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Relationship between polyphenol consumption and metabolic syndrome in adults: a prospective cohort study

Relação entre o consumo de polifenóis e a síndrome metabólica em adultos: uma coorte prospetiva

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TRABALHO DE INVESTIGAÇÃO
1.º CICLO EM CIÊNCIAS DA NUTRIÇÃO | UNIDADE CURRICULAR ESTÁGIO
FACULDADE DE CIÊNCIAS DA NUTRIÇÃO E ALIMENTAÇÃO DA UNIVERSIDADE DO PORTO

TC

Porto, 2021

Abstract

Introduction: The rapid growth of Metabolic syndrome (MetS) prevalence is a major public health concern. Previous studies have reported that total polyphenols intake might be responsible for having a positive impact on MetS. Flavonoids are the most common polyphenols in the human diet, however, there is little evidence of the relationship between specific flavonoid subclasses and MetS.

Aim: To evaluate the association between the intake of total and subclasses of flavonoids and the incidence of MetS.

Methods: A total of 1057 participants were selected from the baseline and the first follow-up of the population-based cohort EPIPorto. At baseline, dietary intake was assessed using a validated food frequency questionnaire covering the previous year and the content of all flavonoid subclasses was obtained from the Phenol-Explorer database. Quartiles of consumption was obtained for flavonoid intake. Incidence of Mets were assessed ate the follow-up using the harmonisation definition. The association between flavonoid intake and the development of MetS was evaluated through logistic regression models, obtaining odds ratios and 95% confidence intervals (OR; 95%CI). An interaction effect was tested for sex and alcohol consumption.

Results: After adjustment in men, lower intake of flavones was associated with a higher incidence of MetS (OR=2.55, 95%CI=1.18,5.52; Q3*vs*.Q4). In women, lower intake of total flavonoids and flavanols was significantly associated with MetS development (OR=2.51; 95%CI=1.32,4.77, OR=1.88; 95%CI=1.01,3.49, Q3*vs*.Q4, respectively).

Conclusions: A higher intake of total flavonoids and some flavonoid subclasses was associated with a lower incidence of MetS, depending on sex.

Keywords: Metabolic Syndrome, Polyphenols, Flavonoids, Cohort Study

Resumo

Introdução: O aumento da prevalência da síndrome metabólica (SM) é um grande problema de saúde pública. Estudos demonstram que a ingestão de polifenóis pode ter um impacto positivo nesta síndrome. Os flavonóides são os polifenóis mais comuns na dieta, no entanto, há pouca evidência da sua relação com a SM.

Objetivo: Avaliar a associação entre a ingestão de flavonóides totais e subclasses e a incidência de SM.

Métodos: Um total de 1057 participantes foi selecionado da coorte de base populacional EPIPorto. No início do estudo, a ingestão alimentar foi avaliada através de um questionário de frequência alimentar validado, referente ao ano anterior, e o conteúdo de todas as subclasses de flavonóides foi calculado através da base de dados *Phenol-Explorer*. Os quartis de consumo foram obtidos para a ingestão de flavonóides. A incidência de SM foi estimada usando a definição harmonizada. A associação entre a ingestão de flavonóides e o desenvolvimento de SM foi avaliada através de modelos de regressão logística, obtendo-se *odds ratios* e intervalos de confiança de 95% (OR; IC95%). Um efeito de interação foi testado para sexo e consumo de álcool.

Resultados: Após ajuste, em homens, menor ingestão de flavonas foi associada a uma maior incidência de SM (OR=2,55, IC95%=1,18,5,52; Q3*vs.*Q4). Nas mulheres, a menor ingestão de flavonóides totais e flavanóis foi significativamente associada ao desenvolvimento de SM (OR=2,51; IC95%=1,32,4,77, OR=1,88; IC95% =1,01,3,49, Q3*vs.*Q4, respetivamente).

Conclusões: Uma maior ingestão de flavonóides totais e de algumas subclasses de flavonóides foi associada a uma menor incidência de SM, dependendo do sexo.

Palavras-chave: Síndrome Metabólica, Polifenóis, Flavonóides, Estudo de Coorte

Acronyms

MetS - Metabolic syndrome

CVD - Cardiovascular Disease

DMT2 - Diabetes Mellitus Type 2

HDL - High-density lipoprotein

BMI - Body Mass Index

mmHg - Millimetres of Mercury

FFQ - Food Frequency Questionnaire

OR - Odds Ratios

CI - Confidence Intervals

USDA - United States Department of Agriculture

DRI - Dietary Reference Intakes

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Introduction

Metabolic syndrome (MetS) is a pathologic condition characterised by abdominal obesity, insulin resistance (alterations in the glucose metabolism), hypertension (high blood pressure), dyslipidemia (low high-density lipoprotein and/or elevated triglycerides)[(1-4)], and a pro-inflammatory and pro-thrombotic state[(1)]. All for these factors increase the risk for cardiovascular disease (CVD), type 2 diabetes mellitus (DMT2)[(2-5)], and cancer incidence[(6, 7)].

The rapid growth of MetS prevalence is a major public health concern[(7, 8)]. The incidence of this syndrome has been increasing throughout the years[(7)], even in parts of the world where undernutrition is a reality[(9, 10)]. It is estimated that 25% of the global population has MetS with a prevalence varying between less than 10% to as high as 84%, depending on the geographic location, socioeconomic conditions, population demographics, and the definition criteria of the syndrome used[(11)]. Particularly, in Portugal, the prevalence rates of MetS affect between 36% to 50% of adults and are higher than in some other European countries and the United States of America[(11, 12)].

Previous studies have reported that the Mediterranean diet might be responsible for positive impact on MetS[(7, 10, 13, 14)], this diet is characterised by an adequately balanced combination of fruit and vegetables, fish, cereals, and olive oil, with reduced consumption of meat and dairy products and moderate intake of alcohol, primarily red wine[(7, 15)]. One of the compounds associated with this dietary pattern[(10)], capable of causing positive effect on MetS, is the polyphenols[(10, 16, 17)]. According to their chemical structures, polyphenols are divided into several families, for instance the flavonoid family. This class is also diversified into six major subclasses: flavones, flavonols, isoflavones, anthocyanins, flavanols, and flavanones[18]. These polyphenolic compounds naturally occur in plant-based foods, being present, through a significant amount, in many commonly consumed fruit, vegetables, grains, chocolate and drinks (including tea, wine, and juices)[19, 20]. These compounds exert their beneficial effect on health through anti-inflammatory, antioxidant, antimicrobial, antiproliferative, pro-apoptotic and hormonal regulatory mechanisms[21-23]. Although there are several studies on the association between flavonoid intake and various diseases, such as DMT2[20, 21, 24, 25] and CVD[20-22, 25], there are few studies on flavonoid subclasses, namely its association with MetS.

Flavonoids from alcoholic beverages include beers, ciders, wines, liquors, brandy, rum, and whiskey[26-28]. Some research suggests that moderate alcohol consumption (≤1 drink/day for women and 1 to 2 drinks/day for men)[29], particularly red wine polyphenols[30-32], can have a protective effect on components of the MetS[29, 33], blood pressure[29], high-density lipoprotein (HDL) cholesterol[33, 34], CVD[35-37], DMT2[34, 38], and mortality[35, 39]. Nevertheless, recent evidence suggests that the benefits of moderate alcohol consumption are outweighed by the increased risk of other health-related harms, including cancer, injuries, and communicable disease[40], so the safest level of drinking is none[40-42].

Considering the gaps in the literature, we conduct a prospective population-based study to analyse the relationship between the intake of each flavonoid subclass and the incidence of MetS.

Aims

This study aimed to analyse the association between the intake of total flavonoids and flavonoid subclasses and the incidence of MetS.

Methods

Study Design

The EPIPorto study is a population-based cohort study[43] with the main aim of assessing the determinants of health in the adult population of Porto, Portugal[44, 45]. For the baseline evaluation (1999–2003), 2485 persons have been repeatedly evaluated over time. The cohort has been followed for more than 15 years, with the first follow-up at 2005-2008. This study included participants selected from the baseline and the first follow-up of the EPIPorto cohort study.

Participants

Participants were non-institutionalised inhabitants of Porto, Portugal, who visit the Department of Hygiene and Epidemiology of the University of Porto Medical School for an interview and examination. The choice of the participants occurred with random digit dialling[46], as part of a health and nutrition survey. The simple random sampling was applied for each household previously selected. Permanent residents were identified according to sex and age, having all more than 18 years old. Refusals were not substituted, and a participation rate of 70% was achieved[47].

For the present study, 1428 participants were excluded: 119 individuals for lack of information in the Mini-Mental State Examination and because they scored less than 24 on this test, which evaluates the cognitive impairment among participants aged over 64 years[48], 775 participants diagnosed with MetS in the baseline, and 534 participants because of the loss to follow-up. Thus, the final sample included 1057 participants (640 women and 417 men) (Appendix A). Included and excluded participants did not differ regarding sex, alcohol consumption and smoking habits, and consumption of total flavonoids and their subclasses. Still, those included were slightly younger (median: 49 *vs*. 50 years, $p<0.001$) and had lower BMI (median: 25 *vs*. 26 kg/m^2, $p<0.001$).

All participants gave their written informed consent to participate. Furthermore, the study was carried out in accordance with the Helsinki Declaration II, and the local institutional ethics committee and the National Individual Data Protection Commission approved the EPIPorto protocol.

Data Collection

During the face-to-face interview, trained staff conducted a structured questionnaire comprising information on sociodemographic, personal, family medical history, and behavioural characteristics applied to all participants. Complete Education was recorded as completed years of schooling. In addition, individuals were categorised according to their smoking status, such as a smoker, which includes both daily (at least one cigarette per day) and occasional smokers (less than a cigarette per day), and non-smoke (a person who never smoked and ex-smokers).

Anthropometric Measurements

Anthropometric measurements were performed with subjects in light clothing and barefoot, after 12 hours of fasting, and under standard procedures. Body weight was measured to the nearest 0.1 kilograms using a digital scale (SECA®, Columbia, USA) and height to the nearest centimetre using a wall stadiometer (SECA®, Hamburg, Germany). The body mass index (BMI) was classified according to World Health Organization standards. In addition, waist circumference was ascertained as the midway between the lower limit of the rib cage and the iliac crest and hip circumference on the maximum circumference over the femoral trochanters; both were measured to the nearest centimetre.

Analytical determinants

Blood pressure was measured on a single occasion using a standard mercury sphygmomanometer with the cuff on the right upper arm. After resting for 10 minutes, the participant measure two times the blood pressure, and the mean of the two readings was calculated as recommended by the American Heart Association[(49)]. If the difference between these two readings was more than five millimetres of mercury (mmHg) for systolic or diastolic blood pressure, a third measurement was taken. For the latter case, the mean was determined with the two closest values. In addition, to measure glucose, total cholesterol, high and low density lipoprotein cholesterol, and triglycerides, blood was sample after a 12-hour overnight, using automatic standard routine enzymatic methods.

Dietary Assessment

Dietary intake was measured at baseline with an 82-item semiquantitative food frequency questionnaire (FFQ) and validated by 7-day food records diaries[(50, 51)] reporting to the previous year. Each subject chose the average frequency of consumption for each food item among nine categories, ranging from “never or less than once a month” to “6 or more times a day”; subjects also reported the average portion consumed (lower, equal, or higher than the average portion size) and the seasonal variation of consumption. Food consumption was converted into total energy intake and nutrients using the software Food Processor Plus® (ESHA Research, Salem-Oregon, 1997), adapted to traditional Portuguese foods.

Estimation of Dietary Flavonoids Intake

The total dietary flavonoid intake and flavonoids subclasses were obtained at baseline by the 82-item FFQs (Appendix B). The estimation of dietary flavonoid intake followed these steps: (1) All foods from the FFQ with no flavonoid content or only traces were excluded, namely all foods belonging to the group of dairy products, meat, fish and eggs; (2) the content of all flavonoid subclasses in 100 g of each food item was obtained from the Phenol-Explorer database (version 3.6)[28, 52, 53]; (3) when an item from the FFQ included several foods (e.g., apples and pears), the proportion of intake was calculated taking into consideration data from national consumption for adults[54]; (4) the individual flavonoid subclasses intake from each food was calculated by multiplying the content of each flavonoid subclasses by the daily consumption of each food; (5) finally, the total flavonoid subclasses intake was calculated as the sum of all individual flavonoid subclasses intakes from the food sources reported in the FFQ. The data used to calculate flavonoid subclasses intake was obtained by chromatography of all the flavonoid compounds, except proanthocyanidins, the content of which was acquired by normal-phase high-performance liquid chromatography[26]. In addition, due to the asymmetric distribution, quartiles of consumptions were calculated for the total and each flavonoid subclass.

Alcoholic beverages consumption

Each participant was asked to report the usual number of alcoholic beverages consumed by day, week, or month as appropriate during previous the year. Information was obtained separately for wine, beer, and spirits. The questions on drinking also inquired on frequency, quantity, duration of consumption. After calculating the mean frequency of consumption, contestants were asked if the average portion consumed was lower, equal, or higher than a glass of 125 ml for wine, a bottle of 330 ml for beer, and a cup of 40 ml for spirits. The total alcohol intake estimated in grams per day was obtained. A photographic album was used to help the decision of the average portion size consumed. Different categories of alcohol intake were defined by the cut points: 10.1g/week for women, and 203.0 g/week for men, according to the median consumption of each sex.

Definition of MetS

The definition of Mets used was the current harmonisation definition. According to this joint statement, a diagnosis of the MetS is made when any three of the five following risk factors are present: enlarged waist circumference with population-specific and country-specific criteria; elevated triglycerides, defined as ≥150 mg/dL, decreased HDL cholesterol, defined as <40 mg/dL in men and <50 mg/dL in women, elevated blood pressure (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg), and high fasting glucose (blood glucose >100 mg/dL), with the inclusion of patients taking medication to manage hypertriglyceridemia, low HDL cholesterol, hypertension, and hyperglycemia[5, 55]. In this definition, obesity is diagnosed using waist circumference and not BMI as waist circumference has been shown to better correlate with visceral adiposity and insulin resistance as well as the development of T2DM and CVD than does BMI. Additionally, in the current Harmonisation definition, ethnic-specific waist circumference cutoff values are used[5, 55].

Statistical Analysis

The Statistical Package for Social Science (IBM® SPSS® Statistics,27.0.1.0) was used for all statistical analyses. Sample characteristics are presented as expressed as number (n) and percentage (%) for categorical variables and median and interquartile range (25th–75th percentile) for continuous variables with markedly skewed distributions. In addition, the normality of the continuous outcomes was assessed with the Kolmogorov-Smirnov (n>50) and Shapiro–Wilk tests (n<50). Comparisons were tested using the Chi-square test for categorical variables and Mann–Whitney test for continuous variables.

Binary logistic regression models analysed associations between dietary intake of flavonoid and MetS development. Consequently, it was possible to determine the differences between the intake quartiles of the flavonoid subclass. Model 1 was adjusted for age and complete education at baseline. Model 2 was additionally adjusted for alcohol intake at baseline. All regression models were adjusted for potential confounders, traditionally considered in studies between MetS and polyphenols.

Smoking habits and total calorie variables were also tested but were not significant for the model, so they were not included. Finally, a potential interaction effect between sex and flavonoid subclasses and between alcohol intake and the flavonoid subclasses were tested by having an interaction term in the final models. As the interaction with sex was statistically significant, all the analyses were conducted separately by sex.

Values are represented according to the 95% confidence interval (CI), and significance for all statistical tests was based on bilateral contrast set at $p < 0.05$.

Results

This study included 1057 participants (60.5% women) over a mean of 4.6 ± 2.6 years of follow-up. During this follow-up period, 209 new cases of MetS were documented (20%). At baseline, the median age was 48 years (P5-P75: 39.0-58.0) for women and 51 years (P5-P75: 40.5-62.0) for men. Table 1 shows the main characteristics of the participants according to sex. Statistically significant differences were found between the two sexes for age, BMI and total energy intake. The median intakes for total flavonoids were 371 mg/day, and for the six subclasses of flavonoids, i.e., flavones, flavanols, flavonols, anthocyanins, flavanones, and isoflavones, were 0.8, 240.2, 39.2, 45.4, 10.9 and 0.1 mg/day, respectively. In men, the consumption of flavonoids was statistically higher in the following subclasses: flavanols, anthocyanins and isoflavones. In women, consumption was statistically higher in flavones (Appendix C).

Table 2 shows no significant differences in flavonoid consumption between participants who developed the MetS and those who did not, except for flavanols in men (Appendix D).

The association between flavonoid consumption and the development of MetS was evaluated using the logistic regression models shown in Table 3 (Appendix E). We found robust associations for men who have a daily intake of 0.67 to 1.03 mg of flavones per day. They have a 2.5 times greater risk of developing MetS than men who consume more flavones (≥ 1.04 mg/day), even after subsequent adjustments (OR = 2.55; 95% CI = 1.18, 5.52, Q3 *vs*. Q4, model 2). Still, regarding the male class, a lower intake of isoflavones (OR=2.05, 95%CI=1.06; 3.98, Q3 *vs*. Q4, model 1) was associated with a higher risk of developing MetS; however, this association was not significant after further adjustments for alcohol intake. In women, after adjustment for age and education (model 1) and additional adjustment for alcohol consumption (model 2), a lower intake of flavanols (240.18 – 364.13 mg/day) was significantly associated (OR = 1.88; 95% CI = 1.01, 3.49, Q3 *vs*. Q4, model 2) with MetS development, comparing to those with higher intakes (≥ 364.14 mg/day). A similar trend was described for total flavonoids (OR = 2.51; 95% CI =1.32,4.77, Q3 *vs*. Q4, model 2).

Discussion

Flavonoids are the most common polyphenols in the human diet and, consequently, the most studied[10]; however, there was little evidence of the relationship between flavonoid subclasses and MetS. Therefore, for the present analysis, we considered the six main flavonoid subclasses commonly consumed in the human diet: flavones, flavonols, isoflavones, anthocyanins, flavanols, and flavanones[23]. In this prospective study, a higher intake of flavonoids and their subclasses, namely, flavones and flavanols, decrease the risk of MetS significantly after controlling for confounding factors. Additionally, the intake of flavonoids was 371 mg/day, which meets the findings of some other countries, such as Spain, which consume around 400 mg/day of flavonoids and Germany, which consume around 380 mg/day[21]. However, in other European countries with very different consumption values, the flavonoid intake might vary between 200 mg/day in Greece to 1000 mg/day in the United Kingdom[56]. A recent analysis of the National Health and Nutrition Examination Survey estimated that the mean flavonoid intake of the United States population was 256 mg/day[57]. The observed differences can be attributed to the study population, ranging from adult and elderly population or just adults, however, some studies include children and adolescents in the sample. Furthermore, how the food intake of flavonoids is assessed is not always the same, varying between 24-hour dietary records and FFQ. In addition, the scientific databases used to estimate flavonoid intake are also different. For example, some studies use the US Department of Agriculture (USDA) database of polyphenols and others using the Phenol-Explorer(21). Finally, the dietary habits and socioeconomic status of diverse study populations should be taken into consideration. For example, in the UK, the higher intake of flavonoids is presumably related to their traditional tea culture(58). In contrast, for Portugal, the consumption of flavonoids may be related to the Mediterranean diet(59) because they are the most abundant polyphenols in this dietary pattern(10).

A few studies demonstrate that flavonoids have a positive impact on MetS(11). This is in line with our results. For example, it has been shown that lower consumption of flavonoids (between 362 mg/day and 540 mg/day) is associated with a 2.5 times higher risk of developing MetS compared to individuals who consume more than 540 mg of flavonoids per day. In literature, only a few estimations of dietary intake of flavonoids subclasses are available. In Denmark, the consumption of flavanones ranged between 7 and 14 mg/day, which is similar to our findings (11 mg/day). On the other hand, our analysis on the consumption of flavonols (41 mg/day) was different from other studies in the Netherlands (23 mg/day)(60). The observed differences can be attributed to the same reasons mentioned for the total flavonoids.

Regarding the flavonoid subclasses, it has been shown that the risk of developing MetS, on men, was 2.5 times lower in participants in the highest flavone quartile of consumption compared to those in the lowest quartile.

Furthermore, in an Iranian population, it was reported that participants in the second tertile compared to those in the lowest tertile had a significant inverse association between flavone intake and the risk of MetS[61], which is in agreement with our finding.

In our sample of Portuguese adults, only flavones and flavanols have shown protective effects against MetS. This could be due to the considerable differences in the absorption of subgroups of flavonoids that may explain their distinct bioactivity and contrasting results among studies[62-64]. According to the literature, the primary food sources of flavones are apricot, cashew, beans, cabbage, apple, mango, and onion. Additionally, in respect to flavanols are tea, fruits, grapes and blackberries, apples, or nuts like beans, almonds, pistachios, and red wine. [60, 65]. Many of these foods are present in the Mediterranean diet since it is a dietary pattern based on the high consumption of olive oil and plant foods (fruits, vegetables, legumes, nuts and non-refined cereals)[7, 14]. This is in line with what is mentioned in the literature. Therefore, the Mediterranean diet may positively impact MetS and that polyphenols play an active role in this effect[10].

Our results differed between sex, with significant developments in some flavonoid subclasses only for men and others only for women. However, literature data are not homogeneous with regard to sex differences in polyphenol intake, and some studies suggest that a higher intake in females compared to males, especially when taking into account the standardisation of intake energy. Moreover, differences in the origin of the selected polyphenol appear to be sex-dependent (e.g., a more significant contribution of fruits and vegetables in women than men who are major consumers of alcoholic beverages and coffee)[21].

Due to our initial hypothesis that the beneficial effect of flavonoids might be different among different levels of alcohol consumption (using as cutoff point the median by sex), a potential impact of interaction was tested between the consumption of each of the flavonoids subclasses and alcohol consumption. No interaction effect was found, and alcohol consumption was considered a confounder (significant in the final model). In the present study, isoflavones lose their effect after adjustment for alcohol consumption, which indicates that the influence of alcohol will be greater than the protective effect of these flavonoids.

The findings of this study should be considered in light of some limitations. First, we only use data from the first follow-up. Nevertheless, the incidence found was still 20% of MetS, and using data from other follow-ups might compromise the results, as we only had consumption information at the baseline. Thus, the participant could have essential changes in food consumption. Second, it is known that dietary data from a self-reported FFQ is subject to bias and overestimation of food intake[66], however, we used a validated FFQ for the Portuguese population, minimising this potential bias. Third, other factors that affect food polyphenol content, such as bioavailability, variety, ripeness, culinary technique, storage, region, and environmental conditions, were not considered. However, other studies also used the same methodology, by estimating flavonoid intake through Phenol-explorer, which is regarded as a more reliable source, as it only contains data from the scientific literature and excludes any data from other sources (e.g., food industry) to ensure full traceability of original data and corresponding documentation on samples and methods of sampling and analysis. Fourth, the possibility of underreporting food consumption by participants who had more MetS features cannot be precluded; for example, underreporting of energy intake has been systematically described in overweight and obese individuals[67]. Fifth, it was not possible to study all classes of polyphenols; only flavonoids were addressed. However, it is the most common group of polyphenolic compounds in the human diet[68]. Finally, the intake of polyphenols may correlate with the consumption of fruits and vegetables and their constituents, i.e., vitamins, folate, and fibre, contributing to the association with chronic diseases. However, when the correlation is too high, it is challenging to ascertain independent effects of dietary components due to multicollinearity.

Nevertheless, this study has important strengths: the prospective design, large sample size relatively, and response rate (70%). Beyond this, the sample was representative of the population of Porto, with individuals aged between 18 and 86 years. Furthermore, when studying polyphenol intakes, the most fundamental problem is the lack of a comprehensive database. Some previous studies have used the USDA database, which is not as comprehensive as the phenol explorer database used in the present study. Additionally, using the phenol explorer database can help clarify the relationships between the consumption of polyphenols and their subclasses and the risk of chronic disease[53]. Moreover, the standardised definition of MetS was used, which is an asset as it allows for international comparisons and facilitates the aetiology with a set of commonly accepted criteria around the world, with cutoff points agreed for different ethnic groups and sex[5]. Lastly, we assessed dietary intake through an FFQ, validated by 7-day food records diaries[50, 51], which has the advantage of determining the usual intake, minimising the effect of the day-to-day variation in food choices.

From a health perspective, the consumption of selected subclasses of flavonoids may be more important than total flavonoid intake(60). Thus, this relationship between flavonoid subclasses and MetS must be confirmed since studies are very scarce. Furthermore, it is essential to understand whether this relationship holds with other classes of polyphenols. In addition, it is crucial to determine whether a nutrient model such as Dietary Reference Intakes (DRI) is applicable to polyphenols, including flavonoids. Therefore, it is essential to develop sufficient data to define a DRI or allow public health recommendations for one or more subclasses of polyphenols. Finally, it is necessary to assess the role of the food matrix in bioavailability. Accordingly, adequately designed human trials are needed, including sex, age and ethnicity subgroups of healthy individuals and individuals with MetS, to achieve, in the early future, safe and effective consumption doses of polyphenols and their classes.

Conclusion

This study provides detailed information on the relationship between polyphenols, namely, flavonoid intake and the development of MetS on an adult population in Porto. A higher intake of total flavonoids and some of their subclasses (such as flavones and flavanols) were associated with a lower incidence of MetS. Thus, although deficiencies in flavonoid intake do not result in specific deficiency diseases, adequate flavonoid intake can provide health benefits, especially with regard to chronic diseases, specifically in MetS.

Acknowledgments

This study was supported by the Epidemiology Research Unit - Institute of Public Health of University of Porto (ISPUP-EPIUnit/ISPUP/UP), (financed by national funding from FCT - UIBD/04750/2020).

I gratefully acknowledge the participants enrolled in EPIPorto for their kindness and all members of the research team for their enthusiasm and perseverance.

To my supervisor and my co-supervisors for all the support given in the elaboration of this work, for the scientific rigour and all the transmission of knowledge.

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Appendix

List of appendices

Appendix A – Figure 1

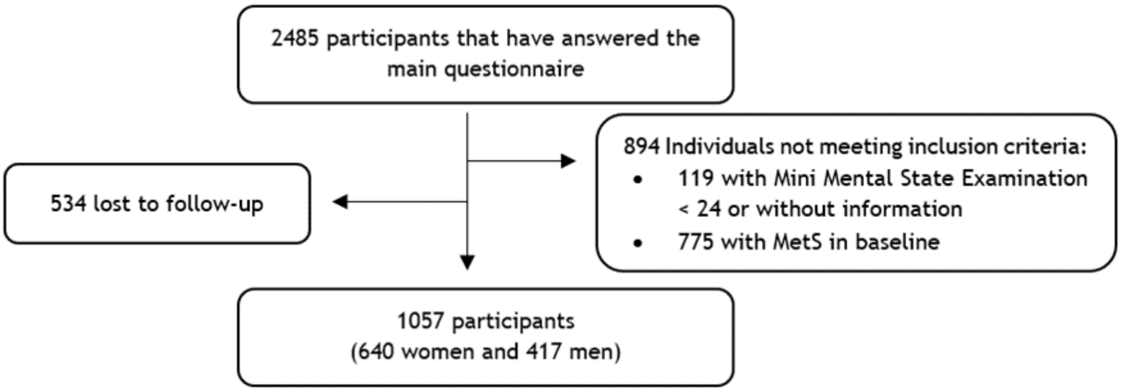

Figure 1 - Flowchart of the participants

Appendix B – Figure 2

INQUÉRITO DE FREQUÊNCIA ALIMENTAR

Agora vou perguntar-lhe sobre os alimentos que costuma consumir. Pense durante o último ano quantas vezes por dia, semana ou mês em média consumiu cada um dos alimentos que vou referindo

I. P. LÁCTEOS	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
1. Leite gordo (1 chávena, 250 ml)			s			d			
2. Leite meio-gordo (1 chávena, 250 ml)			s			d			
3. Leite magro (1 chávena, 250 ml)			s			d			
4. Iogurte (Um, 125 g)			s			d			
5. Queijo curado,semi-curado ou cremoso (Uma fatia, 30g)			s			d			
6. Sobremesas lácteas: pudim flan, pudim de chocolate,etc (Um)			s			d			
7. Gelados (Um, 2 bolas ou copo)			s			d			
II. OVOS, CARNES E PEIXES	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
8. Ovos (Um)			s			d			
9. Frango (1 porção ou 2 peças,150g)			s			d			
10. Perù,coelho (1 porção ou 2 peças,150g)			s			d			
11. Carne vaca, porco, cabrito como prato principal (1 porção, 120g)			s			d			
12. Figado de vaca, porco, frango (1 porção, 130g)			s			d			
13. Lingua,mão de vaca,tripas,chispe,coração,rim (1 porção,100g)			s			d			
14. Fiambre, chouriço, salpicão, presunto,etc (1 porção, 20g)			s			d			
15. Salsichas e similares (Uma média)			s			d			
16. Toucinho, bacon (2 fatias, 50g)			s			d			
17. Peixe gordo: sardinha, cavala, carapau,etc (1 porção, 125g)			s			d			
18. Peixe magro: pescada, faneca, linguado, etc (1 porção, 125g)			s			d			
19. Bacalhau (1 porção, 125g)			s			d			
20. Peixe conserva: atum, sardinhas,etc (1 lata)			s			d			
21. Lulas, polvo (1 porção, 100g)			s			d			
22. Camarão (1 porção,100g) ameijoas, mexilhão,etc. (1/2 chávena)			s			d			
III.ÓLEOS E GORDURAS	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
23. Azeite (1 colher sopa)			s			d			
24. Óleos: girassol, milho, soja (1 colher sopa)			s			d			
25. Margarina (1 colher chá)			s			d			
26. Manteiga (1 colher chá)			s			d			
IV. PÃO, CEREAIS E SIMILARES	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
27. Pão branco ou tostas (Um ou 2 fatias forma, 50g)			s			d			
28. Pão integral ou tostas integrais (Um ou 2 fatias forma, 50g)			s			d			
29. Broa, broa de avintes (1 fatia,80g)			s			d			
30. Arroz cozinhado (Meio prato, 100g)			s			d			
31. Massas: esparguete, macarrão cozinhadas (Meio prato, 100g)			s			d			
32. Batatas fritas (1 porção, 100g)			s			d			
33. Batatas cozidas, assadas (2 batatas médias, 150 g)			s			d			
V. DOCES E PASTEIS	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
34. Bolachas tipo maria ou água e sal (3 bolachas)			s			d			
35. Outras bolachas ou biscoitos (3 bolachas)			s			d			
36. Croissant ou pasteis (Um)			s			d			
37. Chocolate barra (3 quadrados) ou em pó (1 colher sopa)			s			d			
38. Marmelada,compota,geleia,mel (1 colher sobremesa)			s			d			
39. Açucar (1 colher sobremesa ou 1 pacote)			s			d			

V.HORTALIÇAS E LEGUMES	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
40. Couve branca, c. lombarda cozinhadas (1 chávena)			s			d			
41. Penca, tronchuda cozinhadas (1 chávena)			s			d			
42. Couve galega cozinhada (1 chávena)			s			d			
43. Bróculos cozinhados (1 chávena)			s			d			
44. Couve-flor, couve-bruxelas cozinhada (1 chávena)			s			d			
45. Grelos, nabiças, espinafres cozinhados (1 chávena)			s			d			
46. Feijão verde cozinhado (1 chávena)			s			d			
47. Alface, agrião (1 chávena)			s			d			
48. Cebola (uma média)			s			d			
49. Cenoura (uma média)			s			d			
50. Nabo (um médio)			s			d			
51. Tomate fresco (um médio)			s			d			
52. Pimento (meio- médio)			s			d			
53. Pepino (meio- médio)			s			d			
54. Leguminosas cozinhadas: feijão,grão de bico (1 chávena)			s			d			
55. Ervilha grão, fava cozinhadas (meia chávena)			s			d			
VI. FRUTOS	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
56. Maça, pêra (uma média)			s			d			
57. Laranjas (1 média),tangerinas (2 médias)			s			d			
58. Banana (uma média)			s			d			
59. Kiwi (um médio)			s			d			
60. Morangos (1 chávena)			s			d			
61. Cerejas (1 chávena)			s			d			
62. Pêssego (1 médio), ameixa (3 médios)			s			d			
63. Melão, melancia (1 fatia média, 150g)			s			d			
64. Diospiro (1 médio)			s			d			
65. Figo fresco, nêsperas,damascos (3 médios)			s			d			
66. Uvas (1 cacho médio)			s			d			
67. Frutos conserva: pêssego, ananás (2 metades ou rodelas)			s			d			
68. Frutos secos: amêndoas, avelãs, amendoins,etc (meia-chávena)			s			d			
69. Azeitonas (6 unidades)			s			d			
VII. BEBIDAS E MISCELANEAS	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
70. Vinho (1 copo,125 ml)			s			d			
71. Cerveja (1 garrafa ou 1 copo,330 ml)			s			d			
72. Bebidas brancas: aguardente, whisky, brandy, etc. (1cálice,50 ml)			s			d			
73. Refrigerantes: sumol, laranjada,etc (1 garrafa ou 1 copo,330 ml)			s			d			
74. Coca-cola (1 garrafa ou 1 copo,330 ml)			s			d			
75. Café (1 chávena café)			s			d			
76. Chá preto (1 chávena)			s			d			
77. Croquetes, rissois, bolinhos de bacalhau,etc (3 unidades)			s			d			
78. Maionese (1 colher sobremesa)			s			d			
79. Molho de tomate,ketchup (1 colher sopa)			s			d			
80. Pizza (meia pizza-tamanho normal)			s			d			
81. Hamburger (Um médio)			s			d			
82. Sopa de legumes (1 prato)			s			d			

Existe algum alimento que eu não tenha mencionado e que tenha consumido pelo menos 1 vez por semana, mesmo em pequen quantidades, ou numa época em particular. Por ex: flocos de cereais, frutas exóticas, farinha de pau, produtos dietéticos,etc.

ALIMENTOS	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia
			s			d			
			s			d			
			s			d			

Figure 2 - Semiquantitative food frequency questionnaire of EPIPorto

Appendix C – Table 1

Table 1. Characteristics of the study sample, by sex: adults aged 18-86 years (n = 1057) from the EPIPorto adult cohort study, Porto, Portugal (baseline 1999-2003, follow-up 2005-2008)

	Women, n (%) 640 (60.5)	Men, n (%) 417 (39.5)	*P* †
Baseline			
Age (years) [median (P25-P75)]	48 (39.0 - 58.0)	51 (40.5 - 62.0)	**0.003**
Education* (years) [median (P25-P75)]	9 (4.0 - 15.0)	9 (4.0 - 14.0)	0.652
Body Mass Index (kg/m^2) n (%)			
< 18.5	12 (1.9)	4 (1.0)	
18.5 - 24.9	318 (50.4)	172 (41.8)	
25.0 - 29.9	223 (35.3)	198 (48.2)	
≥ 30.0	78 (12.4)	37 (9.0)	**0.036**
Alcohol (g/week) n (%)			
≤ median‡	293 (48.2)	195 (46.8)	
> median‡	315 (51.8)	188 (50.9)	0.362
Smoking Habits n (%)			
Smoker	122 (57.3)	145 (50.0)	
Non-smoker	91 (42.7)	145 (50.0)	0.106
Total energy (kcal/day) [median (P25-P75)]	2000 (1674.1 - 2379.2)	2486 (2146.6 - 2942.2)	**< 0.001**
Total Flavonoids (mg/day) [median (P25-P75)]	349 (241.7 - 501.3)	413 (274.6 - 576.1)	**< 0.001**
Total Flavonoids (mg/day) n (%)			
Q1 (< 245.39)	164 (25.7)	76 (18.3)	
Q2 (245.39 - 362.89)	180 (28.2)	91 (21.9)	
Q3 (362.89 - 540.10)	165 (25.8)	121 (29.2)	
Q4 (≥ 540.10)	130 (20.3)	127 (30.6)	**< 0.001**
Subclasses			
Flavones (mg/day) n (%)			
Q1 (< 0.37)	133 (20.8)	111 (26.7)	
Q2 (0.37 - 0.66)	144 (22.5)	98 (23.6)	
Q3 (0.67 - 1.03)	178 (27.9)	111 (26.7)	
Q4 (≥ 1.04)	184 (28.8)	95 (22.9)	**0.007**
Flavanols (mg/day) n (%)			
Q1 (< 162.03)	170 (26.6)	72 (17.3)	
Q2 (162.03 - 240.17)	189 (29.6)	92 (22.2)	
Q3 (240.18 - 364.13)	146 (22.8)	128 (30.8)	
Q4 (≥ 364.14)	134 (21.0)	123 (29.6)	**< 0.001**
Flavonols (mg/day) n (%)			
Q1 (< 23.89)	165 (25.8)	69 (16.6)	
Q2 (23.89 - 39.16)	150 (23.5)	106 (25.5)	
Q3 (39.17 - 66.41)	146 (22.8)	143 (34.5)	
Q4 (≥ 66.42)	178 (27.9)	97 (23.4)	0.115
Anthocyanins (mg/day) n (%)			
Q1 (< 23.14)	158 (24.7)	63 (15.2)	
Q2 (23.14 - 45.33)	186 (29.1)	74 (17.8)	

Q3 (45.34 - 79.76)	156 (24.4)	131 (31.6)	
Q4 (≥ 79.77)	139 (21.8)	147 (35.4)	**< 0.001**
Flavanones (mg/day) n (%)			
Q1 (< 3.55)	150 (23.5)	88 (21.2)	
Q2 (3.55 - 10.98)	165 (25.8)	119 (28.7)	
Q3 (10.99 - 20.28)	173 (27.1)	105 (25.3)	
Q4 (≥ 20.29)	151 (23.6)	103 (24.8)	0.689
Isoflavones (mg/day) n (%)			
Q1 (< 0.06)	170 (26.6)	76 (18.3)	
Q2 (0.06 - 0.07)	161 (25.2)	82 (19.8)	
Q3 (0.08 - 0.21)	169 (26.4)	139 (33.5)	
Q4 (≥ 0.21)	139 (21.8)	118 (28.4)	**< 0.001**
1° follow up			
Metabolic Syndrome n (%)			
Yes	126 (19.7)	83 (19.9)	
No	514 (80.3)	334 (80.1)	0.496

Abbreviations: Q, quartile; P25–P75, 25th percentile–75th percentile.
† Value was to compare the characteristics across sex using the Mann–Whitney U test and the Chi-square test (for Metabolic Syndrome).
* Number of completed school years.
‡ According to the median of consumption each sex: 10.1g/week for women and 203.0 g/week for men.

Appendix D – Table 2

Table 2. Metabolic syndrome (follow-up) by quartiles of total flavonoids and its subclass intake

	Women, n (%) 640 (60.5)		*p*	Men, n (%) 417 (39.5)		*p*
	Yes	**No**		**Yes**	**No**	
Metabolic syndrome (follow-up)	126 (19.7)	514 (80.3)		83 (19.9)	334 (80.1)	
Total Flavonoids (mg/day)			0.876			0.099
Q1 (< 245.39)	28 (22.2)	136 (26.5)		9 (10.8)	67 (20.2)	
Q2 (245.39 - 362.89)	36 (28.6)	144 (28.1)		20 (24.1)	71 (21.4)	
Q3 (362.89 - 540.10)	44 (34.9)	121 (23.6)		24 (28.9)	97 (29.2)	
Q4 (≥ 540.10)	18 (14.3)	112 (21.8)		30 (36.1)	97 (29.2)	
Subclasses						
Flavones (mg/day) n (%)						
Q1 (< 0.37)	23 (18.3)	110 (21.4)	0.259	20 (24.1)	91 (27.4)	0.833
Q2 (0.37 - 0.66)	26 (20.6)	118 (23.0)		20 (24.1)	78 (23.5)	
Q3 (0.67 - 1.03)	37 (29.4)	141 (27.5)		30 (36.1)	81 (24.4)	
Q4 (≥ 1.04)	40 (31.7)	144 (28.0)		13 (15.7)	82 (24.6)	
Flavanols (mg/day) n (%)			0.536			**0.040**
Q1 (< 162.03)	31 (24.6)	139 (27.1)		8 (9.6)	64 (19.3)	
Q2 (162.03 - 240.17)	33 (26.2)	156 (30.4)		17 (20.5)	75 (22.6)	
Q3 (240.18 - 364.13)	39 (31.0)	107 (20.9)		29 (34.9)	99 (29.8)	
Q4 (≥ 364.14)	23 (18.3)	111 (21.6)		29 (34.9)	94 (28.3)	
Flavonols (mg/day) n (%)			0.633			0.448
Q1 (< 23.89)	29 (23.0)	136 (26.5)		12 (14.5)	57 (17.2)	
Q2 (23.89 - 39.16)	36 (28.6)	114 (22.2)		22 (26.5)	84 (25.3)	
Q3 (39.17 - 66.41)	32 (25.4)	114 (22.2)		26 (31.3)	117 (35.2)	
Q4 (≥ 66.42)	29 (23.0)	149 (29.0)		23 (27.7)	74 (22.3)	
Anthocyanins (mg/day) n (%)			0.061			0.365
Q1 (< 23.14)	36 (28.6)	122 (23.8)		9 (10.8)	54 (16.3)	
Q2 (23.14 - 45.33)	42 (33.3)	144 (28.1)		12 (14.5)	62 (18.7)	
Q3 (45.34 - 79.76)	26 (20.6)	130 (25.3)		33 (39.8)	98 (29.5)	
Q4 (≥ 79.77)	22 (17.5)	117 (22.8)		29 (34.9)	118 (35.5)	
Flavanones (mg/day) n (%)			0.798			0.525
Q1 (< 3.55)	28 (22.2)	122 (23.8)		22 (26.5)	66 (19.9)	
Q2 (3.55 - 10.98)	35 (27.8)	130 (25.3)		22 (26.5)	97 (29.2)	
Q3 (10.99 - 20.28)	31 (24.6)	142 (27.7)		17 (20.5)	88 (26.5)	
Q4 (≥ 20.29)	32 (25.4)	119 (23.2)		22 (26.5)	81 (24.4)	
Isoflavones (mg/day) n (%)						
Q1 (< 0.06)	36 (28.6)	134 (26.1)	0.440	14 (16.9)	62 (18.7)	0.325
Q2 (0.06 - 0.07)	32 (25.4)	129 (25.1)		18 (21.7)	64 (19.3)	
Q3 (0.08 - 0.21)	34 (27.0)	135 (26.1)		35 (42.2)	104 (31.3)	
Q4 (≥ 0.21)	24 (19.0)	115 (22.4)		16 (19.3)	102 (30.7)	

Q, *quartile*

Appendix E – Table 3

Table 3. Logistic regression for metabolic syndrome by quartiles of flavonoids subclass intake

	Women, n (%) 640 (60.5)						Men, n (%) 417 (39.5)					
	Metabolic Syndrome (yes *vs.* no)						Metabolic Syndrome (yes *vs.* no)					
	Crude Model		Model 1		Model 2		Crude Model		Model 1		Model 2	
	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Total Flavonoids (mg/day)												
Q1 (< 245.39)	1.281	0.674; 2.436	1.499	0.761; 2.956	1.364	0.687; 2.705	**0.434**	**0.194; 0.974**	0.633	0.273; 1.467	0.908	0.375; 2.196
Q2 (245.39 - 362.89)	1.556	0.674; 2.436	1.758	0.921; 3.357	1.722	0.898; 3.300	0.911	0.479; 1.733	0.973	0.504; 1.878	1.194	0.595; 2.396
Q3 (362.89 - 540.10)	**2.263**	**1.235; 4.146**	**2.681**	**1.419; 5.066**	**2.514**	**1.324; 4.774**	0.800	0.436; 1.467	0.856	0.461; 1.589	0.861	0.453; 1.636
Q4 (≥540.10)	ref.		ref.		ref.		ref.		ref.		ref.	
Flavones (mg/day)												
Q1 (< 0.37)	0.753	0.426; 1.331	0.779	0.426; 1.427	0.767	0.418; 1.409	1.386	0.649; 2.962	1.444	0.664; 3.141	1.516	0.665; 3.454
Q2 (0.37 - 0.66)	0.793	0.457; 1.375	0.646	0.361; 1.157	0.625	0.347; 1.124	1.617	0.753: 3.472	1.742	0.799; 3.802	1.890	0.840; 4.251
Q3 (0.67 - 1.03)	0.945	0.571; 1.563	0.912	0.538; 1.547	0.867	0.508; 1.480	**2.336**	**1.138; 4.798**	**2.310**	**1.110; 4.844**	**2.549**	**1.177; 5.522**
Q4 (≥ 1.04)	ref.		ref.		ref.		ref.		ref.		ref.	
Flavanols (mg/day)												
Q1 (< 162.03)	1.076	0.594; 1.950	1.385	0.735; 2.607	1.312	0.692; 2.486	**0.405**	**0.174; 0.943**	0.635	0.261; 1.544	0.934	0.368; 2.374
Q2 (162.03-240.17)	1.021	0.569; 1.833	1.283	0.693; 2.376	1.229	0.662; 2.281	0.735	0.376; 1.438	0.856	0.431; 1.703	1.060	0.513; 2.194
Q3 (240.18-364.13)	1.759	0.985; 3.141	**1.965**	**1.066; 3.625**	**1.883**	**1.015; 3.491**	0.949	0.528; 1.708	0.989	0.542; 1.805	1.036	0.555; 1.933
Q4 (≥ 364.14)	ref.		ref.		ref.		ref.		ref.		ref.	
Flavonols (mg/day)												
Q1 (< 23.89)	1.096	0.623; 1.927	1.065	0.582; 1.951	1.042	0.562; 1.932	0.677	0.311; 1.476	0.926	0.412; 2.085	1.173	0.489; 2.818
Q2 (23.89- 39.16)	1.623	0.939; 2.802	1.712	0.964; 3.042	1.750	0.977; 3.135	0.843	0.434; 1.635	0.984	0.497; 1.946	1.100	0.539; 2.244
Q3 (39.17- 66.41)	1.442	0.825; 2.521	1.651	0.913; 2.985	1.654	0.906; 3.018	0.715	0.380; 1.345	0.779	0.408; 1.487	0.665	0.339; 1.307
Q4 (≥ 66.42)	ref.		ref.		ref.		ref.		ref.		ref.	
Anthocyanins (mg/day)												
Q1 (< 23.14)	1.569	0.872; 2.825	1.183	0.636; 2.199	0.984	0.516; 1.875	0.678	0.300; 1.531	0.799	0.347; 1.840	1.271	0.521; 3.100
Q2 (23.14- 45.33)	1.551	0.877; 2.744	1.259	0.691; 2.292	1.116	0.608; 2.048	0.788	0.376; 1.650	0.866	0.407; 1.843	1.202	0.543; 2.658
Q3 (45.34- 79.76)	1.064	0.572; 1.978	0.996	0.523; 1.895	0.928	0.485; 1.775	1.370	0.778; 2.414	1.391	0.780; 2.479	1.668	0.907; 3.064
Q4 (≥ 79.77)	ref.		ref.		ref.		ref.		ref.		ref.	
Flavanones (mg/day)												
Q1 (< 3.55)	0.853	0.484; 1.504	0.761	0.418; 1.387	0.726	0.396; 1.330	1.227	0.625; 2.409	1.252	0.625; 2.510	1.370	0.665; 2.860

Q2 (3.55 - 10.98)	1.001	0.583; 1.718	1.196	0.675; 2.118	1.118	0.628; 1.988	0.835	0.431; 1.616	0.795	0.405; 1.560	0.860	0.428; 1.728
Q3 (10.99- 20.28)	0.812	0.468; 1.408	0.898	0.503; 1.602	0.865	0.480; 1.557	0.711	0.353; 1.434	0.707	0.345; 1.447	0.867	0.413; 1.821
Q4 (≥ 20.29)	ref.		ref.		ref.		ref.		ref.		ref.	
Isoflavones (mg/day)												
Q1 (< 0.06)	1.287	0.726; 2.284	1.302	0.710; 2.387	1.299	0.706; 2.391	1.440	0.657; 3.152	1.499	0.674; 3.336	1.648	0.711; 3.819
Q2 (0.06 - 0.07)	1.189	0.662; 2.136	1.277	0.690; 2.362	1.288	0.695; 2.388	1.793	0.853; 3.767	1.861	0.870; 3.979	2.203	0.999; 4.861
Q3 (0.08 - 0.21)	1.207	0.677; 2.153	1.353	0.738; 2.481	1.344	0.729; 2.478	**2.145**	**1.118; 4.116**	**2.050**	**1.055; 3.983**	1.829	0.918; 3.647
Q4 (≥ 0.21)	ref.		ref.		ref.		ref.		ref.		ref.	

Abbreviations: Q, quartile; CI, confidence interval.
Model 1. Adjusted for age and education.
Model 2. Was additionally adjusted for alcohol intake.
Bold values indicate statistical significance when $p < 0.05$.