Postprandial oxidative stress is modulated by dietary fat in adipose tissue from elderly people

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Abstract We have investigated whether dietary fat modifies the postprandial oxidative stress in adipose tissue of elderly people. Twenty participants received three diets for 4 weeks each: SFA-rich diet, Mediterranean (Med) diet enriched in MUFA with virgin olive oil, and a low-fat, high-carbohydrate diet enriched in *n*-3 PUFA (α -linolenic acid from plant origin) (CHO-PUFA diet). After 12 h of fasting, volunteers received a breakfast

reflecting the fatty acid composition of the diet ingested in the preceding dietary period. Med diet induced higher postprandial *SOD2* and *TlxR* mRNA levels, and CHO-PUFA diet induced higher *GPx1* and *TlxR* mRNA levels compared with SFA-rich diet. Med and CHO-PUFA breakfasts induced a postprandial increase in plasma reduced glutathione (GSH), and a greater postprandial GSH/oxidized glutathione ratio compared to the SFA-rich diet. Our study suggests that the consumption of Med and CHO-PUFA diets may reduce postprandial oxidative stress compared to an SFA-rich diet, which may be due to higher antioxidant enzymes gene expression in adipose tissue.

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Introduction

Several theories have been put forward to explain the aging process. Among these, the free radical theory points towards oxidative stress as the main determinant in the deleterious and cumulative effects in the biology of aging and in many age-associated degenerative diseases (Sohal and Orr 2012; Junqueira et al. 2004; Harman 1956). Additionally, increased oxidative stress has been reported in elderly subjects, involving processes such as an age-related increase in mitochondrial reactive oxygen species (ROS) production and a progressive decline in the reduced glutathione (GSH):oxidized glutathione

(GSSG) ratio and redox potential (Sohal et al. 1987; Rebrin and Sohal 2008).

Although ROS have physiological functions such as their generation by phagocytic cells in host defense mechanisms, muscle adaptation to exercise (Abruzzo et al. 2013; Scheele et al. 2009), signal transducers generated as proliferative response (Finkel 1998), and mitochondrial-derived oxidants which seems to function as signaling molecules (Nemoto et al. 2000; Nishikawa et al. 2000), high levels of ROS influence numerous cellular processes linked to aging and the development of age-related diseases (Finkel and Holbrook 2000).

Oxidative stress occurs when cells are subjected to excessive levels of ROS and/or to a depletion in the antioxidant defenses (Scandalios 2002; Durackova 2009). Obesity is a condition which is also related to cardiovascular disease (CVD), in addition to other pathologies such as insulin resistance (Alberti et al. 2009; Eckel et al. 2010), and is characterized by high oxidative damage, as shown by increased oxidative stress plasma biomarkers (Armutcu et al. 2008; Scandalios 2002). Oxidative stress is defined as the imbalance between the production of ROS and their elimination by the antioxidant system (Durackova 2009). In obesity, it has been demonstrated that systemic oxidative stress is caused by ROS production in the adipose tissue but not in other tissues. This is a consequence of an increase in the expression of NADPH oxidase, an enzyme involved in the production of ROS (Inoguchi and Nawata 2005), and by a decrease in the expression of antioxidant enzymes (Palmieri et al. 2006; Furukawa et al. 2004). In addition, it has been proposed that adipose tissue oxidative stress is responsible for the development of metabolic syndrome (MetS), through dysregulation of the release of adipocytokines and by generating systemic oxidative stress (Palmieri et al. 2006; Furukawa et al. 2004). Therefore, the increased oxidative stress in elderly subjects (Sohal et al. 1987; Rebrin and Sohal 2008) could be involved in the relationship between aging and development of MetS.

In fact, aging is associated with increased prevalence of MetS (Hildrum et al. 2007; Ford et al. 2002), a multicomponent disorder characterized by hypertriglyceridemia, low HDL cholesterol, hyperglycemia, abdominal obesity, and hypertension, and closely linked to CVD and type 2 diabetes mellitus (T2DM) (Eckel et al. 2010). Additionally, age is also a risk

factor for developing hypertension (McEniery et al. 2007). Moreover, aging is also associated to low-grade inflammation, which, together with increased oxidative stress, the key factor responsible for age-induced endothelial dysfunction (Taddei et al. 2001; Hamilton et al. 2001), contribute to the pathophysiological alterations that link aging with CVD (Herrera et al. 2010; Bruunsgaard et al. 2001).

Studies in experimental models have shown the importance of diet in studies on aging, since oxidative stress is directly modulated by the type of dietary fat. In fact, these studies have shown the existence of the relationship between dietary fat intake, aging, and oxidative stress (Sun et al. 2012; Yubero-Serrano et al. 2011; Ochoa et al. 2003). Additionally, it has been demonstrated in animal models that the use of antioxidants as dietary supplements increases their lifespan (Wang et al. 2013).

The etiology of MetS is the result of a complex interaction between genetic, metabolic, and environmental factors, including dietary habits, and probably the quality and quantity of dietary fat (Phillips et al. 2006). Thus, the modulation of oxidative stress by fat composition (Roberts et al. 2002; Roberts et al. 2006) may be the mechanism by which, at least partially, dietary fat could affect MetS development.

However, most of the studies have assessed oxidative stress in the fasting state, and humans spend most of the day in the postprandial state. Postprandial oxidative stress is characterized by an increase in oxidative stress biomarkers after the intake of a meal (Cardona et al. 2008; Devaraj et al. 2008; Ursini and Sevanian 2002), which is extremely important, as humans spend most of their time in the postprandial state (de Koning and Rabelink 2002). In line with this, we previously demonstrated that a MUFA-rich diet improves plasma postprandial oxidative stress parameters compared to an SFA-rich diet, and that low-fat diets have an intermediate effect relative to the MUFA-rich and SFA-rich diets (Perez-Martinez et al. 2010). Additionally, in the same population used in the current study, we have previously demonstrated that the consumption of a Mediterranean (Med) diet reduces endothelial dysfunction (Marin et al. 2010) and postprandial inflammatory response (Camargo et al. 2011) compared to the consumption of SFA-rich and high-carbohydrate diet enriched in *n*-3 PUFA (CHO-PUFA) diets.

In this study, we hypothesized that adipose tissue may be a key tissue in the oxidative stress status of

elderly people and that diet, and specifically the composition of dietary fat, may modulate this process. Our aim was to explore the molecular mechanism that may trigger the diet-induced changes in postprandial oxidative stress, as assessed by the plasma GSH:GSSG ratio, by analyzing the gene expression of NADPH oxidase (involved in ROS production) and the antioxidant enzymes in adipose tissue from elderly people.

Experimental procedures

Participants and recruitment

The study was performed on 20 free-living elderly subjects (10 men and 10 women). Recruitment of the patients took place between 1 January 2006 and 1 January 2007. Informed consent was obtained from all participants, and all underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrolment. None of the subjects showed evidence of chronic illness, such as hepatic, renal, thyroid, or cardiac dysfunction, and they were requested to maintain their regular physical activity and lifestyle and asked to record in a diary any event that could affect the outcome of the study, such as stress, change in smoking habits and alcohol consumption, or intake of foods not included in the experimental design. Of those enrolled, six participants had high blood pressure, two had hyperlipidemia, and three had diabetes mellitus. None of the participants showed evidence of high alcohol consumption or family history of early-onset CVD. None of the participants were active smokers. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by The Human Investigation Review Committee at Reina Sofia University Hospital (Córdoba, Spain).

Randomization and intervention

Participants were randomly assigned to receive, in a crossover design, three diets each for a period of 4 weeks: (1) Med diet enriched in MUFA with virgin olive oil, containing 15 % of energy as protein, 47 % as carbohydrate, and 38 % as fat (24 % MUFA provided by virgin olive oil, 10 % SFA, 4 % PUFA of which 0.4 % was α -linolenic acid); (2) SFA-rich diet, with 15 % of energy as protein, 47 % as carbohydrate, and

38 % as fat (12 % MUFA, 22 % SFA, and 4 % PUFA with 0.4 % α -linolenic acid); (3) low-fat, high-carbohydrate diet enriched in *n*-3 PUFA (CHO-PUFA diet), with 15 % of energy as protein, 55 % as carbohydrate, and 30 % as fat (10 % SFA, 12 % MUFA, and 8 % PUFA with 2 % α -linolenic acid). The cholesterol intake was constant (300 mg/day) throughout the three periods. The *n*-3 PUFA enrichment of the high-CHO and low-fat diet was achieved via the use of natural food components rich in α -linolenic acid of plant origin (based on walnuts; *Juglans regia* L.). Carbohydrate intake from the CHO-PUFA diet was based on the consumption of biscuits and jam and bread. From the Med diet, 80 % of the MUFA was provided by virgin olive oil, which was used for cooking, salad dressing, and as a spread. Butter was used as the main source of SFA during the SFA dietary period. At the end of each dietary intervention period and after a 12-h fast, the subjects were given a fat meal consisting of a breakfast with a composition similar to that consumed in each of the diets, and with 50–60 % of the subject's daily normal intake of energy. The methodology of the fat meal test has been validated and extensively used by our unit (Jimenez-Gomez et al. 2009; Jimenez-Gomez et al. 2010; Lopez-Miranda et al. 2007), and is described next. The composition of the experimental diets was calculated by using the US Department of Agriculture (Human Nutrition Information Service 1987) food tables and Spanish food composition tables for local foodstuffs (Varela 1980). Before the start of the intervention period, the volunteers completed a 3-day weighed food diary and an extensive Food Frequency Questionnaire (FFQ) (31), which allowed the identification of foods to be modified. Fat foods were administered by dietitians in the intervention study. At the start of the intervention period, each patient was provided with a handbook for the diet to which they had been randomly assigned, which included 14 menus elaborated with regular solid foods. Advice was given on the foods to choose and those to avoid when eating outside the home. At baseline, volunteers were provided with a supply of study foods to last for 2 weeks and collected additional study foods every fortnight or when required. At these times, a 24-h recall of the previous day's food intake and a short food use questionnaire based on the study foods were completed to monitor and motivate volunteers to adhere to the dietary advice. A score was used to assess the number of food exchanges achieved in the 24-h recall and additional advice was given if either the 24-h recall or the FFQ

showed inadequate intake of food exchange options. Volunteers were asked to complete 3-day weighed food diaries at baseline, week 2, and week 4. Weighed food intake over two weekdays and one weekend day was obtained by using scales provided by the investigators. A dietary analysis software program (DietSource version 2.0; Novartis, Barcelona, Spain) was used in the nutritional evaluation of the menus. Biochemical laboratory personnel were unaware of the dietary period that each participant was following for each determination.

Collection of blood and urine samples

Venous blood samples were obtained at the end of each dietary intervention period during the fasting state, after a 12-h fast, before ingesting breakfast, and at 2 and 4 h after the ingestion of the breakfast. Samples from the fasting and postprandial states were collected in tubes containing 1 g EDTA/L and were stored in containers with iced water and kept in the dark. Special care was taken to avoid exposure to air, light, and ambient temperature. The plasma was separated from the whole blood by low-speed centrifugation at $1,500 \times g$ for 15 min at 4 °C within 1 h of extraction. Urine samples were obtained at the end of each dietary intervention period during the fasting state, after a 12-h fast, before ingesting breakfast, and at 4 h after the ingestion of the breakfast.

Subcutaneous adipose tissue samples collection

Subcutaneous adipose tissue samples were obtained from the superficial abdominal subcutaneous adipose tissue lateral to the navel with instrument Bard[®] Magnum (ref. MG1522), needles Bard[®] Magnum Core (ref. MN1410) (M & I Medical Sales, Inc., Miami, Florida, USA). Samples were obtained to the study of postprandial adipocyte function at 4 h of administration of the fatty meal. Immediately after extraction, samples were stored in Eppendorf with RNA later until RNA extraction.

RNA isolation from adipose tissue

Adipose tissue was homogenized by Ultra-Turrax T25 (IKA Labortechnik). After lipid removal from the top of the tube, RNA was isolated with a commercial kit RiboPure (Ambion, Applied Biosystem, Austin, TX, USA) that is designed for rapid purification of high-quality RNA, which is collected from the aqueous phase by binding to a glass fiber filter. The quantification

of RNA is made using the spectrophotometer v3.5.2 Nanodrop ND-1000 spectrophotometer (Nanodrop Technology[®], Cambridge, UK).

Gene expression by real-time PCR

Retrotranscription reaction was performed with 1 µg of total RNA by the commercial kit iScript[®] cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer instructions. Real-time PCR reactions were carried out using the OpenArray[™] NT Cyclor system (Applied Biosystems, Carlsbad, CA, USA), according to the manufacturer's instructions. The gene expression analysis was performed in samples from 20 subjects and was duplicated at the postprandial state, 4 h after the meal intake. Primer pairs were selected from the database TaqMan Gene Expression Assays (Applied Biosystems, <https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=601267>) for the following genes: superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2), catalase (CAT), glutathione peroxidase 1 (GPx1), glutathione peroxidase 3 (GPx3), glutathione peroxidase 4 (GPx4), thioredoxin (TXN) and thioredoxin reductase 1 (TrxR), and NADPH oxidase subunits (gp91^{phox}, p22^{phox}, p47^{phox}, p67^{phox} and p40^{phox}). The relative expression for each gene was calculated using the ribosomal protein, large, P0 (*RPLP0*) as housekeeping gene. The data set was analyzed by OpenArray[®] real-time qPCR Analysis Software (Applied Biosystems).

Biomarkers of oxidative stress determination

Levels of GSH and GSSG were calculated to determine the GSH/GSSG ratio in plasma samples using BIOXYTECH[®] GSH-400 (OXIS International Inc., Portland, OR, USA) and GSH-412 Kit (OXIS International Inc.), respectively. The isoprostane content was measured in urine samples by using BIOXYTECH 8-Isoprostane Assay Immunoassay for 8-epi-prostaglandin F2a (OXIS International, Inc.). Lipid peroxidation is a mechanism of cellular and molecular injury. Plasma concentrations of lipid peroxidation products (LPO) were measured by using the Bioxytech LPO-586 Kit (OXIS International Inc.).

Statistical analyses

SPSS statistical software, version 15.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The normal

distribution of variables to characterize the postprandial response was assessed using the Kolmogorov–Smirnov test. The gene expression data were analyzed by using analysis of variance ANOVA. We used ANOVA for repeated measurements to determine the postprandial effect of the fat meal composition, with dietary intervention as the intersubject factor for the GSH and GSH/GSSG ratio, as well as lipoperoxide and isoprostane data. The global p values indicate the following: P1, the effect of the diet and fat meal composition ingested (between-subject effect); P2, the time effect (within-subject effect); and P3, the interaction of both factors (diet by time interaction). Post hoc statistical analysis was completed using Bonferroni's multiple comparisons test. A p value <0.05 was considered significant.

Results

Baseline characteristics

The baseline characteristics of the 20 participants who completed the three dietary intervention periods have already been described (Marin et al. 2011). To sum up, the subjects (10 men and 10 women) had an average age of 67.1 (SEM 4.52) years and an average BMI of 31.9 (SEM 5.50) kg/m^2 .

Diet and NADPH oxidase gene expression in adipose tissue

We analyzed the effect of the three dietary models, Med diet, SFA-rich diet, and CHO-PUFA diet, on the postprandial adipose tissue gene expression of the NADPH oxidase subunits ($p22^{\text{phox}}$, $p40^{\text{phox}}$, $p47^{\text{phox}}$, $p67^{\text{phox}}$, and $gp91^{\text{phox}}$) at 4 h after the administration of a breakfast with a fat composition similar to the dietary period ended (Table 1).

We found that Med diet consumption decreased the postprandial $p47^{\text{phox}}$ mRNA levels compared to the consumption of SFA and CHO-PUFA diets ($p=0.022$ and $p=0.044$, respectively) and increased the postprandial $p22^{\text{phox}}$ subunit mRNA levels compared to the consumption of a CHO-PUFA diet ($p=0.001$). No significant differences were found in the $p40^{\text{phox}}$, $p67^{\text{phox}}$, and $gp91^{\text{phox}}$ subunit mRNA levels between diets.

Diet and antioxidant enzyme gene expression in adipose tissue

In addition, we studied the effect of Med diet, SFA-rich diet, and CHO-PUFA diet consumption on the postprandial gene expression of the antioxidant enzymes *SOD1*, *SOD2*, *CAT*, *GPx1*, *GPx3*, and *GPx4* (Fig. 1).

We observed higher postprandial *SOD2* mRNA levels after the consumption of the Med diet than after the consumption of the SFA-rich diet ($p=0.016$), as well as higher postprandial *GPx3* mRNA levels than after the consumption of the CHO-PUFA diet ($p=0.001$). Moreover, we found that postprandial *GPx1* mRNA levels were higher after the consumption of the CHO-PUFA diet than after the consumption of the SFA-rich diet ($p=0.048$).

No significant differences were found in the *SOD1*, *CAT*, and *GPx4* mRNA levels between diets.

Diet and antioxidant redox-related protein gene expression in adipose tissue

We have also studied the gene expression of *TXN*, a small redox protein involved in ROS detoxification, and *TrxR*, the enzyme that reduces thioredoxin from its oxidized form to its reduced form, which is active in terms of ROS detoxification (Fig. 1). Although we did not observe any significant differences in the *TXN* mRNA levels between diets, we found higher *TrxR* mRNA levels after the consumption of the Med and CHO-PUFA diets than after the consumption of the SFA-rich diet (both $p<0.001$).

Diet and oxidative stress status

In order to assess whether the dietary effect on adipose tissue gene expression also affects oxidative stress status, we determined both the plasma GSH levels and the GSH/GSSG ratio (Fig. 2), which is a parameter that correlates with the biological redox status (Jones 2006).

We did not find any differences in the fasting GSH levels between diets. However, we did find a postprandial increase in the plasma concentration of GSH after the intake of the Med and CHO-PUFA breakfasts ($p<0.05$ between values at 2 and at 4 h after the intake of the breakfasts as compared to the fasting state), whereas we did not observe any change after the SFA-rich breakfasts.

Table 1 Postprandial adipose tissue NADPH oxidase subunits mRNA levels

Genes	SFA-rich diet	Med diet	CHO-PUFA diet	<i>p</i> value
p91phox	0.016±0.004	0.029±0.006	0.017±0.006	0.293
p22phox	0.029±0.007 ^{a,b}	0.062±0.010 ^a	0.016±0.002 ^b	0.001
p47phox	0.003±0.001 ^a	0.000±0.000 ^b	0.002±0.001 ^a	0.008
p40phox	0.012±0.001	0.034±0.004	0.034±0.017	0.205
p67phox	0.003±0.001	0.002±0.001	0.003±0.001	0.756

Values are mean±SEM of the mRNA levels at 4-h after the breakfast with a fat composition similar to the dietary period ended. *p* values are from one-way ANOVA statistical analysis. Row with superscripts without a common letter differ significantly $p<0.05$ between diets in the post hoc analysis by using Bonferroni's multiple comparisons test

SFA-rich saturated fatty acid rich diet, *Med diet* Mediterranean diet enriched in MUFA with virgin olive oil, *CHO-PUFA diet* Low-fat, high-carbohydrate diet enriched in *n-3* PUFA (α -linolenic acid from plant origin)

Furthermore, we observed that the GSH/GSSG ratio increased at 2 h after the intake of the Med breakfast ($p=0.024$), whereas it remained unchanged after the intake of the SFA-rich and CHO-PUFA breakfasts. However, the GSH/GSSG ratio increased at 4 h after the intake of all breakfasts ($p=0.004$, $p=0.002$, and $p=0.004$ after the SFA-rich, Med, and CHO-PUFA diets, respectively). Additionally, the intake of Med and CHO-PUFA breakfasts led to a higher GSH/GSSG ratio, as it increased at 4 h compared with the 2 h ratio ($p=0.025$ and $p=0.010$, respectively).

We also analyzed urinary isoprostane and plasma lipoperoxide levels at the end of each dietary intervention, in fasting and in the postprandial state. We observed that urinary isoprostanes increased in the postprandial state ($p<0.001$). Post hoc statistical analysis showed that taken together, both fasting and postprandial measurements, the consumption of Med diet induced a lower urinary isoprostane level as compared to the consumption of SFA-rich and CHO-PUFA diets ($p=0.001$ and $p=0.025$, respectively). Likewise, we also observed that taken together, both fasting and postprandial measurements, the consumption of the Med diet induced a lower plasma lipoperoxide level as compared to the consumption of the SFA-rich diet ($p=0.031$).

Correlations between postprandial antioxidant enzyme gene expression in adipose tissue, plasma oxidative stress biomarkers

We analyzed the relationship between the postprandial antioxidant enzyme gene expression in adipose tissue (mRNA levels for *CAT*, *SOD1*, *SOD2*, *GPx1*, *GPx3*, *GPx4*, *TXN*, and *TrxR*) and the plasma isoprostane and

lipoperoxide concentrations and GSH/GSSG ratio in the postprandial state (Table 2). We found a negative correlation between the postprandial *GPx3* gene expression with a urinary isoprostane concentration (r , -0.536 ; $p<0.001$), a negative correlation between the postprandial *TrxR* gene expression with a plasma lipoperoxide concentration (r , -0.261 ; $p=0.053$), and a positive correlation between the postprandial *GPx4* gene expression with the GSH/GSSG ratio (r , 0.324 ; $p=0.011$).

Discussion

The postprandial state is a stressful condition in which an increase in oxidative stress takes place (Cardona et al. 2008; Devaraj et al. 2008; Ursini and Sevanian 2002). Postprandial oxidative stress is characterized by an imbalance between the production of ROS and their elimination by the antioxidant system (Durackova 2009). Moreover, the importance of studying the postprandial state also lies in the fact that because of current dietary habits, modern humans spend most of the time in the postprandial state (de Koning and Rabelink 2002). Additionally, oxidative stress modulation by diet (Roberts et al. 2002; Roberts et al. 2006), may be the mechanism by which, at least partially, dietary fat may affect the development of MetS, since it has been shown that oxidative stress may be responsible for the development of MetS (Palmieri et al. 2006; Furukawa et al. 2004).

Our data has shown that the ROS detoxification process in adipose tissue is reduced by SFA-rich diet consumption but not by Med or CHO-PUFA diets in the postprandial state, which therefore leads to an imbalance between ROS production and inactivation and, as a

Fig. 1 Postprandial adipose tissue antioxidant enzyme mRNA levels. Values are mean $\pm$ SEM of the mRNA levels at 4 h after the breakfast with a fat composition similar to the dietary period ended. *SFA-rich* saturated fatty acid rich diet; *Med* Mediterranean diet enriched in MUFA with virgin olive oil; *CHO-PUFA* Low-fat,

high-carbohydrate diet enriched in *n*-3 PUFA (α -linolenic acid from plant origin). One-way ANOVA statistical analysis. * p <0.05 between diets in the post hoc analysis by using Bonferroni's multiple comparisons test

consequence, higher postprandial oxidative stress after the consumption of an SFA-rich diet. Consistent with this, we observed that Med and CHO-PUFA diet consumption reduces postprandial oxidative stress, as assessed by GSH plasma levels and the GSH/GSSG ratio, as compared to an SFA-rich diet.

Although ROS are generated as by-products of metabolism, several enzymes, among which is NADPH oxidase, are also involved in ROS production (Inoguchi and Nawata 2005). Our study showed that the postprandial gene expression of NADPH oxidase subunits was dysregulated by the consumption of the different diets, but there was no defined pattern of upregulation or

downregulation after any specific diet. In fact, although p22^{phox} NADPH oxidase subunit mRNA level were higher after the consumption of Med diet than after the consumption of CHO-PUFA diet, and the p47^{phox} NADPH oxidase subunit mRNA level were lower after the consumption Med diet than after the consumption of SFA-rich and CHO-PUFA diets, we did not find a defined pattern of NADPH oxidase upregulation or downregulation after the consumption of any of the diets. In addition, no gene expression changes were observed in mRNA levels of p91^{phox}, p40^{phox}, p67^{phox} NADPH oxidase subunits between diets. Therefore, an upregulation of the NADPH oxidase subunit adipose

Fig. 2 Oxidative stress parameters. Values are mean $\pm$ SEM of the GSH (in micromolars) and GSH:GSSG ratio increments between time 0 and time 2 (Δ 2 h) and between time 0 and time 4 (Δ 4 h) after the intake of the breakfast with a fat composition similar to the dietary period ended. *SFA-rich* saturated fatty acid rich diet; *Med* Mediterranean diet enriched in MUFA with virgin olive oil; *CHO-PUFA* Low-fat, high-carbohydrate diet enriched in *n*-3

PUFA (α -linolenic acid from plant origin). ANOVA for repeated measures was performed, global *p* values: *P*₁ diet effect; *P*₂ time effect; *P*₃ diet by time interaction. Post hoc statistical analysis using Bonferroni's multiple comparisons test. * $p<0.05$ versus fasting state. \$ $p<0.05$ versus 2-h after meal intake. Different letters: the differences between diets are statistically significant ($p<0.05$)

tissue gene expression may be discarded as the mechanism responsible for the higher oxidative stress observed after the consumption of an SFA-rich diet.

Nevertheless, we observed differences between the diets in the postprandial gene expression of the antioxidant defense system in adipose tissue from elderly people.

The antioxidant enzymes *SOD*, *GPx*, and *CAT* are regarded as the first line of defense against the ROS generated during oxidative stress (Gabielli et al. 2011). *SOD* converts O_2^- into H_2O_2 , which is, in turn, converted into water or molecular oxygen by either *CAT* or *GPx*. Additionally, *GPx4* isoform is mainly involved in the

Table 2 Correlations between postprandial antioxidant enzymes gene expression in adipose tissue and plasma oxidative stress parameter in the MetS patients, 4 h after the administration of the fatty meal with a fat composition similar to that consumed in each of the SFA-rich, Mediterranean, and CHO-PUFA diets. Pearson's linear correlation coefficient

	Isoprostanes	Lipoperoxides	GSH:GSSG
CAT	0.016, $p=0.899$	-0.127, $p=0.355$	0.156, $p=0.234$
SOD1	-0.212, $p=0.102$	0.021, $p=0.878$	-0.025, $p=0.847$
SOD2	0.196, $p=0.133$	-0.143, $p=0.294$	-0.098, $p=0.454$
GPx1	-0.048, $p=0.714$	0.186, $p=0.174$	0.009, $p=0.946$
GPx3	-0.536, $p<0.001$	0.044, $p=0.786$	-0.037, $p=0.816$
GPx4	0.029, $p=0.824$	-0.026, $p=0.845$	0.324, $p=0.011$
TXN	0.019, $p=0.885$	-0.118, $p=0.390$	0.034, $p=0.799$
TrxR	-0.173, $p=0.184$	-0.261, $p=0.053$	0.074, $p=0.570$

detoxification of lipid hydroperoxides generated by the action of O_2^- (Faraci and Didion 2004; Mates et al. 1999; Rahman et al. 2005). Our results showed that the gene expression of the mitochondrial *SOD* isoform, *SOD2*, seems to be modulated by dietary fat rather than by the cytoplasmatic isoform *SOD1*. Although no differences in gene expression between diets were observed for *SOD1* gene expression, we observed higher *SOD2* mRNA levels after the Med diet than after the consumption of an SFA-rich diet. In addition, *SOD2* mRNA levels after the CHO-PUFA diet tended to be higher than after the consumption of an SFA-rich diet although it did not reach the statistical significance.

Taken together, our results showed that both Med and CHO-PUFA diets increase the postprandial gene expression profile of the antioxidant defense system, and therefore the ROS detoxification rate in the adipose tissue, as compared to an SFA-rich diet. In line with the adipose tissue gene expression results, our study showed that the consumption of Med and CHO-PUFA diets reduces postprandial oxidative stress in elderly people, on the basis of the higher values found after the consumption of Med and CHO-PUFA diets as compared to an SFA-rich diet in postprandial plasma GSH levels and the GSH/GSSG ratio, a parameter that correlates with biological redox status (Jones 2006) and decreases as a consequence of glutathione oxidation (Jones 2002). Additionally, the measurement of two other oxidative stress biomarkers, isoprostanes and lipoperoxides, both of which oxidative stress-derived molecules, supports this idea.

The transcription of the antioxidant defense system genes is regulated by the Nrf2 transcription factor (Jaiswal 2004), which in response to oxidative stress migrates to the nucleus and promotes the expression of antioxidant enzymes, controlling the balance between ROS and antioxidant enzymes (Kobayashi and Yamamoto 2005; Xu et al. 2008). Our results suggest that diet, and specifically the composition of dietary fat, may interact with the regulatory mechanisms controlling the transcription of antioxidant genes. In fact, the activation of Nrf2 in response to the postprandial oxidative stress may be inhibited by the consumption of saturated fat.

The relationship between adipose tissue antioxidant enzyme gene expression and redox status is also supported by the fact that the gene expression of *GPx3* and *TrxR* negatively correlates with postprandial urinary isoprostane and plasma lipoperoxide levels, respectively,

and the postprandial gene expression of *GPx4* positively correlates with the GSH/GSSG ratio. Therefore, as the values of the antioxidant enzyme gene expression increase, the values of the oxidative stress biomarkers decrease and the GSH/GSSG ratio increases, showing lower oxidative stress.

In conclusion, our study suggest that the consumption of Med and CHO-PUFA diets may reduce postprandial oxidative stress as compared to an SFA-rich diet in elderly people, and that this reduction may be due to a higher gene expression of the antioxidant defense system in the adipose tissue.

The implications of this study for nutrition, and for the health of the elderly population, lie in the fact that, in addition to the improvement in the endothelial function (Marin et al. 2010) and the anti-inflammatory properties (Camargo et al. 2011) observed after the consumption of a Med diet in the same population in previous studies, the Med diet protects elderly people against postprandial oxidative stress and provides them with an alternative option for aging “more healthily,” which may also protect them against the development of metabolic syndrome.

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