0.948	0.10 (-0.08;0.27)	0.284	0.634
Hydroxyphenylacetic acid																					
Model 1	0.01 (-0.02;0.02)	0.860	0.926	0.02 (-0.01;0.03)	0.066	0.301	-0.01 (-0.02;0.01)	0.696	0.868	-0.09 (-0.82;0.65)	0.818	0.974	0.03 (-0.23;0.29)	0.818	0.950	0.04 (-0.18;0.26)	0.736	0.919	0.00 (-0.05;0.05)	0.986	0.986
Model 2	0.01 (-0.01;0.02)	0.635	0.822	0.02 (-0.01;0.04)	0.066	0.231	-0.01 (-0.02;0.01)	0.587	0.747	-0.08 (-0.89;0.73)	0.847	0.972	0.03 (-0.25;0.3)	0.864	0.956	0.06 (-0.19;0.3)	0.653	0.948	0.01 (-0.05;0.06)	0.855	0.866
Hydroxytyrosol																					
Model 1	0.01 (-0.01;0.02)	0.517	0.666	-0.01 (-0.02;0.02)	0.851	0.976	-0.01 (-0.02;0.01)	0.295	0.626	-0.12 (-0.9;0.67)	0.772	0.974	-0.06 (-0.33;0.22)	0.698	0.950	-0.06 (-0.29;0.18)	0.643	0.919	-0.01 (-0.06;0.05)	0.720	0.775
Model 2	0.01 (-0.01;0.03)	0.347	0.607	-0.01 (-0.03;0.02)	0.445	0.519	-0.01 (-0.03;0.01)	0.247	0.387	-0.03 (-0.89;0.84)	0.954	0.972	-0.26 (-0.55;0.04)	0.090	0.630	-0.04 (-0.3;0.23)	0.789	0.948	0.01 (-0.05;0.07)	0.751	0.866
Protocatechuic acid																					
Model 1	-0.03 (-0.06;0.01)	0.125	0.350	-0.03 (-0.07;0.01)	0.129	0.301	-0.03 (-0.05;0.01)	0.127	0.445	0.01 (-1.6;1.6)	0.998	0.998	0.61 (0.03;1.19)	**0.040**	0.560	0.06 (-0.44;0.55)	0.820	0.919	-0.1 (-0.21;0.02)	0.081	0.271
Model 2	-0.02 (-0.05;0.02)	0.276	0.552	-0.02 (-0.06;0.02)	0.261	0.385	-0.02 (-0.05;0.02)	0.247	0.387	-0.32 (-1.98;1.34)	0.705	0.972	0.52 (-0.07;1.11)	0.083	0.630	0.06 (-0.47;0.58)	0.840	0.948	-0.08 (-0.19;0.04)	0.175	0.613
Syringic acid																					
Model 1	0.01 (-0.01;0.03)	0.194	0.453	0.00 (-0.02;0.02)	0.965	0.976	0.00 (-0.02;0.02)	0.955	0.955	0.69 (-0.18;1.54)	0.118	0.330	-0.14 (-0.44;0.17)	0.387	0.774	0.21 (-0.05;0.45)	0.114	0.798	0.05 (-0.02;0.1)	0.148	0.271
Model 2	0.02 (-0.01;0.03)	0.120	0.480	0.00 (-0.02;0.02)	0.983	0.983	-0.01 (-0.02;0.02)	0.719	0.839	0.56 (-0.38;1.49)	0.245	0.858	-0.1 (-0.41;0.23)	0.572	0.956	0.15 (-0.14;0.43)	0.310	0.948	0.04 (-0.03;0.1)	0.246	0.634
Urolithin A																					
Model 1	-0.01 (-0.02;0.01)	0.090	0.350	-0.01 (-0.02;0.01)	0.112	0.301	-0.01 (-0.02;-0.01)	**0.011**	0.154	-0.1 (-0.57;0.37)	0.686	0.974	0.01 (-0.16;0.18)	0.906	0.950	-0.01 (-0.15;0.14)	0.986	0.986	-0.03 (-0.06;0.01)	0.154	0.271
Model 2	-0.01 (-0.02;0.01)	0.218	0.509	-0.01 (-0.02;0.01)	0.130	0.303	-0.02 (-0.03;-0.01)	**0.003**	**0.042**	-0.02 (-0.52;0.49)	0.957	0.972	-0.02 (-0.2;0.16)	0.839	0.956	0.03 (-0.13;0.19)	0.706	0.948	-0.02 (-0.05;0.02)	0.362	0.634
Urolithin B																					
Model 1	-0.02 (-0.03;-0.01)	**0.003**	**0.042**	-0.01 (-0.03;0.01)	0.229	0.401	-0.01 (-0.03;0.01)	0.104	0.445	-0.55 (-1.17;0.07)	0.081	0.330	0.11 (-0.14;0.36)	0.381	0.774	-0.18 (-0.38;0.03)	0.094	0.798	-0.07 (-0.11;-0.02)	**0.005**	0.070
Model 2	-0.03 (-0.04;-0.01)	**<0.001**	**0.006**	-0.02 (-0.03;0.01)	0.102	0.286	-0.02 (-0.03;-0.003)	**0.013**	0.091	-0.63 (-1.29;0.03)	0.059	0.411	0.11 (-0.16;0.36)	0.425	0.956	-0.25 (-0.47;-0.04)	**0.024**	0.336	-0.08 (-0.12;-0.04)	**0.001**	**0.014**
Vanillic acid																					
Model 1	0.00 (-0.04;0.04)	0.986	0.986	0.02 (-0.03;0.07)	0.423	0.658	-0.01 (-0.05;0.03)	0.604	0.868	-0.2 (-2.42;2.04)	0.864	0.974	0.42 (-0.36;1.19)	0.296	0.774	0.17 (-0.49;0.82)	0.619	0.919	-0.04 (-0.19;0.11)	0.619	0.722
Model 2	0.01 (-0.04;0.05)	0.823	0.886	0.03 (-0.02;0.08)	0.262	0.385	-0.01 (-0.05;0.04)	0.949	0.949	0.05 (-2.3;2.38)	0.972	0.972	0.33 (-0.47;1.12)	0.422	0.956	0.22 (-0.48;0.92)	0.537	0.948	-0.02 (-0.17;0.14)	0.866	0.866

β estimated beta, BG blood glucose CI confidence interval, DBP diastolic blood pressure, HDL-c high-density lipoprotein-cholesterol, MetS metabolic syndrome, MPM microbial phenolic metabolites, SBP systolic blood pressure, TG triglycerides, WC waist circumference. Mixed-effects linear regression models between log-transformed group of MPM (aglycone plus phase II metabolites) and each cardiometabolic health parameter. Fixed effect included two models: model 1 included sex (female/male) and age (continuous, years); Model 2 included model 1 plus Tanner maturation stage (score from I to V), physical activity (≥ 60min / <60min moderate-to-vigorous physical activity), household income (low/medium/high), and energy intake (continuous, Kcal/day). Municipality and schools were considered as random effects. * *p*-adjusted for multiple-testing using the Benjamin-Hochberg procedure considering a false discovery rate < 0.05 as significant. P values < 0.05 were considered significant.

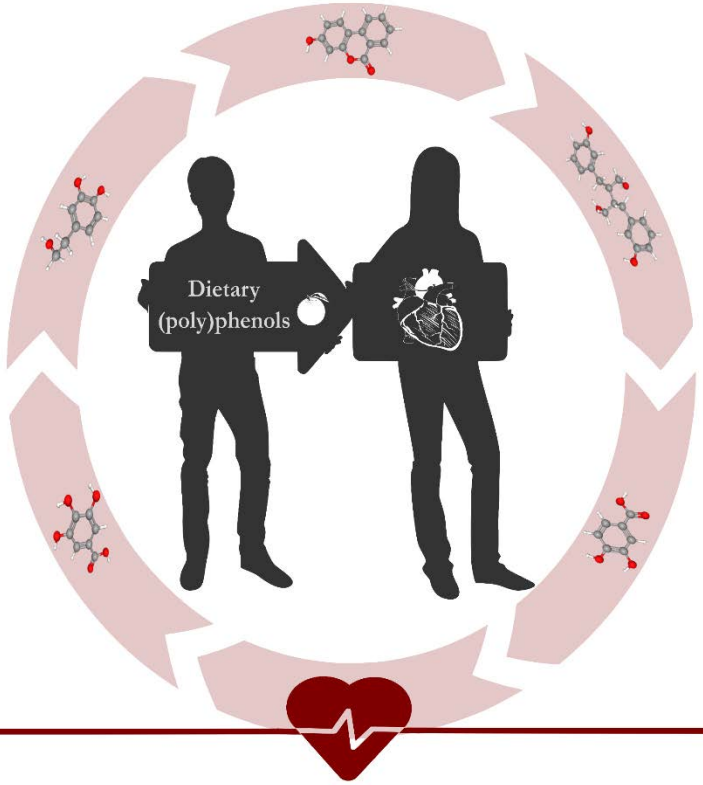
Table S3. Log-transformed group of MPM and MetS and its components.

	WC ≥ 90th			TG ≥ 150 mg/dL			HDL-c ≤ 40 mg/dL			BG ≥ 110 mg/dL			MetS		
	OR (95 %CI)	***p*-value**	***p*-adj***	**OR (95 %CI)**	***p*-value**	***p*-adj***	**OR (95 %CI)**	***p*-value**	***p*-adj***	**OR (95 %CI)**	***p*-value**	***p*-adj***	**OR (95 %CI)**	***p*-value**	***p*-adj***
Coumaric acids															
Model 1	1.06 (0.96;1.17)	0.312	0.485	1.08 (0.90;1.29)	0.399	0.868	1.02 (0.85;1.23)	0.821	0.915	1.02 (0.94;1.11)	0.637	0.811	0.96 (0.79;1.18)	0.714	0.859
Model 2	1.04 (0.93;1.16)	0.580	0.756	1.06 (0.86;1.30)	0.595	0.944	0.96 (0.81;1.15)	0.687	0.802	1.04 (0.94;1.16)	0.430	0.754	0.93 (0.74;1.18)	0.562	0.715
Dihydroxyphenylpropionic acid															
Model 1	0.99 (0.96;1.03)	0.526	0.669	1.00 (0.95;1.06)	0.991	0.991	0.94 (0.89;1.00)	0.050	0.294	0.99 (0.96;1.03)	0.705	0.823	0.98 (0.90;1.06)	0.603	0.859
Model 2	1.00 (0.96;1.04)	0.648	0.756	1.00 (0.94;1.07)	0.944	0.944	0.93 (0.87;0.99)	**0.048**	0.437	1.00 (0.96;1.04)	0.922	0.953	1.01 (0.90;1.13)	0.897	0.897
Dihydroresveratrol															
Model 1	0.99 (0.95;1.03)	0.395	0.553	1.01 (0.94;1.08)	0.854	0.991	0.97 (0.90;1.05)	0.449	0.698	0.98 (0.94;1.02)	0.333	0.666	1.02 (0.91;1.14)	0.736	0.859
Model 2	0.99 (0.94;1.04)	0.455	0.708	1.01 (0.93;1.09)	0.894	0.944	0.99 (0.90;1.09)	0.876	0.943	0.98 (0.94;1.03)	0.431	0.754	1.09 (0.90;1.32)	0.356	0.623
Enterodiol															
Model 1	0.97 (0.91;1.02)	0.180	0.315	1.02 (0.92;1.12)	0.753	0.991	1.04 (0.90;1.20)	0.580	0.812	0.97 (0.92;1.03)	0.308	0.666	1.03 (0.87;1.23)	0.707	0.859
Model 2	0.99 (0.92;1.06)	0.610	0.756	1.01 (0.90;1.13)	0.871	0.944	1.04 (0.89;1.21)	0.653	0.802	0.98 (0.92;1.04)	0.582	0.815	1.02 (0.85;1.23)	0.798	0.859
Enterolactone															
Model 1	0.90 (0.79;1.02)	0.084	0.201	0.83 (0.71;0.98)	0.026	0.364	0.82 (0.68;1.00)	0.052	0.294	0.96 (0.85;1.09)	0.514	0.806	0.78 (0.59;1.02)	0.064	0.299
Model 2	0.90 (0.78;1.05)	0.162	0.397	0.86 (0.72;1.04)	0.116	0.717	0.85 (0.68;1.06)	0.156	0.437	1.01 (0.88;1.16)	0.859	0.953	0.80 (0.59;1.09)	0.165	0.483
Gallic acid															
Model 1	0.96 (0.93;1)	**0.048**	0.201	0.99 (0.93;1.04)	0.618	0.991	1.00 (0.91;1.09)	0.992	0.992	0.97 (0.93;1.01)	0.200	0.666	0.88 (0.81;0.96)	**0.003**	**0.042**
Model 2	0.93 (0.89;0.98)	**0.002**	**0.021**	0.97 (0.91;1.03)	0.345	0.805	0.97 (0.88;1.07)	0.536	0.802	0.96 (0.92;1.00)	0.066	0.308	0.85 (0.77;0.93)	**0.001**	**0.014**
Hydroxybenzoic acid															

Model 1	1.14 (0.99;1.32)	0.086	0.201	0.99 (0.82;1.21)	0.958	0.991	0.98 (0.77;1.24)	0.850	0.915	0.99 (0.86;1.13)	0.852	0.852	0.97 (0.67;1.40)	0.851	0.916
Model 2	1.17 (0.99;1.39)	0.073	0.256	0.98 (0.78;1.24)	0.866	0.944	0.94 (0.71;1.25)	0.657	0.802	1.00 (0.86;1.17)	0.953	0.953	1.06 (0.69;1.64)	0.789	0.859
Hydroxyphenylacetic acid															
Model 1	1.00 (0.96;1.04)	0.713	0.769	0.98 (0.93;1.03)	0.434	0.868	1.05 (0.95;1.16)	0.337	0.674	1.00 (0.97;1.04)	0.819	0.852	0.97 (0.90;1.06)	0.531	0.859
Model 2	1.01 (0.97;1.06)	0.755	0.772	0.97 (0.92;1.02)	0.256	0.717	1.04 (0.93;1.16)	0.492	0.802	1.01 (0.97;1.06)	0.527	0.815	0.97 (0.89;1.06)	0.499	0.699
Hydroxytyrosol															
Model 1	1.00 (0.96;1.05)	0.998	0.998	0.99 (0.93;1.06)	0.829	0.991	0.96 (0.89;1.03)	0.245	0.656	0.99 (0.95;1.03)	0.576	0.806	0.94 (0.86;1.02)	0.115	0.369
Model 2	1.01 (0.96;1.06)	0.772	0.772	0.99 (0.92;1.06)	0.789	0.944	0.94 (0.86;1.02)	0.134	0.437	1.00 (0.95;1.04)	0.886	0.953	0.91 (0.83;0.99)	**0.027**	0.189
Protocatechuic acid															
Model 1	0.93 (0.85;1.02)	0.102	0.204	1.11 (0.98;1.27)	0.102	0.597	0.95 (0.82;1.09)	0.448	0.698	0.93 (0.85;1.01)	0.101	0.471	1.00 (0.79;1.27)	0.982	0.982
Model 2	0.94 (0.85;1.04)	0.170	0.397	1.13 (0.98;1.31)	0.099	0.717	0.95 (0.82;1.10)	0.483	0.802	0.94 (0.85;1.03)	0.182	0.425	0.90 (0.73;1.11)	0.317	0.623
Syringic acid															
Model 1	1.06 (1;1.12)	0.079	0.201	1.10 (0.97;1.25)	0.128	0.597	1.08 (0.94;1.26)	0.281	0.656	1.03 (0.98;1.08)	0.252	0.666	1.17 (0.85;1.61)	0.338	0.676
Model 2	1.11 (1.02;1.21)	**0.019**	0.089	1.07 (0.95;1.21)	0.254	0.717	1.06 (0.92;1.21)	0.427	0.802	1.04 (0.98;1.09)	0.178	0.425	1.13 (0.83;1.52)	0.439	0.683
Urolithin A															
Model 1	0.98 (0.96;1.01)	0.071	0.201	1.00 (0.96;1.03)	0.885	0.991	0.95 (0.90;1.01)	0.104	0.364	0.97 (0.94;0.99)	**0.020**	0.140	0.95 (0.89;1.02)	0.152	0.369
Model 2	0.99 (0.96;1.02)	0.271	0.542	1.00 (0.96;1.05)	0.873	0.944	0.95 (0.89;1.01)	0.103	0.437	0.97 (0.94;0.99)	**0.024**	0.168	0.95 (0.88;1.03)	0.206	0.483
Urolithin B															
Model 1	0.95 (0.92;0.99)	**0.010**	0.140	0.97 (0.92;1.02)	0.248	0.694	0.92 (0.84;1.00)	0.063	0.294	0.93 (0.90;0.97)	**<0.001**	**0.006**	0.88 (0.77;1.00)	0.057	0.299
Model 2	0.94 (0.89;0.98)	**0.003**	**0.021**	0.98 (0.92;1.03)	0.436	0.872	0.93 (0.83;1.03)	0.152	0.437	0.92 (0.88;0.96)	**<0.001**	**0.001**	0.85 (0.73;0.99)	**0.048**	0.224
Vanillic acid															
Model 1	1.03 (0.91;1.17)	0.714	0.769	1.12 (0.92;1.37)	0.248	0.694	1.06 (0.82;1.36)	0.679	0.864	1.04 (0.92;1.17)	0.530	0.806	1.30 (0.90;1.88)	0.158	0.369

Model 2	1.07 (0.92;1.24)	0.436	0.708	1.15 (0.92;1.43)	0.232	0.717	1.00 (0.76;1.32)	0.998	0.998	1.12 (0.96;1.29)	0.143	0.425	1.30 (0.87;1.94)	0.207	0.483

BG blood glucose, CI confidence interval, HDL-c high-density lipoprotein-cholesterol, MetS metabolic syndrome, MPM microbial phenolic metabolites, TG triglycerides, WC waist circumference. Mixed-effects logistic regression models between log-transformed group of MPM (aglycone plus phase II metabolites) and MetS and its components. Fixed effect included two models: model 1 included sex (female/male) and age (continuous, years); Model 2 included model 1 plus Tanner maturation stage (score from I to V), physical activity (≥ 60min / <60min moderate-to-vigorous physical activity), household income (low/medium/high), and energy intake (continuous, Kcal/day). Municipality and schools were considered random effects. High blood pressure was not considered in the statistical analysis as only 2 participants had this condition (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥ 85 mm Hg), and therefore the analysis did not converge. * p-adjusted for multiple-testing using the Benjamin-Hochberg procedure considering a false discovery rate < 0.05 as significant. P values < 0.05 were considered significant.



GLOBAL DISCUSSION

5. GLOBAL DISCUSSION

The prevalence of obesity and metabolic syndrome in adolescents is increasing the risk of a wide range of metabolic disorders like CDVs and T2DM in adulthood, and all-cause mortality (4,7,14–16). The high number of adolescents living with MetS highlights the need for multisectoral interventions to decrease the global burden of MetS and its components, including obesity. Therefore, strategies based on healthy dietary habits and active lifestyles are needed to enhance cardiometabolic health in adolescents, for instance by creating health programs especially designed for them.

Obesity, the main contributor to cardiometabolic complications, is an energy imbalance between calories consumed and calories expended. According to this definition, we could imagine that the main dietetic intervention is based on energy restriction, and of course, the control of food portion size at mealtime. However, more strategies are necessary to avoid obesity (107). Dietary diversity including different plant-based food groups is a great dietary option, like the Mediterranean diet, because of its high content of fiber, vitamins, minerals, and phytochemicals (108,109). In the world of phytochemicals, (poly)phenols are one of the most important because they are widely distributed in most plant foods. Several observational studies and clinical trials conducted in adults have shown the role of (poly)phenols on cardiometabolic health parameters, even with the low risk of CVDs mortality (79,81–84). Nevertheless, the lack of evidence in adolescents generates the question whether (poly)phenols could have an implication on cardiometabolic health in this target population.

Additionally, the diversity and the complexity of (poly)phenols lead to difficulties in their dietary estimation in epidemiological studies. Therefore, diverse approaches have been developed to estimate dietary (poly)phenols, using dietary tools like FFQs, or more recently, identifying biomarkers using analytical methods (45,68,70,72–74,102,110).

This thesis aimed to study the role of dietary (poly)phenol, estimated by different approaches, on cardiometabolic health parameters in a cohort of adolescents aged 11 to 14 years old enrolled in the SI! Program for secondary schools in Spain. Overall, our findings suggested that among adolescents: i) dietary (poly)phenols estimated by FFQs are related to some cardiometabolic and adiposity parameters, ii) Total (poly)phenol in urine as a biomarker of (poly)phenol intake is related to a better cardiovascular profile, iii) LC-LTQ-Orbitrap-HRMS is an accurate analytical

method to evaluate phenolic metabolites in urine samples, iv) some phenolic metabolites are related with better cardiometabolic health.

5.1. Dietary (poly)phenols estimated by FFQs are related to some cardiometabolic and adiposity parameters

Dietary (poly)phenol intake and their main food sources were estimated in 944 adolescents, using Phenol-Explorer database and baseline FFQ, using a standardized method previously described by Tresserra-Rimbau *et al.* and Castro-Barquero *et al.* (79,80). The mean intake of total (poly)phenol intake was 683.2 ± 379.4 mg/day and the median of 598.6 mg/day. This value differed from the median of 326 mg/day reported in a cohort of 2428 European adolescents aged 12.5 to 17.5 years old in 9 European countries within the HELENA study (111). In another study, the mean intake of total (poly)phenols was 455 ± 263.2 mg/day in a cohort of 2045 adolescents aged 11 to 18 years old in the UK (112). The higher values of total (poly)phenols showed in our study compared to the two cohorts, could be due to the difference in flavonoid intake: approx. 206 mg/day in the HELENA cohort, 355.4 ± 230.9 mg/day in the UK cohort, and in our study 530.1 ± 331.3 mg/day. Flavonoids and phenolic acid were the main (poly)phenol subclasses intake in our study, representing 77.6% and 14.3% of total (poly)phenol intake, with similar results found in the two cohorts previously mentioned. Lignans and stilbenes had a lower contribution to total (poly)phenol intake, below 0.6%, likewise to a previous study in a European adolescents' cohort (111). The main food sources of (poly)phenols were fruits or fruit juices, vegetables, cocoa-based products, and nuts. Similar results were shown in the HELENA and UK studies.

The intake of total (poly)phenol flavonoids and phenolic acids was higher in girls. Similar results from HELENA study, girls had more (poly)phenol and flavonoid intake than boys, and no significant differences between sex were found in the other subclasses of (poly)phenols (111). Participants with parents with primary/secondary school education levels had a higher intake of phenolic acids. Contrarily, in the HELENA cohort, higher (poly)phenol intake and flavonoids were reported in adolescents with parents with higher education, but no association was found with phenolic acid intake (111).

One of the important points of estimating habitual (poly)phenol intake through FFQs is to understand the protective effect of long-term dietary (poly)phenol exposure on health (68,70). In **article 2**, higher total (poly)phenol intake and some subclasses of (poly)phenols, especially flavonoids, phenolic acids, stilbenes, and

"other (poly)phenols" were associated with lower values of WC z-score, MAP z-score, DBP z-score, BG, TG, and HDL-c (except for stilbenes, which a direct association was observed), after the adjustment of anthropometric, lifestyle, sociodemographic and dietary covariates. Some of these findings agree or are contradictory with other observational studies. In the HELENA study, no association was observed between quartiles of total energy-adjusted (poly)phenol intake with WC z-score, SBP z-score, DBP z-score, BG, and TG in 657 European adolescents (100). Although both studies are conducted in adolescents, there are some differences in polyphenol intake (the median (poly)phenol intake in the HELENA study was 347.2 mg/day, lower than in our study: 683.2 ± 379.4 mg/day), probably by differences in dietary patterns. In the HELENA study, about 70% of the adolescents were from non-Mediterranean countries (100). Other studies conducted in adult populations with higher MetS risk and higher dietary (poly)phenol intake support some of our findings. In a cohort of Poland and Italian adults, higher total (poly)phenol intake was associated with lower values of WC, BP, and better lipid profile (lower TG and higher HDL), although some results varied according to sex (113,114). In the PREDIMED-Plus cohort, higher total (poly)phenol intake (mean of 846 ± 318 mg/day) was related to higher values of HLD-c in 6,633 adults aged 65.0 ± 4.9 years old with MetS in Spain (79). By contrast, we found an inverse association with HDL-c, probably because our sample population is young and mostly healthy while the PREDIMED-plus population has MetS.

Flavonoid, phenolic acid, lignan, stilbene, and "others (poly)phenol" intake; were analyzed separately due to the heterogeneity in their chemical structures and their different bioavailability. We found that a higher intake of flavonoids was associated with a lower WC z-score, DBP z-score, and HDL-c compared to the lowest intake. Similar findings were shown in adults with metabolic complications within the TOSCA.IT and PREDIMED-Plus cohort (79,114). By contrast, in the HELENA study, higher flavonoid intake was only associated with BMI z-score. Although in our study, BMI z-score was considered as a covariate in all cardiometabolic health parameters evaluated, except in WC, in a separate analysis, we showed that the high flavonoid intake was associated with a lower BMI z-score (β=-0.09, P= <0.001), likely to HELENA study (100). One plausible mechanism to explain the relationship between dietary (poly)phenols and BP is that flavonoids regulate NO levels by increasing the activation of NO synthase and endothelial NO synthase, influencing BP (115). Regarding obesity, flavonoids could inhibit the differentiation of adipocytes, promote fatty acid oxidation, increase thermogenesis and energy expenditure, and decrease the synthesis of fatty acids, all of them implicated in

weight maintenance and lipid profile (116). Prebiotic properties of flavonoids also might affect microbiota metabolism. We found that high phenolic acid intake was associated with lower values of WC z-score, BG, and TG. These results agree with observational studies conducted on adults (114,117). In the HELENA study, two phenolic acids (ferulic acid and hydroxycinnamic acid) were inversely associated with WC (100). Phenolic acids are implicated in several pathways related to obesity, lipid, and glucose metabolism. They suppress lipid accumulation in 3T3-L1 adipocytes and regulate insulin, leptin, adiponectin, and proinflammatory cytokines (118,119). Higher stilbene intake was associated with a better lipid profile (lower TG and higher HDL-c levels). In contrast, in HELENA study, no association was found between stilbenes and cardiometabolic health parameters in European adolescents (111). We also found some contradictions with flavonoid intake because its consumption was inversely associated with HDL-c levels, maybe because our sample population is young and healthy. Further longitudinal studies are needed to clarify the true direction of these associations. Contrary to other studies in adults, we did not find significant associations between lignans and cardiometabolic health parameters.

In **article 3** we analyzed the relationship between flavonoids from cocoa-based products and adiposity parameters in adolescents. The rationale of this paper was the large set of evidence about cocoa and obesity in adults shown in several systematic reviews, but the lack of studies conducted in adolescents (120–124). As mentioned, cocoa-based products are one of the main contributors to dietary (poly)phenol and flavonoid intake in adolescents. The main findings showed that participants with a higher intake of flavonoids from cocoa-based products had lower values of BMI z-score, and WHtR, and a lower probability of abdominal obesity. In the case of flavonoids from cocoa powder, an inverse association was found with BMI z-score, WC z-score, WHtR, fat mass percentage (%FM), and fat mass index (FMI) z-score. Concerning dark chocolate, an inverse association was observed only with WC z-score. Additionally, no association was found between flavonoids from milk chocolate intake and any adiposity parameters, probably because of its very low flavonoid content.

As observed, not all phenolic compounds have the same associations with all cardiometabolic health parameters evaluated. Therefore, promoting a healthy and diverse dietary pattern in adolescents based on a higher intake of (poly)phenol-rich foods (fruits, vegetables, nuts, cocoa-based products, etc.), could be the way to improve cardiometabolic health in this population.

5.2. Total (poly)phenol in urine as a biomarker of (poly)phenol intake is related to a better cardiovascular profile

In **article 4**, we showed that the highest excretion of TPE, measured by Folin-Ciocalteu spectrophotometric method, was associated with lower body fat percentage, TG, TC, and LDL-c cholesterol in boys, after adjusting for all confounder variables. In girls, higher TPE was associated with less values of SBP and SBP, and TC. The present finding is consistent with a previous study conducted by our research group in adults with high CVDs risk, where TPE was inversely associated with BP, possibly by stimulating the formation of vasoprotective factors such as NO in plasma (125). Due to the previous association observed between TPE and individual cardiometabolic health parameters, in **article 5**, we described the association between TPE and cardiovascular health (CVH) score for the first time. In this study, the CVH score was calculated using the cut-offs established by the American Heart Association based on health behaviours (smoking status, BMI, physical activity, and healthy diet) and health factors (BP, total cholesterol, and blood glucose) data. Our main findings were that higher TPE was associated with higher (healthier) CVH scores, ideal TC, but also with other aspects of a healthy lifestyle, such as ideal smoking status and physical activity. The association between TPE and CVH score was observed only in boys, after a separate analysis by sex. The beneficial role of dietary (poly)phenols, measured by TPE, on cardiovascular health has been described in several observational and clinical trials and reported throughout this discussion section (55,79,81,82,88,100,114,117,125–129).

5.3. LC-LTQ-Orbitrap-HRMS is an accurate analytical method to evaluate phenolic metabolites in urine samples

The development and application of new technologies to determine phenolic compounds and their metabolites in biological samples is increasing. We developed an analytical method to identify and quantify 54 urinary phenolic metabolic (mainly microbial phenolic metabolites) even aglycones and phase II metabolites by LC-LTQ-Orbitrap HRMS in a large epidemiological study (601 urine samples from adolescents at baseline of the SI! Program for secondary school) (**Article 6**).

As mentioned, LC-LTQ-Orbitrap HRMS is extensively used for accurate identification of (poly)phenols in nutrimetabolomic studies. According to the results of different publications, HRMS is a fully-appropriate approach for quantitative LC-MS analysis due to its versatility and robustness characteristics (74–77). Kaufmann *et al.* reported similar performance in terms of sensitivity, selectivity, linearity,

accuracy, and precision, between HRMS and MS/MS methods in the analysis of 240 pesticide residues in different matrices (78). Grund et al., compared HRMS and QqQ systems for the determination of protease inhibitors, tyrosine kinase inhibitors, metanephrines, and steroids (compounds routinely measured in hospital laboratories), reporting similar values with both techniques in terms of limit of detection (LOD), precision, accuracy, and curve calibration (77). Bruce *et al.* compared the LC-HRMS and LC-MS/MS systems for the quantification of vitamin D metabolites in human serum, obtaining excellent correlations between both methods (130). Herrero *et al.* also compared the capabilities of HRMS with an Orbitrap analyzer with respect to the MS/MS with QqQ analyzer to quantify organic contaminants in sewage, obtaining for both methods similar limit of quantification (LOQ), LOD, linear range, and repeatability for glucocorticoids, and better LOD and LOQ for polyether ionophores with the HRMS method (131).

Although several publications have evaluated the comparison between HRMS and MS/MS quantitative methods, most of them are related to drugs, hormones, or contaminants. There are few studies that evaluated this comparison in (poly)phenols. Cavaliere *et al.* reported the comparison between HRMS and QqQ systems in the quantification of (poly)phenols in wine, showing better performance of HRMS approach in terms of sensitivity, linearity range, matrix effect, and precision (132). Vallverdú-Queralt *et al.* also reported that a linear ion trap Orbitrap mass spectrometer (LC-LTQ-Orbitrap-HRMS) was the technique that presented more sensitivity and better resolution of mass spectra compared to QqQ (LC-MS/MS) to identify phenolic compounds in tomato samples (76). In addition to this, LC/HRMS has been extensively applied to the quantification of phenolic compounds in leafy vegetables, foods, and beverages (76,132–136), but fewer in biological samples.

Apart from our study (**article 6**), only two studies validated a quantitative method using HRMS in biological samples, and our study is the only method applied in large human samples. Ordónez *et al.* validated an HPLC-HRMS method to determine microbiota-derived phenolic acids and aromatic compounds in urine, reporting that HPLC-HRMS was suitable for the analysis of phase II metabolites, providing complete information on MPM-mediated by degradation of orange juice (poly)phenols (137). Pereira-Caro *et al.* also developed a method UHPLC-HRMS to identify and quantify flavanol metabolites and microbial-derived catabolites in urine, plasma, and feces samples of rats (138).

We also investigated the correlation between microbial phenolic metabolites and dietary (poly)phenols, finding that whole grain intake was correlated with lignans (enterodiol, enterolactone, and their derivatives), and nut intake with urolithins (A and B and their derivatives), in agreement with previous studies (139,140). Thus, suggesting these microbial phenolic metabolites are candidate biomarkers for intake of these food groups in adolescents.

5.4. Some urinary phenolic metabolites are related to better cardiometabolic health

In **article 7**, we explained the cross-sectional results of a sub-study conducted in 560 adolescents within the SI! Program cohort and we observed that higher urinary phenolic metabolites (UPM), namely gallic acid, urolithin A and B and their derivatives, were associated with lower values of WC z-score, DBP z-score, MetS score, and lower probability of having MetS, as well as some of its clinical components (abdominal obesity and high BG), after adjustment for sex, age, Tanner maturation stage, physical activity, household income, and energy intake.

We found that higher gallic acid (aglycone, glucuronide, and sulfate form) was associated with lower odds of having MetS and abdominal obesity. Gallic acid in its aglycone form, is easily absorbed in the stomach and small intestine. This phenolic compound is widely distributed in fruits and vegetables as aglycone or esterified, but it is also a final UPM derived from the metabolism of flavonoids, the most abundant class of (poly)phenol, as tannins (141,142), likely by the action of gut microbiota. Plausible mechanisms explain the action of gallic acid on cardiometabolic disorders, especially with body weight maintenance, lipid, and glucose metabolism (142,143). Gallic acid is one of the final products of gut microbiota metabolism of galloylated catechins (epigallocatechin gallate, epicatechin gallate, and epicatechin) (141), which modulate gut dysbiosis influencing obesity (144). In animal models, gallic acid and epicatechin gallate induce thermogenesis and browning in adipocytes (145), through the activation of enzymes involved in energy homeostasis (142,143). In the Caco2-cell line, gallic acid inhibits glucose absorption through the inhibition of sodium-dependent SGLT1 transporters (146).

Additionally, higher levels of urinary urolithin A and B (aglycone plus glucuronide and sulfate form) were associated with lower MetS score, WC z-score, and/or DBP z -score, and with lower probability of having abdominal obesity and high BG (only for urolithin B). Urolithins are the final product of ellagitannins metabolism (45,141,147). Ellagitannins are widely distributed in nuts and red fruits. Ellagitannins are broken down into ellagic acids, which are further metabolized into

urolithins by gut microbiota action (98,141,147). *In vitro*, animal, and some human clinical studies have shown the protective effect of urolithins on cardiometabolic diseases, even obesity (147–149). Istas G. *et al.* suggested the beneficial effect of plasma urolithins on vascular function in healthy individuals, after the consumption of raspberries (150). The inverse association between urolithin B and abdominal obesity found in our study could be explained because urolithins enhance thermogenesis, lipolysis, and fatty oxidation (147). We observed an inverse association between urolithin B and the probability of having high BG. In contrast with this, Selma *et al.* showed that only urinary urolithin A was inversely correlated with fasting glucose in MetS patients (149). Likely, because urolithins could act by inhibiting inflammatory signaling pathways, activating autophagy, and maintaining mitochondrial function in pancreatic cells (98).

In our cross-sectional study, hydroxycinnamic acids (*p*-, *o*-, and *m*-coumaric acids), dihydroxyphenylacetic acid, dihydroresveratrol, enterodiol, enterolactone, hydroxybenzoic acids (3- and 4-hydroxybenzoic acids), hydroxyphenylacetic acid, hydroxytyrosol, protocatechuic acid, syringic acid, and vanillic acid; were not associated with MetS and its clinical features in adolescents after the full-adjustment for confounder variables. These results are in contrast with the literature reported in previous studies (45,89,95,151,152).

5.5. Limitations and strengths

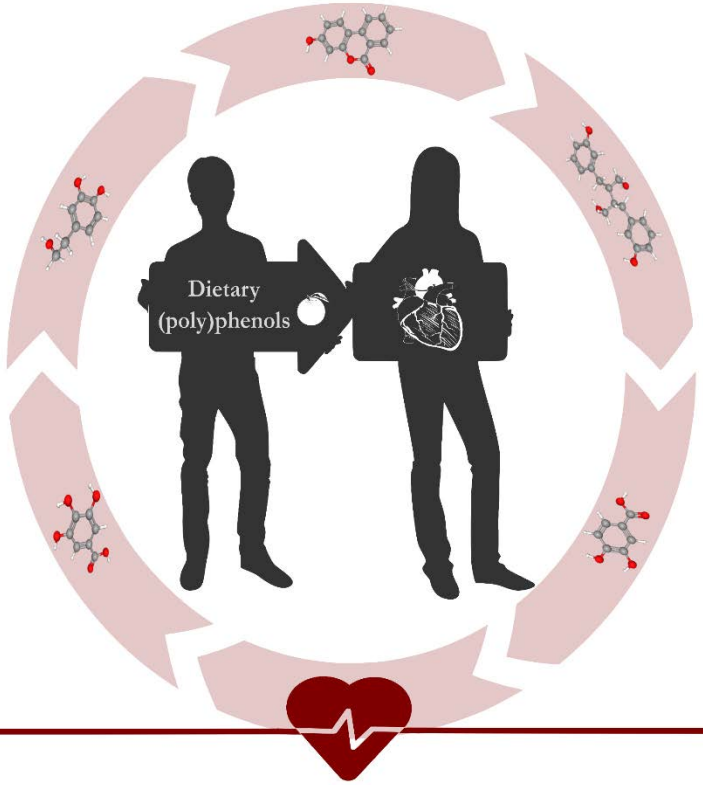
There are some limitations to be considered in the present thesis. One of them is its cross-sectional design, which precludes causal assumptions about dietary (poly)phenol and differences in cardiometabolic health parameters.

Regarding (poly)phenol intake measurement by FFQs, some biases are prone because misreporting is common in dietary self-assessment in adolescents. Although we exclude FFQ with extremely low and high energy intake (less than 803 or above 4013 kcal/day for boys, and less than 502 or above 3511 kcal/day for girls) (153,154), adolescents with high values of BMI tend to report lower energy intake, and this is more likely in adolescents with self-image dissatisfaction (155). Additionally, although (poly)phenol intake was estimated using a comprehensive database (Phenol-explorer) and a standardized technique, information about some foods widely consumed in Spain or specific areas in Spain is still scared because they have not been characterized or are poorly characterized in the Phenol-Explorer database, and in consequence, there is some variation in the estimation of phenolic content. In addition to this, environmental and food processing conditions can alter

the (poly)phenol content in a way that we cannot estimate (68). Finally, as we pointed out in the introduction section, the bioavailability of dietary (poly)phenol is influenced by several biological factors which included interaction with food matrix, nutrients, gut microbiota, genetics (polymorphisms), plasma proteins, age, sex, etc. Although age and sex, and some foods and or nutrients have been considered in our regression models as covariates, the interindividual variation associated with all these relevant factors cannot be estimated. Therefore, the association between dietary (poly)phenol intake and cardiometabolic health parameters might be distorted by dietary data bias.

Regarding urinary (poly)phenols, although Folin-Ciocalteu is a rapid and easy method to estimate total (poly)phenols content in urine and in consequence total dietary (poly)phenol intake; it is not possible to calculate the weight of individual phenolic compounds in the relationship with cardiometabolic parameters. Therefore, the metabolomic technique used in this thesis is relevant to find these individual associations. However, some limitations are needed to be acknowledged in this topic. Our study did not aim to elucidate the underlying molecular mechanisms to understand these associations. Additionally, several factors affect the direct bioavailability of phenolic metabolites, being one of them the gut microbiota profile, which was not contemplated in our study.

Strengths of the present study include the large sample size and the standardization of measures performed in the SI! Program for Secondary Schools trial to reduce bias. Up to this date, this is one of the few studies about dietary (poly)phenol intake and cardiometabolic health parameters in adolescents, and this is the first study evaluating the several phenolic metabolites associated with cardiometabolic parameters in this target population, highlighting the importance to generate more evidence. Another strength is the precise identification and quantification of urinary phenolic metabolites through a validated analytical method. Finally, all the analyses were conducted in the same cohort, therefore, we could generate a global conclusion about this Spanish study.



CONCLUSION

6. CONCLUSION

According to the stated objectives and the results described in the articles presented in this thesis, the main conclusions were as follow:

General conclusion:

Higher dietary (poly)phenol intake and its metabolites were associated with better cardiometabolic health and its parameters like body weight/composition, blood pressure, fasting glucose, and lipid profile; in adolescents aged 11 to 14 years old from a Spanish cohort.

Specifics conclusions:

- Higher total (poly)phenol intake and some subclasses of (poly)phenols, especially flavonoids, phenolic acids, stilbenes, and "other (poly)phenols" were associated with lower values of WC, BP, BG, TG, and HDL-c (except for stilbenes, which directly association was observed).

- Higher flavonoids from cocoa-based products, especially cocoa powder and dark chocolate, were associated with adiposity parameters and with lower probability of having abdominal obesity. However, these associations were not observed in case of flavonoids from milk chocolate.

- Total (poly)phenols in urine analyzed by the Folin-Ciocalteu spectrophotometric method and used as a marker of (poly)phenol intake, was inversely associated with BP, TC, and with better or healthier cardiovascular health.

- We developed an analytical method to identify and quantify 54 urinary phenolic metabolites (mainly microbial phenolic metabolites) including aglycones and phase II metabolites through LC-LTQ-Orbitrap-HRMS approach in a large epidemiological study. We identified lignans (enterodiol, enterolactone) and urolithins (A and B) as candidate marker of whole grains and nuts intake, respectively.

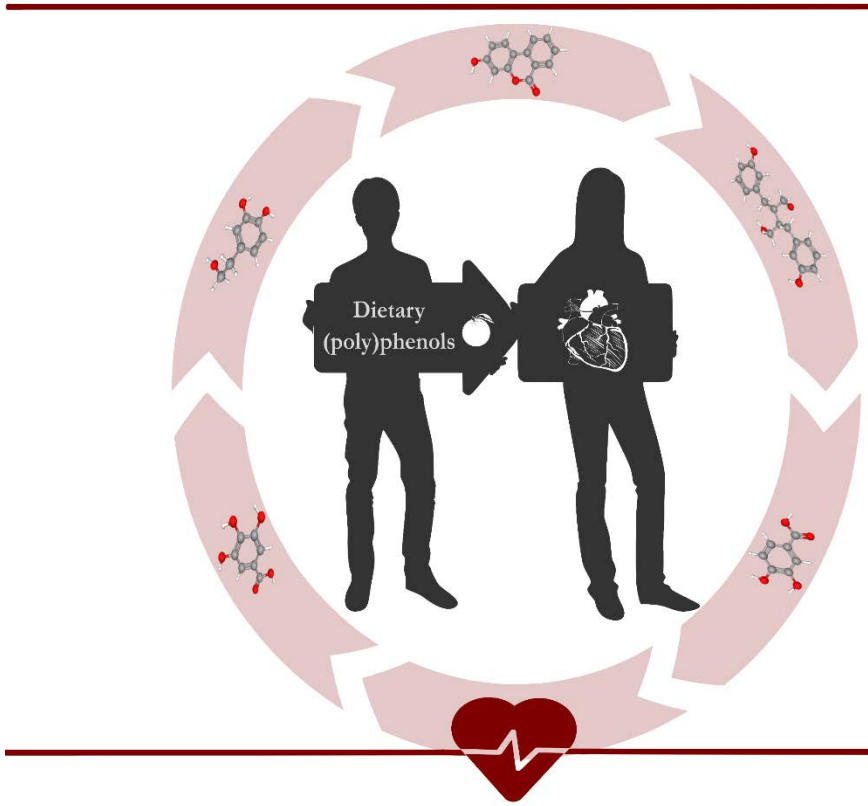
- Higher urinary excretion of phenolic metabolites (gallic acid, urolithin A and B) was associated with lower values of WC, DBP, MetS score, and lower probability of having MetS, abdominal obesity and high BG.

Future perspectives

This thesis helps to collect dietary data which supports the development of nutritional guidelines including safe doses of (poly)phenol, and the development of educational and health policies targeting dietary habits based on a high intake of (poly)phenol-rich food groups to protect cardiometabolic health in adolescents.

In a future perspective, further studies (longitudinal and clinical trials) are needed to expand knowledge on dietary (poly)phenol intake and its metabolites with cardiometabolic health in adolescents. In particular:

- Longitudinal studies are needed to observe the direction of the relationship between dietary (poly)phenols and cardiometabolic health continuing over time, having a relevant clinical change in the same cohort.
- Clinical trials for a long time and at a large scale are needed to elucidate the effect on cardiometabolic parameters of safe dose-response of a dietary (poly)phenol intervention during adolescence and its consequence in adulthood.
- Interindividual variation studies considering gut microbiota profile and polymorphisms evaluated in adolescents are needed to understand if this variable affects the relationship between dietary (poly)phenols and cardiometabolic health parameters.



BIBLIOGRAPHY

BIBLIOGRAPHY

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019. J Am Coll Cardiol. 2020 Dec 22;76(25):2982–3021.
2. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J. 2016 Nov 7;37(42):3232–45.
3. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep 7;42(34):3227–337.
4. Wilson PWF, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation. 2005 Nov 15;112(20):3066–72.
5. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The Metabolic Syndrome and Cardiovascular Risk: A Systematic Review and Meta-Analysis. J Am Coll Cardiol. 2010 Sep 28;56(14):1113–32.
6. Guembe MJ, Fernandez-Lazaro CI, Sayon-Orea C, Toledo E, Moreno-Iribas C, Cosials JB, et al. Risk for cardiovascular disease associated with metabolic syndrome and its components: a 13-year prospective study in the RIVANA cohort. Cardiovasc Diabetol. 2020 Dec 1;19(1):1–14.
7. Lakka HM. The Metabolic Syndrome and Total and Cardiovascular Disease Mortality in Middle-aged Men. JAMA. 2002 Dec 4;288(21):2709.
8. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA: The Journal of the American Medical Association. 2001 May 16;285(19):2486–97.
9. Alberti KGMM, Zimmet PZ. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications Part 1: Diagnosis and Classification of Diabetes Mellitus Provisional Report of a WHO Consultation. 1997;(8).
10. Bonora E. The metabolic syndrome and cardiovascular disease. Ann Med. 2006 Jan 8;38(1):64–80.
11. Kahn R. Metabolic syndrome—what is the clinical usefulness? The Lancet. 2008 Jun 7;371(9628):1892–3.
12. Noubiap JJ, Nansseu JR, Lontchi-Yimagou E, Nkeck JR, Nyaga UF, Ngouo AT, et al. Global, regional, and country estimates of metabolic syndrome

burden in children and adolescents in 2020: a systematic review and modelling analysis. Lancet Child Adolesc Health. 2022 Mar 1;6(3):158–70.

13. Noubiap JJ, Nansseu JR, Lontchi-Yimagou E, Nkeck JR, Nyaga UF, Ngouo AT, et al. Geographic distribution of metabolic syndrome and its components in the general adult population: A meta-analysis of global data from 28 million individuals. Diabetes Res Clin Pract. 2022 Jun 1;188:109924.
14. Asghari G, Hasheminia M, Heidari A, Mirmiran P, Guity K, Shahrzad MK, et al. Adolescent metabolic syndrome and its components associations with incidence of type 2 diabetes in early adulthood: Tehran lipid and glucose study. Diabetol Metab Syndr. 2021 Dec 1;13(1):1–9.
15. Magnussen CG, Koskinen J, Chen W, Thomson R, Schmidt MD, Srinivasan SR, et al. Pediatric Metabolic Syndrome Predicts Adulthood Metabolic Syndrome, Subclinical Atherosclerosis, and Type 2 Diabetes Mellitus but Is No Better Than Body Mass Index Alone. Circulation. 2010 Oct 19;122(16):1604–11.
16. Morrison JA, Friedman LA, Wang P, Glueck CJ. Metabolic Syndrome in Childhood Predicts Adult Metabolic Syndrome and Type 2 Diabetes Mellitus 25 to 30 Years Later. J Pediatr. 2008 Feb 1;152(2):201–6.
17. Agudelo GM, Bedoya G, Estrada A, Patiño FA, Muñoz AM, Velásquez CM. Variations in the Prevalence of Metabolic Syndrome in Adolescents According to Different Criteria Used for Diagnosis: Which Definition Should Be Chosen for This Age Group? Metab Syndr Relat Disord. 2014 May 17;12(4):202–9.
18. Ford ES, Li C. Defining the Metabolic Syndrome in Children and Adolescents: Will the Real Definition Please Stand Up? J Pediatr. 2008 Feb 1;152(2):160-164.e13.
19. Reisinger C, Nkeh-Chungag BN, Fredriksen PM, Goswami N. The prevalence of pediatric metabolic syndrome—a critical look on the discrepancies between definitions and its clinical importance. International Journal of Obesity 2020 45:1. 2020 Nov 18;45(1):12–24.
20. Zimmet P, Alberti GKMM, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents – an IDF consensus report. Pediatr Diabetes. 2007 Oct 1;8(5):299–306.
21. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a Metabolic Syndrome Phenotype in Adolescents: Findings From the Third National Health and Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med. 2003 Aug 1;157(8):821–7.

22. Ford ES, Ajani UA, Mokdad AH. The Metabolic Syndrome and Concentrations of C-Reactive Protein Among U.S. Youth. Diabetes Care. 2005 Apr 1;28(4):878–81.
23. de Ferranti SD, Gauvreau K, Ludwig DS, Neufeld EJ, Newburger JW, Rifai N. Prevalence of the Metabolic Syndrome in American Adolescents. Circulation. 2004 Oct 19;110(16):2494–7.
24. Heshmat R, Heidari M, Ejtahed HS, Motlagh ME, Mahdavi-Gorab A, Ziaodini H, et al. Validity of a continuous metabolic syndrome score as an index for modeling metabolic syndrome in children and adolescents: the CASPIAN-V study. Diabetol Metab Syndr. 2017 Dec 9;9(1):89.
25. Prochotska K, Kovacs L, Vitariusova E, Feber J. Is arterial stiffness predicted by continuous metabolic syndrome score in obese children? Journal of the American Society of Hypertension. 2016 Jan 1;10(1):47–54.
26. Hesse MB, Young G, Murray RD. Evaluating health risk using a continuous metabolic syndrome score in obese children. Journal of Pediatric Endocrinology and Metabolism. 2016 Jan 1;29(4):451–8.
27. Pandit D, Chiplonkar S, Khadilkar A, Kinare A, Khadilkar V. Efficacy of a continuous metabolic syndrome score in Indian children for detecting subclinical atherosclerotic risk. International Journal of Obesity 2011 35:10. 2011 Jul 19;35(10):1318–24.
28. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. The Lancet. 2005 Apr 16;365(9468):1415–28.
29. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. The Lancet. 2017 Dec 16;390(10113):2627–42.
30. de Bont J, Bennett M, León-Muñoz LM, Duarte-Salles T. The prevalence and incidence rate of overweight and obesity among 2.5 million children and adolescents in Spain. Revista Española de Cardiología (English Edition). 2022 Apr 1;75(4):300–7.
31. Santos-Beneit G, Fernández-Jiménez R, de Cos-Gandoy A, Rodríguez C, Carral V, Bodega P, et al. Lessons Learned From 10 Years of Preschool Intervention for Health Promotion: JACC State-of-the-Art Review. J Am Coll Cardiol. 2022 Jan 25;79(3):283–98.
32. Arcila-Agudelo, Ferrer-Svoboda, Torres-Fernàndez, Farran-Codina. Determinants of Adherence to Healthy Eating Patterns in a Population of

Children and Adolescents: Evidence on the Mediterranean Diet in the City of Mataró (Catalonia, Spain). Nutrients. 2019 Apr 15;11(4):854.

33. Esquius L, Aguilar-Martínez A, Bosque-Prous M, González-Casals H, Bach-Faig A, Colillas-Malet E, et al. Social Inequalities in Breakfast Consumption among Adolescents in Spain: The DESKcohort Project. Nutrients. 2021 Jul 22;13(8):2500.
34. Fornari E, Maffeis C. Treatment of Metabolic Syndrome in Children. Front Endocrinol (Lausanne). 2019 Oct 15;10:702.
35. Kornet-van der Aa DA, Altenburg TM, van Randeraad-van der Zee CH, Chinapaw MJM. The effectiveness and promising strategies of obesity prevention and treatment programmes among adolescents from disadvantaged backgrounds: a systematic review. Obesity Reviews. 2017 May 1;18(5):581–93.
36. Friedman F, Mozaffarian D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity. Circulation. 2016 Jan 12;133(2):187–225.
37. Fernandez-Jimenez R, Santos-Beneit G, Tresserra-Rimbau A, Bodega P, de Miguel M, de Cos-Gandoy A, et al. Rationale and design of the school-based SI! Program to face obesity and promote health among Spanish adolescents: A cluster-randomized controlled trial. Am Heart J. 2019 Sep 1;215:27–40.
38. Tsao R. Chemistry and Biochemistry of Dietary Polyphenols. Nutrients. 2010 Dec 10;2(12):1231–46.
39. Hano C, Tungmunnithum D. Plant Polyphenols, More than Just Simple Natural Antioxidants: Oxidative Stress, Aging and Age-Related Diseases. Medicines. 2020 May 9;7(5):26.
40. Laoué J, Fernandez C, Ormeño E. Plant Flavonoids in Mediterranean Species: A Focus on Flavonols as Protective Metabolites under Climate Stress. Plants. 2022 Jan 10;11(2):172.
41. del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid Redox Signal. 2013 May 10;18(14):1818–92.
42. Cohen SD, Kennedy JA. Plant Metabolism and the Environment: Implications for Managing Phenolics. Crit Rev Food Sci Nutr. 2010 Jul 30;50(7):620–43.
43. Polia F, Pastor-Belda M, Martínez-Blázquez A, Horcajada MN, Tomás-Barberán FA, García-Villalba R. Technological and Biotechnological Processes To Enhance the Bioavailability of Dietary (Poly)phenols in Humans. J Agric Food Chem. 2022 Feb 23;70(7):2092–107.

44. Laveriano-Santos EP, López-Yerena A, Jaime-Rodríguez C, González-Coria J, Lamuela-Raventós RM, Vallverdú-Queralt A, et al. Sweet Potato Is Not Simply an Abundant Food Crop: A Comprehensive Review of Its Phytochemical Constituents, Biological Activities, and the Effects of Processing. Antioxidants. 2022 Aug 25;11(9):1648.
45. Marhuenda-Muñoz M, Laveriano-Santos EP, Tresserra-Rimbau A, Lamuela-Raventós RM, Martínez-Huélamo M, Vallverdú-Queralt A. Microbial Phenolic Metabolites: Which Molecules Actually Have an Effect on Human Health? Nutrients. 2019 Nov 1;11(11): 2725.
46. Bohn T, McDougall GJ, Alegría A, Alminger M, Arrigoni E, Aura A, et al. Mind the gap—deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites—a position paper focusing on carotenoids and polyphenols. Mol Nutr Food Res. 2015 Jul 3;59(7):1307–23.
47. Bohn T. Dietary factors affecting polyphenol bioavailability. Nutr Rev. 2014 Jul 1;72(7):429–52.
48. Teng H, Chen L. Polyphenols and bioavailability: an update. Crit Rev Food Sci Nutr. 2019 Jul 20;59(13):2040–51.
49. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004 May 1;79(5):727–47.
50. Konishi Y, Zhao Z, Shimizu M. Phenolic acids are absorbed from the rat stomach with different absorption rates. J Agric Food Chem. 2006 Oct 4;54(20):7539–43.
51. Piskula MK, Yamakoshi J, Iwai Y. Daidzein and genistein but not their glucosides are absorbed from the rat stomach. FEBS Lett. 1999 Mar 26;447(2–3):287–91.
52. Passamonti S, Vrhovsek U, Vanzo A, Mattivi F. The stomach as a site for anthocyanins absorption from food. FEBS Lett. 2003 Jun 5;544(1–3):210–3.
53. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005 Jan 1;81(1):230S-242S.
54. Manach C, Hubert J, Llorach R, Scalbert A. The complex links between dietary phytochemicals and human health deciphered by metabolomics. Mol Nutr Food Res. 2009 Oct 1;53(10):1303–15.
55. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary Polyphenols and the Prevention of Diseases. Crit Rev Food Sci Nutr. 2005 Jun;45(4):287–306.

56. Scalbert A, Williamson G. Dietary Intake and Bioavailability of Polyphenols. J Nutr. 2000 Aug 1;130(8):2073S-2085S.
57. Morand C, de Roos B, Garcia-Conesa MT, Gibney ER, Landberg R, Manach C, et al. Why interindividual variation in response to consumption of plant food bioactives matters for future personalised nutrition. Proceedings of the Nutrition Society. 2020 May 4;79(2):225–35.
58. Peters CM, Green RJ, Janle EM, Ferruzzi MG. Formulation with ascorbic acid and sucrose modulates catechin bioavailability from green tea. Food Research International. 2010 Jan 1;43(1):95–102.
59. Neilson AP, Sapper TN, Janle EM, Rudolph R, Matusheski N v, Ferruzzi MG. Chocolate matrix factors modulate the pharmacokinetic behavior of cocoa flavan-3-ol phase II metabolites following oral consumption by Sprague-Dawley rats. J Agric Food Chem. 2010 Jun 9;58(11):6685–91.
60. Tuck KL, Freeman MP, Hayball PJ, Stretch GL, Stupans I. The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. J Nutr. 2001 Jul 1;131(7):1993–6.
61. Cebeci F, Şahin-Yeşilçubuk N. The matrix effect of blueberry, oat meal and milk on polyphenols, antioxidant activity and potential bioavailability. Int J Food Sci Nutr. 2014 Feb 15;65(1):69–78.
62. Urpi-Sarda M, Llorach R, Khan N, Monagas M, Rotches-Ribalta M, Lamuela-Raventos R, et al. Effect of Milk on the Urinary Excretion of Microbial Phenolic Acids after Cocoa Powder Consumption in Humans. J Agric Food Chem. 2010 Apr 28;58(8):4706–11.
63. Piazza C, Privitera MG, Melilli B, Incognito T, Marano MR, Leggio GM, et al. Influence of inulin on plasma isoflavone concentrations in healthy postmenopausal women. Am J Clin Nutr. 2007 Sep 1;86(3):775–80.
64. Larkin TA, Price WE, Astheimer LB. Increased probiotic yogurt or resistant starch intake does not affect isoflavone bioavailability in subjects consuming a high soy diet. Nutrition. 2007 Oct 1;23(10):709–18.
65. Tamura M, Nakagawa H, Tsushida T, Hirayama K, Itoh K. Effect of pectin enhancement on plasma quercetin and fecal flora in rutin-supplemented mice. J Food Sci. 2007 Nov 1;72(9):S648-51.
66. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev. 1998 Nov 1;56(11):317–33.
67. Koioe T, Isemura M, Suzuki Y, Ooani S. Evidence for the Interaction between (−)-Epigallocatechin Gallate and Human Plasma Proteins

Fibronectin, Fibrinogen, and Histidine-rich Glycoprotein. OUP. 2014;60(8):1317–9.

68. Probst Y, Guan V, Kent K. A systematic review of food composition tools used for determining dietary polyphenol intake in estimated intake studies. Food Chem. 2018 Jan 1;238:146–52.
69. Lachat C, Hawwash D, Ocké MC, Berg C, Forsum E, Hörnell A, et al. Strengthening the Reporting of Observational Studies in Epidemiology – nutritional epidemiology (STROBE-nut): An extension of the STROBE statement. Nutr Bull. 2016 Sep 1;41(3):240–51.
70. Xu Y, Le Sayec M, Roberts C, Hein S, Rodriguez-Mateos A, Gibson R. Dietary Assessment Methods to Estimate (Poly)phenol Intake in Epidemiological Studies: A Systematic Review. Advances in Nutrition. 2021 Mar 3;12(5):1781–801.
71. Rothwell JA, Perez-Jimenez J, Neveu V, Medina-Remon A, M'Hiri N, Garcia-Lobato P, 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. 2013 Oct 7;2013(0):bat070–bat070.
72. Medina-Remón A, Tresserra-Rimbau A, Arranz S, Estruch R, Lamuela-Raventos RM. Polyphenols excreted in urine as biomarkers of total polyphenol intake. Bioanalysis. 2012 Nov;4(22):2705–13.
73. López-Yerena A, Domínguez-López I, Vallverdú-Queralt A, Pérez M, Jáuregui O, Escribano-Ferrer E, et al. Metabolomics Technologies for the Identification and Quantification of Dietary Phenolic Compound Metabolites: An Overview. Antioxidants. 2021 May 25;10(6):846.
74. Laveriano-Santos EP, Marhuenda-Muñoz M, Vallverdú-Queralt A, Martínez-Huélamo M, Tresserra-Rimbau A, Miliarakis E, et al. Identification and Quantification of Urinary Microbial Phenolic Metabolites by HPLC-ESI-LTQ-Orbitrap-HRMS and Their Relationship with Dietary Polyphenols in Adolescents. Antioxidants. 2022 Jun 14;11(6):1167.
75. Sasot G, Martínez-Huélamo M, Vallverdú-Queralt A, Mercader-Martí M, Estruch R, Lamuela-Raventós RM. Identification of phenolic metabolites in human urine after the intake of a functional food made from grape extract by a high resolution LTQ-Orbitrap-MS approach. Food Research International. 2017 Oct 1;100(Pt 3):435–44.
76. Vallverdú-Queralt A, Jáuregui O, Medina-Remón A, Andrés-Lacueva C, Lamuela-Raventós RM. Improved characterization of tomato polyphenols using liquid chromatography/electrospray ionization linear ion trap quadrupole Orbitrap mass spectrometry and liquid

chromatography/electrospray ionization tandem mass spectrometry. Rapid Communications in Mass Spectrometry. 2010 Sep 22;24(20):2986–92.

77. Grund B, Marvin L, Rochat B. Quantitative performance of a quadrupole-orbitrap-MS in targeted LC–MS determinations of small molecules. J Pharm Biomed Anal. 2016 May 30;124:48–56.
78. Kaufmann A, Dvorak V, Crüzer C, Butcher P, Maden K, Walker S, et al. Study of High-Resolution Mass Spectrometry Technology as a Replacement for Tandem Mass Spectrometry in the Field of Quantitative Pesticide Residue Analysis. J AOAC Int. 2012 Mar 1;95(2):528–48.
79. Castro-Barquero S, Tresserra-Rimbau A, Vitelli-Storelli F, Doménech M, Salas-Salvadó J, Martín-Sánchez V, et al. Dietary Polyphenol Intake is Associated with HDL-Cholesterol and A Better Profile of other Components of the Metabolic Syndrome: A PREDIMED-Plus Sub-Study. Nutrients. 2020 Mar 4;12(3):689.
80. Tresserra-Rimbau A, Medina-Remón A, Pérez-Jiménez J, Martínez-González MA, Covas MI, Corella D, et al. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study. Nutr Metab Cardiovasc Dis. 2013;23(10):953–9.
81. Tresserra-Rimbau A, Castro-Barquero S, Vitelli-Storelli F, Becerra-Tomas N, Vázquez-Ruiz Z, Díaz-López A, et al. Associations between Dietary Polyphenols and Type 2 Diabetes in a Cross-Sectional Analysis of the PREDIMED-Plus Trial: Role of Body Mass Index and Sex. Antioxidants. 2019 Nov 8;8(11):537.
82. Rienks J, Barbaresko J, Nöthlings U. Association of Polyphenol Biomarkers with Cardiovascular Disease and Mortality Risk: A Systematic Review and Meta-Analysis of Observational Studies. Nutrients. 2017 Apr 22;9(4):415.
83. Grosso G, Micek A, Godos J, Pajak A, Sciacca S, Galvano F, et al. Dietary Flavonoid and Lignan Intake and Mortality in Prospective Cohort Studies: Systematic Review and Dose-Response Meta-Analysis. Am J Epidemiol. 2017 Jun 15;185(12):1304–16.
84. Liu X ming, Liu Y jian, Huang Y, Yu H jie, Yuan S, Tang B wen, et al. Dietary total flavonoids intake and risk of mortality from all causes and cardiovascular disease in the general population: A systematic review and meta-analysis of cohort studies. Mol Nutr Food Res. 2017 Jun 1;61(6):1601003.
85. Micek A, Godos J, del Rio D, Galvano F, Grosso G. Dietary Flavonoids and Cardiovascular Disease: A Comprehensive Dose-Response Meta-Analysis. Mol Nutr Food Res. 2021 Mar 1;65(6):e2001019.

86. Crowe-White KM, Evans LW, Kuhnle GGC, Milenkovic D, Stote K, Wallace T, et al. Flavan-3-ols and Cardiometabolic Health: First Ever Dietary Bioactive Guideline. Advances in Nutrition. 2022 Oct 3;nmac105.
87. Wang X, Qi Y, Zheng H. Dietary Polyphenol, Gut Microbiota, and Health Benefits. Antioxidants. 2022 Jun 20;11(6):1212.
88. Liu K, Luo M, Wei S. The Bioprotective Effects of Polyphenols on Metabolic Syndrome against Oxidative Stress: Evidences and Perspectives. Oxid Med Cell Longev. 2019 Nov 30;2019:1–16.
89. Springer M, Moco S. Resveratrol and Its Human Metabolites—Effects on Metabolic Health and Obesity. Nutrients. 2019 Jan 11;11(1):143.
90. Ma G, Chen Y. Polyphenol supplementation benefits human health via gut microbiota: A systematic review via meta-analysis. J Funct Foods. 2020 Mar 1;66:103829.
91. Rajha HN, Paule A, Aragonès G, Barbosa M, Caddeo C, Debs E, et al. Recent Advances in Research on Polyphenols: Effects on Microbiota, Metabolism, and Health. Mol Nutr Food Res. 2022 Jan 2;66(1):2100670.
92. García-Villalba R, Giménez-Bastida JA, Cortés-Martín A, Ávila-Gálvez MÁ, Tomás-Barberán FA, Selma MV, et al. Urolithins: a Comprehensive Update on their Metabolism, Bioactivity, and Associated Gut Microbiota. Mol Nutr Food Res. 2022 Nov 15;66(21):2101019.
93. Tomás-Barberán FA, González-Sarrías A, García-Villalba R, Núñez-Sánchez MA, Selma M v., García-Conesa MT, et al. Urolithins, the rescue of "old" metabolites to understand a "new" concept: Metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. Mol Nutr Food Res. 2017 Jan 1;61(1):1500901.
94. Esteban-Torres M, Santamaría L, Cabrera-Rubio R, Plaza-Vinuesa L, Crispie F, de Las Rivas B, et al. A Diverse Range of Human Gut Bacteria Have the Potential To Metabolize the Dietary Component Gallic Acid. Appl Environ Microbiol. 2018 Oct 1;84(19) e01558-18.
95. Frankenfeld CL. Cardiometabolic Risk Factors Are Associated with High Urinary Enterolactone Concentration, Independent of Urinary Enterodiol Concentration and Dietary Fiber Intake in Adults. J Nutr. 2014 Sep 1;144(9):1445–53.
96. Mele L, Mena P, Piemontese A, Marino V, López-Gutiérrez N, Bernini F, et al. Antiatherogenic effects of ellagic acid and urolithins in vitro. Arch Biochem Biophys. 2016 Jun 1;599:42–50.
97. Spigoni V, Mena P, Cito M, Fantuzzi F, Bonadonna R, Brighenti F, et al. Effects on Nitric Oxide Production of Urolithins, Gut-Derived Ellagitannin

Metabolites, in Human Aortic Endothelial Cells. Molecules. 2016 Aug 2;21(8):1009.

98. Li K, Xiao Y, Bian J, Han L, He C, El-Omar E, et al. Ameliorative Effects of Gut Microbial Metabolite Urolithin A on Pancreatic Diseases. Nutrients. 2022 Jun 20;14(12):2549.
99. Yang J, Guo Y, Henning SM, Chan B, Long J, Zhong J, et al. Ellagic Acid and Its Microbial Metabolite Urolithin A Alleviate Diet-Induced Insulin Resistance in Mice. Mol Nutr Food Res. 2020 Oct 23;64(19):2000091.
100. Wisnuwardani RW, de Henauw S, Forsner M, Gottrand F, Huybrechts I, Knaze V, et al. Polyphenol intake and metabolic syndrome risk in European adolescents: the HELENA study. Eur J Nutr. 2020 Mar 22;59(2):801–12.
101. Wisnuwardani RW, de Henauw S, Béghin L, Mesana MI, Gottrand F, Moreno LA, et al. Changes in (poly)phenols intake and metabolic syndrome risk over ten years from adolescence to adulthood. Nutrition, Metabolism and Cardiovascular Diseases. 2022 Aug 1;32(8):1830–40.
102. Medina-Remón A, Barrionuevo-González A, Zamora-Ros R, Andres-Lacueva C, Estruch R, Martínez-González MÁ, et al. Rapid Folin–Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. Anal Chim Acta. 2009 Feb 16;634(1):54–60.
103. Chandler JL, Brazendale K, Beets MW, Mealing BA. Classification of physical activity intensities using a wrist-worn accelerometer in 8-12-year-old children. Pediatr Obes. 2016 Apr 1;11(2):120–7.
104. UNESCO. International Standard Classification of Education (ISCED) 2011. International Standard Classification of Education (ISCED) 2011. UNESCO Institute for Statistics; 2012.
105. INE: Instituto Nacional de Estadística. Encuesta anual de estructura salarial. https://www.ine.es/jaxiT3/Datos.htm?t=9948.
106. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976 Mar 1;51(3):170–9.
107. Camacho S, Ruppel A. Is the calorie concept a real solution to the obesity epidemic? Glob Health Action. 2017 May 9;10(1): 1289650.
108. Castro-Barquero S, Lamuela-Raventós R, Doménech M, Estruch R. Relationship between Mediterranean Dietary Polyphenol Intake and Obesity. Nutrients. 2018 Oct 17;10(10):1523.
109. Archero F, Ricotti R, Solito A, Carrera D, Civello F, di Bella R, et al. Adherence to the Mediterranean Diet among School Children and

Adolescents Living in Northern Italy and Unhealthy Food Behaviors Associated to Overweight. Nutrients. 2018 Sep 18;10(9):1322.

110. Ulaszewska MM, Weinert CH, Trimigno A, Portmann R, Andres Lacueva C, Badertscher R, et al. Nutrimetabolomics: An Integrative Action for Metabolomic Analyses in Human Nutritional Studies. Mol Nutr Food Res. 2019 Jan 1;63(1):1800384.
111. Wisnuwardani RW, de Henauw S, Androutsos O, Forsner M, Gottrand F, Huybrechts I, et al. Estimated dietary intake of polyphenols in European adolescents: the HELENA study. Eur J Nutr. 2019 Sep 30;58(6):2345–63.
112. Ziauddeen N, Rosi A, del Rio D, Amoutzopoulos B, Nicholson S, Page P, et al. Dietary intake of (poly)phenols in children and adults: cross-sectional analysis of UK National Diet and Nutrition Survey Rolling Programme (2008–2014). Eur J Nutr. 2019 Dec 17;58(8):3183–98.
113. Grosso G, Stepaniak U, Topor-Mądry R, Szafraniec K, Pająk A. Estimated dietary intake and major food sources of polyphenols in the Polish arm of the HAPIEE study. Nutrition. 2014 Nov 1;30(11–12):1398–403.
114. Vitale M, Vaccaro O, Masulli M, Bonora E, del Prato S, Giorda CB, et al. Polyphenol intake and cardiovascular risk factors in a population with type 2 diabetes: The TOSCA.IT study. Clinical Nutrition. 2017 Dec 1;36(6):1686–92.
115. Mena P, Domínguez-Perles R, Gironés-Vilaplana A, Baenas N, García-Viguera C, Villaño D. Flavan-3-ols, anthocyanins, and inflammation. IUBMB Life. 2014 Nov 1;66(11):745–58.
116. Cercato LM, Oliveira JP, Souza MTS, Andrade N, Martel F, Camargo EA. Effect of flavonoids in preclinical models of experimental obesity. PharmaNutrition. 2021 Jun 1;16:100260.
117. Grosso G, Stepaniak U, Micek A, Stefler D, Bobak M, Pająk A. Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study. Eur J Nutr. 2017 Jun 25;56(4):1409–20.
118. Fujimaki T, Sato C, Yamamoto R, Watanabe S, Fujita H, Kikuno H, et al. Isolation of phenolic acids and tannin acids from *Mangifera indica* L. kernels as inhibitors of lipid accumulation in 3T3-L1 cells. Biosci Biotechnol Biochem. 2022 Apr 21;86(5):665–71.
119. Ibitoye OB, Ajiboye TO. Dietary phenolic acids reverse insulin resistance, hyperglycaemia, dyslipidaemia, inflammation and oxidative stress in high-fructose diet-induced metabolic syndrome rats. Arch Physiol Biochem. 2018 Oct 20;124(5):410–7.

120. Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. Am J Clin Nutr. 2012 Mar 1;95(3):740–51.
121. Kord-Varkaneh H, Ghaedi E, Nazary-Vanani A, Mohammadi H, Shab-Bidar S. Does cocoa/dark chocolate supplementation have favorable effect on body weight, body mass index and waist circumference? A systematic review, meta-analysis and dose-response of randomized clinical trials. Crit Rev Food Sci Nutr. 2019 Aug 22;59(15):2349–62.
122. Lin X, Zhang I, Li A, Manson JAE, Sesso HD, Wang L, et al. Cocoa Flavanol Intake and Biomarkers for Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Nutr. 2016;146(11):2325–33.
123. Sun Y, Zimmermann D, de Castro CA, Actis-Goretta L. Dose-response relationship between cocoa flavanols and human endothelial function: a systematic review and meta-analysis of randomized trials. Food Funct. 2019 Oct 1;10(10):6322–30.
124. González-Sarrías A, Combet E, Pinto P, Mena P, Dall'Asta M, Garcia-Aloy M, et al. A Systematic Review and Meta-Analysis of the Effects of Flavanol-Containing Tea, Cocoa and Apple Products on Body Composition and Blood Lipids: Exploring the Factors Responsible for Variability in Their Efficacy. Nutrients. 2017 Jul 13;9(7):746.
125. Medina-Remón A, Tresserra-Rimbau A, Pons A, Tur JA, Martorell M, Ros E, 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. 2015 Jan 1;25(1):60–7.
126. Ghosh D, Scheepens A. Vascular action of polyphenols. Mol Nutr Food Res. 2009 Mar 1;53(3):322–31.
127. Cheng C, Li Z, Zhao X, Liao C, Quan J, Bode AM, et al. Natural alkaloid and polyphenol compounds targeting lipid metabolism: Treatment implications in metabolic diseases. Eur J Pharmacol. 2020 Mar 5;870:172922.
128. Farhat G, Drummond S, Al-Dujaili EAS. Polyphenols and Their Role in Obesity Management: A Systematic Review of Randomized Clinical Trials. Phytotherapy Research. 2017 Jul 1;31(7):1005–18.
129. de Bruyne T, Steenput B, Roth L, de Meyer GRY, dos Santos CN, Valentová K, et al. Dietary Polyphenols Targeting Arterial Stiffness: Interplay of

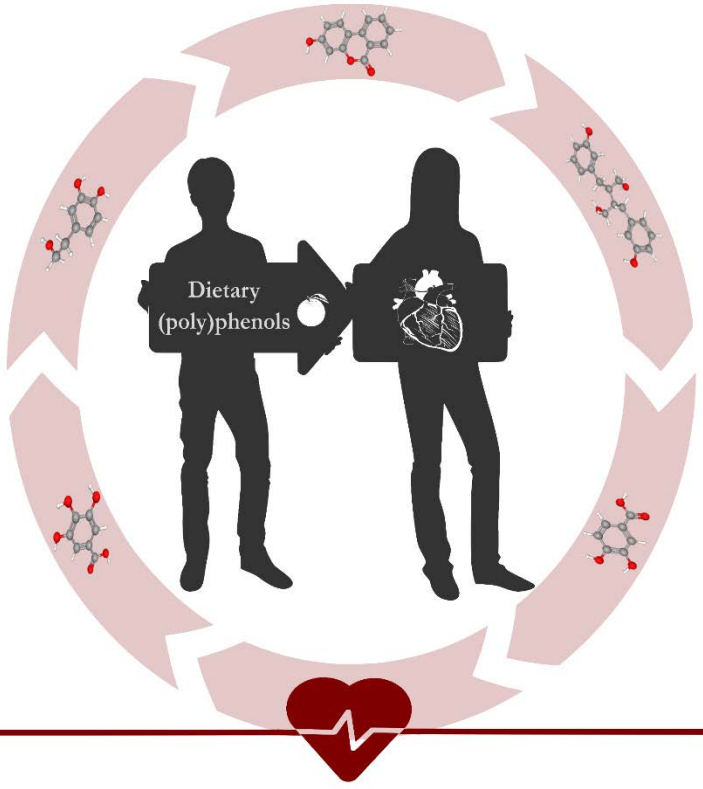
Contributing Mechanisms and Gut Microbiome-Related Metabolism. Nutrients. 2019 Mar 8;11(3):578.

130. Bruce SJ, Rochat B, Béguin A, Pesse B, Guessous I, Boulat O, et al. Analysis and quantification of vitamin D metabolites in serum by ultra-performance liquid chromatography coupled to tandem mass spectrometry and high-resolution mass spectrometry – a method comparison and validation. Rapid Communications in Mass Spectrometry. 2013 Jan 15;27(1):200–6.
131. Herrero P, Cortés-Francisco N, Borrull F, Caixach J, Pocurull E, Marcé RM. Comparison of triple quadrupole mass spectrometry and Orbitrap high-resolution mass spectrometry in ultrahigh performance liquid chromatography for the determination of veterinary drugs in sewage: benefits and drawbacks. Journal of Mass Spectrometry. 2014 Jul 1;49(7):585–96.
132. Cavaliere C, Antonelli M, Capriotti AL, la Barbera G, Montone CM, Piovesana S, et al. A Triple Quadrupole and a Hybrid Quadrupole Orbitrap Mass Spectrometer in Comparison for Polyphenol Quantitation. J Agric Food Chem. 2019 May 1;67(17):4885–96.
133. Zhang G, Chen S, Zhou W, Meng J, Deng K, Zhou H, et al. Rapid qualitative and quantitative analyses of eighteen phenolic compounds from Lycium ruthenicum Murray by UPLC-Q-Orbitrap MS and their antioxidant activity. Food Chem. 2018 Dec 15;269:150–6.
134. Giambanelli E, Filippo D'Antuono L, Romero-González R, Garrido Frenich A. Identification and quantification of phenolic compounds in edible wild leafy vegetables by UHPLC/Orbitrap-MS. J Sci Food Agric. 2018 Feb 1;98(3):945–54.
135. Barnaba C, Dellacassa E, Nicolini G, Nardin T, Malacarne M, Larcher R. Identification and quantification of 56 targeted phenols in wines, spirits, and vinegars by online solid-phase extraction – ultrahigh-performance liquid chromatography – quadrupole-orbitrap mass spectrometry. J Chromatogr A. 2015 Dec 4;1423:124–35.
136. Barbosa S, Pardo-Mates N, Hidalgo-Serrano M, Saurina J, Puignou L, Núñez O. Detection and Quantitation of Frauds in the Authentication of Cranberry-Based Extracts by UHPLC-HRMS (Orbitrap) Polyphenolic Profiling and Multivariate Calibration Methods. J Agric Food Chem. 2018 Sep 5;66(35):9353–65.
137. Ordóñez JL, Pereira-Caro G, Ludwig I, Muñoz-Redondo JM, Ruiz-Moreno MJ, Crozier A, et al. A critical evaluation of the use of gas chromatography-and high performance liquid chromatography-mass spectrometry techniques

for the analysis of microbial metabolites in human urine after consumption of orange juice. J Chromatogr A. 2018 Nov 9;1575:100–12.

138. Pereira-Caro G, Ordóñez JL, Ludwig I, Gaillet S, Mena P, del Rio D, et al. Development and validation of an UHPLC-HRMS protocol for the analysis of flavan-3-ol metabolites and catabolites in urine, plasma and feces of rats fed a red wine proanthocyanidin extract. Food Chem. 2018 Jun 30;252:49–60.
139. Milder IEJ, Kuijsten A, Arts ICW, Feskens EJM, Kampman E, Hollman PCH, et al. The Journal of Nutrition Nutritional Epidemiology Relation between Plasma Enterodiol and Enterolactone and Dietary Intake of Lignans in a Dutch Endoscopy-Based Population 1,2. J Nutr. 2007;137:1266–71.
140. Garcia-Aloy M, Hulshof PJM, Estruel-Amades S, Osté MCJ, Lankinen M, Geleijnse JM, et al. Biomarkers of food intake for nuts and vegetable oils: an extensive literature search. Genes Nutr. 2019 Mar 19;14(7).
141. Mosele JI, Macià A, Motilva MJ. Metabolic and Microbial Modulation of the Large Intestine Ecosystem by Non-Absorbed Diet Phenolic Compounds: A Review. Molecules. 2015 Sep 1;20(9):17429.
142. Kosuru RY, Roy A, Das SK, Bera S. Gallic Acid and Gallates in Human Health and Disease: Do Mitochondria Hold the Key to Success? Mol Nutr Food Res. 2018 Jan 1;62(1):1700699.
143. Yang CS, Zhang J, Zhang L, Huang J, Wang Y. Mechanisms of body weight reduction and metabolic syndrome alleviation by tea. Mol Nutr Food Res. 2016 Jan 1;60(1):160–74.
144. Cheng M, Zhang X, Miao Y, Cao J, Wu Z, Weng P. The modulatory effect of (-)-epigallocatechin 3-O-(3-O-methyl) gallate (EGCG3"Me) on intestinal microbiota of high fat diet-induced obesity mice model. Food Research International. 2017 Feb 1;92:9–16.
145. Kim HS, Moon JH, Kim YM, Huh JY. Epigallocatechin Exerts Anti-Obesity Effect in Brown Adipose Tissue. Chem Biodivers. 2019 Oct 1;16(10):e1900347.
146. Eid HM, Wright ML, Anil Kumar N v., Qawasmeh A, Hassan STS, Mocan A, et al. Significance of Microbiota in Obesity and Metabolic Diseases and the Modulatory Potential by Medicinal Plant and Food Ingredients. Front Pharmacol. 2017 Jun 30;8:387.
147. Hasheminezhad SH, Boozari M, Iranshahi M, Yazarlu O, Sahebkar A, Hasanpour M, et al. A mechanistic insight into the biological activities of urolithins as gut microbial metabolites of ellagitannins. Phytotherapy Research. 2022 Jan 20;36(1):112–46.

148. Romo-Vaquero M, Cortés-Martín A, Loria-Kohen V, Ramírez-de-Molina A, García-Mantrana I, Collado MC, et al. Deciphering the Human Gut Microbiome of Urolithin Metabotypes: Association with Enterotypes and Potential Cardiometabolic Health Implications. Mol Nutr Food Res. 2019 Feb 1;63(4): e1800958.
149. Selma M v., González-Sarrías A, Salas-Salvadó J, Andrés-Lacueva C, Alasalvar C, Örem A, 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. Clin Nutr. 2018 Jun 1;37(3):897–905.
150. Istas G, Feliciano RP, Weber T, Garcia-Villalba R, Tomas-Barberan F, Heiss C, et al. Plasma urolithin metabolites correlate with improvements in endothelial function after red raspberry consumption: A double-blind randomized controlled trial. Arch Biochem Biophys. 2018 Aug 1;651:43–51.
151. Torres-Fuentes C, Suárez M, Aragonès G, Mulero M, Ávila-Román J, Arola-Arnal A, et al. Cardioprotective Properties of Phenolic Compounds: A Role for Biological Rhythms. Mol Nutr Food Res. 2022 Nov 28;66(21):2100990.
152. Fraga LN, Coutinho CP, Rozenbaum AC, Tobaruela E de C, Lajolo FM, Hassimotto NMA. Blood pressure and body fat % reduction is mainly related to flavanone phase II conjugates and minor extension by phenolic acid after long-term intake of orange juice. Food Funct. 2021 Nov 15;12(22):11278–89.
153. Juton C, Castro-Barquero S, Casas R, Freitas T, Ruiz-León AM, Crovetto F, et al. Reliability and Concurrent and Construct Validity of a Food Frequency Questionnaire for Pregnant Women at High Risk to Develop Fetal Growth Restriction. Nutrients. 2021 May 12;13(5):1629.
154. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997 Apr 1;65(4):1220S-1228S.
155. Jones L, Ness A, Emmett P. Misreporting of Energy Intake From Food Records Completed by Adolescents: Associations With Sex, Body Image, Nutrient, and Food Group Intake. Front Nutr. 2021 Dec 13;8:970.



APPENDIX

APPENDIX

Other publications

Reviews

Health Effects of Resveratrol: Results from Human Intervention Trials.
Ramírez-Garza SL, **Laveriano-Santos EP,** Marhuenda-Muñoz M, Storniolo CE, Tresserra-Rimbau A, Vallverdú-Queralt A, Lamuela-Raventós RM.
Nutrients. 2018 ;10(12):1892.

Sweet potato is not simply an abundant food crop: a comprehensive review of its phytochemical constituents, biological activities, and the effects of processing.
Emily P. Laveriano-Santos, Anallely López-Yerena, Carolina Jaime-Rodríguez, Johana González-Coria, Rosa M. Lamuela-Raventós, Anna Vallverdú-Queralt, Joan Romanyà, Maria Pérez.
Antioxidants. 2022; 11, 1648.

Original articles

Urinary Nitric Oxide Levels Are Associated with Blood Pressure, Fruit and Vegetable Intake and Total Polyphenol Excretion in Adolescents from the SI! Program.
Ramírez-Garza SL, **Laveriano-Santos EP**, Arancibia-Riveros C, Carrasco-Jimenez JC, Bodega P, Cos-Gandoy Ad, Miguel Md, Santos-Beneit G, Fernández-Alvira JM, Fernández-Jiménez R, Martínez-Gómez J, Estruch R, Lamuela-Raventós RM, Tresserra-Rimbau A
Antioxidants. 2022; 11(11):2140.

Effect of differentiated organic fertilization on tomato production and phenolic content in traditional and high-yielding varieties.
Johana Gonzalez Coria; Julián Lozano-Castellón; Carolina Jaime-Rodriguez; Alexandra Olmo-Cunillera; **Emily Laveriano-Santos**; Maria Pérez; Rosa M. Lamuela-Raventos; Jordi Puig; Anna Vallverdu-Queralt; Joan Romanyà.
Antioxidants. 2022; 11(11):2127.

One-Year Changes in Urinary Microbial Phenolic Metabolites and the Risk of Type 2 Diabetes—A Case-Control Study.
Marhuenda-Muñoz, María, Inés Domínguez-López, **Emily P. Laveriano-Santos**, Isabella Parilli-Moser, Cristina Razquin, Miguel Ruiz-Canela, Francisco Javier Basterra-Gortari, Dolores Corella, Jordi Salas-Salvadó, Montserrat Fitó, José Lapetra, Fernando Arós, Miquel Fiol, Lluis Serra-Majem, Xavier Pintó, Enrique Gómez-Gracia, Emilio Ros, Ramon Estruch, and Rosa M. Lamuela-Raventós. 2022. Antioxidants. 2022;11(8):1540.

Moderate Consumption of Beer (with and without Ethanol) and Menopausal Symptoms: Results from a Parallel Clinical Trial in Postmenopausal Women.
Trius-Soler M, Marhuenda-Muñoz M, **Laveriano-Santos EP,** Martínez-Huélamo M, Sasot G, Storniolo CE, Estruch R, Lamuela-Raventós RM, Tresserra-Rimbau A. Nutrients. 2021;13(7):2278.

Prevalence and correlates of cardiovascular health among early adolescents enrolled in the SI! Program in Spain: a cross-sectional analysis.
Fernandez-Jimenez R, Santos-Beneit G, de Cos-Gandoy A, Fernández-Alvira JM, Tresserra-Rimbau A, Storniolo C, Domènech M, **Laveriano-Santos EP**, Bodega P, de Miguel M, Rodríguez C, Carvajal I, Ibañez B, Estruch R, Lamuela-Raventós RM, Fuster V.
Eur J Prev Cardiol. 2020;29(1):e7-e10.

Communication in scientific meetings

Poster

Poster 1. Flavonoids from cocoa-base products and obesity among Spanish adolescents enrolled in the SI! Program: a cross-sectional study.
Emily P. Laveriano-Santos, Camila Arancibia-Riveros, Anna Tresserra-Rimbau, Ana María Ruiz-León, Sara Castro-Barquero, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Vanesa Carral, Gloria Santos-Beneit, Juan M. Fernández-Alvira, Rodrigo Fernández-Jiménez, Valentín Fuster, and Rosa M. Lamuela-Raventós
VI Workshop Anual del INSA-UB, 2021

Poster 2. Relationship between cacao flavonoids, adiposity indicators, and blood pressure in Spanish adolescents.

Emily P. Laveriano-Santos, Camila-Arancibia, Sonia L. Ramírez-Garza, Anna Tresserra-Rimbau, Sara Castro-Barquero, Ana Ruiz, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Xavier Orrit, Gloria Santos-Beneit, Juan Miguel Fernández-Alvira, Rodrigo Fernández-Jiménez, Valentín Fuster, Rosa M. Lamuela-Raventós.

XII Symposium Ciber Fisiopatología de la Obesidad y Nutrición. October 26- 28, 2021.

Poster 3. Polifenoles en orina y su relación con factores de riesgo cardiovascular en adolescentes españoles del Programa Si! en Educación Secundaria.

Emily P. Laveriano-Santos, Isabella Parilli-Moser, Sonia L. Ramírez-Garza, Anna Tresserra-Rimbau, Ramón Estruch, Gloria Santos-Beneit, Juan Miguel Fernández-Alvira, Rodrigo Fernández-Jiménez, Valentín Fuster, Rosa M. Lamuela-Raventós.

IV Virtual Congress FESNAD. November 03-06, 2020

Poster 4. Relationship between (poly)phenols and body weight in adolescents, pilot study.

Laveriano-Santos EP, Treserra-Rimbau A, Domenech M, Estruch R , Santos-Beneit G, Fernández-Alvira JM, Fuster V, Lamuela-Raventós RM.

XVII Congreso de la Sociedad Española de Nutrición. Barcelona, June 27-29, 2018. ANNALS OF NUTRITION AND METABOLISM 73, 34-35

Poster 5. Relationship between (poly)phenols and cardiovascular risk factors in adolescents, pilot study.

Emily P. Laveriano-Santos, Sonia Ramírez-Garza, Carolina Storniolo, Anna Treserra-Rimbau, Monica Domenech, Ramon Estruch, Ana Ruiz, Patricia Bodega, Mercedes de Miguel, Gloria Santos-Beneit, Juan Miguel Fernández-Alvira, Rodrigo Fernández-Jiménez, Valentín Fuster, Rosa M. Lamuela-Raventós.

XII International Conference Mediterranean Diet. Barcelona, April 18-19, 2018.

Poster 6. Nutritional status and total urinary (poly)phenols in adolescents: picture from a pilot study.

Emily P. Laveriano-Santos, Sonia L. Ramírez-Garza, Carolina E. Storniolo, Anna Tresserra-Rimbau, Mònica Domenech, Ramon Estruch, Patricia Bodega, Mercedes de Miguel, Gloria Santos-Beneit, Juan Miguel Fernández-Alvira, Rodrigo Fernández-Jiménez, Valentín Fuster.

IV Workshop INSA-UB. Barcelona, November 15, 2018.

Oral communication

Communication 1. Microbial phenolic metabolites and their relationship with (poly)phenol-rich foods in Spanish adolescents enrolled in the SI! Program for secondary schools: a cross-sectional study.

Emily P. Laveriano-Santos, María Marhuenda-Muñoz, Anna Vallverdú-Queralt, Anna Tresserra-Rimbau, Camila Arancibia-Riveros, Ana María Ruiz-León, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Vanessa Carral, Gloria Santos-Beneit, Juan M. Fernández-Alvira, Rodrigo Fernández-Jiménez, Valentín Fuster, and Rosa M. Lamuela-Raventós

The 3rd International Conference on Food Bioactives and Health. June 21-24, 2022, Parma, Italy.

Communication 2. Microbial phenolic metabolites and metabolic syndrome score in adolescents: preliminary results

Emily P. Laveriano-Santos, Paola Quifer-Rada, María Marhuenda-Muñoz, Camila Arancibia-Riveros, Anna Vallverdú-Queralt, Anna Tresserra-Rimbau, Ana María Ruiz-León, Rosa Casas, Ramón Estruch, Patricia Bodega, Mercedes de Miguel, Amaya de Cos-Gandoy, Jesús Martínez-Gómez, Gloria Santos-Beneit, Juan M. Fernández-Alvira, Rodrigo Fernández-Jiménez, and Rosa M. Lamuela-Raventós.

The "Scientific Antipasti before FBHC", Satellite meeting at the 3rd International Conference on Food Bioactives & Health. June 20, Parma – Italy

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Other communication activities

Invited Speaker- Short conference: "Polifenoles del cacao y obesidad en adolescentes españoles"

Emily Laveriano-Santos

22 Congreso-Foro AdENyD, 09-10 june 2022, Vitoria-Gasteiz, Spain.

Awards

Best poster. XII International Congress of Mediterranean Diet. Barcelona, April 18-19, 2018. "Relationship between (poly)phenols and cardiovascular risk factors in adolescents, pilot study".

Best poster. IV Workshop INSA-UB. Barcelona, November 15, 2018. Nutritional status and total urinary (poly)phenols in adolescents: picture from a pilot study

Project Participation

2017–2021 School-based Behavioral Intervention to Face Obesity and Promote Cardiovascular Health Among Spanish Adolescents.
Principal investigators: Valentin Fuster, Ramon Estruch, Rosa M. Lamuela, Gloria Santos, Juan Fernández, Rodrigo Jiménez.
Fundació la Marató de TV3 (Barcelona, Barcelona) https://www.clinicaltrials.gov/ct2/show/NCT03504059

2020-2022 FoodPhyt Project: Food phytochemicals matter for cardiometabolic health.
Principal investigator: Dr. Manach Claudine (INRAE- NutriVasc Research Group-France).
https://www6.inrae.fr/foodphyt/

Pre-doctoral stay

National Research Institute for Agriculture, Food and the Environment (INRAE) – Nutrition Unit NutriVasc Research Group- France). January – April 2022. Supervisor: Dr. Manach Claudine
Untargeted metabolomic analysis to identify urinary biomarkers of cocoa intake using high-resolution mass spectrometry, management of plant-food phytochemical online-database (PhytoHub), and systematic-review methodology to conduct a study about raw and processed tomato with cardiometabolic health parameters based on human clinical trials.