Postprandial oxidative stress is modified by dietary fat: evidences from a human intervention study

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Title: Postprandial oxidative stress is modified by dietary fat: evidences from a human intervention study

AUTHORS: Pablo Perez-Martinez[*], Jose Maria Garcia-Quintana[*], Elena M. Yubero-Serrano[*], Inmaculada Tasset-Cuevas[†], Isaac Tunez[†], Antonio Garcia-Rios[*], Javier Delgado-Lista[*], Carmen Marin[*], Francisco Perez-Jimenez[*], Helen M Roche[‡], Jose Lopez-Miranda[*]

Affiliations: [*] Lipids and Atherosclerosis Unit, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC)/Hospital Universitario Reina Sofía/Universidad de Córdoba and Ciber Fisiopatologia Obesidad y Nutricion, Instituto Salud Carlos III. Spain; [†]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC)/Hospital Universitario Reina Sofía/Universidad de Córdoba, Spain and [‡]Nutrigenomics Research Group, UCD Conway Institute, School of Public Health & Population Science, University College Dublin, Belfield, Dublin 4, Ireland.

Key words: oxidative stress; metabolic syndrome; dietary fat; postprandial lipemia; LIPGENE study.

Short title: postprandial oxidative stress and dietary fat

Author for correspondence: Jose Lopez-Miranda. Reina Sofia University Hospital, Lipids and Atherosclerosis Research Unit. Avda. Menéndez Pidal, s/n. 14004 Córdoba, Spain. Phone: +34-957010947. FAX: +34-957218250. e-mail: jlopezmir@uco.es.

Abstract

Previous evidences support the concept that increased oxidative stress may play an important role in the metabolic syndrome (MetS)-related manifestations. Dietary fat quality has been proposed to be critical in oxidative stress and the pathogenesis of the MetS. We investigated whether oxidative stress parameters are affected by diets with different fat quantity and quality during the postprandial state in subjects with the MetS. Patients were randomly assigned to one of four isoenergetic diets distinct in fat quantity and quality: high-saturated fatty acid (HSFA); high-monounsaturated fatty acid (HMUFA) and two low-fat, high complex carbohydrate (diets, supplemented with 1.24 g/day of long chain n-3 polyunsaturated fatty acid (LFHCC n-3) or with placebo (LFHCC) for 12 weeks. HMUFA diet enhanced postprandial reduced glutathione levels and the glutathione/oxidized glutathione ratio, compared to the other three diets. In addition, after the HMUFA diet postprandial lipid peroxides levels, protein carbonyls concentration, superoxide dismutase activity and plasma hydrogen peroxide plasma levels were lower compared to subjects adhering to the HSFA diet. Both LFHCC diets had an intermediate effect relative to the HMUFA and HSFA diets. Our data support the notion that the HMUFA diet improves postprandial oxidative stress in patients with the MetS. These findings suggest that the postprandial state is important for understanding possible cardio-protective effects associated with monounsaturated dietary fat particularly in subjects with the MetS (NCT00429195)

Introduction

The metabolic syndrome (MetS) refers to the aggregation of cardiometabolic risk factors, including insulin resistance, dyslipidemia, hyperglycemia, and hypertension[1]. It is often characterized by oxidative stress, a condition in which an imbalance results between the production and inactivation of reactive oxygen species (ROS)[2]. Previous studies support the concept that increased oxidative stress may play an important role in MetS related manifestations, including atherosclerosis, hypertension and type 2 diabetes mellitus (T2DM)[3]. Patients with MetS have elevated oxidative damage, as evidenced by decreased antioxidant protection, and increased lipid peroxidation, protein carbonyls, and hydrogen peroxide (H_2O_2) plasma levels[4]. Nevertheless, although some of the constituent characteristics of the MetS are known to share common pathogenic mechanisms of damage, the impact of hereditary predisposition and the role of environment and dietary habit in determining inflammatory process-triggered oxidation are still unclear.

Recent dietary intervention studies have demonstrated that altering fat composition can significantly reduce oxidative stress in the MetS[5,6]. A number of clinical trials examined short- or intermediate-term effects of the Mediterranean diet on different circulating markers of oxidative stress[7-10]. Recently, Dai et al. reported a robust inverse association between adherence to the Mediterranean diet and oxidative stress as measured by reduced glutathione/oxidized glutathione (GSH/GSSG) ratio, independent of a wide range of known cardiovascular disease risk factors[11]. Other trials, however, yielded conflicting results. Given the potential of altering dietary fat composition (i.e n-3 polyunsaturated fatty acids (PUFA) or low fat diets) in the prevention and therapy of a number of chronic diseases including MetS[12] and the inconsistencies found so far, this subject is in need of more in depth investigation.

Much of our knowledge of the relationship between lipids, lipoprotein metabolism and the development of atherosclerosis and cardiovascular disease is based on characterising fasting metabolic markers. However humans spend the majority of time in a nonfasting, postprandial state, with a continual fluctuation in the degree of lipemia throughout the day. In line with this notion, oxidative stress has received considerable attention over the past several years in the fasting state; however there is a paucity of data on postprandial oxidative stress. With regard to the postprandial state, several previous studies have demonstrated that a breakfast enriched in saturated fat resulted in an increase in biomarkers of oxidative stress[13-15]. Based in these previous evidences, we investigated whether oxidative stress is affected by diets with different fat quantity and quality during the postprandial state in MetS patients from the LIPGENE study.

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Material and Methods

Design

The current study was conducted within the framework of the LIPGENE study ("Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis"), a Framework 6 Integrated Projected funded by the European Union. A subgroup of patients with the MetS was randomly stratified to one of four dietary interventions. MetS was defined by published criteria[16,17], which conformed to the LIPGENE inclusion and exclusion criteria[18]. Pre- and post-intervention was administered a fatty meal with a fat composition similar to that consumed in each of the diets (**Figure 1**). The intervention study design and the dietary strategy protocol have been previously described in detail by Shaw et al[19].

Participant and recruitment

75 patients were included. All participants gave written, informed consent and underwent a comprehensive medical history, physical examination and clinical chemistry analysis before enrolment. This study was carried out in the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, from February 2005 to April 2006. The experimental protocol was approved by the local ethic committee, according to the Helsinki Declaration. The study was registered with The US National Library of Medicine Clinical Trials registry (NCT00429195).

Randomization and intervention

Each volunteer was randomly stratified to one of four dietary interventions for 12 weeks (**Figure 1**). Randomisation was completed centrally, according to age, gender and fasting plasma glucose concentration using the MINIM (Minimisation Programme for Allocating patients to Clinical Trials, Dept of Clinical Epidemiology, The London Hospital Medical College, UK) randomisation programme. The composition of the four diets was as follow:

- High-fat (38% energy), SFA-rich diet (16% SFA, 12 % MUFA 6% PUFA) (HSFA).
- High-fat (38% energy), MUFA-rich diet (8% SFA, 20% MUFA, 6% PUFA) (HMUFA).
- Low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11%; MUFA; 6% PUFA), with 1g/d high oleic sunflower oil (placebo) (LFHCC)
- Low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g/d LC n-3 PUFA (LFHCC n-3).

Post-intervention (week 12), we performed a postprandial challenge with the same fat composition as consumed on the assigned dietary period (**Figure 1**). Patients presented at the clinical centre at 8:00 a.m following a 12 h fast, refrained from smoking during the fasting period and abstained from alcohol intake during the preceding 7 days, in the laboratory and, after canulation a fasting blood sample was taken before the test meal, which then was ingested under supervision within 20 min. The test meal reflected fatty acid composition of each subject chronic dietary intervention. Subsequent blood samples were drawn at 2 and 4 hours. Test meals provided an equal amount of fat (0.7 g/kg body weight), cholesterol (5 mg/kg of body weight) and vitamin A (60,000 IU/m^2 body surface area). The test meal provided 65% of energy as fat, 10% as protein and 25% as carbohydrates. During the postprandial assessment subjects rested, did not consume any other food for 9 hours, but were allowed to drink water. The composition of the breakfasts was as follow: HSFA breakfast (38% SFA, 21% MUFA 6% PUFA); HMUFA breakfast (12% SFA, 43% MUFA,10% PUFA); LFHCC breakfast with placebo capsules (21% SFA, 28% MUFA, 16% PUFA); LFHCC with LC n-3 PUFA (21% SFA, 28%; MUFA, 16% PUFA (1.24 g/d of LC n-3 PUFA).

Measurements

Blood was collected in tubes containing ethylene diaminetetraacetic acid (EDTA) to give a final concentration of 0.1% EDTA. Plasma was separated from red cells by centrifugation at 1500 × *g* for 15 min at 4 ºC.

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Biomarkers determined in frozen samples were realized by laboratory investigators who were blinded to the interventions. Lipid parameters were assessed with a DDPPII Hitachi modular analyzer (Roche, Basal, Switzerland), using specific reagents (Boehringer-Mannheim, Mannheim, Germany). Plasma triglycerides (TG) and cholesterol concentrations were assayed by enzymatic procedures[20,21]. Apolipoprotein (Apo) A-I and apo B were determined by turbidimetry[22]. HDL-C was measured by precipitation of a plasma aliquot with dextran sulphate-Mg2+, as described by Warnick et al[23]. Low density lipoprotein-cholesterol (LDL-C) was calculated by using the following formula; plasma cholesterol - (high density lipoprotein-cholesterol (HDL-C) + large triacylglycerol rich lipoprotein (TRL)-C+ small TRL-C). Plasma glucose concentrations were measured using the Architect-CG16000 analyzer (Abbott Diagnostics, Tokio, Japan) by the exoquinase method and plasma insulin concentrations were measured by chemoluminescence with an Architect-I2000SR analyzer (Abbott Diagnostics, Tokio, Japan). Plasma fatty acid composition was determined by the enzymatic colorimetric assay for the determination of free fatty acids, Half-micro test (Roche Diagnostic GmbH).

Determination of oxidative stress biomarkers

Lipid peroxides (LPO) in plasma were estimated using the method of Eldermeier et al.[24]. This method uses a chromatogenic reagent which reacts with the lipid peroxidation products malondialdehyde and 4-hydroxyalkenals at 45 ± 1ºC, yielding a stable chromophore with maximum absorbance 586 nm. Protein carbonyls content was carried out in plasma samples using the method of Levine[25]. Samples were incubated with 500ml of a 10 mM solution of 2,4-dinitrophenylhydrazine in 2N HCl for 60 min. Subsequently, the proteins were precipitated from the solutions using 500 ml of 20% trichloroacetic acid. The proteins were then washed three times with a solution of ethanol and ethylacetate (1:1 vol/vol) and dissolved in 1 ml of 6M guanidine (containing 20 mM phosphate buffer, pH 2,3, in trifluoroacetic acid) at 37º C. The carbonyls were evaluated in a spectrophotometer (UV-1603 Shimadzu, Kyoto, Japan) at wavelength 360 nm[26]. Antioxidant capacity in plasma was measured using BIOXYTECH® AOP-490 TM (OXIS International). This assay is based upon the reduction of $Cu2^{+}$ to Cu^{+} by the combined action of all antioxidants present in the sample. Thus a chromogenic ragent forms a complex with Cu^{+} which has an absorbance at 490 nm[27]. Total glutathione (data non-shown), reduced glutathione (GSH) and GSH/oxidized glutathione (GSSG) ratio were carried out in plasma samples using BIXYTECH® GSH-420, GSH-400 and GSH/GSSG-412 kits, respectively. The determination of total glutathione levels is based on the formation of a chromophoric thione which has an absorbance at 420 nm. GSH concentration is based on reaction which leads to the formation of a chromophore with absorbance at 400 nm. The GSH-412 method uses the thiol-scavenging reagent and this technique is based on Tietze method [28]. To determine GSSG levels we used the following equation: GSSG = A-B; where A= total glutathione and B= GSH. Plasma hydrogen peroxide (H_2O_2) concentration was measured using the BIOXYTECH® H_2O_2-560 Assay (OXIS International). This kit allows for colorimetric quantitative determination of hydrogen peroxide (total hydroperoxides) in aqueous samples. This assay is based on method of ferric-xylenol orange hydroperoxide assay[29,30].

Antioxidant enzyme activity

Total superoxide dismutase (SOD; E.C: 1.15.1.1) activity was determined by colorimetric assay in plasma at wavelengths of 525 nm according to the laboratory method described by McCord and Nebot[31,32]. Glutathione peroxidase (GPx; E.C.: 1.11.1.9) activity was evaluated in plasma using the Glutathione Peroxidase Assay Kit (Cayman Chemical). This assay is based on the oxidation of NADPH to NAD^{+}, catalysed by a limiting concentration of glutathione reductase, with maximum absorbance at 340 nm. The GPx activity was measured based on the formation of GSSG from GPx-catalyzed oxidation of GSH by H_2O_2, coupled with NADPH consumption in the presence of exogenously added glutathione reductase, with maximum absorbance at 340 nm. This assay is on based in method of Flohé[34].

Although GSH/GSSG ratio and antioxidant enzymes activities may be measured in erythrocyte, their plasma measurements provide an approach to the blood state, reflecting the similar change at the intracellular level [34].

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Statistical Analyses

All data presented in the text and tables are expressed as mean values and standard deviations. SSPS 17 for Windows (SSPS INC., Chicago) was used for the statistical analysis. The data were analyzed using repeated measures analysis of variance and Student *t* test for paired data analysis and analysis of variance for repeated measures. In this analysis we studied: 1. the postprandial time points; 2. the effect of the type of fat meal ingested, independent of time (represented by dietary fat); and 3. the interaction of both factors, indicative of the degree of the postprandial response in each group of subjects with each breakfast (represented by time*dietary fat interaction). Post hoc statistical analysis was completed by using the protected least-significant-difference test to identify significant differences between dietary treatments. The contrast statistic used when the sphericity assumption was not satisfied was Huynh-Feldt. $P<0.05$ was considered statistically significant.

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Results

Dietary compliance was good, with close attainment of the dietary intervention targets **(Table 1)**. There were no significant differences in dietary composition at baseline between the four diet groups. However, during the intervention period we observed significant differences between the different diets. The HSFA diet group consumed 17.9% of energy as saturated fat compared to the HMUFA group who consumed 21% as monounsaturated fat, while carbohydrate consumption fell to 38.2% and 40.7% respectively. The LFHCC diet group decreased their intake of total fat to 27% and the LFHCC n-3 diet to 26.5%, while carbohydrate consumption rose to 51.2% and 54% respectively. The LFHCC n-3 diet group attained their target intake of EPA and DHA at 1.8g/day. In addition, analysis of plasma fatty acids obtained after each dietary period showed good adherence in the different intervention stages. During the HSFA diet, we observed an increase in plasma C16:0 palmitic (11%), C18:0 stearic (20%) and C14:0 myristic acids (90%) compared with the baseline state. Consistently, the chronic intake of the LFHCC n-3 diet induced an increase in the C22:6n3 docosahexaenoic (96%) and C20:5n3 eicosapentaenoic acids (206%). Finally, in our typically Mediterranean population, subjects consumed a high intake of C18:1 oleic acid at baseline (21.6% energy from MUFA) and no significantly increase were observed at the end of the HMUFA period (21.1% energy from MUFA).

Table 2 shows the age, baseline BMI and lipid related risk factors of the 75 subjects with MetS randomized to each dietary intervention.

The effect of dietary fat quality and quantity on postprandial plasma TG, glucose, insulin, glutathione (GSH), glutathione/oxidized glutathione (GSH/GSSG) ratio, lipoperoxides (LPO), protein carbonyl, hydrogen peroxide (H_2O_2), superoxide dismutase activity (SOD), total antioxidant capacity, and glutathione peroxidase (GPx) were assessed post-intervention. Postprandial glucose and insulin levels after the intervention were not significantly different between diets (data not shown). We observed a significantly increment in the area under the curve (AUC) for postprandial TG during the HSFA (5231.87 mmol x min/L) diet compared with the HMUFA (2095.32 mmol x min/L) and LFHCC n-3 (2354.65 mmol x min/L) diets (HSFA vs HMUFA, $P<0.013$ and HSFA vs LFHCC n-3, $P<0.018$).

Figure 2A shows that postprandial plasma GSH levels were higher 2 h after the intake of the HMUFA diet ($P<0.05$), compared to the other three diets. Plasma GSH levels remained significantly higher 4 h after the HMUFA diet compared with the HSFA diet ($P<0.05$). In addition, at 2 h after the HSFA diet we observed lower GSH plasma levels ($P<0.05$) compared to the LFHCC and LFHCC n-3 diets. As an indicator of redox status we measured the GSH/GSSG ratio. Interestingly we showed a consistent postprandial increase in GSH/GSSG ($P=0.002$) ratio 2 h after the HMUFA diet ($P<0.001$), compared to the other three diets (**Figure 2B**). GSH/GSSG remained significantly higher 4 h after the HMUFA diet compared with the LFHCC and HSFA diets ($P<0.003$). Consistently, postprandial plasma GSSG levels were lower 2 h after the HMUFA diet compared with the other 3 diets ($P<0.004$) and remained significantly lower at 4 h compared with the LFHCC and HSFA diets (**Figure 2C**). Moreover, GSSG levels were significant higher 4 h after the HSFA diet compared with the LFHCC n-3 ($P<0.05$). We also measured lipid peroxidation given the important potential effect of fat composition. Interestingly, plasma LPO levels were significantly higher 2 h after the HSFA diet, compared to subjects adhering to the HMUFA and LFHCC n-3 diets ($P<0.05$), (**Fig 3A**). Moreover, plasma LPO level remained lower later in the postprandial phase 4 h after the HMUFA diet, compared to all other diets (**Figure 3A**). In the case of postprandial protein carbonyls concentration, a significant time * diet interaction ($P< 0.02$) confirmed that levels were significantly higher 2 h after the HSFA diet but lower 2h following the HMUFA diet (**Figure 3B**). Consistent with this effect postprandial SOD was significantly lower 2 h and 4 h after the HMUFA diet, compared with the HSFA and LFHCC diets ($P<0.001$) (**Figure 3C**). Again, an intermediate effect was found after the intake of the two LFHCC diets compared with the HSFA (**Figure 3C**). Postprandial plasma H_2O_2 levels were significant higher 2 h after the HSFA diet compared with the other three diets (**Figure 3D**). Postprandial total antioxidant capacity (**Figure 3E**) and plasma GPx levels were not significantly different between diets (**Figure 3F**).

On the other hand, with the aim of identifying the effects of long-term consumption of the four diets, we also explored the effect of dietary fat on GSH, GSH/GSSG ratio, GSSG, LPO, protein carbonyls, H_2O_2, SOD, total antioxidant capacity, and GPx at baseline (before the test meal) . In this context we found significantly differences related to the chronic dietary intervention for GSH, protein carbonyl and SOD plasma levels. Thus, the results of our study demonstrated that the long-term consumption of the HMUFA diet increased the GSH plasma levels compared with the HSFA, LFHCC and LFHCC n-3 diets ($P<0.002$) (**Figure 2A**). Moreover GSH levels remained significantly lower after the HSFA diet compared with the LFHCC diet ($P<0.05$) (**Figure 2A**). Protein carbonyls concentrations were significantly lower after the HMUFA diet compared with the other 3 diets ($P<0.001$) (**Figure 3B**). Finally, SOD was significantly higher after the HSFA diet, compared with the HMUFA ($P<0.002$) and LFHCC n-3 diets ($P<0.043$) (**Figure 3C**).

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Discussion

Oxidative stress has been associated with all the individual components and with the onset of cardiovascular complications in subjects with the MetS. Although some aspects of diet have been linked to individual features of the MetS[35], the role of diet in the etiology of the syndrome is poorly understood. Our study supports the notion that the HMUFA diet improves postprandial oxidative stress parameters as measured by GSH and GSH/GSSG ratio. In addition, the HMUFA diet induced lower postprandial plasma levels of LPO, protein carbonyls concentration and SOD compared to subjects adhering to the other three diets. Furthermore, postprandial plasma H_2O_2 levels were unfavourable increased during the HSFA diet compared to the other three diets.

Fasting is not the typical physiological state of the modern human being, which spends most the time in the postprandial state. Therefore, the assessment of the postprandial lipemic response may be more relevant to identify disturbances in metabolic pathways related to oxidative stress than measures taken in the fasting state. Although previous studies demonstrated that patients with the MetS have greater levels of oxidative stress after a fat overload[14,15], the current well controlled intervention study is in our knowledge, the first to examine the effect of four isoenergetic diets distinct in fat quantity and quality during the postprandial state.

One biomarker used to evaluate oxidative stress in humans is the redox state GSH/GSSG and this quotient appears to correlate with the biological status of the cell[36]. Biochemically, GSH/ GSSG redox decreases lipid hydroperoxides by reducing these peroxides into alcohols and suppressing their generation[37]. Moreover, a lower GSH/GSSG ratio may result in protein glutathionylation and oxidatively altered GSH/GSSG redox signalling and associated gene expression and apoptosis, which may contribute to atherosclerosis[38]. In our study, the HMUFA diet produced a postprandial increase in GSH plasma levels and GSH/GSSG ratio compared to the other three diets (HSFA and LFHCC diets). Thus, our data carried out in the postprandial state support previous evidences in the fasting state suggesting that dietary patterns similar to those of the Mediterranean-style diet exert positive effects on GSH/GSSG ratio[11]. Additionally, Fitó et al. found that olive oil caused increases in glutathione reductase plasma [39]. This data supports the idea that HMUFA may trigger the increase of GSH and GSH/GSSG ratio, together with a reduction of GSSG by mean the stimulation GR activity. The major levels of GSH may help to regenerate the most important antioxidant such as ascorbic acid, alpha-tocopherol and others [40]. This situation may explain the highest antioxidant effect of HMFA than LFHCC and LFHCC n-3 on oxidative stress characterized by a reduction in lipid peroxidation, carbonylated protein and hydrogen peroxide levels. On the other hand a possible mechanism to explain the increased plasma GSH/GSSG ratio observed after the intake of the HMUFA diet could be through decreased GSSG concentrations and enhanced GSH levels, like it is suggested by our data. Initially, GSH is oxidized into GSSG by the enzyme GPx; in this process, GSH quenches peroxides. GSSG reverts to GSH via gluthathione reductase with concomitant oxidation of NADPH[41]. Diverse nutrients and biofactors in foods characteristic of the Mediterranean diet may provide higher NADPH[42] and up-regulate gluthathione reductase activity[43], which may lead to a decrease in GSSG and a resulting in higher GSH/GSSG ratio. Interestingly the effects observed by the present study were the results of the consumption of diets over a period of 12 weeks. As a result, chronic ingestion results in a more faithful translation of the effects of the different dietary models, in that meal consumption is not an isolated phenomenon, and in the type of design that employs only acute ingestion of fats it is impossible to separate out the potential effects of the background diet on the oxidative stress status. In this line, we demonstrated that the long-term consumption of the HMUFA diet increased the GSH plasma levels and decreased protein carbonyls concentrations compared with the HSFA, LFHCC and LFHCC n-3 diets. These findings suggest that background dietary MUFA consumption may influence the nature and extent of postprandial oxidative stress. Our results are important, particularly in view of the lack of randomized controlled trials assessing the effects of diets rich in MUFA typical for the traditional Mediterranean diet on glutathione redox pathways in the general population and in patients with metabolic syndrome.

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Emerging evidence thus points to the notion that oxidative stress may contribute to insulin resistance, the key characteristic of MetS[44]. Mitochondria are generally considered a major source of ROS, which can lead to lipid peroxidation[45]. In particular, the mitochondrial matrix is sensitive to peroxide-induced oxidative damage and must be protected against the formation and accumulation of lipids and lipid peroxides, where free fatty acids (FFA) can modulate signaling functions of ROS[46]. In the current study, the HMUFA diet induced lower postprandial plasma levels of LPO and protein carbonyl concentration compared to subjects adhering to the other three diets. Although not explored, and beyond the remit of our study, a probable mechanistic explanation for the observed effects lies in the high intakes of MUFA together with natural antioxidants present in the Mediterranean diet that could prevent against the accumulation of the lipid peroxides. According with our results a recent PREDIMED sub-study demonstrated that adherence to the Mediterranean diet was associated with a reduced oxidative status, as shown by lower serum concentrations of oxidized LDL compared with the low-fat diet[10]. On the other hand, our data are in agreement with previous postprandial studies, suggesting that the acute intake of fat load enriched in saturated fat results in increased postprandial oxidative stress in patients with MetS[14-15].

During the past few years, several studies have shown that n-3 PUFA have potentially cardioprotective effects, especially in high-risk patients with dyslipidemia, and might therefore be expected to be of benefit in T2DM. In our study, as compared with the HSFA diet the effect of the two LFHCC diets was favourable intermediate. In the same line, a previous study showed that daily supplementation of EPA and DHA for three months reduced levels of 8-iso-$PGF_{2\alpha}$ measured in plasma, but not 15-keto-dihydro-$PGF_{2\alpha}$, compared to controls among healthy subjects in the KANWU study[47]. An isoprostane reducing effect of n-3 PUFA has also been observed in treated-hypertensive type 2 diabetic patients[48]. Contrary to these previous evidences, PUFA has been suggested to be more prone to oxidation than SFA and MUFA[49,50]. Although only suggestive, these results need to be confirmed and extended.

In response to oxidative stress, cells attempt to fortify the antioxidant arsenal as the first line of defence. The natural antioxidant system consists of a series of antioxidant enzymes and numerous endogenous and dietary antioxidant compounds that react with and inactive ROS. Superoxide is converted to H_2O_2 by a group of enzymes known as SOD. H_2O_2, in turn, is converted to water and molecular oxygen by either catalase or GPx. In addition, GPx can reduce lipid hydroperoxides and other organic hydroperoxides. Previous studies[51] show that the expression of SOD is upregulated by superoxide so the decrease in the SOD after consumption of the HMUFA could be explained to a lower generation of SOD in this diet. Moreover, it has been reported that several genes encoding antioxidant enzymes, such as SOD, contain antioxidant response elements sequences in its promoter that bind the transcription factor Nrf2 in response to increased ROS, promoting an increase in the expression of these enzymes (52). In our study, the long-term consumption of HSFA diet also increased the SOD compared with the HMUFA and LFHCC n-3 diets. Therefore, the higher SOD activity obtained after the chronic intake of the HSFA diet could be explained by increased activation of Nrf2 which we believe could be a direct consequence of a higher production of O^{2-} in this situation. In our study, GPx plasma levels and total antioxidant capacity were not significantly different among the four diets during the postprandial period. One reason for this finding could be explained because some pathways but not others may be differentially influenced by dietary fat in the postprandial state. However it is also interesting to emphasize that although we did not find significantly differences for these paramenters a trend was observed between the HSFA vs HMUFA diet. Interestingly, our results also expand our recent finding in this same population, of an inverse association between HMUFA diet and inflammation[18], since inflammation and oxidative stress are tightly inter-dependent. A synergistic relationship exists between oxidative damage and inflammation, and both of these are related to endothelial dysfunction[18].

Hyperlipidemia and hyperglycemia have been associated with increased oxidative damage affecting lipoproteins and the antioxidant status (53). Previous evidences suggest that prolonged elevations in TRL, associated with decreased chylomicron clearance, result in a restriction in vitamin E transfer to LDL and HDL along with greater susceptibility to oxidation (53). In this context, we have demonstrated in a recent study that a HMUFA diet can lower postprandial lipoprotein abnormalities associated with the MetS[54]. Also the adverse TG

raising effects of the long-term LFHCC diets were avoided by concomitant LC n-3 PUFA supplementation. These findings suggest that the beneficial effects observed during the consumption of the HMUFA diet could be mediated at least in part by a more efficient clearance of the postprandial lipoproteins which is accompanied by the improvement of the postprandial oxidative stress observed in these patients. However, the detailed relations among hyperlipidemia, hyperglycemia, hyperinsulinemia, and oxidative stress are still under research.

Our study presents some limitations. Ensuring adherence to dietary instructions is difficult in a feeding trial. However, adherence to recommended dietary patterns was good, as judged by measurements. On the other hand, our design has the strength of reproducing real-life conditions with home-prepared foods, reflecting usual practice.

In summary, our results support the notion that the HMUFA diet improves postprandial oxidative stress parameters in patients with the MetS. Both LFHCC diets had an intermediate effect relative to the HMUFA and HSFA diets. These findings suggest that the postprandial state is important for understanding possible cardio-protective effects associated with the Mediterranean diet particularly in subject with the MetS. In addition, these findings support recommendations to consume a HMUFA diet as a useful tool to prevent cardiovascular diseases in MetS patients.

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List of Abbreviations

Apo: apolipoprotein; EDTA: ethylene diaminetetraacetic acid; FFA: free fatty acids; GPx: glutathione peroxidise; GSH: reduced glutathione; GSH/GSSG: reduced glutathione/oxidized glutathione; GSSG: oxidized glutathione; HDL-C: high density lipoprotein-cholesterol; H_2O_2: hydrogen peroxide; HMUFA: high monounsaturated fatty acid rich-rich diet; HSFA: high saturated fatty acid-rich diet; LDL-C: low density lipoprotein-cholesterol; LFHCC: low fat, high-complex carbohydrate diet; LPO: lipid peroxides; MetS: metabolic syndrome; PUFA: polyunsaturated fatty acid; ROS: reactive oxygen species; SOD: superoxide dismutase activity; T2DM: type 2 diabetes mellitus; TRL: triacylglycerol rich lipoprotein.

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Figure Legends

Figure 1: Intervention study design. Each volunteer was randomly stratified to one of four dietary interventions for 12 weeks. Pre- and post-intervention was administered a fatty meal with a fat composition similar to that consumed in each of the diets.

Figure 2: (A) Reduced glutathione (GSH), (B) reduced glutathione/oxidized glutathione (GSH/GSSG) ratio, and (C) oxidized glutathione (GSSG) in the postprandial state at the end of each dietary period. n=75. Results are expressed as mean ± SEM. Data were analysed using ANOVA for repeated measures.
*p<0.05: HMUFA vs HSFA, LFHCC and LFHCC n-3; † p<0.05: HSFA vs HMUFA, LFHCC and LFHCC n-3; # p<0.05: HSFA vs LFHCC; ‡ p<0.05: HSFA vs LFHCC n-3; ¶ p<0.05: HMUFA vs HSFA and LFHCC.

HSFA: high SFA-rich diet; HMUFA: high MUFA-rich diet; LFHCC: low fat, high-complex carbohydrate diet with 1g/d high oleic sunflower oil (placebo); LFHCC n-3: Low fat, high-complex carbohydrate with 1.24 g/d LC n-3 PUFA diet.

Figure 3: (A) Lipid peroxides, (B) protein carbonyls, (C) superoxide dismutase, (D) hydrogen peroxide, (E) total antioxidant capacity and (F) glutathione peroxidase in the postprandial state at the end of each dietary period. n=75. Results are expressed as mean ± SEM. Data were analysed using ANOVA for repeated measures.
*p<0.05: HMUFA vs HSFA, LFHCC and LFHCC n-3; †p<0.05: HSFA vs HMUFA, LFHCC and LFHCC n-3; ‡ p<0.05: HSFA vs LFHCC n-3; §p<0.05: MUFA vs SFA; ¶ p<0.05: HMUFA vs HSFA and LFHCC.

HSFA: high SFA-rich diet; HMUFA: high MUFA-rich diet; LFHCC: low fat, high-complex carbohydrate diet with 1g/d high oleic sunflower oil (placebo); LFHCC n-3: Low fat, high-complex carbohydrate with 1.24 g/d LC n-3 PUFA diet.

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TABLE 1: Dietary intake at baseline and end of intervention period, alongside dietary targets.

	HSFA	HMUFA	LFHCC	LFHCC n-3
Baseline				
n	17	18	20	20
Energy (MJ/d)	8.81 ± 0.5[a]	8.12 ± 0.4[a]	8.48± 0.4[a]	8.84 ± 0.4[a]
%E from fat	43.3 ± 1.3[a]	42.8 ± 1.2[a]	41.4 ±1.1[a]	45.5 ± 1.4[a]
%E from SFA	11.6 ± 0.5[a]	10.7 ± 0.5[a]	10.2 ± 0.4[a]	12.0 ± 0.4[a]
%E from MUFA	20.9 ± 0.9[a]	21.6 ± 0.7[a]	20.4 ± 0.7[a]	22.7 ± 0.9[a]
%E from PUFA	4.45 ± 0.2[a]	4.84 ± 0.2[a]	4.39 ± 0.2[a]	4.80 ± 0.2[a]
%E from CHO	37.5 ± 1.3[a]	38.9 ± 1.0[a]	40.9 ± 1.3[a]	36.5 ± 1.7[a]
%E from protein	17.2 ± 0.5[a]	17.6 ± 0.7[a]	16.6 ± 0.6[a]	16.8 ± 0.5[a]
Total EPA and DHA (g/d)	0.39 ± 0.06[a]	0.42 ± 0.1[a]	0.36 ± 0.07[a]	0.43 ± 0.06[a]
Target				
%E from fat	38	38	28	28
%E from SFA	16	8	8	8
%E from MUFA	12	20	11	11
%E from PUFA	6	6	6	6
Total EPA and DHA (g/d)				1.24
End of intervention				
n	17	18	20	20
Energy (MJ/d)	8.2 ± 0.4[a]	7.7 ± 0.4[a]	7.7 ± 0.4[a]	9.2 ± 0.5[a]
%E from fat	40.3 ± 0.5[a]	40.2 ± 0.7[a]	27.1 ± 0.5[b]	26.5 ± 0.5[b]
%E from SFA	17.9 ± 0.3[a]	9.1 ± 0.4[b]	6.6 ± 0.3[c]	6.4 ± 0.3[c]
%E from MUFA	12.8 ± 0.3[a]	21.1 ± 0.4[b]	11.5 ± 0.3[c]	11.1 ± 0.3[c]
%E from PUFA	6.1 ± 0.3[a]	5.7 ± 0.2[a]	5.3 ± 0.2[a]	5.0 ± 0.2[a]
%E from CHO	38.3 ± 1.0[a]	40.7 ± 1.0[a]	51.2 ± 1.1[b]	54.1 ± 0.9[b]
%E from protein	19.2 ± 0.7[a]	19.2 ± 0.7[a]	21.2 ± 1[b]	18.8 ± 0.6[a,b]
Total EPA and DHA (g/d)	0.42 ± 0.1[a]	0.41 ± 0.1[a]	0.47 ± 0.08[a]	1.83 ± 0.1[b]

Abbreviations: %E, percentage energy; HSFA, SFA-rich diet; HMUFA, MUFA-rich diet; LFHCC, Low fat, high-complex carbohydrate diet with 1g/d high oleic sunflower oil (placebo); LFHCC n-3, Low fat, high-complex carbohydrate diet with 1.24 g/d LC n-3 PUFA diet. Differences ($P<0.05$) between diet groups were assessed by one-way ANOVA. [a,b,c] Mean values within a row with unlike superscript letters were significantly different. Values presented are the mean ± SEM of each diet group.

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TABLE 2: Baseline characteristics of subjects with the MetS assigned to each diet.

Characteristics	**HSFA**	**HMUFA**	**LFHCC**	**LFHCC n-3**	*P value*
n	17	18	20	20	
Age, years	58.58 (1.9)	54.61 (1.8)	56.35 (1.8)	55.30 (1.4)	0.449
BMI, Kg/m2	35.27 (0.8)	34.45 (0.8)	35.48 (0.6)	35.15 (0.7)	0.798
Total Cholesterol, mg/dL	200.02 (9.8)	189.13 (6.9)	207.16 (10.3)	189.49 (8.0)	0.405
Total TG, mg/dL	171.13 (13.3)	143.74 (13.4)	144.48 (13.0)	138.43 (14.3)	0.654
LDL-C, mg/dL	136.06 (7.7)	131.64 (5.6)	147.58 (8.5)	131.47 (7.6)	0.374
HDL-C, mg/dL	43.2 (2.5)	44.6 (2.3)	44.5 (2.3)	42.1 (1.9)	0.834
ApoB, mg/dL	92.1 (4.1)	89.06 (3.8)	101 (5.6)	90.7 (5.05)	0.291
ApoA-1, mg/dL	133.5 (4.02)	135.03 (5.7)	135.1 (5.3)	130.2 (4.3)	0.876

Abbreviations: HSFA, SFA-rich diet; HMUFA, MUFA-rich diet; LFHCC, Low fat, high-complex carbohydrate diet with placebo; LFHCC n-3, Low fat, high-complex carbohydrate diet with 1.24 g/d LC n-3 PUFA diet; BMI, body mass index; TG, triglycerides; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; apo, apolipoprotein.
*Values presented are the mean ± SEM of each diet group.

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Figure 1.

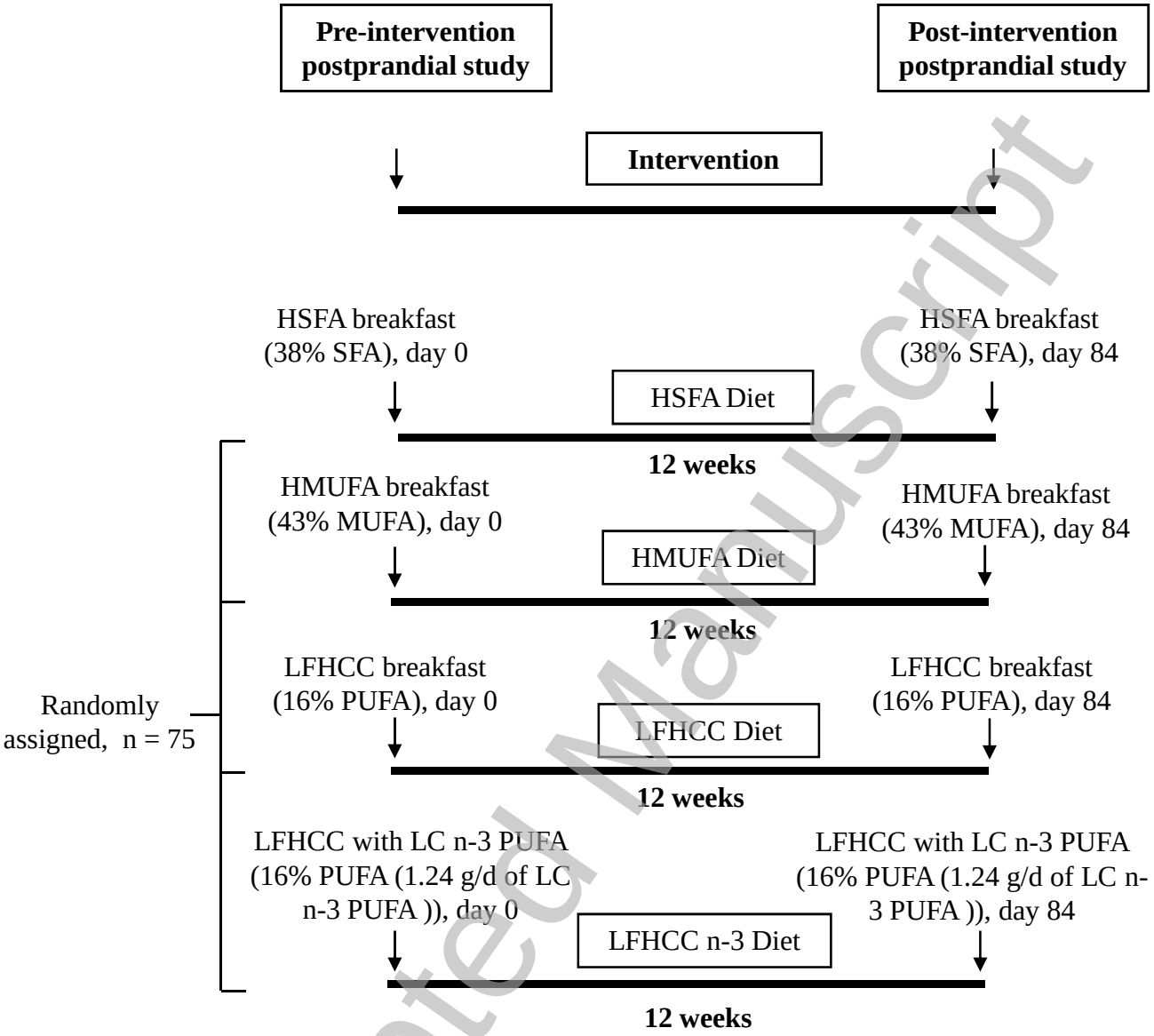

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Figure 2

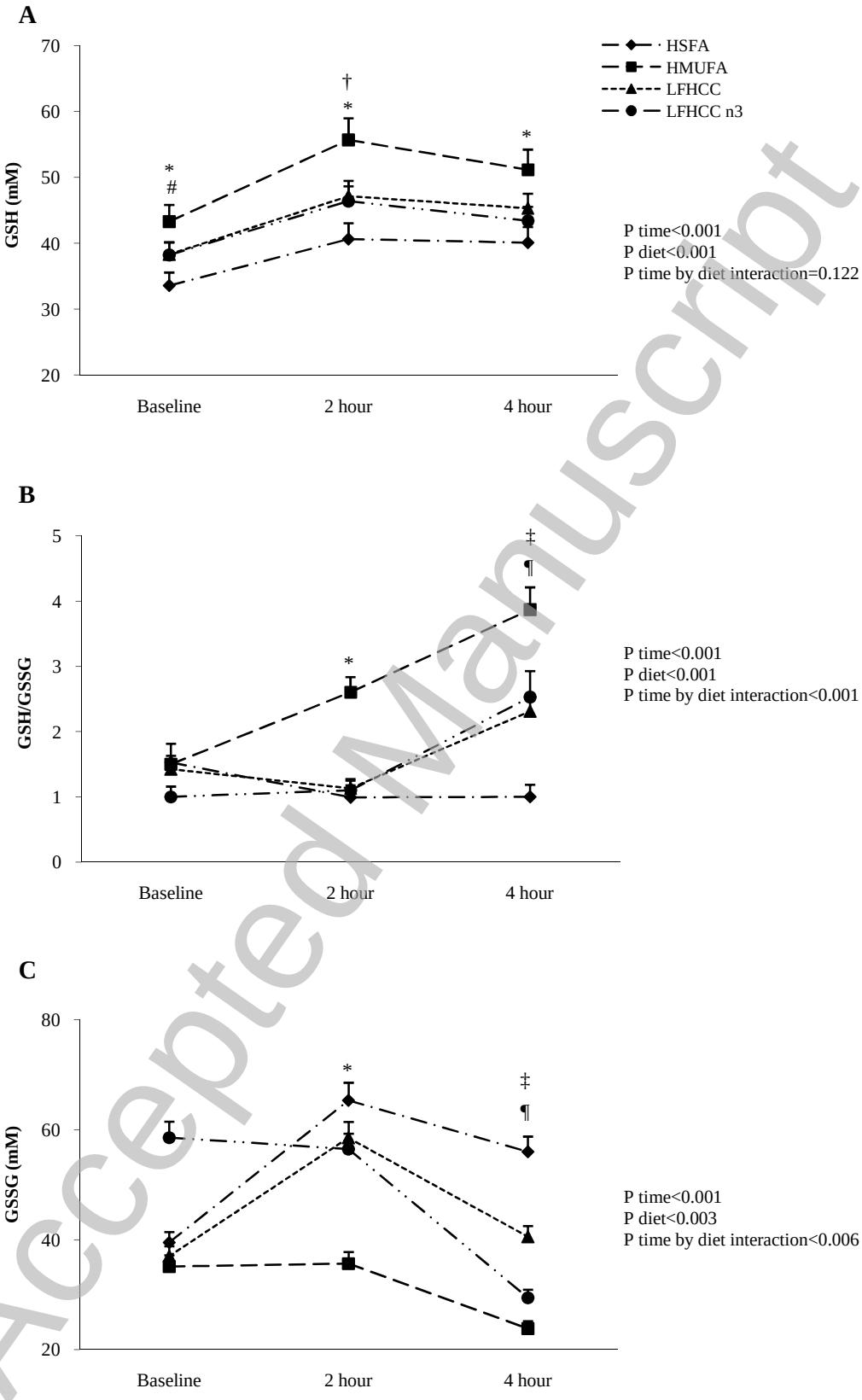

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Figure 3

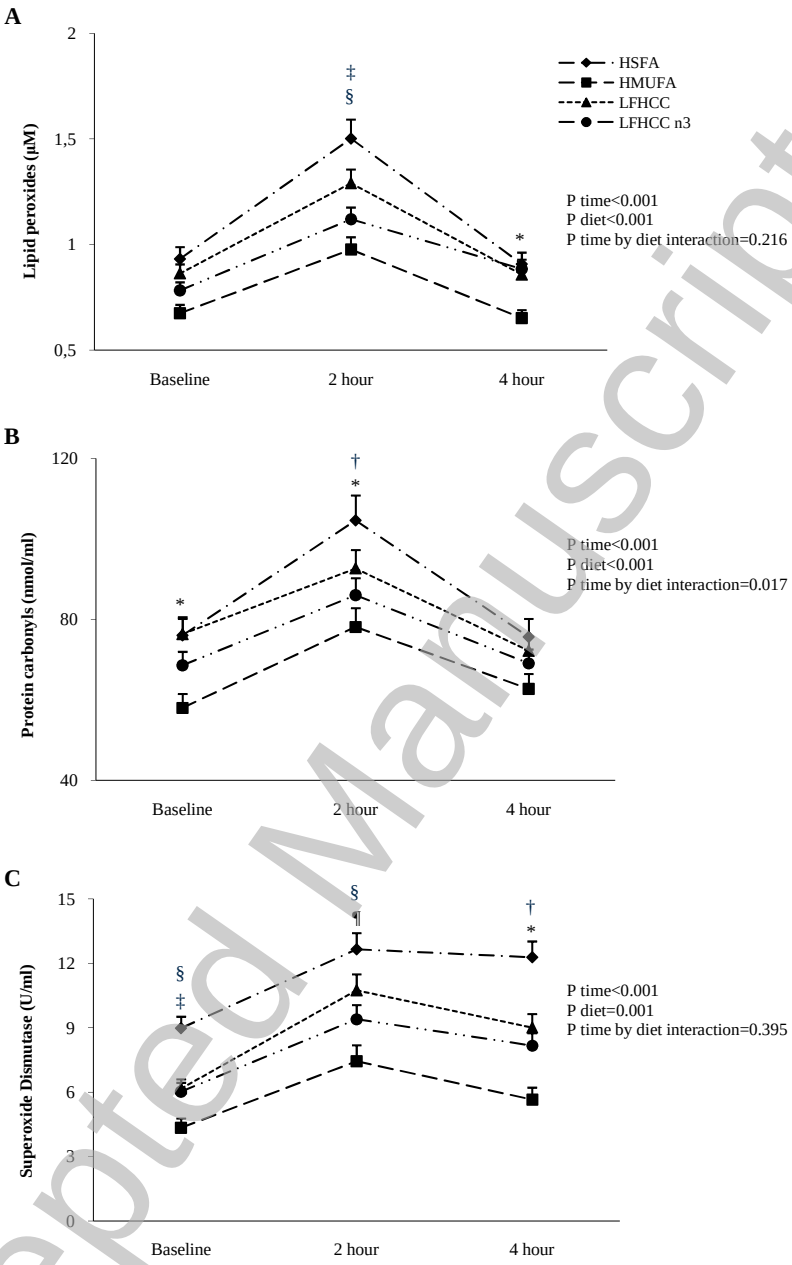

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Figure 3