Interuniversity master in Nutrition and Metabolism Investigation

Final Project

An increase in the excretion of polyphenols in urine is associated with better cardiovascular health in Spanish adolescents from Program SI! for Secondary Schools

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ABSTRACT

Background: Epidemiological studies have shown an association exists between increased consumption of foods high in polyphenols and a more favorable cardiovascular risk profile. Although cardiovascular disease is the leading cause of death in adults worldwide few studies have yet to identify the incidence of cardiovascular risk factors in adolescents. Adolescence-specific incidence in obesity, diabetes mellitus, hypertension, nutrient-poor diets, sedentary lifestyle, higher tobacco use, and excessive alcohol consumption may also be associated with more undesirable cardiovascular risks outcomes. Therefore, the aim of this study is to evaluate the association between high urinary polyphenol excretion and a better cardiovascular health in adolescents.

Methods: This program is designed as a cluster-randomized controlled intervention trial in secondary schools in Spain. The trial involved 1194 adolescents (12 to 16 years of age) who participated in the study to evaluate a score of health criteria. The score of health criteria included body mass index (BMI), eating habits, physical activity, blood pressure, cholesterol levels, glucose levels, and smoking to establish an Ideal Cardiovascular Health (ICH). A follow-up assessment took place at 2 and 4 years after initiation of study. The Folin-Ciocalteu method was used for the determination of Total Polyphenol Excretion (TPE) in fasting urine samples, with previous solid-phase extraction. TPE was categorized into tertiles (T1< 86.3; T2 86.3-141.3, T3 >141.3) mg Gallic Acid Equivalent (GAE)/ g creatinine. Linear regression models at different levels for an ordinal categorical response were used to assess the association of tertiles of TPE with ICH score which was evaluated from 0 to 7.

Results: Amongst the participants those with the highest excretion of polyphenols in total urine had a better lipid profile; total cholesterol ($p = 0.004$), triglycerides ($p = 0.036$), and glucose ($p = 0.028$) compared to the first tertile. In addition, an inverse association was found between the highest tertiles of TPE and triglycerides, level of sexual maturation, age, parental education and socioeconomic level specifically in men in the adjusted models.

Conclusion: A higher concentration of TPE in urine is related to ideal cardiovascular health in adolescents.

Key Words: Polyphenols, Urine Polyphenols, Cardiovascular disease, ideal cardiovascular health, Mediterranean diet, Adolescents.

INTRODUCTION

Cardiovascular diseases (CVDs) is used to refer a range of diseases which affects the heart and blood vessels.These includes hypertension; coronary heart disease; cerebrovascular disease; heart failure. Cardiovascular disease is the leading cause of death in the world, according to World Health Organization (WHO) [1]. In 2017 17.79 million people died as a consequence of CVDs, which is the 31,7% of all deaths registered in the world[2].

Obesity, diabetes mellitus, and hypertension are cardiovascular risk factors (CVRFs) that could be prevented by promotion of healthy dietary habits, smoking cessation, and physical activity. Unhealthy eating habits and excessive alcohol consumption can exacerbate these risks [3,4]. Cardiovascular health studies are often focused on the adult population. However, evidence suggests that the promotion of an ideal cardiovascular health regimen from birth through childhood to young adulthood, and beyond can help prevent this disease earlier and lead to better outcomes[5]. CVRFs can already be identified in adolescents, and with development of autonomy these progress in teens to unhealthy habits such as a poor diet, physical inactivity, alcohol consumption, and tobacco use, these factors mentioned above are related to obesity [5–9]. In 2016, a study reported that more than 124 million children and adolescents worldwide from ages 5-19 suffer from obesity, and the prevalence in Spain is among the highest of European countries [10]. These risk factors can be reduced or prevented with the acquisition of a healthy diet, such as the Mediterranean Diet (MD) [11].

MD is considered one of the healthiest dietary patterns, and has a protective effect against overweight and obesity[11–13]. This diet involves much more than just an eating pattern; it is a cultural heritage which incorporates a balanced lifestyle with a collection of recipes. It is a celebration of traditions and customs involving a style of cooking with typical ingredients and daily activities. Characterized by its qualities; like the type of fat used (olive oil, fish, and nuts), the portions of main nutrients such as cereals and vegetables a Mediterranean diet is garnished with seasonal vegetables, aromatic herbs and seasonings is rich in micronutrients [14].

In a study involving 1135 adolescents in Sicily (Italy), it was shown that some factors can influence the adherence to this diet such as the environment and socioeconomic status; a higher adherence to the MD was associated with high economic status and high physical activity, whereas lower adherence was associated with living in an urban environment and being obese. KIDMED (Mediterranean Diet Quality Index for children and teenager) scores, which indicate whether adherence to the Mediterranean diet is low (from 0 to 3), medium (from 4 to 7) or high (from 8 to 12) showed that adolescents in urban areas eat less fruit and consumed more meat, sweetened drinks, and fast food than the adolescents who live in rural settings who preferred to eat more sweets and snacks [15]. A separate study completed in Spain, involving 3850 children and youths revealed geographical differences according to the location, socio-economic status and diet. The Northeastern population showed the most favorable outcome in relation to their diet compared to those in the North. A lower percentage of high quality-diet was associated with low socio-economic groups compared to those in the middle and upper-income cohorts[11]. A scientific review of articles published over a span of 15 years, revealed poor adherence to a MD in approximately half of the populations in Spain, Greece, and Italy, where social and demographic factors were major determinants of adherence to MD [16].

Mediterranean Diet (MD) is characterized by its effects on preventing CVRFs. Evidence suggests that bioactive compounds in the MD, such as polyphenols, are responsible for its benefits[3]. Phenolic compounds represent the largest group of substances present in plant foods, they are widely found in vegetables, fruits, seeds, coffee, wine, and tea. Phenolic compounds are further divided into different groups: phenolic acids, flavonoids, lignans, and a diet rich in these compounds can improve health and reduce the incidence of CVDs [3,17,18].

The capacity of polyphenols to modulate the activities of enzymes, cell signaling mechanisms and different cellular processes, in part, are due to the phytochemical characteristics of these compounds, which allow them to participate in cellular metabolic oxidation-reduction reactions[17,19,20]. These activities are characterized by their vasodilatory and vasoprotective, antithrombotic, antilipemic, anti-atherosclerotic, anti-inflammatory, and anti-apoptotic actions[6,17,19–22]. The high consumption, synergism, and bioavailability of polyphenols can have beneficial health effects. Studies have shown an inverse relationship between the risk of myocardial infarction and the high intake of polyphenols [23,24].

To quantify polyphenol intake there are different tools such as Food Frequency Questionaries (FFQs), diet histories, diet diaries and nutritional biomarkers. The first ones rely on self-reporting of dietary patterns by the study participants, which can be uncertain because foods that are perceived unhealthy are commonly not reported, and vegetables and fruits can be over-reported[25]. However, it is difficult to quantitatively establish the benefit afforded by polyphenols because there is a significantly great diversity of polyphenol content in foods. There is limited data concerning the polyphenol content of some specific foods, to properly quantify those in the habitual food intake or the absorption of polyphenols ingested [26]. Quantification of some specific polyphenol biomarkers that are accessible on fluids and tissues are better than the traditional dietary assessment, providing valuable information on the polyphenol intake in humans. For example, the Folin-Ciocalteu (F—C) assay, which is commonly used for the analysis of total polyphenol (TP) content in foods as well as in urine samples, is rapid, simple and allows the simultaneous determination of TP in a larger number of samples. It is potentially useful for evaluating the effectiveness of urinary polyphenols as markers of intake, bioavailability and accumulation in the body. [26,27]

Therefore, the quantification of nutritional biomarkers is necessary to associate a higher consumption of polyphenols in a diet to a better cardiovascular health score. This study aims to identify if a correlation exists between polyphenol excretion in the urine and a favorable cardiovascular health score in adolescents.

MATERIALS AND METHODS

Study Design, population and randomization process

Program SI! is a school intervention program aimed at boys and girls 12 to 16 years of age to demonstrate that the acquisition of healthy habits during adolescence with educational intervention from school, students and family can reduce the risks of cardiovascular disease and improve the quality of life in adulthood (8). This program is designed as a cluster-randomized controlled intervention trial in which the units of randomization include Secondary schools in Spain, with adolescents ranging from 12 to 16 years of age. The schools selected must meet the inclusion criteria such as being public school located in Barcelona (and surroundings) or Madrid, provision of education from grades 1 through 4. The intervention was carried out in 24 secondary schools in Spain (17 in Barcelona and 7 in Madrid), with a sample of 1326 adolescents at baseline, where schools were randomized (1: 1: 1) to receive a short-term (2 years) or long-term (4 years) educational program, or to receive the usual curriculum (control). Participants had already been evaluated at the beginning of 2017 (baseline), after the second year, and after 4 years of follow up(8).

The study was approved by the Committee for Ethical Research (CEI) from the *Instituto de Salud Carlos III* in Madrid (CEI PI 35_2016) and by the CEI of *Fundació Unió Catalana d'Hospitals* in Barcelona (CEI 16/41) and the University of Barcelona Bioethics Committee (IRB0000309).

Information was collected and handled according to the Spanish Law 15/1999 on the Protection of Personal Data, safeguarding the confidentiality of all participants. The study is registered at ClinicalTrials.gov, number NCT03504059. The reporting of this study protocol adheres to the SPIRIT guideline[9].

For the current Final Master Work, 1194 participants were included from the Si! Program baseline data after excluding participants with unavailable data of total polyphenol excretion in urine (TPE), diagnosis of diabetes or hypertension, and having taken any drugs or supplements the day before the data collection at baseline, according to Laveriano-Santos *et. al.* [3].

Ideal Cardiovascular Health metrics

The ideal cardiovascular health index and its components were calculated considering seven health metrics outlined by American Heart Association (AHA) [5] using the cut-off values for adolescents, and categorized as non-ideal or ideal. The seven cardiovascular health metrics are split into four health behaviors (smoking status, physical activity, dietary patterns, and BMI) and three health factors (fasting total cholesterol, blood glucose, and blood pressure). Table 1 details specific definitions of non-ideal and ideal cardiovascular health for children and adolescents aged 12 to 16 years[5].

Table 1. Ideal and non-ideal: Health Metrics in Children and Adolescents

Metric	**Non-ideal**	**Ideal**
Smoking	Smoked one or more cigarettes	Never smoked, or tried
Body Mass Index	≥ 85^{th} percentile	< 85^{th} percentile
Physical activity	< 60 min/day moderate or vigorous activity every day or non-physical activity	≥ 60 min/day moderate or vigorous activity every day
Healthy Diet Score*	0-3 components	4-5 components
Total Cholesterol	≥ 170 mg/dL	<170 mg/dL
Blood pressure	≥ 90^{th} percentile	< 90^{th} percentile
Fasting blood glucose	≥ 100 mg./dL	< 100 mg/dL

*The Healthy Diet Score is based on adherence to the following dietary recommendations: fruits and vegetables, ≥4.5 cups per day; fish, 2 or more 3.5-oz servings per week; sodium, ≤1500 mg/d; sugar-sweetened beverages, ≤450 kcal (36 oz) per week; and whole grains, ≥3 servings a day scaled to a 2000-kcal/d diet. Reprinted from Lloyd-Jones et al.1 Copyright © 2010, American Heart Association, Inc. Downloaded from http://ahajournals.org by on April 22, 2021

Health Behaviors

Smoking status was assessed via self-reported questionnaires. Adolescents who reported that they had never smoked were categorized as having an ideal smoking status and those who reported having smoked one or more cigarettes were categorized as presenting a poor smoking status.

Dietary pattern was evaluated using an updated version of the validated 157-items semi-quantitative food frequency questionnaire (FFQ) and also a validated Children's Eating Habits Questionnaire (CEHQ)[28,29]. The healthy diet score used for ideal dietary profiling included thresholds of fruits and vegetables ≥4.5 servings/day, fish ≥2 servings/week, fiber-rich whole grains ≥1 servings/day, and sugar-sweetened beverages ≤1 L/ week scaled to a 2000 kcal/d diet.

Physical activity was estimated with a triaxial Actigraph wGT3X-BT accelerometer (ActiGraph Corporation, Pensacola, FL, USA) on the non-dominant wrist for 7 days, excluded when bathing or swimming. The cut-off points of Chandler were applied to estimate total activity and minutes spent in moderate-to-vigorous physical activity[30].

Weight was measured using digital scale (OMRON BF511, precision of 0.1 kg), and height to the nearest 0.1 cm with a stadiometer (SECA scale 213) participants wore light clothing and no shoes. Body mass index (BMI) was calculated in kilograms divided by square height meters (kg/m^2). BMI z-scores and BMI percentiles were calculated according to age and gender specific median of the Centers for Disease Control (CDC) standards and National Health and Nutrition Examination Survey (NHANES) [31,32].

Health factors

Blood pressure (BP) was measured in a sitting position using a digital monitor OMRON M6 (OMRON HEALTHCARE Co., Muko, Kyoto, Japan). The additional measurements were taken at 2-to-3-minute intervals after the subject relaxed. Average values were calculated for the final systolic blood pressure (SBP) and diastolic blood pressure (DBP) [9]. These two z-scores were calculated according to the High Blood Pressure Working Group of the National Blood Pressure Education Program for children and adolescents[33].

Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), and glucose profile were analyzed using a portable CardioCheck®Plus (PTS Diagnostic, Indianapolis, IN, USA) biochemical analyzer in finger-prick capillary whole blood samples (approximately 40 μL), all data taken after overnight fasting. Coefficients of variation of TC were 4.9%, HDL-C 8,7% and glucose 4.0%[34].

Ideal cardiovascular health score

Using the metrics and criteria for individual ideal health factors and behaviors, we calculated the ideal cardiovascular health score corresponding to the AHA's concept[5]. To create the score, a value of 1 was assigned for each metric if the criterion for ideal cardiovascular health was met. If the criterion was not met, a value of 0 was assigned. The range of scores was thus 0 to 7, with a higher score indicating a better cardiovascular health profile. In analyses, we used the ideal cardiovascular health index as an ordinal variable (from 0 to 7), as well as categorized as poor, intermediate, and ideal ICH in children and adolescents.

Total Polyphenol Excretion (TPE) in Urine

Available data of TPE in urine was obtained from the F-C spectrophotometric method realized in 2018 at the laboratory of Antioxidants Research Group reported by Laveriano-Santos *et. al.* This F-C 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)[35]. Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) was used for curve calibration[35]. The analysis of creatinine in urine samples was followed by an adapted Jaffé alkaline picrate method for microtiter 96-well plates[35]. TPE was expressed as mg gallic acid equivalent (GAE)/g creatinine and the categorized into tertiles of TPE.

Covariate assessment

Sociodemographic factors included as covariates are age, sex, puberty, parent education, and income. Puberty development was assessed according to Tanner maturation stages[36], participants self-reported it using pictograms. Parents or legal guardians had to complete a questionnaire to assess sociodemographic factors which included place of birth, house income, and education level [37]. Parental education level was categorized low when families had no studies whatsoever or primary studies, categorized medium if they had secondary studies and professional training, and high which means university studies. These parameters were evaluated according to the International Standard Classification of Education (ISCED)[38]. House income was categorized in low, medium, and high based on the annual survey of salary for the Spanish population[39].

Statistical analysis

Participant's characteristics were conferred as means and standard deviations (SD) for continuous variables and frequencies and percentages for categorical variables. To identify the relationship between TPE and ICH scores, this cross-sectional analysis was organized into tertiles of TPE (mg GAE/g creatinine): T1(<86.3), T2 (86.3-141.3), T3 (>141.3). One-way analysis variance (ANOVA) and Chi-Square analysis were used to compare the unadjusted samples means and qualitative variables across TPE tertiles respectively.

Linear regression models at different levels for an ordinal categorical response were used to assess the association of tertiles of TPE with ICH score which was evaluated from 0 to 7. TPE was unadjusted in model number 1; age(continuous), fasting (yes/no), triglycerides (continuous), tanner stage (I to V), and sex (girls/boys) were introduced in model number 2; and model number 3 was adjusted by highest parental education (yes/no) and house income (low, medium and high). The association between ICH score and TPE was evaluated by the comparison of the highest and lowest tertile of TPE.

Barcelona and Madrid municipalities and also schools were included as a random effect. The potential effect modification in the association between quartiles of TPE and ICH by sex were evaluated by an interaction analysis using cross-product term between TPE and sex. It was also analyzed by girls and boys to evaluate the potential modification.

Statistical analysis was managed using Stata statistical software package version 16.0 (StataCorp, College Station, Tx, USA). Statistical tests were two-sided, and p-values under 0.05 were considered significant.

RESULTS

The 1194 participants are shown in Table 2 summarizing the baseline gender-stratification characteristics which were included in this study. It is shown that 48% of the cohort were girls with a mean age of 12.0 ± 0.4, 52% represented boys with a mean age of 12.1 ± 0.5. According to WC, there was a significantly higher value in boys compared to girls. Age and sex-adjusted BMI calculated as ideal, intermediate, and poor with percentiles <85% percentile, 80-95% percentile, >95% percentile respectively, a significantly higher value was identified in males than females. There was no significant difference in blood pressure, both systolic and diastolic between the two sexes. In comparison, glucose and TG were significant (P-value= 0.002 and 0.004 respectively) between males and females, with higher values of TG in females and glucose in males. There were no differences between the values of total cholesterol, HDL-C, and LDL-C between the two genders. It was observed that females performed more vigorous physical activity per minute per day than males. When analyzing the dietary section, it was found that there are no relevant differences between the two sex groups; however, there is a higher consumption of sugar-sweetened beverages among males.

Table 2. Baseline characteristics of Study Sample by sex.

	N	Boys (N= 624)	Girls (N=570)	P-value
Age (y)	1194	12.1 (0.5)	12.0 (0.4)	**0.002**
Anthropometric measurements				
Weight (kg)	1193	49.2 (11.9)	48.6 (10.4)	0.327
Height (kg)	1194	155.2 (7.8)	155.3 (6.8)	0.667
WC (cm)	1194	73.1(11.1)	70.5 (9.0)	**<0.001**
BMI (kg/m2)				
BMI, >95th percentile	109	62 (56.9)	47 (43.1)	**<0.001**
BMI 85-95th percentile	210	136 (64.8)	74 (35.2)	
BMI <85th percentile	874	425 (48.6)	449 (51.4)	
Blood pressure				
SBP (mmHg)				
SBP >95th percentile,	79	41 (51.9)	38 (48.1)	0.983
SBP 90-95th percentile,	72	37 (51.4)	35 (48.6)	
SBP <90th percentile,	1040	545 (52.4)	495 (47.6)	
DBP (mmHg)				
DBP >95th percentile,	53	22 (41.5)	31 (58.5)	0.125
DBP 90-95th percentile,	58	26 (44.8)	32 (55.2)	
DBP <90th percentile,	1080	575 (53.2)	505 (46.8)	
Biochemistry analytes				
Glucose (mg/dL)	1155	103.9 (11.7)	101.8 (11.7)	**0.002**
TC (mg/dL)	1155	151.1 (36.0)	154.3 (32.5)	0.109
HDL-C (mg /dL)	1153	62.9 (17.2)	62.8 (14.3)	0.849
LDL-C (mg/dL)	893	77.5 (26.9)	78.3 (24.8)	0.641
TG (mg/dL)	1154	73.2(43.5)	80.2 (37.8)	**0.004**

Smoking				
MVPA(min/day)	1121	72.2 (23.7)	77.1 (22.9)	**<0.001**
Dietary variables				
Fruit and vegetables (serv/day), mean (SD)	1156	4.1 (2.7)	4.2 (2.8)	0.525
Whole grain (serv/day)	1153	0.4 (0.6)	0.4 (0.6)	0.875
Fish (serv/week)	850	4.0 (3.4)	3.7 (2.2)	0.122
Sweet beverages (serv/week)	850	2.1 (3.5)	1.5 (2.4)	**0.003**
Sociodemographic				
Parents education,				
Low	226	112 (49.6)	114 (50.4)	0.561
Medium	466	239 (51.3)	227 (48.7)	
High	443	238 (53.7)	205 (46.2)	
Socioeconomic status,				
Low	366	185 (50.6)	181 (49.5)	0.745
Medium	348	180 (51.7)	168 (48.3)	
High	392	209 (53.3)	183 (46.7)	
Municipality,				
Barcelona	845	434 (51.4)	411 (48.6)	0.332
Madrid	349	190 (54.4)	159 (45.6)	

Data are expressed as the mean (SD) or as percentage. Abbreviations: N number; SD standard deviation, TPE total polyphenol excretion, WC waist circumference, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, TC total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, TG triglycerides. Statistical analyses were undertaken using the t-test for continuous variables and the chi-square test for categorical variables. p-values refer to differences between boys' and girls' values < 0.05; values shown in bold are statistically significant.

Table 3 shows the relationship of TPE with the different components adjusted by sex with the same number of participants where it was observed that among the statistics found, the group with the highest polyphenol excretion had a lower weight (47.9 kg SD 11.2) than the lowest group (49.8 kg); the same for waist circumference where the T3 group had a lower circumference (71.3 cm) than the T1 group (72.8 cm). BMI also reveals that within the overweight and obese group (95th percentile) there is a lower percentage (28.4%) of girls, which presented higher polyphenol excretion and 33.8% of the participants were within the ideal weight (percentile <85%). Both systolic and diastolic blood pressure also indicated a change between groups. However, none of these data were significant.

In the biochemical analysis a significant level of glucose was found among participants, the group that excreted more polyphenols had a lower level of glucose (mg/dL) compared to the T1 group, as well as TC and TG. This indicates that there is a relationship between TPE and a possible reduction in certain biochemical levels. It is also interesting to note that in the municipality of

Barcelona a higher excretion of polyphenols was found compared to Madrid; nor was a difference in TPE found between sociodemographic and economic levels among parents of the participants.

Table 3. General characteristics of Study Sample at baseline by tertiles of TPE

	N	T1 < 86.3	T2 (86.3-141.3)	T3 (>141.3)	P-value*
Girls,	570	192	197	181	
Age (y)	1194	12.0 (0.5)	12.1 (0.4)	12.0 (0.5)	0.078
Anthropometric measurements					
Weight (kg)	1193	49.8 (12.0)	48.9 (10.3)	47.9 (11.2)	0.056
Height (kg)	1194	155.2 (7.4)	155.5 (7.1)	155.0 (7.6)	0.251
WC (cm)	1194	72.8 (11.1)	71.5 (9.4)	71.3 (10.1)	0.072
BMI (kg/m2)					
BMI, >95th percentile	109	44 (40.4)	34 (31.2)	31 (28.4)	0.314
BMI 85-95th percentile	210	76 (36.2)	63 (30.0)	71 (33.81)	
BMI <85th percentile	874	278 (31.8)	301 (34.4)	295 (33.8)	
Blood pressure					
SBP (mmHg)					
SBP >95th percentile	79	35 (44.3)	24 (30.4)	20 (25.3)	0.234
SBP 90-95th percentile	72	25 (34.7)	21 (29.2)	26 (36.1)	
SBP <90th percentile	1040	337 (32.4)	352 (33.9)	351 (33.75)	
DBP (mmHg)					
DBP > 95th percentile	53	21 (39.6)	16 (30.2)	16 (30.2)	0.627
DBP 90-95th percentile	58	21 (36.2)	15 (25.9)	22 (37.9)	
DBP <90th percentile	1080	355 (32.9)	366 (33.9)	359 (33.2)	
Biochemistry analytes					
Glucose (mg/dL)	1155	104.0 (11.9)	103.1 (12.2)	101.6 (11.1)	**0.028**
TC (mg/dL)	1155	155.6 (32.6)	156.0 (37.5)	146.3 (32.2)	**0.004**
HDL-C (mg /dL)	1153	63.4 (15.8)	63.9 (17.1)	61.2 (14.7)	0.362
LDL-C (mg/dL)	893	78.4 (24.3)	80.5 (28.1)	74.6 (24.4)	0.207
TG (mg/dL)	1154	78.5 (45.2)	78.8 (39.3)	77.3 (38.2)	**0.036**
Smoking					
MVPA(min/day)	1121	74.3 (23.8)	74.0 (22.9)	75.6 (23.6)	0.457
Dietary variables					
Fruit and vegetables (serv/day)	1156	4.3 (3.0)	4.0 (2.7)	4.1 (2.6)	0.495
Whole grain (serv/day)	1153	0.4 (0.7)	0.3 (0.5)	0.4 (0.6)	0.525
Fish (serv/week)	850	3.9 (2.5)	4.0 (3.6)	3.8 (2.4)	0.587
Sweet beverages (serv/week)	850	2.0 (3.0)	1.7 (3.2)	1.7 (2.8)	0.733
Sociodemographic					
Parent education					
Low	226	77 (34.1)	81 (35.8)	68 (30.1)	0.843
Medium	466	154 (33.1)	154 (33.1)	158 (33.9)	
High	443	148 (33.4)	114 (32.5)	151 (34.1)	

Socioeconomic status,					
Low	366	126 (34.4)	129 (35.3)	111 (30.3)	0.600
Medium	348	118 (33.9)	119 (34.2)	111 (31.9)	
High	392	122 (31.1)	130 (33.2)	140 (35.7)	
Municipality					
Barcelona	845	296 (35.0)	291 (34.4)	258 (30.5)	**0.006**
Madrid	349	102 (29.2)	107 (30.7)	140 (40.1)	

Data are expressed as the mean (SD) or as percentage. Abbreviations: N number; SD standard deviation, TPE total polyphenol excretion, T tertiles of TPE, WC waist circumference, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, TC total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, TG triglycerides. Statistical analyses were undertaken using ANOVA for continuous variables and the chi-square test for categorical variables. p-values refer to differences between tertiles of TPE values < 0.05; values shown in bold are statistically significant. *Adjusted for sex

Table 4 shown the analyses made for ideal cardiovascular health index as an ordinal variable from 0 to 7, as well as categorized as poor, intermediate, and ideal ICH in children and adolescents and the association to TPE in tertiles. Overall, the ICH prevalence had a significant difference between the tertiles, showing that there is a clear relation between more excretion of total polyphenols and ICH variables. As mentioned above, the health factors for ideal cardiovascular health as defined by AHA include smoking habits (no smoking), which in the results was found to be lower in T3 in both classifications (ideal and poor). Body mass index (BMI), showed an inverse relation with TPE in the ideal group, even though in the poor group there was less percentage of participants on the group that had a higher TPE.

Physical activity, which was observed in a higher number of participants in the group that excreted more polyphenols. In health diet score there was no major difference between de 3 groups. None of these values mentioned before were significant. In the biochemistry analysis the results showed a significant difference in total cholesterol (P-value= 0.003) decreased in those with TPE in both the ideal total cholesterol (36.2%) and a lower percentage of participants with poor cholesterol with TPE (33.4%) compared to T1 (31.5% and 38.9%). Glucose and blood pressure although there was a change between T1, T2 and T3 the values were not significant.

Table 4. ICH Components (AHA) by tertiles of TPE

	Total	T1 (<86.3)	T2 (86.3-141.3)	T3 (>141.3)	P-value
N	1194	398	398	398	
Overall ICH prevalence					
Ideal	126	44 (34.9)	36 (28.6)	46 (36.5)	**0.019**
Intermediate	780	237 (30.4)	279 (35.8)	264 (33.9)	
Poor	288	117 (40.6)	83 (28.8)	88 (30.6)	

BMI percentile					
Ideal	874	278 (31.81)	301 (34.4)	295 (33.8)	0.155
Non ideal	319	120 (37.6)	97 (30.4)	102 (32.0)	
Smoking status					
Ideal	1097	365 (33.8)	364 (33.2)	368 (33.6)	0.795
Non ideal	96	33 (34.4)	34 (35.4)	29 (30.2)	
Physical activity					
Ideal	807	265 (32.8)	271 (33.6)	271 (33.6)	0.860
Non ideal	314	108 (34.4)	101 (32.2)	105 (33.4)	
Health diet score					
Ideal	32	12 (37.5)	10 (31.3)	10 (31.3)	0.879
Non ideal	1162	386 (33.2)	388 (33.4)	388 (33.4)	
Total cholesterol					
Ideal	813	256 (31.5)	263 (32.4)	294 (36.2)	**0.003**
Non ideal	342	133 (38.9)	120 (35.1)	89 (26.0)	
Blood pressure					
Ideal	975	315 (32.3)	338 (34.7)	322 (33.0)	0.095
Non ideal	216	82 (38.0)	59 (27.3)	75 (34.7)	
Glucose					
Ideal	432	135 (31.3)	146 (33.8)	151 (35.0)	0.375
Non ideal	723	254 (35.1)	237 (32.8)	232 (32.1)	

Data are expressed as percentage Abbreviations: N number; TPE total polyphenol excretion, T tertiles of TPE, ICH ideal cardiovascular health, BMI body mass index. Statistical analyses were undertaken using the chi-square test. p-values refer to differences between tertiles of TPE values < 0.05; values shown in bold are statistically significant.

The relationship between tertiles of TPE and ICH was estimated using linear regression and shown in Table 5, this evaluates the tendency of each factor evaluated in the groups which excreted more polyphenols in urine (T3) compared to the least excreted polyphenol in urine group. The association among tertiles of TPE and model 3 which is adjusted with sex, age, tanner stage, triglycerides, and parent education and income are lineal meaning that the tendency of more TPE does make a better ICH overall as shown in Figure 1. There's a significant association between boys and ICH in all regression adjusted models.

Table 5. Linear regression model to assess the relationship between ICH overall according to tertiles of TPE

ICH overall	**Models (B, IC)**	**n**	**T3 vs. T1**	*p- value*	*p- trend*
	Model 1	1194	0.52 (0.13;0.91)	**0.008**	0.483

All participants	Model 2	1148	0.41 (0.10;0.73)	**0.011**	0.519
	Model 3	1054	0.34 (0.03;0.66)	**0.033**	0.551
Girls	Model 1	570	0.04 (-0.97;1.05)	0.941	0.941
	Model 2	543	0.08 (-0.90;1.06)	0.875	0.876
	Model 3	502	0.05 (-0.87;0.97)	0.921	0.921
Boys	Model 1	624	0.47(0.14;0.80)	**0.006**	**0.006**
	Model 2	605	0.36 (0.14;0.58)	**0.001**	**0.001**
	Model 3	552	0.34 (0.10;0.58)	**0.006**	**0.006**

TPE: Total polyphenol excretion in urine. T: Tertile of TPE. ICH: ideal cardiovascular health. B: Non-standardized coefficient. IC: Confidence interval. All participants Model 1: unadjusted. Model 2: adjusted with sex (also interaction), age, tanner stage, triglycerides. Model 3: adjusted with model 2 plus parent education and income. p-value T3 vs. T1 of TPE, and p-trend < 0.05; values shown in bold are statistically significant.

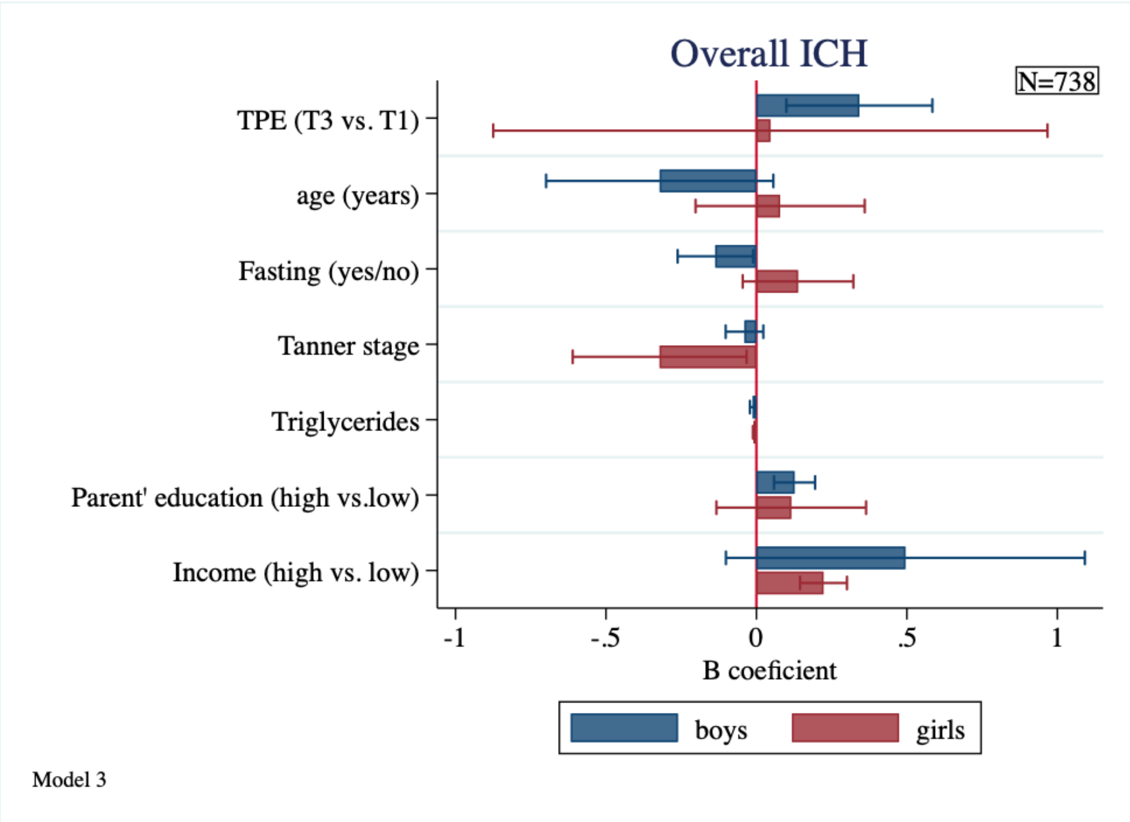

Figure 1. Lineal generalized model to observe association between ICH (continuous) and TPE (Highest and lowest quartile of TPE) in girls and boys, adjusted by fasting (yes/no), age (continuous), Tanner stage (scale 1-7), moderate and vigorous physical activity (MVPA) (quartiles), energy intake (quartiles), parent education (low, medium, and high), and income (low, medium, and high). B. no standardized coefficient.

DISCUSSION

The SI! Program for health promotion in secondary schools seek to provide the necessary educational elements for long-term lifestyle changes among adolescents. Especially targeting the increased risk of cardiovascular risk factors [37,40] As previously mentioned, intervention at an early age can result in a better cardiovascular profile. Therefore, answering to the initial hypothesis in this study conducted in 1194 adolescents ages 12 to 16 showed a relationship between the increase of total polyphenol excretion in urine and an association with cardiovascular health indicators such as weight, waist circumference, glucose, and lipid profile.

Previous studies have reported the association between higher polyphenol excretion and a better cardiovascular risk factor profile, although there are differences between gender. In the PREDIMED (Prevention with Mediterranean Diet) group study conducted in a cohort of elderly with a higher risk of cardiovascular disease, they observed that TPE in urine was associated with low values of weight, BMI, and waist-to-height ratio[41]. According to the data analyzed, the association between waist circumference (WC) and polyphenol excretion was significantly different between the two genders, with greater circumference prevailing in men than women. As in the PREDIMED study where a direct negative relationship was found with certain parameters related to body composition ((BMI z-score, WC z-score, and body fat percentage) which showed a sex-dependent relationship (especially among boys) which suggests that polyphenols may be a secondary factor of the Mediterranean Diet for weight loss; that shows similar results in our study, where the highest tertile showed better BMI and WC scores.

In the linear regression models that assessed the association of TPE tertiles with ICH score evaluated in the range from 0 to 7 showed interesting results, for example, boys in all models showed significant differences between the highest and lowest tertile. Model number 3 which was adjusted by highest parental education and house income suggested a difference in eating behaviors indicating better cardiovascular health in those who had parents with higher education levels and higher economic income. This can be supported by a study carried out in Seville, Spain involving 6,851 adolescents 11 to 16 years of age; where it was evaluated how the socioeconomic status of the parents, among other factors, can affect the eating habits of the adolescents. An association was found, being the highest and most direct between the level of education of the parents and the eating behaviors of the adolescents, Parents who presented a higher education were significantly ($p < 0.01$) more likely to eat fruit daily than those who presented a lower educational level (OR 1.29)[42].

Additionally, this study analyses ideal cardiovascular health as a concept that is beneficially associated with vascular health already in adolescence, supporting the relevance of targeting these metrics as part of primordial prevention. In the biochemical analysis the results showed a significant difference in total cholesterol (P-value= 0.003) where it decreased in those with TPE in both the ideal total cholesterol (36.2%) and a lower percentage of participants with poor cholesterol with TPE (33.4%) compared to T1 (31.5% and 38.9%), Supporting these results, in Italy a cross-sectional study carried out in 2573 males and females showed a relationship between a higher polyphenol intake and a better lipid profile values [43]. The PREDIMED study showed an association with higher consumption of polyphenols and blood pressure, fasting plasma glucose, and triglycerides[44]. Also, in a randomized double-blind, placebo-controlled study on 71 moderately obese male and female participants where they evaluated the efficacy of the consumption of polyphenols extracted from apples and hop bract, and they observed a decrease not only in visceral fat but also total cholesterol and LDL-cholesterol levels [45].

Some studies have shown beneficial effects of polyphenol intake and blood pressure reduction. In an intervention in 92 participants they concluded that an increase in polyphenol intake through diet results in an improvement in cardiovascular risk markers in hypertensive participants[46]. Another study showed that a polyphenol-rich diet can have a pleiotropic effect on cardiometabolic risk factors specifically on lipid response, glucose response, and insulin secretion regardless of BMI, gender, and age[47]. Despite the evidence found, in our study, there was no significant relationship between total polyphenol excretion and blood pressure. However, changes in other factors like total cholesterol or triglycerides, glucose, and body composition in the largest tertile do show a relationship in better cardiovascular health overall. What can be concluded is that most of these studies were performed on participants who already had hypertension or some cardiovascular problem due to advanced age; compared to the participants in this study, who, because they are adolescents, are normotensive.

In the HELENA Study, 526 adolescents (54% girls); had presented data on inflammatory biomarkers and polyphenol intake from 2 nonconsecutive 24-h recalls via matching with the Phenol-Explorer database. They used multilevel linear models to test the association between polyphenol intake with a pro/anti-inflammatory biomarker ratio as well as with separate inflammatory biomarkers, adjusted for sociodemographic variables, BMI z-score serum triglycerides and diet inflammation index. They observed that inflammation biomarkers were significantly associated with sex, country, alcohol use, BMI z-score and serum triglyceride. Higher polyphenol consumption was related to a lower serum pro/anti-inflammatory biomarker ratio. Participants with higher total polyphenol intakes were often girls, had a higher puberty status and a lower Dietary Inflammatory Index (DII)[48]. In comparison to our study, which reported to significant difference in polyphenol consumption of fruits, vegetables, fish or whole grains between the two sexes. In relation to the biomarkers of this study, a small decrease in blood pressure could be seen among the groups which excreted more polyphenols, but not significantly as in the HELENA study mentioned above. However, our study presented significant changes in other data, such as triglycerides and glucose, which were not significant in the HELENA study. Which in conclusion it is important to mention that in both studies a higher consumption and excretion on polyphenols may contribute to the prevention of inflammation-related chronic diseases as it is related to a reduce pro/anti-inflammatory biomarker ratio.

The knowledge of the effects that polyphenols have on the lipid profile is not quite clear, this is due to the lack of information on the active metabolites and their relationship with the intestinal microbiome. In addition to environmental factors such as the place of development, climate change, stressful environment, etc. However, there is a favorable causal relationship between polyphenol intake and an improved lipid profile; among these an increase in lipolysis, stimulation of fatty acid β-oxidation, reduction of lipogenesis, inhibition of adipocyte differentiation and growth, inhibition of the expression and secretion of pro-inflammatory molecules, reduction of oxidative stress and production of free radicals in adipose tissue[49]. Lipid digestion and absorption can be due because of the decrease in the activity of digestive enzymes and lipid emulsification that polyphenols can cause. Catechins, resveratrol and curcumin have shown a reduction in the accumulation of fat in adipocytes by activating adenosine monophosphate-activated protein kinase (AMPK) and because of the down-regulation of the expression of lipogenic genes. Moreover, these polyphenols can also increase lipolysis and by upregulating hormone-sensitive lipase they stimulate fatty acid β-oxidation [50].

Strengths and Limitations

The results of the study could have been affected because of the participants dropouts throughout the period of time, even though it was unrelated to the intervention. Also, the lack of adjustment for multiple comparisons could have affected some results. Our main strength is that this study is a cluster-randomized controlled designed which allows isolation of the outcomes of the SI! Program Intervention, although this can be a limitation as well because it is not possible to see a causal effect of polyphenols on ICH. Moreover, another strength of our study is that there are very few articles in ICH and polyphenols which help acquire knowledge about key components of healthy life style. Even though, our findings are limited to Spanish adolescents, this study proves that some strategies can be successful at young ages.

CONCLUSION

The results of this study suggest that a higher concentration of polyphenols excreted in urine, which refers to a higher consumption of polyphenols, is associated with ideal cardiovascular health scores in adolescents. Being more specific, there is a direct relationship between lower body weight, fat mass, total cholesterol, glucose and LDL-cholesterol with higher TPE. In addition, for future recommendations, it would be important to consider determining which type of polyphenols could be more related to ICH and each component, so we could identify if there is any polyphenol biomarker of a specific food intake related to ICH.

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