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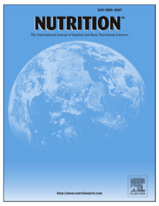

Applied nutritional investigation

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

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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

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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.

A stepwise multiple regression analysis also was used to identify the key foods (FV food group) consumed by the participants. Calorie consumption outliers were defined by dispersing interquartile as previously described [47]. Outliers were excluded (five individuals with caloric intake greater than the upper limit ≥2.640 kcal/d) followed by all statistical analyzes previously described. The results maintained the same trend with all participants included. Data processing and analysis were performed using the software STATA version 9.1 (Stata Corp., College Station, TX, USA), considering $P < 0.05$.

Results

Anthropometric, clinical, and lifestyle characteristics were assessed according to tertiles of FV intake (Table 1). The diastolic blood pressure was higher ($P = 0.040$) in the first than third tertile. There were lower percentages of participants with metabolic syndrome ($P = 0.027$), individuals in technical administrative positions A, B, and C ($P = 0.002$) and current smokers in the highest tertiles ($P = 0.003$).

An estimate of inadequacy for FV intake of 76.4% was observed compared with that recommended by the WHO (≥400 g/d) [12]. In turn, the most commonly consumed vegetables were lettuce, carrot/pumpkin, and tomato, which together explained 85.4% of the variability in this food group, whereas orange and apple were important items that together explained 84.3% of total variability in fruit intake. Other consumed vegetables and fruits explained only 14.6% and 15.7% of the total variability in intake of these food groups, respectively.

The assessment of dietary habits in relation to tertiles of FV intake demonstrated that the consumption of monounsaturated fatty acids ($P = 0.005$), saturated fatty acids ($P = 0.010$), and cholesterol ($P = 0.031$) were lower, whereas fiber and magnesium intakes were higher ($P < 0.001$) in the third tertile compared with the first and second. Additionally, carbohydrate intake was higher ($P = 0.015$), whereas lipid intake was lower ($P = 0.020$) in men allocated in the third tertile compared with those in the first tertile. Finally, vitamin C intake was higher ($P < 0.001$) in the highest tertile compared with the other tertiles (Table 2).

Considering the oxidative stress markers, men in the highest tertile of FV intake demonstrated lower values of ox-LDL (P for trend $= 0.050$), urinary 8-iso-PGF2 α (P for trend $= 0.003$), and 8-OHdG (P for trend $= 0.028$) regardless of confounding variables. The inclusion of the covariate “current smoker” (yes/no) in the linear regression model attenuated the statistical significance in relation to oxidative stress markers (Table 3).

Sample categorization into tertiles based on the content of fiber, vitamin C, and magnesium from FV, revealed that ox-LDL concentrations were negatively associated with fiber (P for trend $= 0.013$) and magnesium (tendency, P for trend $= 0.060$). The urinary values of 8-iso-PGF2 α also were inversely associated with the content of fiber (P for trend $= 0.034$), vitamin C (P for trend $= 0.007$), and magnesium (P for trend $= 0.018$) from FV. Lower urinary concentrations of 8-OHdG were found in participants with higher vitamin C intake from FV (P for trend $= 0.050$), regardless of the adjustment covariates. There was attenuation of statistical significance between oxidative stress markers and nutrient intake from FV after addition of the covariate “current smoker” in the linear regression model (Table 4).

Discussion

The first relevant finding of this study was the inverse association between FV consumption and the assessed biomarkers of oxidative stress (8-iso-PGF2 α, 8-OHdG, and ox-LDL) in middle-aged men. Similarly, in a cross-sectional study involving young adults, there was a negative association between FV intake and concentrations of ox-LDL [29]. Another study with adults reported that a diet rich in FV was also inversely associated with the values of 8-iso-PGF2 α in both transversal and longitudinal analysis [15]. Additionally, intervention studies with diets rich in FV (9.2 and 12 portions/d) demonstrated a reduction in concentrations of urinary 8-iso-PGF2 α [14] and 8-OHdG [48].

In this study, vegetable intake resulted in greater negative association with ox-LDL and 8-OHdG levels than fruit intake. In fact, the most consumed vegetables (lettuce, carrot/pumpkin, and

Table 1

Anthropometric, clinical, and lifestyle characteristics, according to tertiles (T) of energy-adjusted fruit and vegetable consumption (g/d)

	T1 <200.2 n = 98	T2 200.2–341.1 n = 98	T3 ≥341.1 n = 100	P-value[*]
Age (y)	52 (47–54)	52 (46–54)	52 (47–54)	1.000
BMI (kg/m^2)	25.7 (23.0–28.0)	25.2 (23.0–28.4)	25.1 (23.8–27.5)	0.914
WC (cm)	91.5 (83.2–97.7)	89.9 (82.7–97.0)	88.5 (83.5–94.9)	0.893
TBF (%)	23.8 (16.4–28.8)	22.3 (17.8–27.9)	23.7 (19.2–26.9)	0.845
Android fat (%)	27.4 (18.4–35.1)	25.6 (18.1–35.8)	27.7 (21.7–33.5)	0.400
SBP (mm Hg)	127.2 (116–135)	124.2 (115–130)	125.7 (116–132)	0.090
DBP (mm Hg)	81 (76–86)[†]	80 (73–87)	80 (72–85)	0.040
MetS (%)	33.6	20.4	20.0	0.027
FFA (mmol/L)	0.80 (0.6–1.1)	0.70 (0.6–0.9)	0.75 (0.5–0.9)	0.104
TC (mg/dL)	216.5 (187–244)	210.0 (192–236)	207.0 (172–241)	0.266
HDL-C (mg/dL)	43 (37–53)	46 (40–55)	44 (37–51)	0.120
TAGs (mg/dL)	114.0 (84–172)	116.5 (80–160)	114.5 (81–161)	0.485
Glucose (mg/dL)	87.5 (82.0–95.0)	88.0 (83.0–95.0)	89.0 (83.0–95.0)	0.831
Insulin (μUI/mL)	5.3 (3.3–8.5)	5.0 (3.1–7.8)	5.4 (3.5–8.7)	0.664
HOMA-IR	1.1 (0.7–1.9)	1.1 (0.7–1.6)	1.2 (0.8–1.9)	0.651
HPA (steps/d)	10 808 (8693–13 100)	10 797 (8309–14 079)	10 501 (8384–13 924)	0.716
Excessive alcohol consumption (%)	33.3	36.8	29.8	0.666
Work position ABC (%)	78.6	62.2	58.0	0.002
Current smoker (%)	21.4	12.2	7.0	0.003

ANOVA, analysis of variance; BMI, body mass index; DBP, diastolic blood pressure; FFA, free fatty acid; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HPA, habitual physical activity; MetS, metabolic syndrome; SBP, systolic blood pressure; TBF, total body fat; TC, total cholesterol; TAGs, triacylglycerols; WC, waist circumference; work position ABC, technical-administrative positions A, B, or C

Data are median (25th–75th quartile) or (%)

[*] P-value from one-factor ANOVA test or χ^2 test for continuous or categorical variables, respectively.

[†] Significantly different from T3 ($P < 0.05$, from the Bonferroni post hoc test).

Table 2
Food and nutrient consumption, according to tertiles (T) of energy-adjusted fruit and vegetable consumption (g/d)

	T1 $<$200.2 n = 98	T2 200.2–341.1 n = 98	T3 $\geq$341.1 n = 100	P-value[*]
Energy intake (kcal)	1354.7 (1009.8–1777.4)	1373.2 (1092.5–1737.6)	1365.1 (1098.4–1727.3)	0.986
Carbohydrate (g/d)	172.3 (128.2–224.3)[†]	181.6 (144.7–227.9)	196.3 (156.4–240.8)	0.015
Protein (g/d)	65.8 (54.5–85.5)	65.4 (54.4–83.2)	64.2 (54.3–80.1)	0.729
Lipids (g/d)	44.7 (31.0–59.8)[†]	42.8 (32.9–54.8)	35.9 (28.4–49.3)	0.020
MUFA (g/d)	14.3 (10.3–19.4)[†]	13.9 (10.5–18.5)[‡]	12.2 (8.8–15.8)	0.005
PUFA (g/d)	6.4 (4.7–9.5)	6.5 (4.9–8.7)	5.9 (4.4–7.7)	0.179
SFA (g/d)	15.4 (10.3–19.8)[†]	15.3 (11.0–19.6)[‡]	12.6 (9.7–16.5)	0.010
Cholesterol (mg/d)	207.9 (154.0–294.6)[†]	212.6 (165.2–270.0)[‡]	180.5 (131.2–243.4)	0.031
Fiber (g/d)	18.1 (15.2–22.0)[†]	20.8 (15.8–25.3)[‡]	24.0 (19.0–27.7)	$<$0.001
Magnesium (mg/d)	159.8 (119.5–189.7)[†]	169.9 (138.9–209.6)[‡]	185.5 (158.9–215.3)	$<$0.001
Vitamin C (mg/d)	35.0 (23.4–48.7)[§]	74.9 (56.4–110.4)[‡]	134.8 (96.4–197.2)	$<$0.001

ANOVA, analysis of variance; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid
Data are median (25th–75th quartile)

[*] P-value from one-factor ANOVA test.

[†] Significantly different from T3 ($P < 0.05$, from the Bonferroni post hoc test).

[‡] Significantly different from T3 ($P < 0.05$, from the Bonferroni post hoc test).

[§] Significantly different from T2 and T3 ($P < 0.05$, from the Bonferroni post hoc test).

tomato) have higher fiber content than the most consumed fruits (orange and apple) [40], thus fiber consumption from FV has been inversely associated with ox-LDL [29]. In this context, an intervention study with apparently healthy men found that a higher consumption of vegetables (200 g/d) for 4 wk was associated with decreased levels of substances involved in the pathways of ox-LDL [49]. Moreover, a cross-sectional study with healthy participants demonstrated an inverse association of urinary 8-OHdG with polyphenol intake from vegetable, but not from fruit [27].

Interestingly, in the present study, 70% of participants included in the third tertile of FV (participants with lower oxidative stress levels) consumed 400 g/d or more of FV, which is in concert with the WHO's daily recommendation [12]. These outcomes reinforce the benefits of adequate consumption of FV, including against oxidative stress, an important risk factor for chronic diseases.

Another relevant finding of this study was that men allocated in the highest tertile of vitamin C intake (from FV) presented with lower concentrations of urinary 8-iso-PGF2 α and 8-OHdG. Vitamin C is an important reduction agent that converts the reactive species of oxygen and nitrogen into harmless species acting as an in vivo antioxidant [50,51]. Increased FV consumption has been associated with increased intake of vitamin C, which is related to higher antioxidant capacity and reduced oxidative stress [29,32]. In fact, consumption of a diet rich in vitamin C (500 mL of gazpacho), over 14 d, reduced the concentrations of 8-iso-PGF2 α in healthy participants [32].

Fiber intake of the FV group was associated with lower concentrations of lipid peroxidation biomarkers (ox-LDL and 8-iso-PGF2 α) in this study. A previous cross-sectional study with adult men and women also reported that higher fiber consumption from FV was associated with lower plasma concentrations of ox-LDL [29]. In this context, diets rich in fiber reduced lipid peroxidation in obese adult mice when compared with animals fed a standard diet [52]. The action of certain fibers on the sequestration of bile salts and/or the partial blockage of the

Table 3
Oxidative stress markers with respect to tertiles (T) of energy-adjusted fruit and vegetable consumption

	Ox-LDL (U/L)	8-iso-PGF2α (ng/mg Crn)	8-OHdG (ng/mg Crn)
Energy-adjusted fruit intake (g/d)			
T1 ($<$134.1)	56.8 (45.8–65.5)	1.21 (0.9–1.9)	8.5 (6.3–11.3)
T2 (134.1–248.0)	55.9 (42.9–66.6)	1.14 (0.8–1.6)	7.9 (5.8–9.9)
T3 ($\geq$248.0)	55.9 (42.3–63.2)	1.10 (0.6–1.6)	7.4 (5.9–9.9)
P for trend[*]	0.090	0.040	0.192
P for trend[†]	0.107	0.120	0.250
Energy-adjusted vegetable intake (g/d)			
T1 ($<$47.4)	57.3 (48.2–68.9)	1.34 (0.9–1.9)	8.7 (6.9–11.6)
T2 (47.4–84.1)	57.4 (43.9–66.6)	1.01 (0.6–1.6)	8.2 (6.1–10.3)
T3 ($\geq$84.1)	52.6 (42.2–61.0)	1.18 (0.8–1.6)	6.7 (5.7–9.0)
P for trend[*]	0.006	0.103	$<$0.001
P for trend[†]	0.006	0.098	$<$0.001
Energy-adjusted fruit and vegetable intake (g/d)			
T1 ($<$200.2)	57.1 (45.2–66.6)	1.29 (0.9–2.1)	8.6 (6.5–11.6)
T2 (200.2–341.1)	56.4 (44.9–64.8)	1.16 (0.8–1.7)	7.9 (5.9–9.8)
T3 ($\geq$341.1)	54.4 (41.1–62.3)	1.08 (0.7–1.5)	7.1 (5.7–9.6)
P for trend[*]	0.050	0.003	0.028
P for trend[†]	0.062	0.013	0.040

Crn, creatinine; HOMA-IR, homeostasis model assessment-estimated insulin resistance; ox-LDL, oxidized low-density lipoprotein; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-iso-PGF2α, 8-iso-prostaglandin F2α
Data are median (25th–75th quartile)

[*] P for trend from the linear regression model, adjusted for android fat, habitual physical activity, work position, excessive alcohol consumption, energy intake, free fat acid, and HOMA-IR.

[†] P for trend from the linear regression model, adjusted for same variables above plus current smoker.

Table 4
Oxidative stress markers with respect to tertiles (T) of energy–adjusted dietary fiber, vitamin C, and magnesium from fruit and vegetable intake

	Ox–LDL (U/L)	8–iso–PGF2α (ng/mg Crn)	8–OHdG (ng/mg Crn)
Energy–adjusted fiber from fruit and vegetable intake (g/d)			
T1 (<4.0)	57.2 (45.8–69.9)	1.20 (0.9–1.8)	8.6 (6.5–11.8)
T2 (4.0–6.5)	56.8 (45.0–64.8)	1.19 (0.8–1.8)	7.8 (5.7–9.6)
T3 (≥6.5)	50.3 (41.9–61.9)	1.10 (0.6–1.6)	7.3 (5.7–9.9)
P for trend*	0.013	0.034	0.100
P for trend†	0.016	0.085	0.140
Energy–adjusted vitamin C from fruit and vegetable intake (mg/d)			
T1 (<56.8)	57.3 (45.8–66.8)	1.15 (0.8–1.7)	8.3 (6.4–11.1)
T2 (56.8–98.0)	53.9 (43.2–66.3)	1.30 (0.8–2.1)	8.2 (5.8–10.6)
T3 (≥98.0)	55.8 (43.3–62.3)	1.00 (0.6–1.3)	7.1 (5.7–9.0)
P for trend*	0.201	0.007	0.050
P for trend†	0.230	0.019	0.070
Energy–adjusted magnesium from fruit and vegetable intake (mg/d)			
T1 (<30.4)	57.4 (45.2–69.7)	1.30 (1.0–2.1)	8.4 (6.6–11.5)
T2 (30.4–48.9)	56.2 (43.6–65.8)	1.10 (0.8–1.7)	7.9 (5.7–10.1)
T3 (≥48.9)	52.3 (42.5–61.9)	1.00 (0.6–1.6)	7.4 (5.7–9.6)
P for trend*	0.060	0.018	0.136
P for trend†	0.070	0.048	0.178

Crn, creatinine; 8-iso-PGF2α, 8-iso-prostaglandin F2α; ox-LDL, oxidized low-density lipoprotein; 8-OHdG, 8-hydroxy-2′-deoxyguanosine
Data are median (25th–75th quartile)

* *P* for trend from the linear regression model, adjusted for android fat, habitual physical activity, work position, excessive alcohol consumption, energy intake, and free fatty acid.

† *P* for trend from the linear regression model, adjusted for same variables above plus current smoker.

enterohepatic cycle that avoids the recycling of bile acids and increases the expression of the limiting cholesterol enzyme 7-α hydroxylase [53] may help to explain the reduction of the circulating LDL cholesterol molecules susceptible to oxidation.

Furthermore, the higher intake of magnesium from FV was associated with lower lipid peroxidation (ox-LDL and 8-iso-PGF2 α). In this context, an experimental study found higher antioxidant biomarkers levels and lower lipid peroxidation biomarkers levels in rats that consumed higher and lower quantities of magnesium, respectively [31]. Thus, the magnesium deficiency (which can be caused by its low intake [31]) may contribute to the release of inflammatory cytokines along with the excessive production of free radicals and promotion of oxidative stress. Moreover, the inflammation provoked by magnesium deficiency could promote proatherogenic changes in lipoprotein metabolism, thrombosis, endothelial dysfunction, and other metabolic disorders [54]. Therefore, we suggest the high consumption of FV could help minimize oxidative stress.

The statistical attenuation observed between oxidative stress biomarkers and food variables evaluated after adding "current smoker" as covariate in the models, could be explained by higher values of 8-iso-PGF2 α (2.29 ± 1.69 versus 1.36 ± 1.47 ng/mg creatinine; $P < 0.001$) and 8-OHdG (9.54 ± 4.29 versus 8.25 ± 2.90 ng/mg creatinine; $P = 0.080$) found among smokers compared with nonsmokers in the present study. In fact, oxidative stress was found high among smokers [55], probably because cigarette smoke contains potent oxidants, such as acrolein, hydroxyl radicals, and organic radicals [56]. Thus, smoking may have a negative effect on oxidative stress even in participants who consume foods such as FV with antioxidant capacity.

Finally, this study indicate that FV consumption, close (341.1–399 g/d) or similar (≥400 g/d) to that recommended by the WHO, could reach lower oxidative stress status, a recognized risk condition for cardiovascular diseases [2] and cancer [3]. Furthermore, the selection of FV as source of vitamin C, fiber, and magnesium also could help minimize oxidative stress. In this context, the implementation of public policies and adoption of multiple nutritional educational plans to stimulate high FV intake among middle-aged men should be encouraged in Brazil.

The limitations of this study relate to the cross-sectional nature of the investigation, for which we cannot prove the reported associations are causal. However, potential confounding variables were controlled as much as possible. We were unable to distinguish between cooking techniques used to prepare FV, which might influence the bioavailability of bioactive compounds, including vitamin C [57]. However, FV consumption analyses from FFQ have been related with oxidative stress markers in previous cross-sectional studies [29,30]. Further studies involving analysis of others contents of FV with potential antioxidant capacity (e.g., polyphenols and carotenoids) may clarify the role of FV on the redox status.

Conclusion

Fruit and vegetable intake was inversely associated with oxidative stress markers in middle-aged men. The vitamin C, fiber, and magnesium content from this food group seem to contribute to beneficial effects on the redox balance.

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