Modulators of erythrocyte glutathione peroxidase activity in healthy adults: An observational study

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The aim of this study was to investigate the potential modulators of erythrocyte glutathione peroxidase (GPx) activity in young and apparently healthy individuals. One hundred one individuals (53 women and 48 men) were evaluated for anthropometric measurements, biochemical markers, clinical, dietary, and endogenous and exogenous components of the antioxidant defense system. Statistical analysis was performed to detect differences among subjects by the median of GPx activity. A linear regression model and Spearman correlation coefficients were used to screen the associations between GPx activity and interest variables. Individuals with higher GPx enzymatic activity were older and higher circulating levels of oxidized low-density lipoprotein (ox-LDL) values, but conversely lower nail concentrations of selenium and copper ($P < 0.05$). The GPx activity was positively correlated to truncal fat percentage values ($r = 0.24$, $P = 0.016$), circulating levels of ox-LDL ($r = 0.28$, $P = 0.004$), and daily vitamin C intake ($r = 0.28$, $P = 0.007$), and negatively correlated to the nail concentration of selenium ($r = -0.24$, $P = 0.026$). Interesting, it was noticed that the truncal fat percentage and circulating levels of ox-LDL explained 5.9 and 7.4% of the GPx enzymatic activity. Thus, preventive measures such as adequate antioxidant intake and proper fat percentage would be a priority in the nutritional care of young and apparently healthy individuals.

Keywords: Erythrocyte GPx activity, Oxidative stress, Antioxidant capacity, Dietary intake, Biomarkers, Antioxidant assessment

Introduction

An imbalance between antioxidant defenses and toxic compounds such as reactive oxygen species (ROS) may activate oxidative stress (OS).¹ In physiological conditions, cells have several antioxidative mechanisms to alleviate the effects of this biological process.² The OS can be assayed by measuring markers of the oxidative damage or by measuring levels of antioxidants.

Circulating erythrocytes are exposed to a high partial pressure of oxygen, have membranes rich in polyunsaturated fatty acids and contain large amounts of iron.³ Thus, erythrocyte glutathione peroxidase (GPx) activity has been reported as an important marker of the efficiency of the antioxidant

enzymatic system,⁴ whose integrated and synergistic actions determine the removal of ROS, as well as controlling or repairing any accompanying oxidative injuries.⁵

Damage related to OS has been associated with the onset of some processes involved in metabolic syndrome features.⁴ Some studies claim that OS plays an important role in endothelial pathogenesis⁶ and inflammatory conditions.^{7,8} However, it is not clear whether these processes are cause or consequence of these interactions. Furthermore, little is known about the limit at which the physiological response to the occurrence of ROS becomes adverse or pathological.

Thus, this study aimed to investigate the potential role of existing mediators of the erythrocyte GPx activity in young and apparently healthy individuals. We investigated the putative relationships among

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erythrocyte GPx activity and anthropometrical, biochemical, clinical, and dietary measurements including some endogenous (serum levels of uric acid and ceruloplasmin) and exogenous (concentration of antioxidant minerals: copper, zinc, and selenium) components of the antioxidant defense system. Additionally, the impact on GPx of dietary intake of specific antioxidant compounds (vitamin C, vitamin A, zinc, and copper) and other markers of OS (circulating levels of oxidized low-density lipoprotein (ox-LDL) and antioxidant capacity of the plasma) were also verified.

Materials and methods

Subjects

One hundred and one individuals aged between 18 and 35 years were recruited to participate in the study (53 women and 48 men; age: 23.1 ± 3.4 years; body mass index: 22.0 ± 2.7 kg/m²). Initial enrollment screening included evaluations to exclude subjects with evidence of any disease related to OS, chronic inflammation, fluid imbalance, changes of body composition, and nutrient absorption or metabolism. Other exclusion criteria were drug or nutritional treatment that affects energy balance, dietary intake, lipid profile, insulin levels, and glucose metabolism; contraceptive use up to 2 months before participation in this study; recent follow-up of weight-loss diets or unstable weight in the past six months. The study was approved by the Human Research Ethics Committee of the Federal University of Viçosa, Brazil (protocol number 009/2006) in accordance with the principles of the Helsinki Declaration.

Anthropometric and body composition assessments

Anthropometric and body composition were assessed by validated procedures. Thus, 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 using an electronic microdigital scale (Tanita[®] TBF-300A model, Tokyo, Japan). Body mass index was calculated by the quotient between body weight and squared height (kg/m²). Waist circumference was measured midway between the lowest rib and the iliac crest, and hip circumference was measured at the maximal hip circumference without gluteal contraction. Both circumferences were measured with an inelastic and flexible tape to the nearest 0.1 cm. The waist-to-hip ratio was also calculated. Triceps, biceps, subscapular, and suprailiac skinfold thicknesses (ST) were measured to the nearest 1 mm using a skinfold caliper (Lange caliper, Cambridge Scientific Industries Inc.[®], Cambridge, MD, USA). Total body fat percentage, body fat mass, and free fat body mass

were measured using a body composition analyzer (Biodynamics 310 model[®], Washington, USA). Truncal fat percentage was calculated as the sum of subscapular and suprailiac ST divided by the sum of four ST.⁹

Blood pressure assessment

Systolic and diastolic blood pressures were measured with a mercury sphygmomanometer (BIC[®], São Paulo, SP, Brazil) following World Health Organization Criteria (2004).

Analyses of biological samples

Blood samples were drawn by vein puncture after a 12-hour overnight fast. The ethylenediaminetetraacetic acid plasma, heparin plasma, and serum samples were separated from whole blood by centrifugation at $2200 \times g$ at 5°C for 15 minutes (Eppendorf AG[®], 5804R model, Hamburg, Germany) and immediately stored at -80°C until analyzed.

Lipid and glucose profile

Serum glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triacylglycerol 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, MG, Brazil). Low-density lipoprotein cholesterol (LDL-c) data were calculated by the Friedewald equation.¹⁰ The total cholesterol-to-HDL-c ratio was also assessed.¹¹ Plasma insulin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) kit (Linco Research[®], St. Charles, USA). Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance.¹²

Antioxidant biomarkers

GPx activity (nmol/(ml/minute)) was measured in erythrocytes by a commercially available kit (Cayman Chemical[®], Ann Arbor, MI, catalog no. 703102). This assay measures GPx activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione produced upon reduction of hydroperoxide by GPx is recycled to its reduced state by GR and nicotinamide adenine dinucleotide phosphate (NADPH). The oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A₃₄₀ is directly proportional to the GPx activity in the sample. TAC was assessed by a colorimetric assay, which relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2,2'-Azino-di-(3-ethylbenzthiazoline sulphonate)) to ABTS•+ by metmyoglobin, which is subsequently quantified as a

mmol Trolox equivalent (mM) as described by the supplier (Cayman Chemical[®], MI, USA, catalog no. 709001). Plasma ox-LDL concentrations were determined by an ELISA assay kit (Mercodia[®], Uppsala, Sweden). 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[®], MG, Brazil).

Trace elements in the nails

Nail samples were collected at the time of interview and stored at room temperature in clean polypropylene bags. Fingernail 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 the measurement of selenium at 196 nm with a spectral band width of 2.0 nm.¹³ An electrodeless discharge lamp (Perkin Elmer) was used as the 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 microgram per gram ($\mu\text{g/g}$) of nail for copper and zinc and nanogram per gram of nail for selenium.

Dietary intake and lifestyle assessments

A 72-hour food record was used to collect information about antioxidant nutrient intake. Individuals who did not provide all food records were excluded from the dietary analyses. A booklet was given to participants to record daily food and drink intake over a period of three non-consecutive days, including a weekend day. Dietary intake data were computed using a specific computer program (DietPro, version 5.0, AS Systems[®]). Detailed instructions were given by the interviewer to the participant to record all possible information including additions, such as sugar in coffee and amount, no matter how small they were, cooking method, type, and brand names of industrial foods. Household measures such as cups, bowls, and spoons were provided to help the recording and quantification process. Geometric food models were also provided to help in recording portion sizes.

Lifestyle co-variables such as vitamin supplementation users, smoking status (smokers or non-smokers), number of cigarettes per day, regular practice of physical activity (yes or no), and volume of physical activity were also collected. To quantify the volume of physical activity, an activity metabolic equivalent was used.¹⁴ This index represents the ratio of energy expended during each specific activity to resting metabolic rate. Briefly, the time spent in each activity is multiplied by an metabolic equivalent score specific to that activity and then summed over all activities, obtaining a mean value for the week overall, expressed as hours per day.

Statistical analyses

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 subjects with GPx activity higher and lower than the median value (cutoff: 522.63 nmol/(ml/minute)). Dichotomous variables were analyzed by chi-squared (χ^2) test. The Spearman correlation coefficients were used to screen the statistical associations between GPx activity and variables of interest. Linear regression models were fitted for co-variables (gender, age, smoking status, regular practice of physical activity and energy intake) to identify the predictors of GPx activity when they showed significant effects. Results are presented as a mean $\pm$ standard deviation). Confidence intervals (95% CIs) were used to describe linear regression coefficient (β), and a $P < 0.05$ was considered statistically significant. Statistical analyses were performed using an SAS system version 8.0 for Windows (SAS Institute Inc.[®], Cary, NC, USA).

Results

Individuals with higher GPx enzymatic activity were older and evidenced higher circulating levels of ox-LDL values (Table 1), but conversely lower nail concentrations of selenium and copper ($P < 0.05$). For the daily antioxidant nutrient intake, only vitamin C (Table 2) demonstrated a positive association with GPx enzymatic activity ($P = 0.010$). No other biochemical, anthropometric, lifestyle or physical composition parameters differed between the two groups categorized according the media of GPx activity (≤ 522.63 nmol/(ml/minute) versus > 522.63 nmol/(ml/minute)).

The GPx enzymatic activity was analyzed for potential associates (see Supplement 1). It was positively correlated with truncal fat percentage values ($r = 0.24$, $P = 0.016$), circulating levels of ox-LDL ($r = 0.28$, $P = 0.004$) and daily vitamin C intake ($r = 0.28$, $P = 0.007$) and negatively correlated to the nail

Table 1 Anthropometric, clinical, and biochemical data (mean $\pm$ SD) for young adults categorized by erythrocyte GPx activity (cutoff: 522.63 nmol/(ml/minute))

	All participants	Lower GPx activity (n = 51)	Higher GPx activity (n = 50)	P value
Age (years)	23.1 $\pm$ 3.4	22.7 $\pm$ 3.8	23.5 $\pm$ 2.9	0.026 [#]
Body weight (kg)	62.5 $\pm$ 10.1	62.9 $\pm$ 11.4	63.5 $\pm$ 10.8	0.357
BMI (kg/m ²)	22.0 $\pm$ 2.7	22.1 $\pm$ 2.8	21.9 $\pm$ 2.6	0.281
Waist circumference (cm)*	78.1 $\pm$ 8.3	78.2 $\pm$ 8.6	78.3 $\pm$ 8.5	0.973
Hip circumference (cm)*	95.2 $\pm$ 6.1	95.9 $\pm$ 6.0	94.9 $\pm$ 6.2	0.434
Waist-to-hip ratio*	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.487
Tricipital ST (mm)*	17.1 $\pm$ 6.9	18.0 $\pm$ 7.0	16.8 $\pm$ 6.7	0.369
Bicipital ST (mm)	7.3 $\pm$ 3.8	7.6 $\pm$ 4.5	6.5 $\pm$ 3.5	0.123
Suprailiac ST (mm)	17.0 $\pm$ 7.6	17.6 $\pm$ 8.4	17.2 $\pm$ 9.4	0.269
Subscapular ST (mm)	16.1 $\pm$ 5.3	16.5 $\pm$ 6.4	17.0 $\pm$ 7.2	0.359
Sum of 4 STs (mm)	58.2 $\pm$ 21.0	59.8 $\pm$ 22.8	57.5 $\pm$ 25.1	0.210
Truncal fat (%)*	58.3 $\pm$ 6.6	57.0 $\pm$ 6.8	59.3 $\pm$ 5.9	0.068
Total body fat (%)*	22.3 $\pm$ 5.7	23.2 $\pm$ 6.4	21.9 $\pm$ 6.1	0.286
Body fat mass (kg)	13.0 $\pm$ 4.1	14.5 $\pm$ 4.6	13.9 $\pm$ 5.0	0.142
Body free fat mass (kg)	48.7 $\pm$ 8.1	48.4 $\pm$ 10.0	49.6 $\pm$ 9.1	0.220
Systolic blood pressure (mmHg)	10.9 $\pm$ 0.9	11.1 $\pm$ 0.9	11.0 $\pm$ 0.9	0.229
Diastolic blood pressure (mmHg)	7.4 $\pm$ 0.6	7.4 $\pm$ 0.7	7.4 $\pm$ 0.6	0.428
Glucose (mg/dl)*	90.3 $\pm$ 6.7	90.5 $\pm$ 6.4	90.7 $\pm$ 6.6	0.884
Insulin (μ U/ml) [†]	10.2 $\pm$ 5.3	10.8 $\pm$ 5.1	9.9 $\pm$ 6.5	0.105
HOMA-IR [†]	2.3 $\pm$ 1.3	2.4 $\pm$ 1.2	2.2 $\pm$ 1.3	0.137
Total cholesterol (mg/dl)*	152.0 $\pm$ 29.3	156.4 $\pm$ 30.2	148.3 $\pm$ 21.8	0.129
HDL-c (mg/dl)*, [‡]	43.2 $\pm$ 11.1	45.6 $\pm$ 10.0	42.4 $\pm$ 10.2	0.119
LDL-c (mg/dl)	90.2 $\pm$ 22.4	94.4 $\pm$ 28.9	88.1 $\pm$ 18.7	0.178
Triacylglycerol (mg/dl)	94.2 $\pm$ 36.8	95.2 $\pm$ 30.0	93.5 $\pm$ 37.1	0.221
Total cholesterol-to-HDL-c ratio [‡]	3.5 $\pm$ 36.2	3.5 $\pm$ 0.7	3.6 $\pm$ 1.0	0.209
Uric acid (mg/dl)*	3.6 $\pm$ 1.2	3.8 $\pm$ 1.2	3.5 $\pm$ 1.0	0.319
Ceruloplasmin (mg/dl)	35.8 $\pm$ 7.6	36.8 $\pm$ 7.9	35.2 $\pm$ 7.0	0.138
Selenium (ng/g of nail)*, [§]	387.6 $\pm$ 80.2	393.8 $\pm$ 84.5	360.1 $\pm$ 64.4	0.045 [#]
Zinc (μ g/g of nail) [§]	129.5 $\pm$ 64.3	125.3 $\pm$ 48.4	133.2 $\pm$ 72.9	0.370
Copper (μ g/g of nail) [§]	6.9 $\pm$ 5.9	7.9 $\pm$ 5.6	6.3 $\pm$ 4.4	0.046 [#]
Total antioxidant capacity (mM) [‡]	1.7 $\pm$ 1.1	1.8 $\pm$ 1.0	1.6 $\pm$ 1.0	0.109
oxLDL (U/L)*, [¶]	75.2 $\pm$ 34.2	70.4 $\pm$ 31.3	83.9 $\pm$ 31.2	0.033 [#]

GPx, glutathione peroxidase; BMI, body mass index; ST, skinfold thickness; HOMA-IR, homeostasis model assessment of insulin resistance; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; ox-LDL, oxidized low-density lipoprotein cholesterol.

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

[†]*n* = 48 for GPx activity > 522.63 nmol/(ml/minute).

[‡]*n* = 48 for GPx activity $\leq$ 522.63 nmol/(ml/minute); *n* = 49 for GPx activity > 522.63 nmol/(ml/minute).

[§]*n* = 41 for GPx activity $\leq$ 522.63 nmol/(ml/minute); *n* = 41 for GPx activity > 522.63 nmol/(ml/minute).

[¶]*n* = 50 for GPx activity $\leq$ 522.63 nmol/(ml/minute).

[#]Significant difference: *p* < 0.05.

concentration of selenium ($r = -0.24$, $P = 0.026$) as depicted (Fig. 1). No associations were found for life-style variables, such as number of cigarettes/day or caloric expenditure of physical activity (data not presented in table).

The nail selenium concentrations positively correlated with variables associated with antioxidant defense, such as total plasma antioxidant capacity ($r = 0.29$, $P = 0.008$) and the levels of antioxidant minerals, such as zinc ($r = 0.36$, $P = 0.001$) and copper ($r = 0.38$, $P = 0.001$), in the nails (data not presented in table).

By means of regression analysis, it was noticed that the addition of 1 unit of truncal fat percentage (1%) and of ox-LDL plasma concentration (1 U/l) was able to determine an increase of 10.665 and 2.430 nmol/(ml/minute) in GPx activity, respectively (Table 3). It is worth emphasizing that the truncal fat percentage and circulating levels of ox-LDL explained

through the r^2 values about 5.9 and 7.4% of the enzymatic activity variation, which was statistically significant despite its magnitude.

Discussion

The GPx enzymatic activity is commonly analyzed in human erythrocytes as related to hemoglobin auto-oxidation as well as to the continuous production of ROS and hydrogen peroxide.¹⁵ However, age, gender, and other lifestyle features, such as tobacco use and dietary supplements, seem to determine the antioxidant enzyme activity in human erythrocytes, especially of the GPx enzymatic activity.¹⁶ In agreement with our results, some studies demonstrated a positive correlation between GPx activity and age.^{16,17} The increase in GPx activity may consist of an adaptative mechanism since hydrogen peroxide concentrations in erythrocytes tend to increase with aging.¹⁸ Regarding the tobacco effect and antioxidant

Table 2 Lifestyle variables and daily nutrient intake (mean $\pm$ SD) concerning young adults categorized by erythrocyte GPx activity (cutoff: 522.63 nmol/(ml/minute))

	Lower GPx activity (n = 51)	Higher GPx activity (n = 50)	P value
Lifestyle variables			
Vitamin supplementation users (%) [*]	1.9	5.2	0.178
Smokers (%) ^{*,†}	11.6	13.6	0.316
Regular practice of sport (%) ^{*,‡}	76.7	72.7	0.736
Smoking (cigarettes/d) [‡]	1.2 $\pm$ 3.9	2.5 $\pm$ 7.1	0.262
MET (h/d) [‡]	30.9 $\pm$ 10.6	31.1 $\pm$ 9.3	0.340
Daily antioxidant nutrient intake [§]			
Vitamin A (mg) [†]	780.9 $\pm$ 383.8	868.5 $\pm$ 556.2	0.412
Vitamin C (mg/d)	118.9 $\pm$ 82.9	136.2 $\pm$ 71.3	0.010[#]
Copper (mg)	1.2 $\pm$ 0.7	1.1 $\pm$ 0.58	0.471
Selenium (μ g)	97 $\pm$ 22	108 $\pm$ 19	0.071
Zinc (mg) [†]	9.0 $\pm$ 3.4	7.7 $\pm$ 3.7	0.089

MET, metabolic activity equivalent, GPx, glutathione peroxidase.

To measure the nutrient intake was used the software DietPro, version 5.0, AS Systems.

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

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

[‡]*n* = 43 for GPx activity $\leq$ 522.63 nmol/(ml/minute); *n* = 44 for GPx activity > 522.63 nmol/(ml/minute).

[§]*n* = 39 for GPx activity $\leq$ 522.63 nmol/(ml/minute); *n* = 44 for GPx activity > 522.63 nmol/(ml/minute).

[#]Significant difference: *p* < 0.05

supplement therapy, studies have reported that GPx activity is higher in non-smoking individuals¹⁹ and that vitamin supplement use may improve dysregulated oxidant and antioxidant status.^{20,21} However, in this study, these variables were not associated with

GPx activity, as reported previously.^{22,23} The small incidence of smokers (10.8%) and consumers of vitamin supplements (5.9%) may have been a determinant for the lack of association between such variables and GPx activity.

Figure 1 Significant Spearman's coefficient correlations between erythrocyte GPx activity and anthropometric, biochemical, and daily nutrient intake data. Truncal fat (%), oxLDL (U/L), Selenium (ng/g of nail), *n* = 101; Vitamin C (mg/day), *n* = 83.

Table 3 Possible predictors of erythrocyte GPx activity by regression analysis*

	β coefficient (95% IC)	R^2	P value
Age (years) [†]	4.860 (−11.576 to 21.297)	0.003	0.558
Truncal fat (%) [†]	10.665 (2.214 to 19.117)	0.059	0.013[#]
Selenium (ng/g of nail) [‡]	−0.649 (−1.455 to 0.157)	0.030	0.113
Copper (μg/g of nail) [‡]	−10.586 (−22.686 to 1.514)	0.036	0.085
ox-LDL (U/l) [§]	2.430 (0.714 to 4.146)	0.074	0.006[#]
Vitamin C (mg/d) [¶]	0.236 (−0.314 to 0.786)	0.008	0.395

ox-LDL, oxidized low-density lipoprotein cholesterol.

*Adjusted for gender, age, smoking status, regular practice of physical activity, and energy intake, when they showed significant effect.

[†]n = 101, [‡]n = 82, [§]n = 100, [¶]n = 83.[#]Significant difference: p < 0.05.

Previous investigations have shown that individuals bearing any pathological conditions associated with the increase of ROS generation showed higher erythrocyte GPx activity, revealing that GPx activity increase can be induced by the onset of OS resulting from associated pathologies.^{15,24} This study demonstrated that circulating levels of ox-LDL were positively related to GPx, even in individuals without pathological conditions. In the face of such findings, it can be suggested that there is an increase in the activity of the GPx in an attempt to counteract the damage caused by the oxidant conditions (levels of ox-LDL). In other words, there is a modulatory mechanism aiming to restore the redox system balance. The action of such a modulating mechanism is likely the determining factor of the positive associations found between truncal fat percentage and GPx enzymatic activity. The accumulation of adipose tissue, especially visceral, predisposes to ROS generation,^{6,8} and truncal fat percentage is an indirect predictor of such accumulation.⁹ Additionally, the elevated levels of fatty acids in accumulated fat of abdominal obesity are associated with higher NADPH oxidase activity and increased systemic oxidative status.²⁵ Taking into account these facts, the truncal fat percentage and circulating levels of ox-LDL seem to be activators of the measured enzyme activity.

The effect of selenium on the increase of GPx activity also has been highlighted in some studies.^{26,27} This mineral has received special attention as it is an essential component inserted in the GPx catalytic center, covalently bound to a cysteine residue, changing into selenocysteine.²⁸ The nutritional status of selenium was verified by concentrations in the nail, as the marker is able to reflect the historical nutritional status for a period of 6–18 preceding months.²⁹ The nail selenium level has higher applicability when associated with retrospective and chronic effects, without being affected by its recent dietary intake, emphasizing that alterations in the standard dietary intake of this mineral are reflected in nails only three months later.³⁰ Moreover, the GPx enzymatic activity verified in erythrocytes is limited by a maximum

period of 120 preceding days, the half-life of such cells.³¹ These facts may explain why nail selenium concentration was negatively associated with GPx activity in our study. Another possible view is that this mineral is mainly recruited to supply metabolic demand,³² which in part refers to its insertion in the GPx catalytic center.²⁸ As discussed in this paper, our study showed associations among antioxidant enzymatic activity and oxidative conditions.

The control of the antioxidant micronutrient levels is very important to the redox system balance. For instance, copper deficiency or excess is associated with specific clinical manifestations related to OS.³³ This mineral is fixed to the active site of several enzymes such as SOD that have a functional role in redox reactions.³⁴ The levels of SOD were not measured in our study. Nevertheless, some studies found synergistic actions of this mineral with SOD and GPx.^{35,36} Therefore, the possible explanation for the lower nail copper concentration in the individuals with the highest GPx activity follows the same trend as the results that have been found for selenium.

The positive association between daily vitamin C intake and GPx activity corroborates the synergistic action of this vitamin on other compounds of the antioxidant defense system.^{36,37} The precise mechanism(s) is not known; however, some data have suggested that this vitamin can significantly affect antioxidant systems.³⁷ Vitamin C is a water-soluble antioxidant that besides having a direct effect on the elimination of ROS is able to act to potentialize the activity of other antioxidants.^{38,39} Free radical self-amplifying chain reactions can be broken by vitamin C, which also acts as a key cofactor for several enzymes that catalyze hydroxylation reactions.⁴⁰ Moreover, the administration *in vitro* of vitamin C prevents haemoglobin reduction in erythrocytes.⁴¹ However, it is important to highlight that the metabolic pathways between vitamin C and GPx have so far not been shown to occur *in vivo*.

The biological variability of the GPx activity may be a limiting factor.⁴² This fact could explain the

absence of associations between the erythrocyte GPx activity and other components of the antioxidant system, such as serum levels of uric acid, ceruloplasmin and TAC. The lack of interactions among some antioxidant compounds demonstrates the complex network that maintains oxidative status.

There are some potential limitations in this study. Due to the cross-sectional design, this study can only evaluate associations but not definitive causation. Furthermore, participants provided temporal dietary information via 3-day food records, which could partly explain the inconsistency of some serum status and dietary antioxidant intake relationships. Therefore, the associations observed should be checked with care and type I/II errors cannot be ruled out as well as the involvement of other mechanisms in the maintenance of the oxidative status. A recent study in mice showed that even if OS is an accompanying factor of the cardiovascular derangement, the commonly used OS biomarkers cannot be used for the assessment of cardiovascular dysfunction.⁴³

To sum up, oxidative situations such as circulating levels of ox-LDL and truncal fat percentage were able to predict the increase in the erythrocyte GPx activity. This increase in GPx may be able to act as a compensatory mechanism in the presence of these conditions. Thus, for health OS equilibrium, preventive measures such as an adequate antioxidant intake and physiological body fat percentage maintenance would be a priority in the nutritional care of young and apparently healthy individuals.

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Disclaimer statements

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co-leader of the project and was responsible for follow up, design, financial management, and the editing of the manuscript. All of the authors actively participated in manuscript preparation, and they all read and approved the final manuscript.

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Conflicts of interest The final manuscript 'Modulators of erythrocyte glutathione peroxidase activity in healthy adults: an observational study' has been evaluated and approved by all authors and we have taken due care to ensure the integrity of our work and our personal scientific reputation. This work does not have any potential conflicts of interest, or any information on prior or duplicate publication.

Ethics approval The study was approved by the Human Research Ethics Committee of the Federal University of Viçosa, Brazil (protocol number 009/2006).

References

- Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol* 2006;141(2): 312–22.
- Gultekin F, Delibas N, Yasar S, Kilinc I. *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. *Arch Toxicol* 2001;75(2):88–96.
- Hebbel RP. Erythrocyte antioxidants and membrane vulnerability. *J Lab Clin Med* 1986;107(5):401–4.
- Ziobro A, Duchnowicz P, Mulik A, Koter-Michalak M, Broncel M. Oxidative damages in erythrocytes of patients with metabolic syndrome. *Mol Cell Biochem* 2013;378(1–2):267–73.
- 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;142(2): 231–55.
- Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. *Life Sci* 2009;84(21–22):705–12.
- Harrison DG, Gongora MC, Guzik TJ, Widder J. Oxidative stress and hypertension. *J Am Soc Hypertens* 2007;1(1):30–44.
- Hermesdorff 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.
- 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;84(3):505–12.
- Tremblay AJ, Morrisette 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; 37(9):785–90.
- Castelli WP. Cholesterol and lipids in the risk of coronary artery disease – the Framingham Heart Study. *Can J Cardiol* 1988; 4(Suppl A):5A–10A.
- 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;28(7):412–9.
- Navarro-Blasco I, Alvarez-Galindo JI. 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;17(4): 277–89.

- 14 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; 32(9 Suppl):S498–504.
- 15 Ordóñez FJ, Rosety-Rodríguez M. Correlation between glutathione peroxidase activity and anthropometrical parameters in adolescents with Down syndrome. *Res Dev Disabil* 2007;28(1):105–8.
- 16 Pavao M, Cordeiro C, Costa A, Raposo J, Santos M, Neve J, *et al*. Comparison of whole-blood glutathione peroxidase activity, levels of serum selenium, and lipid peroxidation in subjects from the fishing and rural communities of 'Rabo de Peixe' village, San Miguel Island, the Azores' Archipelago, Portugal. *Biol Trace Elem Res* 2003;92(1):27–40.
- 17 Rousseau AS, Margaritis I, Arnaud J, Faure H, Roussel AM. Physical activity alters antioxidant status in exercising elderly subjects. *J Nutr Biochem* 2006;17(7):463–70.
- 18 Ceballos-Picot I, Trivier JM, Nicole A, Sinet PM, Thevenin M. Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. *Clin Chem* 1992;38(1):66–70.
- 19 Kim SH, Kim JS, Shin HS, Keen CL. Influence of smoking on markers of oxidative stress and serum mineral concentrations in teenage girls in Korea. *Nutrition* 2003;19(3):240–3.
- 20 Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, *et al*. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J Physiol* 2003;549(Pt 2):645–52.
- 21 Guo CH, Wang CL. Effects of zinc supplementation on plasma copper/zinc ratios, oxidative stress, and immunological status in hemodialysis patients. *Int J Med Sci* 2013;10(1):79–89.
- 22 Dragsted LO, Pedersen A, Hermetter A, Basu S, Hansen M, Haren GR, *et al*. The 6-a-day study: effects of fruit and vegetables on markers of oxidative stress and antioxidative defense in healthy nonsmokers. *Am J Clin Nutr* 2004;79(6):1060–72.
- 23 Wahlqvist ML. Antioxidant relevance to human health. *Asia Pac J Clin Nutr* 2013;22(2):171–6.
- 24 Al-Gadani Y, El-Ansary A, Attas O, Al-Ayadhi L. Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children. *Clin Biochem* 2009;42(10–11):1032–40.
- 25 Skalicky J, Muzakova V, Kandar R, Meloun M, Rousar T, Palicka V. Evaluation of oxidative stress and inflammation in obese adults with metabolic syndrome. *Clin Chem Lab Med* 2008;46(4):499–505.
- 26 Elango N, Samuel S, Chinnakkannu P. Enzymatic and non-enzymatic antioxidant status in stage (III) human oral squamous cell carcinoma and treated with radical radio therapy: influence of selenium supplementation. *Clin Chim Acta* 2006;373(1–2):92–8.
- 27 Mishra V, Baines M, Perry SE, McLaughlin PJ, Carson J, Wenstone R, *et al*. Effect of selenium supplementation on biochemical markers and outcome in critically ill patients. *Clin Nutr* 2007;26(1):41–50.
- 28 Steinbrenner H, Sies H. Protection against reactive oxygen species by selenoproteins. *Biochimica et Biophysica Acta* 2009; doi:10.1016/j.bbagen.2009.02.014.
- 29 Al-Saleh I, Billede G. Determination of selenium concentration in serum and toenail as an indicator of selenium status. *Bull Environ Contam Toxicol* 2006;77(2):155–63.
- 30 Little C, Olinescu R, Reid KG, O'Brien PJ. Properties and regulation of glutathione peroxidase. *J Biol Chem* 1970;245(14):3632–6.
- 31 Rea HM, Thomson CD, Campbell DR, Robinson MF. Relation between erythrocyte selenium concentrations and glutathione peroxidase (EC 1.11.1.9) activities of New Zealand residents and visitors to New Zealand. *Br J Nutr* 1979;42(2):201–8.
- 32 Ovaskainen ML, Virtamo J, Alfthan G, Haukka J, Pietinen P, Taylor PR, *et al*. Toenail selenium as an indicator of selenium intake among middle-aged men in an area with low soil selenium. *Am J Clin Nutr* 1993;57(5):662–5.
- 33 Bremner I. Manifestations of copper excess. *Am J Clin Nutr* 1998;67(5 Suppl):1069S–73S.
- 34 Squitti R, Polimanti R. Copper phenotype in Alzheimer's disease: dissecting the pathway. *Am J Neurodegener Dis* 2013; 2(2):46–56.
- 35 Mansour SA, Mossa AT, Heikal TM. Effects of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: in vitro studies. *Toxicol Ind Health* 2009;25(8): 557–63.
- 36 Karajibani M, Hashemi M, Montazerifar F, Bolouri A, Dikshit M. The status of glutathione peroxidase, superoxide dismutase, vitamins A, C, E and malondialdehyde in patients with cardiovascular disease in Zahedan, Southeast Iran. *J Nutr Sci Vitaminol (Tokyo)* 2009;55(4):309–16.
- 37 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;107(8):1119–27.
- 38 Rodrigo R, Guichard C, Charles R. Clinical pharmacology and therapeutic use of antioxidant vitamins. *Fundam Clin Pharmacol* 2007;21(2):111–27.
- 39 Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. *Diabetes Obes Metab* 2007;9(6):813–39.
- 40 Isogawa A, Yamakado M, Yano M, Shiba T. Serum superoxide dismutase activity correlates with the components of metabolic syndrome or carotid artery intima-media thickness. *Diabetes Res Clin Pract* 2009;86(3):213–8.
- 41 Eshginia S, Marjani A. The effect of vitamin C on the erythrocyte antioxidant enzymes in intoxicated-lead rat offsprings. *J Clin Diagn Res* 2013;7(6):1078–81.
- 42 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;30(6):449–54.
- 43 Mladenka P, Zatloukalova L, Filipky T, Vavrova J, Holeckova M, Palicka V, *et al*. Common biomarkers of oxidative stress do not reflect cardiovascular dys/function in rats. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2013;157(2): 146–52.