



Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrn.com

Applied nutritional investigation

Low energy and carbohydrate intake associated with higher total antioxidant capacity in apparently healthy adults



Kiriaque Barra Ferreira Barbosa Ph.D.^a, Ana Carolina Pinheiro Volp Ph.D.^b,
 José Luiz Marques-Rocha B.Sc.^{c,d}, Sônia Machado Rocha Ribeiro Ph.D.^c,
 Iñigo Navarro-Blasco Ph.D.^e, Maria Ángeles Zulet Ph.D.^{d,f},
 J. Alfredo Martínez Ph.D.^{d,f,*}, Josefina Bressan Ph.D.^c

^a Nutrition Center, Universidade Federal de Sergipe, Aracaju, Brazil^b Department of Social Clinical and Nutrition, Universidade Federal de Ouro Preto, Ouro Preto, Brazil^c Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Brazil^d Department of Nutrition, Food Science and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain^e Department of Chemistry and Soil Science, Navarra University, Pamplona, Spain^f CIBERobn, Physiopathology of Obesity, Carlos III Institute, Madrid, Spain

ARTICLE INFO

Article history:

Received 11 November 2013

Accepted 30 March 2014

Keywords:

Oxidative stress

Total antioxidant capacity

Dietary intake

Biomarkers

Antioxidant assessment

ABSTRACT

Objectives: The aim of this study was to investigate the associations between plasma total antioxidant capacity (TAC) and anthropometric, biochemical, clinical, and dietary measurements in young and apparently healthy individuals.

Methods: We evaluated 156 individuals (91 women and 65 men; ages 23.1 ± 3.5 y; body mass index 22 ± 2.9 kg/m²) for anthropometrics, biochemical markers, clinical, dietary, and some components of the antioxidant defense system, including the plasma TAC. Statistical analyses were performed to detect differences between individuals with TAC higher and lower than the mean value and to screen the associations between TAC and variables of interest. A linear regression model was fitted to identify TAC predictors.

Results: Daily caloric intake and macronutrient consumption were lower in individuals who exhibited the highest TAC values ($P < 0.05$). Linear regression analysis showed that daily calories and carbohydrate intake was a possible negative TAC predictor ($P < 0.05$). Nevertheless, there was no difference in the values of oxidized low-density lipoprotein in the individuals separated by means of TAC. In contrast, individuals whose plasma TAC values were above the mean showed higher low-density lipoprotein cholesterol concentrations, total cholesterol/high-density lipoprotein cholesterol values, and selenium in nails ($P < 0.05$).

Conclusions: In physiological conditions, the caloric intake level seems to be an important factor to act in the modulation of plasma TAC, before establishing anthropometric impairments of body or metabolic composition, or both. Additionally, the plasma TAC increase may be able to act as a compensatory mechanism.

© 2014 Elsevier Inc. All rights reserved.

This study was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG CDS 303/06). KB-B and AC-V contributed to the design and fieldwork, data collection, analysis, and drafting of the manuscript. JL-R, SM-R, IN-B, and MA-Z were involved in the design and the fieldwork as well as in the critical reading of the manuscript. JB was responsible for general coordination, follow-up, design, financial management, and the editing of the manuscript. JAM was a co-leader of the project and was responsible for follow-up, design, financial management, and editing of the manuscript. All of the authors actively participated in manuscript preparation, and all read and approved the final manuscript.

* Corresponding author: Tel.: +34 94 842 5600; fax: +34 94 842 5619.

E-mail address: jalfmtz@unav.es (J. A. Martínez).

Introduction

The continuous production of reactive oxygen species (ROS) during metabolic processes culminates in the activation of antioxidant defense mechanisms [1]. These protective mechanisms include some enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx); macromolecules such as ceruloplasmin; and other compounds such as the ascorbic acid, α -tocopherol, β -carotene, uric acid, selenium, copper, and zinc [2]. The total antioxidant capacity (TAC)

includes consideration of the occurrence of a synergic action of all antioxidants present in organic fluids, providing an integrative system between such compounds [3]. Thus, TAC has a higher predictive capacity and biological relevance when compared with the activity of a single antioxidant.

Several previous studies have shown that increasing the intake of antioxidant nutrients (e.g., vitamins C and E) or foods (e.g., tea, fruits, and vegetables) may have cardioprotective effects by reducing inflammation, improving vascular reactivity, and lowering oxidative stress (OS) [4–6]. However, the extent to which dietary compounds can have a significant influence on OS remains controversial [7].

In vitro studies have shown the capacity of diet antioxidant in removing the ROS or inhibiting their deleterious action [8,9]. However, in vivo studies have revealed divergent results regarding the effect of dietary antioxidants on TAC [10,11]. In this context, it is assumed that the complex interactions existing between several antioxidants limit the extension of evidences originating from in vitro studies.

Prior studies demonstrated a significant TAC reduction by several pathologic conditions associated with OS [12,13]. This reduction may be seen in obese individuals [14,15]; especially, those with increased visceral adiposity [16]. Moreover, there seem to be no doubts as for such participation in important alterations associated with metabolic syndrome [17–19], suggesting that OS precedes chronic diseases in some cases [20].

The antioxidant defense system capacity is a determining factor in health maintenance in prevention of diseases. Furthermore, the role of the diet and some nutrients on OS and its related diseases remains unclear [21,22]. Therefore, the present study investigated the possible existing associations between plasma TAC and anthropometrical, biochemical, clinical, and dietary measurements in young and apparently healthy individuals. Moreover, our study analyzed associations between plasma TAC and some endogenous (serum levels of uric acid and ceruloplasmin, GPx enzymatic activity) and exogenous (concentration of antioxidant minerals in nails: copper, zinc, and selenium) components of the antioxidant defense system and dietary intake of antioxidant compounds (vitamins C and A, zinc, and copper). Additionally, the TAC association with circulating levels of oxidized low-density lipoprotein (ox-LDL), another marker of OS, was investigated.

Material and methods

Participants

We recruited 156 individuals between the ages of 18 and 35 y to participate in the study (91 women and 65 men; ages 23.1 ± 3.5 y; and body mass index [BMI] 22 ± 2.9 kg/m²). Initial screening excluded individuals with evidence of any disease related to OS; chronic inflammation, hydric balance disorders, changes in body composition and nutrient absorption or metabolism. Other exclusion criteria were as follows: drug or nutritional treatment that affects energy balance, dietary intake, lipid profile, insulin levels, or glucose metabolism, contraceptive use up to 2 mo before participation in the study and weight loss diet follow-up or unstable weight in the past 6 mo. In agreement with the principles of the Helsinki Declaration and following a clear explanation of the study protocol, each participant signed a written informed consent form. The study was approved by the Human Research Ethics Committee of the Federal University of Viçosa, Brazil (protocol no. 009/2006). Participant recruitment occurred between January 2009 and February 2010.

Anthropometric and body composition assessments

Height was measured with a stadiometer (Seca 206 model, Hamburg, Germany) to the nearest 0.1 cm. Body weight was measured to the nearest 0.1 kg by using an electronic micro digital scale (Tanita TBF-300 A model, Tokyo, Japan). BMI was calculated by the quotient between body weight and square height (kg/m²).

Waist and hip circumference was measured with an inelastic and flexible tape to the nearest 0.1 m [23]. Triceps, biceps, subscapular and suprailiac skinfold thicknesses were measured to the nearest 1 mm by using a skinfold caliper (Lange caliper, Cambridge Scientific Industries Inc., Cambridge, MD, USA) [24]. The sum of skinfold thickness was calculated. Total body fat percentage was measured to the nearest 0.1% using a body composition analyzer (Biodynamics 310 model, Seattle, WA, USA). Body fat mass and body free fat mass were also estimated using the same body composition analyzer [25]. Truncal fat percentage was computed as the sum of subscapular and suprailiac skinfold thicknesses divided by the sum of four skinfold measurements [26].

Blood pressure assessment

Systolic and diastolic blood pressures were measured by a mercury sphygmomanometer (BIC, SP, Brazil) following World Health Organization criteria [27].

Analyses of biological samples

Blood samples were drawn by venous puncture after a 12-h overnight fast. The ethylenediaminetetraacetic acid plasma, heparin plasma, and serum samples were separated from whole blood by centrifugation at 3500g at 5°C for 15 min (Eppendorf AG, 5804 R model, Hamburg, Germany) and immediately stored at -80°C until analyzed.

Lipid and glucose profile

Serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations (mg/dL) were assessed by an automated colorimetric assay (BS-200, Shenzhen Mindray Bio-medical Electronics Co., Nanshan, China) using specific commercially available kits (Bioclin, Quibasa, Minas Gerais, Brazil). LDL cholesterol (LDL-C) data were calculated by the Friedewald equation as previously described [28] and validated [29]. The ratio of TC to HDL-C was also assessed [30]. Plasma insulin concentrations (sensitivity 2 $\mu\text{U/mL}$) were measured by an enzyme-linked immunosorbent assay kit as described by the supplier (Linco Research, St. Charles, MO, USA). Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance calculated as fasting glucose (nmol/L) \times fasting insulin ($\mu\text{U/mL}$)/22.5 [31].

Antioxidant biomarkers

Plasma TAC was assessed by a colorimetric assay, which relies on the ability of antioxidants in the sample to inhibit the oxidation of 2,2'-Azino-di-(3-ethylbenzthiazoline) sulphonate (ABTS) to ABTS^{•+} by metmyoglobin. The amount of ABTS^{•+} produced was monitored by reading the absorbance at 750 nm. Under the reaction conditions used, the antioxidants in the sample caused suppression of absorbance at 750 nm to a degree, which is proportional to their concentration. The capacity of antioxidants in the sample to prevent ABTS oxidation was compared with that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble tocopherol analog, and quantified as millimolar Trolox equivalents (709001, Cayman Chemical, Ann Arbor, MI, USA). Plasma ox-LDL concentrations (sensitivity < 6.56 U/L) were determined by an enzyme-linked immunosorbent assay kit (Merckodia, Uppsala, Sweden). GPx activity (nmol/[mL/min]) was measured in erythrocytes by a commercially available kit (703102, Cayman Chemical). Uric acid and ceruloplasmin concentrations (mg/dL), were assessed by an automated colorimetric assay (BS-200, Shenzhen Mindray Bio-medical Electronics Co., Nanshan, China) using specific commercially available kits (Bioclin, Quibasa, Minas Gerais, Brazil).

Trace elements in the nails

Nail samples were collected at the time of interview and stored at room temperature in clean polypropylene bags. Finger- and toenail samples were treated with sub-boiling nitric acid in a high-pressure Teflon digestion vessel using a microwave digestion system (Ethos Plus, Milestone, Sorisole, Italy). A Perkin Elmer Analyst 800 atomic absorption spectrometer (Norwalk, CT, USA), equipped with transverse-heated graphite atomizer, Zeeman background corrector, and AS-800 autosampler, was used for measurement of selenium at 196.0 nm with a spectral band width of 2.0 nm [32]. An electrodeless discharge lamp (Perkin Elmer) was used as a light source operated at 280 mA. Pyrolytic-coated graphite tubes with end caps supplied by Perkin Elmer were used. Zinc and copper concentrations in digested acid solutions were analyzed by flame atomic absorption spectrophotometry (Perkin Elmer). Zinc and copper hollow cathode lamps (Perkin Elmer) provided resonance lines of 213.9 and 324.8 and were operated at 15 mA with a slit width set at 0.7 nm. The measured concentration values were adjusted for the sample weight and expressed as $\mu\text{g/g}$ of nail for copper and zinc and ng/g of nail for selenium.

Dietary intake and lifestyle assessments

A 72-h food record was used to collect information about energy and nutrient intake. A booklet was given to the participants to record everything they ate or drank over a period of 3 non-consecutive d, including a weekend day. Dietary intake data was computed using a specific computer program (DietPro[®], version 5.0, AS Systems). Detailed instructions were given by the interviewer to the participants to record all possible information, such as amount of sugar in coffee, cooking method, type of food, and brand names of industrial foods. Household measures such as cups, bowls, spoons, etc. were provided to help the recording and quantification process. Geometric food models were also provided to help recording portion sizes.

Covariables about lifestyle such as vitamin supplementation users, smoking status (smokers or non-smokers), number of cigarettes smoked per day, regular physical activity (yes or no), and volume of physical activity were also collected. To quantify the volume of physical activity, an activity metabolic equivalent (MET) was used [33]. This index represents the ratio of energy expenditure during each specific activity to resting metabolic rate. METs were computed by a multiple of resting metabolic rate (MET score) to each activity. The MET scores were provided by Compendium of Physical Activities, a coding scheme that classifies specific physical activity by rate of energy expenditure. METs were calculated by multiplying time spent on each activity by a specific MET score to that activity. The scores were then summed over all activities to obtain a mean value of overall week, expressed in h/d.

Statistical analysis

The Kolmogorov-Smirnov normality test was used to determine variable distribution. Accordingly, the parametric Student's *t* test or nonparametric Mann-Whitney *U* test was performed to detect differences between individuals' TAC higher and lower than the median value (cutoff: 1.60 mM for anthropometric, clinical, biochemical, and lifestyle features data and cutoff: 1.62 mM for dietary intake data). Dichotomous variables were analyzed by χ^2 test. The Spearman correlation coefficients were used to screen the statistical associations between TAC and interest variables. Linear regression model, used to identify the predictors of TAC, was adjusted for covariables such as sex, age, smoking status, and regular physical activity, when they showed significant effect. Results are presented as mean \pm SD. Confidence intervals (95% CIs) were used to describe linear regression coefficient (β). $P < 0.05$ was considered statistically significant. Statistical analyses were performed by using SAS software system version 8.0 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

The results showed that individuals whose plasma TAC values were above the mean demonstrated higher serum LDL-C levels and higher relative TC-to-HDL-C ratio ($P < 0.05$) (see Table 1). The same occurred for selenium concentrations in the nails. No other biochemical or clinical parameter differed between the two groups with regard to plasma TAC cutoff criteria (≤ 1.60 versus > 1.60 mm). Likewise, no anthropometric measurements or body composition differences were found (Table 1).

Plasma TAC values were associated positively with selenium concentrations in the nails, and to the ratio of TC to HDL-C and to LDL-C serum levels. Nevertheless, there was no difference in the values of ox-LDL between the individuals separated by means of TAC (Table 1).

The daily macronutrients (g/kg) and calories (kcal/kg) intake values were lower among individuals who showed higher plasma TAC values ($P < 0.05$) (Table 2). The absolute carbohydrate ($P = 0.017$) and lipid ($P = 0.018$) daily intake (g) were also lower among individuals who showed higher plasma TAC values. The same tendency was also seen in the absolute daily calorie ($P = 0.056$) and protein intake ($P = 0.055$).

There was a positive correlation between the plasma TAC and selenium concentrations in nails ($r = 0.21$; $P = 0.015$) (not presented in the Table). The values of daily calorie and macronutrient intake correlated inversely to plasma TAC. However, these correlations were not statistically significant. No association was found for lifestyle variables such as number of cigarettes/d or

Table 1

Anthropometric, clinical, and biochemical data (mean \pm SD) for young adults categorized by TAC concentrations (1.60 mM)

	TAC ≤ 1.60 mM (n = 78)	TAC > 1.60 mM (n = 78)	P-value
Age (y)	23.5 \pm 3.5	22.8 \pm 3.6	0.055
Body weight (kg)	62.2 \pm 11.4	62.7 \pm 11.0	0.320
BMI (kg/m ²)	21.7 \pm 2.9	22.3 \pm 2.9	0.110
Waist circumference (cm)*	77.6 \pm 8.6	78.8 \pm 8.6	0.391
Hip circumference (cm)*	95.6 \pm 7.6	95.5 \pm 5.8	0.872
Truncal fat (%)	57.7 \pm 6.5	59.0 \pm 6.7	0.206
Total body fat (%)	23.1 \pm 6.7	24.3 \pm 6.5	0.190
Systolic blood pressure (mm Hg)	11.0 \pm 0.9	10.9 \pm 0.9	0.471
Diastolic blood pressure (mm Hg)	7.4 \pm 0.7	7.3 \pm 0.7	0.213
Glucose (mg/dL)	91.4 \pm 7.4	89.8 \pm 6.0	0.112
Insulin (μ U/mL) [†]	10.4 \pm 5.8	10.1 \pm 5.3	0.412
HOMA-IR [‡]	2.3 \pm 1.3	2.3 \pm 1.3	0.338
Total cholesterol (mg/dL)*	156.6 \pm 33.4	163.9 \pm 29.3	0.151
HDL-C (mg/dL) [§]	47.4 \pm 11.7	45.7 \pm 11.1	0.189
LDL-C (mg/dL)	91.1 \pm 25.9	100.4 \pm 27.1	0.012 [#]
Triglyceride (mg/dL)	96.7 \pm 36.9	103.4 \pm 51.5	0.340
TC-to-HDL-C ratio [‡]	3.4 \pm 0.7	3.7 \pm 0.9	0.006 [#]
Uric acid (mg/dL)*	3.5 \pm 1.1	3.6 \pm 1.1	0.413
Ceruloplasmin (mg/dL)	37.2 \pm 8.5	37.3 \pm 8.4	0.336
Selenium (ng/g of nail) [§]	372.8 \pm 74.2	398.4 \pm 92.4	0.040 [#]
Zinc (μ g/g of nail) [§]	118.5 \pm 27.5	140.0 \pm 86.2	0.126
Copper (μ g/g of nail) [§]	7.0 \pm 5.4	7.0 \pm 3.9	0.124
GPx activity (nmol/[mL/min])	608.3 \pm 300.4	552.5 \pm 262.5	0.237
Ox-LDL (U/L)*, [¶]	77.3 \pm 30.1	70.5 \pm 29.4	0.157

BMI, body mass index; GPx, glutathione peroxidase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; ox-LDL, oxidized low-density lipoprotein; ST, skinfold thickness; TAC, total antioxidant capacity; TC, total cholesterol

* Student's *t* test for variables with normal distribution. The remaining variables were analyzed by Mann-Whitney *U* test.

[†] n = 77 for TAC ≤ 1.60 mM; n = 75 for TAC > 1.60 mM.

[‡] n = 76 for TAC ≤ 1.60 mM; n = 73 for TAC > 1.60 mM.

[§] n = 67 for TAC ≤ 1.60 mM; n = 64 for TAC > 1.60 mM.

^{||} n = 54 for TAC ≤ 1.60 mM; n = 43 for TAC > 1.60 mM.

[¶] n = 77 for TAC ≤ 1.60 mM.

[#] $P < 0.05$.

physical activity energy expenditure related to the resting metabolic rate (not presented in the Table).

In the linear regression analysis, the daily calorie and carbohydrate intake had a negative predictive effect on plasma TAC (see Table 3). The decrease of 1 unit in energy (1 kcal/kg) and carbohydrate (1 g/kg) intake determined an increase of 0.019 and 0.113 mm, respectively, in plasma TAC.

Discussion

The capacity of antioxidants in vivo to scavenging free radicals is determined by several factors [34]. Most of the assays partially failed in obtaining a good reproducibility when using plasma because it is composed of a large number of substances, some of which possess masking features [35,36]. In this sense, the current work investigated different types of markers of antioxidant status to evaluate some of the important components of this protective system.

In physiological conditions, a great part of TAC appears to be due to the endogenous component activity (nearly 85%), as opposed to the participation of dietary antioxidant [37,38]. The ineffectiveness of dietary antioxidant in increasing plasma TAC is further proven by the findings of this study. Other studies demonstrated that supplementation with vitamins and antioxidant minerals was not able to increase biological fluid TAC [11],

Table 2

Lifestyle features and daily nutrient intake (mean \pm SD) for young adults categorized by TAC concentrations (cutoff: 1.60 mM for lifestyle features and 1.62 mM for daily nutrient intake)

	TAC \leq 1.60 mM (n = 78)	TAC > 1.60 mM (n = 78)	P-value
Lifestyle features			
Vitamin supplementation users (%) [*]	5.2	7.7	0.711
Smokers (%) ^{*,†}	11.9	13.0	0.762
Regular practice of sport (%) ^{*,†}	71.6	73.9	0.901
Smoking (cigarettes/d) [†]	1.6 \pm 4.8	0.8 \pm 2.7	0.484
MET (h/d)	30.3 \pm 9.0	31.2 \pm 10.0	0.232
	TAC \leq 1.62 mM (n = 66)	TAC > 1.62 mM (n = 66)	P-value
Daily nutrient intake			
Total calorie (kcal) [‡]	2843.2 \pm 748.8	2595.4 \pm 742.4	0.056
Carbohydrate (g)	376.9 \pm 114.1	332.5 \pm 97.7	0.017 [#]
Protein (g)	106.7 \pm 34.5	99.1 \pm 34.5	0.055
Lipid (g) [‡]	103.2 \pm 33.7	90.5 \pm 26.0	0.018 [#]
Total calorie (kcal/kg) [‡]	46.5 \pm 12.5	41.7 \pm 11.3	0.024 [#]
Carbohydrate (g/kg)	6.1 \pm 1.7	5.3 \pm 1.4	0.009 [#]
Protein (g/kg)	1.7 \pm 0.5	1.5 \pm 0.5	0.034 [#]
Lipid (g/kg)	1.6 \pm 0.6	1.4 \pm 0.4	0.013 [#]
Fiber (g)	27.5 \pm 14.4	26.1 \pm 16.3	0.160
Cholesterol (g)	234.7 \pm 103.2	209.9 \pm 93.4	0.081
SFAs (g)	23.4 \pm 9.9	21.4 \pm 9.4	0.143
MUFAs (g)	17.1 \pm 11.2	16.0 \pm 6.8	0.416
PUFAs (g)	14.7 \pm 9.6	13.7 \pm 7.5	0.264
Vitamin A (mg)	886.6 \pm 507.6	921.7 \pm 537.4	0.321
Vitamin C (mg)	136.5 \pm 116.9	107.7 \pm 64.0	0.083
Iron (mg)	16.5 \pm 6.5	19.6 \pm 30.3	0.068
Copper (mg)	1.2 \pm 0.6	1.4 \pm 1.5	0.252
Zinc (mg) [‡]	8.5 \pm 3.8	8.1 \pm 3.3	0.550
Selenium (μ g)	100 \pm 26	117 \pm 23	0.099

MET, activity metabolic equivalent; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TAC, total antioxidant capacity Dietary intake data was computed using DietPro®, version 5.0, AS Systems

^{*} χ^2 test for dichotomous variables.

[†] n = 67 for TAC \leq 1.60 mM; n = 69 for TAC > 1.60 mM.

[‡] Student's *t* test for variables with normal distribution. The remaining variables were analyzed by Mann-Whitney *U* test.

[#] *P* < 0.05.

even with the increase of plasma or serum levels of such compounds [39,40]. The biological variability of some components of the antioxidant system may be a limiting factor of its application as a predictor of physiopathological events [41]. This fact could explain the absence of associations between TAC and other components of the antioxidant system, such as serum levels of

Table 3

Possible predictors of TAC concentrations by a linear regression analysis^{*}

	β coefficient (95% CI)	P-value
LDL-C (mg/dL) [†]	0.004 (–0.003 to 0.012)	0.273
TC-to-HDL-C ratio [†]	0.016 (–0.012 to 0.043)	0.075
Selenium (ng/g of nail) [‡]	0.002 (–0.001 to 0.004)	0.139
Caloric intake (kcal/d) [‡]	0.001 (–0.000 to 0.002)	0.058
Carbohydrate (g/d) [§]	–0.031 (–0.003 to 0.001)	0.059
Protein (g/d) [§]	–0.008 (–0.015 to 0.000)	0.057
Lipid (g/d) [§]	–0.009 (–0.019 to 0.002)	0.077
Daily caloric intake (kcal/kg) [§]	–0.019 (–0.024 to –0.002)	0.036 [#]
Daily carbohydrate intake (g/kg) [§]	–0.113 (–0.195 to –0.037)	0.009 [#]
Daily protein intake (g/kg) [§]	–0.201 (–0.414 to –0.000)	0.050
Daily lipid intake (g/kg) [§]	–0.479 (–1.582 to 0.123)	0.105

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAC, total antioxidant capacity; TC, total cholesterol

^{*} Analysis was adjusted by sex and age.

[†] n = 149.

[‡] n = 131.

[§] n = 132.

[#] *P* < 0.05.

uric acid, ceruloplasmin, and erythrocyte GPx activity. The lack of association among some antioxidant compounds demonstrates the complex interaction to maintain oxidative status.

The most important antioxidant enzymes in erythrocytes are the SOD, CAT, and GPx, which play an important role in ROS elimination [42]. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, whereas CAT converts hydrogen peroxide into water (2). The major function of GPx, which uses glutathione as a substrate, is to reduce soluble hydrogen peroxide and alkyl peroxides [43]. These enzymes work together to prevent oxidative damage to the cell [44]. In this sense, we choose the one with more data in the scientific literature to evaluate this pathway of the antioxidant system.

This study showed that the selenium concentrations occurring in the nails were related to TAC but the selenium intake was not. The antioxidant activity of this mineral is in part due to its participation as a GPx enzymatic cofactor [45]. The selenium quantification in the nails is explained by the capacity of maintaining a long-term nutritional status based on consumption over the preceding 6 to 18 mo [46]. Meanwhile, its blood dosage is limited to a short-term nutritional status from a few preceding weeks [47]. The fact that selenium dosage in the nails is an indicator of its usual dietetic intake possibly has been a determinant of the positive association observed between the concentrations of this mineral in the nails and plasma TAC. On the other hand, the variability of selenium content in food sources and consumption tables, which depends on geographic areas, soil, climate factors, or the elaboration of dishes, among others, may explain why there is no correlation between TAC and selenium intake.

Caloric restriction has been associated with the modulation of OS [48]. Intervention studies showed positive effects of calorie restriction on the oxidation of lipids, proteins [49], and DNA [50], as well as an effect on some markers of the antioxidant defense system [51]. Some studies showed that these protective effects on the onset of OS are mainly attributed to weight reduction [6, 48,52]. However, the present study demonstrated that in healthy and predominantly eutrophic individuals (BMI < 25 kg/m²; 84%), the calorie and macronutrient intake were lower among individuals with higher plasma TAC, after normalized by body weight. For the regression analysis, normalized by sex and age, it was demonstrated that that 3.7% and 5.1% of the increase in plasma TAC was due to the effect of calorie and carbohydrate intake, respectively. Nevertheless, the statistical significance is lost when these analysis are made normalizing the data by the energy intake.

A plausible explanation is that in addition to a decrease in energy content, changes in the content and the amount of macronutrients consumed affected systemic oxidative balance. It would be viable to consider that with lower anion superoxide radical generation resulting from lower availability of oxidizable substrates, there would be less recruitment of enzymatic defense systems, consequently preserving higher plasma TAC. Indeed, previous research has shown that lipid, carbohydrate [53], and protein intake [54] leads to an increase of OS and inflammation status, respectively.

When the generation of ROS overcomes the antioxidant defense systems, the OS condition is evidenced [55], which has relevant implications for a number of pathologic conditions [56, 57]. In physiological conditions, the biological system recruits regulation mechanisms that aim to limit intracellular levels of such reactive species and to control the incidence of oxidative damage [58]. Such regulation consists of negative feedback mechanisms through which the biological system activates gene expression and enzyme pathways to restore homeostatic balance [58,59].

Based on these findings, it is possible to justify that in the present study, the highest plasma TAC was associated with higher LDL-C circulating levels and higher ratios of TC to HDL-C. The LDL-C is the substrate for the oxidation reaction, mediated by the substitution of approximately 60 lysine residues in apolipoprotein B100, which results in the generation of ox-LDL [60]. Moreover, the atherosclerotic process is intimately associated with the onset of OS [61]. In the presence of pathological conditions associated with OS, the increase of TAC as a compensatory mechanism is not feasible. The substantial increase of free radicals, resulting from pathologic processes, exceeds the compensative increased capacity of antioxidant defenses [12,13]. However, it is important to highlight that the present study was conducted with young, apparently healthy individuals, with no physiopathological condition associated with OS and according to the cutoff points of the IV Brazilian Guidelines on dyslipidemia and atherosclerosis prevention [62], predominantly normolipidemic (TC < 200 mg/dL, 92.3%; LDL-C < 160 mg/dL, 97.4%). Thus, it is possible to consider the increase of plasma TAC as a compensatory system, with the purpose of restoring homeostatic balance. This suggests that TAC may be a link between the redox state and atherogenesis at early stage.

The action of OS associated with visceral adipose tissue results in the increase of adipokine expression, inflammatory signaling, and induction of insulin resistance. These factors predispose the generation of ROS and consequent onset of OS [57,63]. In the present study, the small number of obese individuals (BMI ≥ 30 kg/m², 1.3%), as well as elevated waist circumference values (102 cm for men and 88 for women, 4.8%), [64] might have determined the absence of associations between plasma TAC and these anthropometric parameters as opposed to other adiposity measurements such as skinfolds, percent body fat, and body fat mass.

It is important to note that various measurement methods have been developed and improved for total antioxidant status. To date and to our knowledge, the ABTS is the most common method used in the field and many recent studies were published using this method to evaluate the plasma TAC [5,65–67]. Additionally, some studies found good reproducibility and correlation between the results measured by ABTS and other methods [68–71]. However, there is not yet an accepted reference method and there are some limitations in all of them. Therefore, the results from this method need to be analyzed carefully, taking into account the debate about the actual meaning of antioxidants and the evaluation of their overall activity; it is known that capacity of free radical scavenging does not always correlate well with the capacity to inhibit oxidation [34].

This study presents some limitations that should be considered. The dietary assessment software did not provide important information to discuss the data deeply. The software used does not provide quantitatively different types of carbohydrates, fiber, and starches consumed by participants. With the data available, it was not possible to calculate the glycemic index and glycemic load. Furthermore, this is a cross-sectional study and the associations observed should be checked carefully and further studies should be performed to confirm these interesting results.

Conclusions

In young and apparently healthy individuals, the calorie intake level seems to be the first line of defense in the modulation of plasma TAC, before establishing anthropometric impairments of body and/or metabolic composition. In physiological conditions,

the plasma TAC increase may be able to act as a compensatory mechanism in the presence of oxidation.

Acknowledgments

The authors acknowledge Sérgio O. de Paula, D.S., Leandro L. de Oliveira, D.S., Antônio Policarpo S. Carneiro, D.S., and Neuza M. B. Costa, D.S., for technical assistance, and Łukasz M. Grześkowiak (funded by CAPES), for language review. Elisângela Lessa, Damiana D. Rosa, and Carolina O. Resende helped with data collection. We thank all volunteers for their participation in the study. Also CIBERobn and Linea Especial LE/97 from the University of Navarra are gratefully credited.

References

- [1] Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;1:47–95.
- [2] Valdecantos MP, Perez-Matute P, Martinez JA. Obesity and oxidative stress: role of antioxidant supplementation. *Rev Invest Clin* 2009;2:127–39.
- [3] Serafini M, Del Rio D. Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool? *Redox Rep* 2004;3:145–52.
- [4] Floegel A, Chung SJ, von Ruesten A, Yang M, Chung CE, Song WO, et al. Antioxidant intake from diet and supplements and elevated serum C-reactive protein and plasma homocysteine concentrations in US adults: a cross-sectional study. *Public Health Nutr* 2011;11:2055–64.
- [5] Hermsdorff HH, Barbosa KB, Volp AC, Puchau B, Bressan J, Zulet MA, et al. Vitamin C and fibre consumption from fruits and vegetables improves oxidative stress markers in healthy young adults. *Br J Nutr* 2012;8:1119–27.
- [6] de la Iglesia R, Lopez-Legarrea P, Celada P, Sanchez-Muniz FJ, Martinez JA, Zulet MA. Beneficial effects of the RESMENA dietary pattern on oxidative stress in patients suffering from metabolic syndrome with hyperglycemia are associated to dietary TAC and fruit consumption. *Int J Mol Sci* 2013;4:6903–19.
- [7] Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radic Biol Med* 2006;12:1727–46.
- [8] Serafini M, Laranjinha JA, Almeida LM, Maiani G. Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. *J Nut Biochem* 2000;11–12:585–90.
- [9] Duarte TL, Jones GD. Vitamin C modulation of H₂O₂-induced damage and iron homeostasis in human cells. *Free Radic Biol Med* 2007;8:1165–75.
- [10] Karlén A, Retterstol L, Laake P, Kjolsrud-Bonh S, Sandvik L, Blomhoff R. Effects of a daily intake of one glass of red wine on biomarkers of antioxidant status, oxidative stress and inflammation in healthy adults. *e-SPEN* 2007;1–2:127–33.
- [11] Navas-Carretero S, Cuervo M, Abete I, Zulet MA, Martinez JA. Frequent consumption of selenium-enriched chicken meat by adults causes weight loss and maintains their antioxidant status. *Biol Trace Elem Res* 2011;1:8–19.
- [12] Demirbag R, Yilmaz R, Kocycigit A. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. *Mut Res* 2005;2:197–203.
- [13] Kurban S, Mehmetoglu I, Oran B, Kiyici A. Homocysteine levels and total antioxidant capacity in children with acute rheumatic fever. *Clin Biochem* 2008;1–2:26–9.
- [14] Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond)* 2006;3:400–18.
- [15] Krzystek-Korpacka M, Patryn E, Boehm D, Berdowska I, Zielinski B, Noczynska A. Advanced oxidation protein products (AOPPs) in juvenile overweight and obesity prior to and following weight reduction. *Clin Biochem* 2008;12:943–9.
- [16] Chrysohoou C, Panagiotakos DB, Pitsavos C, Skoumas I, Papademetriou L, Economou M, et al. The implication of obesity on total antioxidant capacity in apparently healthy men and women: the ATTICA study. *Nutr Metab Cardiovasc Dis* 2007;8:590–7.
- [17] Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovasc Ther* 2012;1:49–59.
- [18] Hermsdorff HH, Puchau B, Volp AC, Barbosa KB, Bressan J, Zulet MA, et al. Dietary total antioxidant capacity is inversely related to central adiposity as well as to metabolic and oxidative stress markers in healthy young adults. *Nutr Metab (Lond)* 2011;8:59.
- [19] Bondia-Pons I, Ryan L, Martinez JA. Oxidative stress and inflammation interactions in human obesity. *J Physiol Biochem* 2012;4:701–11.
- [20] Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 2004;5:816–23.

- [21] Barbosa KB, Bressan J, Zulet MA, Martinez Hernandez JA. Influence of dietary intake on plasma biomarkers of oxidative stress in humans. *An Sist Sanit Navar* 2008;3:259–80.
- [22] Hollman PC, Cassidy A, Comte B, Heinonen M, Richelle M, Richling E, et al. The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. *J Nutr* 2011;5:989–1009.
- [23] Salas-Salvado J, Rubio MA, Barbany M, Moreno B. Consensus for the evaluation of overweight and obesity and the establishment of therapeutic intervention criteria. *Med Clin (Barc)* 2007;5:184–96.
- [24] Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: Measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 1974;1:77–97.
- [25] Barbosa KB, Volp AC, Hermsdorff HH, Navarro-Blasco I, Zulet MA, Martinez JA, et al. Relationship of oxidized low density lipoprotein with lipid profile and oxidative stress markers in healthy young adults: a translational study. *Lipids Health Dis* 2011;10:61.
- [26] Warnberg J, Nova E, Moreno LA, Romeo J, Mesana MI, Ruiz JR, et al. Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. *Am J Clin Nutr* 2006;3:505–12.
- [27] Whitworth JA, Chalmers J. World health organisation-international society of hypertension (WHO/ISH) hypertension guidelines. *Clin Exp Hypertens* 2004;7–8:747–52.
- [28] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;6:499–502.
- [29] Tremblay AJ, Morrissette H, Gagne JM, Bergeron J, Gagne C, Couture P. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. *Clin Biochem* 2004;9:785–90.
- [30] Castelli WP. Cholesterol and lipids in the risk of coronary artery disease—the Framingham Heart Study. *Can J Cardiol* 1988;4:5–10.
- [31] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;7:412–9.
- [32] Navarro-Blasco I, Alvarez-Galindo JL. Selenium content of Spanish infant formulae and human milk: Influence of protein matrix, interactions with other trace elements and estimation of dietary intake by infants. *J Trace Elem Med Biol* 2004;4:277–89.
- [33] Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 2000;9:498–504.
- [34] Niki E. Antioxidant capacity: which capacity and how to assess it? *J Ber Res* 2011;4:169–76.
- [35] Blauz A, Pilaszek T, Grzelak A, Dragan A, Bartosz G. Interaction between antioxidants in assays of total antioxidant capacity. *Food Chem Toxicol* 2008;7:2365–8.
- [36] Bompadre S, Leone L, Politi A, Battino M. Improved FIA-ABTS method for antioxidant capacity determination in different biological samples. *Free Radic Res* 2004;8:831–8.
- [37] Kampa M, Nistikaki A, Tsaousis V, Maliraki N, Notas G, Castanas E. A new automated method for the determination of the total antioxidant capacity (TAC) of human plasma, based on the crocin bleaching assay. *BMC Clin Pathol* 2002;1:3.
- [38] Crews H, Alink G, Andersen R, Braesco V, Holst B, Maiani G, et al. A critical assessment of some biomarker approaches linked with dietary intake. *Br J Nutr* 2001;1:5–35.
- [39] Huang HY, Appel LJ, Croft KD, Miller ER, Mori TA, Puddey IB. Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial. *Am J Clin Nutr* 2002;3:549–55.
- [40] Dunstan JA, Breckler L, Hale J, Lehmann H, Franklin P, Lyons G, et al. Supplementation with vitamins C, E, beta-carotene and selenium has no effect on anti-oxidant status and immune responses in allergic adults: a randomized controlled trial. *Clin Exp Allergy* 2007;2:180–7.
- [41] Bolzan AD, Bianchi MS, Bianchi NO. Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: influence of sex, age and cigarette smoking. *Clin Biochem* 1997;6:449–54.
- [42] Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006;4:601–23.
- [43] Bebe FN, Panemangalore M. Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats. *J Environ Sci Health B* 2003;3:349–63.
- [44] Eroglu S, Pandir D, Uzun FG, Bas H. Protective role of vitamins C and E in diclorvos-induced oxidative stress in human erythrocytes in vitro. *Biol Res* 2013;1:33–8.
- [45] Steinbrenner H, Sies H. Protection against reactive oxygen species by selenoproteins. *Biochim Biophys Acta* 2009;11:1478–85.
- [46] Al-Saleh I, Billedo G. Determination of selenium concentration in serum and toenail as an indicator of selenium status. *Bull Environ Contam Toxicol* 2006;2:155–63.
- [47] Longnecker MP, Stampfer MJ, Morris JS, Spate V, Baskett C, Mason M, et al. A 1-y trial of the effect of high-selenium bread on selenium concentrations in blood and toenails. *Am J Clin Nutr* 1993;3:408–13.
- [48] Buchowski MS, Hongu N, Acra S, Wang L, Warolin J, Roberts LJ 2nd. Effect of modest caloric restriction on oxidative stress in women, a randomized trial. *PLoS One* 2012;10. e47079.
- [49] Johnson JB, Summer W, Cutler RG, Martin B, Hyun DH, Dixit VD, et al. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radic Biol Med* 2007;5:665–74.
- [50] Saiki A, Nagayama D, Ohhira M, Endoh K, Ohtsuka M, Koide N, et al. Effect of weight loss using formula diet on renal function in obese patients with diabetic nephropathy. *Int J Obes (Lond)* 2005;9:1115–20.
- [51] Skrha J, Kunesova M, Hilgertova J, Weiserova H, Krizova J, Kotlikova E. Short-term very low calorie diet reduces oxidative stress in obese type 2 diabetic patients. *Physiol Res* 2005;1:33–9.
- [52] Lopez-Legarrea P, de la Iglesia R, Abete I, Bondia-Pons I, Navas-Carretero S, Forga L, et al. Short-term role of the dietary total antioxidant capacity in two hypocaloric regimes on obese with metabolic syndrome symptoms: the RESMENA randomized controlled trial. *Nutr Metab (Lond)* 2013;1:22.
- [53] Tsai WC, Li YH, Lin CC, Chao TH, Chen JH. Effects of oxidative stress on endothelial function after a high-fat meal. *Clin Sci (Lond)* 2004;3:315–9.
- [54] Lopez-Legarrea P, de la Iglesia R, Abete I, Navas-Carretero S, Martinez JA, Zulet MA. The protein type within a hypocaloric diet affects obesity-related inflammation: the RESMENA project. *Nutrition* 2014;30(4):424–9.
- [55] Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol* 2004;2:231–55.
- [56] Galili O, Versari D, Sattler KJ, Olson ML, Mannheim D, McConnell JP, et al. Early experimental obesity is associated with coronary endothelial dysfunction and oxidative stress. *Am J Physiol Heart Circ Physiol* 2007;2:H904–11.
- [57] Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. *Life Sci* 2009;21–22:705–12.
- [58] Munoz A, Costa M. Nutritionally mediated oxidative stress and inflammation. *Oxid Med Cell Longev* 2013;2013:610950.
- [59] Hicks JJ, Torres-Ramos YD, Sierra-Vargas MP. Estrés oxidante. Concepto y clasificación. *R Endoc Nutr* 2006;4:223–6.
- [60] Holvoet P. Obesity, the metabolic syndrome, and oxidized LDL. *Am J Clin Nutr* 2006;6:1438.
- [61] Katakami N, Sakamoto K, Kaneto H, Matsuhisa M, Shimizu I, Ishibashi F, et al. Combined effect of oxidative stress-related gene polymorphisms on atherosclerosis. *Biochem Biophys Res Commun* 2009;4:861–5.
- [62] SBC. Sociedade Brasileira de Cardiologia, IV Diretriz Brasileira sobre dislipidemias e prevenção de aterosclerose. *Arq Bra Card* 2007;88:2–19.
- [63] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004;12:1752–61.
- [64] NCEP-ATPIII. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;19:2486–97.
- [65] Yucel H, Ozyaydin M, Dogan A, Erdogan D, Icli A, Sutcu R. Evaluation of plasma oxidative status in patients with slow coronary flow. *Kardiol Pol* 2013;6:588–94.
- [66] Kamodyova N, Tothova L, Celec P. Salivary markers of oxidative stress and antioxidant status: influence of external factors. *Dis Markers* 2013;5:313–21.
- [67] Khajehnasiri F, Mortazavi SB, Allameh A, Akhondzadeh S, Hashemi H. Total antioxidant capacity and malondialdehyde in depressive rotational shift workers. *J Environ Public Health* 2013;2013:150693.
- [68] Bahadoran Z, Golzarand M, Mirmiran P, Shiva N, Azizi F. Dietary total antioxidant capacity and the occurrence of metabolic syndrome and its components after a 3-year follow-up in adults: Tehran Lipid and Glucose Study. *Nutr Metab (Lond)* 2012;1:70.
- [69] Rendo-Urteaga T, Puchau B, Chueca M, Oyarzabal M, Azcona-Sanjulian MC, Martinez JA, et al. Total antioxidant capacity and oxidative stress after a 10-week dietary intervention program in obese children. *Eur J Pediatr* 2013;173:609–16.
- [70] Puchau B, Zulet MA, de Echavarri AG, Hermsdorff HH, Martinez JA. Dietary total antioxidant capacity is negatively associated with some metabolic syndrome features in healthy young adults. *Nutrition* 2010;5:534–41.
- [71] Puchau B, Zulet MA, de Echavarri AG, Hermsdorff HH, Martinez JA. Dietary total antioxidant capacity: A novel indicator of diet quality in healthy young adults. *J Am Coll Nutr* 2009;6:648–56.