Nutrition 32 (2016) 566–572

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Dietary flavonoid intake, total antioxidant capacity and lipid oxidative damage: A cross-sectional study of Iranian women

Beitolah Alipour Ph.D. [a], Bahram Rashidkhani M.D., M.P.H., Ph.D. [b], Sareh Edalati M.Sc. [a,*]

[a] Department of Community Nutrition, Nutrition Faculty, Tabriz Medical University, Tabriz, Iran
[b] Department of Community Nutrition, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 2 January 2015
Accepted 18 November 2015

Keywords:
Total antioxidant capacity
Dietary antioxidant
Flavonoids
Lipid oxidative damage

ABSTRACT

Objectives: Although strong evidence supports the antioxidant potential of flavonoids in vitro, the effect of flavonoids at physiological concentrations on the overall antioxidant status in humans is inconsistent. The aim of this study was to examine cross-sectional associations between total flavonoid consumption, serum total antioxidant capacity (TAC), and malondialdehyde (MDA) levels in apparently healthy women.

Methods: Through a multistage cluster sampling, 170 women ages 20 to 48 y were recruited. The usual dietary flavonoid intake was estimated using a semiquantitive food frequency questionnaire (FFQ) by matching food items with the US Department of Agriculture flavonoid databases. General linear models were used to compare the biochemical parameters across tertiles of flavonoid intakes.

Results: As dietary anthocyanin intake rose from the lowest to the highest tertile, the multivariate-adjusted mean TAC concentrations significantly increased from 1.08 to 1.28 ($P_{trend} = 0.01$). This association was still significant after adjustment for fruit and vegetable intake and antioxidant vitamins ($P_{trend} = 0.03$). The highest tertile of total flavonoid intake and theaflavins had higher mean concentrations of TAC than did the lowest tertile, but there was no linear trend ($P < 0.05$). There were statistically significant positive relationships between dietary intake of grapes and eggplant as main food sources of anthocyanins and serum TAC ($P = 0.02$ and 0.04, respectively). No significant associations were found between MDA and flavonoids intakes ($P > 0.05$).

Conclusions: The findings of the present study support the attribution of anthocyanins to overall antioxidant status. However, further research is needed to confirm these observed associations.

Introduction

Flavonoids are a group of polyphenolic compounds ubiquitously found in plant-derived foods, such as fruits, vegetables, tea, wine, and coca products [1]. They have received considerable interest due to their possible contribution to reducing risk for several chronic diseases [2,3]. The protective effects of flavonoids may be mediated by improvements in antioxidant status [4]. Oxidative stress is characterized by an imbalance between the production of reactive oxygen species and the antioxidant defenses [5] and it has been suggested to be correlated with the development of a range of chronic diseases [6].

Flavonoids appear to be a part of antioxidants that contribute to the high antioxidant capacity of fruits and vegetables and some are found to be several times stronger on the basis of molar concentration than vitamins E and C [7,8]. The direct role of flavonoids in reducing oxidative damage has been demonstrated in many in vitro studies [9–12]. However, direct antioxidant

This work was supported by funding from the Tabriz Medical University. All laboratory measurements were carried out at the research laboratory of Research Institute for Endocrine Sciences of Shahid Beheshti Medical University. BA was involved in designing the study, advising on procedures, and project supervision. BR gave statistical advice and was involved in revising the manuscript. SE carried out the research, collected data, and wrote the initial manuscript. All the authors have read and approved the final manuscript. The authors have no conflicts of interest to declare.

* Corresponding author. Tel.: +98 411 335 7582; fax: +98 411 334 0634.
E-mail address: saraedalati@yahoo.com (S. Edalati).

http://dx.doi.org/10.1016/j.nut.2015.11.011
0899-9007/

benefits of flavonoids, at physiological concentration in vivo, have not been completely determined [13]. Short-term intervention studies examining the effects of individual flavonoids or flavonoid-rich foods on circulating oxidative stress biomarkers in vivo have been inconsistent, with some showing no effect [14–16], whereas others show some benefits [17–20]. To our knowledge, evidence published linking dietary flavonoid intake to antioxidant status is limited and to date, few observational studies have addressed this effect. One observational study has been conducted on the association of habitual dietary flavonoid intake to antioxidant status, and in that study, dietary flavonol intakes were inversely associated with malondialdehyde (MDA) levels in elderly men in Spain [21]. However, in regard to flavonoid intake, no study to our knowledge has focused on premenopausal women and has assessed the marker of total antioxidant capacity (TAC).

The objectives of the present study were to estimate the intake of individual flavonoids and their major sources and to evaluate their association with MDA, a known oxidative damage biomarker, and TAC levels in a subsample of Iranian premenopausal women. The study aimed to accomplish this objective using the most comprehensive updated flavonoids databases.

Methods

Study population

A cross-sectional study design was used. Apparently healthy premenopausal women ages 20 to 48 y (N = 170) who had been referred to eight health centers (from different regions in Tehran) affiliated with the Medical University of Shahid Beheshti, were randomly selected using a multistage cluster random sampling method.

A woman was defined as premenopausal if she had menstruated in her usual pattern in the previous 3 mo and had not experienced removal of the uterus and/or ovaries [22].

Exclusion criteria were as follow: history of cancer or autoimmune, liver, or renal dysfunction; diabetes; hypertension; hypo- or hyperthyroidism; infection; pregnancy and lactating; experiencing menopause; use of any medications and supplements known to influence the variables studied (lipid-lowering drugs, contraceptive drugs, herbal and antioxidant supplements, probiotic supplements, antibiotics, hormone replacement therapy); on a restrictive diet or experiencing any change in dietary patterns. After exclusions, 170 women were available for participation in the study. All participants gave written consent after receiving verbal and written information about the study. The procedures of this study were approved by the medical Ethics Committee of Tabriz University of medical sciences as well as Shahid Beheshti University of medical sciences.

Assessment of dietary and flavonoid intake

Usual dietary intake was estimated using a semiquantitive food frequency questionnaire (FFQ). This questionnaire was developed and validated for use in a broad range of diet and health studies in Iran, and there were good correlation coefficients for foods that were primary contributors to flavonoid intake between the FFQ and diet records [23].

This FFQ consists of a list of 168 food items, and during an in-person interview, each participant was asked to report how often, on average, she had consumed each food item on a daily, weekly, monthly, or yearly basis over the previous year. The questionnaire asked about the amount consumed each time in standard or household units. Additional questions asked about beverage consumption (green tea) and spices not included in the original FFQ. The inclusion of these food items were based on a pilot study that was performed in 40 individuals who were not included in the main study. To help participants estimate the more accurate amount of food eaten, we used quantitative tools including household scales (e.g., cup, tablespoon, teaspoon plate, glass, small bowl, and spatula) and a validated food album [24].

For the estimation of flavonoid intake, we developed a flavonoid food composition table by matching FFQ food items with the flavonoid values of the updated and expanded US Department of Agriculture (USDA) databases on flavonoids [25] and isoflavones [26]. For mixed dishes, total flavonoid values were determined by calculating the percent weight contribution of each ingredient multiplied by its flavonoids content per 100 g and then summing up all ingredients. For FFQ food items not included in the USDA database, we checked phenol explorer [27]. When data for cooked foods were unavailable, retention factors for all flavonoid classes, except isoflavones were used (70%, 35%, and 25% after frying, cooking in a microwave oven, and boiling, respectively) [28]. A 25% retention factor for fresh fruits was used for dried fruit [29].

Portion sizes of consumed foods were converted to grams by using household measures. We multiplied the reported daily gram of each food consumed by the flavonoid content of the food (mg/100 g) and summed flavonoid intakes across all foods and beverages. Intakes were derived for the following six main subclasses as follows: flavonol (quercetin, kaempferol, myricetin, isorhamnetin), flavones (luteolin, apigenin), flavanones (eriodictyol, hesperetin, naringenin), flavan-3-ols (catechins and epicatechins), theaflavins, anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin), and isoflavones. Total flavonoid intake was calculated by summing up intakes of the six component subclasses. Flavonoid intake was adjusted for energy intake by the residual method [30].

Daily total energy intake and specific nutrients were computed by multiplying the frequency of consumption of an item by the nutrient content of specified portions using the Iranian food composition table (FCT) [31] and USDA's FCT. All the dietary questionnaire data were rechecked to minimize potential bias in coding.

Biochemical measurement

Blood samples were drawn after 12 to 14 h of fasting and were centrifuged for 10 min at 3000*g* to separate serum. Serum aliquots were stored at −70°C and were analyzed in the research laboratory of Endocrine research Center of Shahid Beheshti Medical University. Fasting plasma glucose was measured by the enzymatic colorimetric method using glucose oxidase. Triacylglycerol (TG) level was measured by enzymatic colorimetric analysis with glycerol phosphate oxidase. Serum total cholesterol, TG, and high-density lipoprotein cholesterol, were assayed using commercial kits (Pars Azmoon Inc, Tehran, Iran) by enzymatic methods and a Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands). Low-density lipoprotein cholesterol was calculated by Friedewald formula [32]. TAC was measured by a commercially available method (LDN, Nordhorn, Germany). MDA, a terminal product of lipid peroxidation, was measured by the thiobarbituric acid reduction method using a commercially available kit (QuantiChrom TBARS Assay kit, BioAssay Systems, Hayward, CA, USA). The intraassay coefficient of variance for MDA was 5.7%.

Assessment of anthropometric variables

Height was measured with a stadiometer (Seca, model 206, Hamburg, Germany) fixed to the wall; the women were without shoes and headdress, and the Frankfort Plane technique was used [33]. Weight was determined to the nearest 0.1 kg using a digital scale (Seca 707, Hamburg, Germany) with the participant standing in light clothing and barefoot. Body mass index (BMI) was calculated by dividing body weight by height squared (kg/m^2). Waist and hip circumference were measured using a Seca 201 measuring tape according to the World Health Organization (WHO) protocol [34]. The waist-to-hip ratio (WHR) was calculated as waist circumference (in cm) divided by hip circumference (in cm). Percentage of body fat were estimated by using a hand-to-foot bioelectrical impedance scale (Omron, model HBF 511), which recorded impedance from hand to foot and consequently, calculated fat mass and skeletal muscle percentage from the impedance value and the participant's weight, height, age, and sex.

Physical activity and other variables

Baseline information such as age, smoking habits, menopausal status, socioeconomic status, use of herbal and vitamin supplements, use of contraceptive drugs or hormone replacement therapy, per-day dieting, and history of any diseases were also asked through a face-to-face interview. Physical activity was determined using a self-reported questionnaire [35] and expressed as metabolic equivalent hours per day (MET-h/d) [36].

Statistical analysis

Statistical analyses were performed with SPSS 21 for Windows (SPSS Inc., Chicago, IL, USA). Data are reported as mean ± SD for normally distributed variables or as the number and percentage (for qualitative variables). Geometric means and 95% confidence interval (CI) were used for variables with skewed distribution. Baseline characteristic among tertiles of flavonoids intake were compared using χ^2 tests for categorical variables, analysis of variance for normally distributed variables, and by nonparametric tests (Mann-Whitney *U* test or Kruskall-Wallis test) for non-normally distributed variables. Energy-adjusted flavonoid intake through residual method, for six main subclasses of flavonoids, were assigned as tertile intakes, based on their 33th, 66th, 99th values, and generalized linear models (GLM) were used to compare mean (±SE) values of TAC (dependent variables) across these groups (fixed factor). Three models with TAC as the outcome variable were developed. Model 1 was adjusted for age, BMI,