Nutrition 30 (2014) 660–665

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Fruit and vegetable intake and related nutrients are associated with oxidative stress markers in middle-aged men

Paula G. Cocate M.Sc. [a], Antônio José Natali Ph.D. [b], Alessandro de Oliveira M.Sc. [a], Giana Z. Longo Ph.D. [a], Rita de Cássia G. Alfenas Ph.D. [a], Maria do Carmo G. Peluzio Ph.D. [a], Eliziária C. dos Santos M.Sc. [c], Jéssica M. Buthers R.D. Student [a], Leandro L. de Oliveira Ph.D. [c], Helen Hermana M. Hermsdorff Ph.D. [a,*]

[a] Department of Nutrition and Health, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil
[b] Departament of Physical Education, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil
[c] Departament of General Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 18 July 2013
Accepted 28 October 2013

Keywords:
Fruit and vegetable
Vitamin C
Magnesium
Fiber
Oxidized LDL
8-iso-prostaglandin F2 α
8-hydroxy-2′-deoxyguanosine

ABSTRACT

Objective: The aim of this cross-sectional study was to assess the potential relationships between fruit and vegetable (FV) intake and oxidative stress markers in middle-aged men, with an emphasis on vitamin C, fiber, and magnesium content.
Methods: The study was conducted with 296 healthy men, age 50.5 ± 5.0 y, and body mass index (BMI) of 25.8 ± 3.5 kg/m^2. Dietary intake, anthropometry, blood pressure, lifestyle features, and blood and urine biochemical data were assessed with validated procedures. The oxidative stress markers selected were plasma oxidized low-density lipoprotein (ox-LDL), urinary 8-iso-prostaglandin F2 α (8-iso-PGF2 α) and 8-hydroxy-2′-deoxyguanosine (8-OHdG).
Results: The men included in the highest tertile of FV intake (≥341.1 g/d) displayed lower concentrations of ox-LDL, 8-iso-PGF2 α and 8-OHdG (P for trend < 0.05), regardless of confounding factors. Concentrations of ox-LDL were negatively associated with fiber from the FV intake (P for trend < 0.05) regardless of confounding factors. ox-LDL and 8-OHdG concentrations tended to be lower in the higher tertile of magnesium (P for trend = 0.06) and vitamin C from FV intake (P for trend = 0.05), respectively. Additionally, concentrations of 8-iso-PGF2 α were lower in men in the highest tertile of fiber (≥6.5 g/d; P for trend = 0.034), vitamin C (≥98.0 mg/d; P for trend = 0.007), and magnesium (≥48.9 mg/d; P for trend = 0.018) from the FV-group intake.
Conclusions: Greater FV intake was independently associated with reduced ox-LDL, 8-OHdG, and 8-iso-PGF2 α in middle-aged men. Fiber, vitamin C, and magnesium from FV seem to contribute to this beneficial relationship.

Introduction

Free radicals, when produced in adequate proportion, have relevant biological functions such as gene activation and participation in the body's defense mechanisms against infections [1]. However, when the production of free radicals and/or reactive species exceeds the antioxidant defense, it may favor the oxidation of biomolecules such as lipids, proteins, and DNA, resulting in cell damage and loss of biological function [1]. In this sense, oxidative stress may play a decisive role in the pathogenesis and progression of several chronic diseases, including cardiovascular disease [2] and cancer [3].

During lipid oxidation, the peroxidation of the arachidonic acid (lipid present in body cell membranes) produces F2 isoprostane. Increases in this biomarker have been positively related to coronary artery disease [4] and diabetes mellitus [5], as well as other diseases [6]. Reactive oxygen species within blood vessels also can promote oxidative modification of the

PGC designed the study, analyzed and collected the data, wrote the initial draft, and assembled the final version of the manuscript. AO, JMB, and ECS collected the data. RCGA, GZL, MCGP, and LLO designed the study and analyzed the data. AJN and HHMH designed the study, analyzed the data and helped assemble the manuscript. All authors assisted in editing the manuscript as well as reading and approving the final manuscript.

* Corresponding author. Tel.: +55 31 3899; fax: +55 31 2541.
E-mail address: helenhermana@ufv.br (H. H. M. Hermsdorff).

0899-9007/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2013.10.015

low-density lipoprotein (LDL) molecule generating oxidized LDL (ox-LDL) [7], which is directly related to atherosclerotic events [8]. Moreover, oxidative damage to DNA may occur via oxidation of deoxyguanosine, producing 8-hydroxy-2′-deoxyguanosine (8-OHdG) [9], which is considered a risk factor for cancer, atherosclerosis, and diabetes mellitus [10].

Eating habits can also significantly influence the development of most chronic diseases, affecting the health of individuals throughout life. Thus, a healthy diet including fruits and vegetables (FV) can play an important role in the prevention of these diseases in middle-aged individuals [11]. It has been reported that the daily intake of adequate amounts of FV is inversely associated with chronic disease [12], cardiovascular risk factors [13], and oxidative stress [14,15] probably due to their potential anti-inflammatory [16,17] and antioxidant effects [18–21]. However, to our knowledge, studies investigating the relationship of FV intake with lipid and/or DNA oxidation biomarkers in middle-aged men are still scarce [22–24], especially considering the dietary habits of the Brazilians whose FV intake is nearly 90% lower [25] than that recommended by the World Health Organization (WHO) [12]

Fruits and vegetables are sources of nutritional components with high antioxidant capacity such as carotenoids [26] and polyphenols [27,28]. Additionally, although this food group is an important source of vitamin C, fiber, and magnesium, nutrients that have been negatively associated with oxidative stress events [29–33], this relationship has yet to be clarified.

Therefore, the aim of this cross-sectional study was to assess the relationship between FV intake and the concentrations of ox-LDL, 8-iso-prostaglandin F2 α (8-iso-PGF2 α), and 8-OHdG in middle-aged men, with an emphasis on vitamin C, fiber, and magnesium content.

Materials and methods

Participants

This cross-sectional study was carried out between March and December 2011. The sample size was calculated [34] considering the total number of male staff at Federal University of Viçosa (UFV), Viçosa city, Brazil in February 2011, ages between 40 and 59 y (1744 individuals), confidence level of 95%, and 24.4% expected prevalence of metabolic syndrome (a metabolic condition highly prevalent with oxidative stress [35]) in Brazilian middle-aged men [36] and 4.5% sampling error, resulting in 293 participants as a minimum sample size required.

Participants were recruited by systematic sampling. We excluded those individuals who self-declared body weight alterations >3 kg in the 3 mo preceding the study, altered levels of physical activity and eating habits in the 3 mo preceding the study, thyroid disease, heart failure, cerebrovascular diseases, infectious diseases, inflammatory diseases, gastrointestinal diseases, liver disease, chronic kidney disease or a history of kidney stones, cancer in the previous 10 y, eating disorders (anorexia and bulimia), and food allergies. Individuals using vitamin supplements and those taking diuretics or drugs that alter food intake and/or the metabolism of nutrients, pacemaker and/or prosthetic limbs users, and elite athletes also were excluded.

We interviewed 848 men and eliminated 548 by the exclusion criteria. Of the 300 individuals selected, 4 did not answer the food frequency questionnaire (FFQ); thus the final sample comprised 296 individuals.

Each participant signed a written informed consent, which was approved by the Ethics Committee in Human Research of the Federal University of Viçosa (reference no. 069/2010) in accordance with principles of the Declaration of Helsinki.

Lifestyle factors and habitual dietary intake

Information about lifestyle factors including work position, current smoking status, and alcohol consumption was collected using a questionnaire. The criteria for classification of work position were previously described [37], whereas excessive alcohol consumption was defined as intake >21 units/wk [38].

The habitual physical activity was estimated by the mean number of daily steps (7 consecutive d) measured by Digi-Walker SW-200 pedometer (Yamax Corporation, Tokyo, Japan), according to the instructions previously described [37].

A quantitative FFQ with 105 food items validated for the Brazilian population [39] was used to assess the usual dietary intake of the participants during the previous 6 mo. Daily food consumption was estimated as frequency × portion × size for each consumed food item. The intake of each nutrient, such as fiber, vitamin C, magnesium were assessed using the software Dietpro version 5.5 i (AS Systems, Viçosa, Brazil), using mainly two Brazilian nutritional composition tables [40,41]. When the needed nutritional information was not observed in these tables, the U.S. Department of Agriculture table [42] was used.

The FV intake assessed from the data in the FFQ included the evaluation of 8 fruits (only fresh): Orange, banana, apple, papaya, watermelon/melon, pear, and other fruits (grapes and pineapple) and 10 vegetables (fresh or cooked): Lettuce, watercress/kale/spinach (dark green leaves), cabbage, cauliflower/broccoli, carrot/pumpkin, tomatoes, beets, chayote/zucchini, okra, and cucumber. Juice intake was not considered in this study due to the joint determination of FFQ for sugar-sweetened and unsweetened juices.

Anthropometric and biomedical data collection

Anthropometric determinations such as weight, height, and waist circumference were taken using standard measurement procedures, as previously described [37]. Body mass index was calculated as weight (kg) divided by height squared (m^2). Total body fat percentage was determined by total body scanning with a dual energy x-ray absorptiometry (GE/Lunar, Madison, WI; enCORE software version 13.31) and the percentage of fat in the android region was determined using the "region of interest" program, according to the manufacturer's instructions. Systolic and diastolic blood pressures were measured using VI Brazilian Guidelines on Hypertension [43].

A venous blood sample was taken after 12-h overnight fast for measuring glucose, insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TAGs), free fatty acid (FFA), and ox-LDL. Glucose was measured by the glucose oxidase method (Cobas Mira Plus, Roche Diagnostics, GmbH, Montclair, NJ, USA) and insulin by electrochemiluminescence using the Modular Analytics (E170, Roche Diagnostics, GmbH, Mannheim, Germany). Using a previously described formula [44], the Index of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was utilized to estimate insulin resistance. Then, serum TC, HDL-C, and TAGs were determined by the enzymatic colorimetric method (Cobas Mira Plus - Roche Diagnostics GmbH, Montclair, NJ, USA). FFA concentrations were determined by the kinetic spectrophotometry method using the kit EnzyChromFree Fatty AcidAssay (Bioassay Systems, Hayward, CA, USA). The metabolic syndrome was diagnosed by Alberti et al. criteria [45]. Finally, plasma concentrations of ox-LDL were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden) based on the direct sandwich technique.

Urinary biomarkers of oxidative stress

Urine was collected in sterile tubes (after 12-h overnight fasting) for measuring the oxidative stress biomarkers, 8-iso-PGF2 α and 8-OHdG.

Competitive ELISA was used to determine urinary concentrations of 8-iso-PGF2 α (Oxford, MI, USA) and 8-OHdG (Cayman, MI, USA). The analyses were performed according to manufacturer's instructions. Although the Cayman's kit recognizes the 8-OHdG from DNA, the ELISA values are always higher than liquid chromatography–mass spectrometry inasmuch as this method also detects 8-hydroxyguanosine and 8-hydroxyguanine from either DNA or RNA. The values of urinary 8-iso-PGF2 α and 8-OHdG were normalized by mg of urinary creatinine, measured by a kinetic colorimetric method, using a Bioclin commercial kit (Cobas Mira Plus, Roche Diagnostics GmbH, Montclair, NJ, USA).

Statistical analysis

Normal distribution of the data was determined by the Shapiro-Wilk test. Non-normally distributed variables were log-transformed before statistical analysis. To evaluate the association between FV intake, oxidative stress, and other variables, participants were categorized into tertiles based on this food group consumption adjusted by daily energy intake using the residual method. The quantiles cutoff criteria have been previously applied [16,29] and are based on a valid and reliable method to assign two or more groups of risk in nutritional epidemiology studies [46]. A comparison between the three groups was performed by analysis of variance followed by a Bonferroni post hoc test. A χ^2 test for linear trend was used to compare proportions between FV intake and categorical variables.

Linear trends were assessed by assigning the average value to each tertile of FV intake and modeling those values as a continuous variable. Initially, it was used a model controlled by android fat (%), habitual physical activity, work position, excessive alcohol consumption, daily caloric intake (kcal/d), FFA concentrations (mmol/L) and HOMA-IR. Then, it was developed by another model controlled by the same variables associated with the covariate "current smoker." The same procedures were performed to assess the relationship between dietary fiber, vitamin C, and magnesium (from FV intake) and markers of oxidative stress.