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

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ABSTRACT

Previous evidence supports the concept that increased oxidative stress may play an important role in MetS (metabolic syndrome)-related manifestations. Dietary fat quality has been proposed to be critical in oxidative stress and the pathogenesis of the MetS. In the present study, 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 for 12 weeks: a high-saturated-fatty-acid (HSFA) diet, a high-mono-unsaturated-fatty-acid (HMUFA) diet 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 1 g/day of sunflower oil high in oleic acid (LFHCC) as placebo]. The HMUFA diet enhanced postprandial GSH (reduced glutathione) levels and the GSH/GSSH (oxidized glutathione) ratio, compared with the other three diets. In addition, after the HMUFA-rich diet postprandial lipid peroxide levels, protein carbonyl concentrations, SOD (superoxide dismutase) activity and plasma H₂O₂ levels were lower compared with subjects adhering to the HSFA-rich diet. Both LFHCC diets had an intermediate effect relative to the HMUFA and HSFA diets. In conclusion, 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 the possible cardioprotective effects associated with mono-unsaturated dietary fat, particularly in subjects with the MetS.

Key words: dietary fat, LIPGENE study, metabolic syndrome, oxidative stress, postprandial lipaemia.

Abbreviations: Apo, apolipoprotein; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; HDL(-C), high-density lipoprotein(-cholesterol); LC, long-chain; LDL(-C), low-density lipoprotein(-cholesterol); LFHCC diet, low-fat/high-complex-carbohydrate diet with 1 g/day of sunflower oil high in oleic acid; LPO, lipid peroxide; MetS, metabolic syndrome; MUFA, mono-unsaturated fatty acid; HMUFA diet, high-fat MUFA-rich diet; NEFA, non-esterified ‘free’ fatty acid; Nrf2, nuclear factor-erythroid 2-related factor 2; PGF₂α, prostaglandin F₂α; PUFAs, polyunsaturated fatty acids; LFHCC n−3 diet, low-fat/high-complex-carbohydrate diet with 1.24 g/day of long-chain n−3 PUFAs; ROS, reactive oxygen species; SFA, saturated fatty acid; HSFA diet, high-fat SFA-rich diet; SOD, superoxide dismutase; T2DM, Type 2 diabetes mellitus; TG, triacylglycerol; TRL, TG-rich lipoprotein.

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INTRODUCTION

The MetS (metabolic syndrome) refers to the aggregation of cardiometabolic risk factors, including insulin resistance, dyslipidaemia, hyperglycaemia and hypertension [1]. It is often characterized by oxidative stress, a condition in which an imbalance results between the production and inactivation of ROS (reactive oxygen species) [2]. Previous studies support the concept that increased oxidative stress may play an important role in MetS-related manifestations, including atherosclerosis, hypertension and T2DM (Type 2 diabetes mellitus) [3]. Patients with the MetS have elevated oxidative damage, as evidenced by decreased antioxidant protection, and increased lipid peroxidation, protein carbonyls and plasma H\textsubscript{2}O\textsubscript{2} 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 the environment and dietary habits in determining inflammatory-process-triggered oxidation are still uncertain.

Previous dietary intervention studies have demonstrated that altering fat composition can significantly reduce oxidative stress in the MetS [5,6]. A number of clinical trials have examined short- or intermediate-term effects of the Mediterranean diet on different circulating markers of oxidative stress [7–10]. Recently, Dai et al. [11] reported a robust inverse association between adherence to the Mediterranean diet and oxidative stress as measured by the GSH (reduced glutathione)/GSSG (oxidized glutathione) ratio, independent of a wide range of known cardiovascular disease risk factors [11]. Other trials, however, have yielded conflicting results. Given the potential of altering dietary fat composition [i.e. \(n-3\) PUFAs (polyunsaturated fatty acids) or low-fat diets] in the prevention and treatment of a number of chronic diseases, including the MetS [12], and the inconsistencies found so far, this subject is in need of a 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 characterizing fasting metabolic markers. However, humans spend the majority of time in a non-fasting postprandial state, with a continual fluctuation in the degree of lipaemia 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 oxidative stress biomarkers [13–15]. On the basis of previous findings, in the present study, we have investigated whether oxidative stress is affected by diets with different fat quantity and quality during the postprandial state in patients with the MetS from the LIPGENE study.

MATERIAL AND METHODS

Design

The present 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 VI Integrated Project funded by the European Union. A subgroup of patients with the MetS was randomly stratified to one of four dietary interventions. The MetS was defined according to published criteria [16,17], which conformed to the LIPGENE inclusion and exclusion criteria [18]. Pre- and post-intervention, a fatty meal was administered 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 described previously in detail by Shaw et al. [19].

Participants and recruitment

A total of 75 patients were included. All participants gave written informed consent and underwent a comprehensive medical history, physical examination and clinical chemistry analysis before enrolment. The 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 ethics 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). Randomization was completed centrally, according to age, gender and fasting plasma glucose concentration using the MINIM (Minimisation Programme for Allocating patients to Clinical Trials; Department of Clinical Epidemiology, The London Hospital Medical College, London, U.K.) randomization program. The composition of the four diets was as follows: (i) HSFA (high-fat (38 % energy) SFA (saturated fatty acid)-rich diet [16 % SFA, 12 % MUFA (mono-unsaturated fatty acid) and 6 % PUFA]); (ii) HMUFA [high-fat (38 % energy) MUFA-rich diet (8 % SFA, 20 % MUFA and 6 % PUFA)]; (iii) LFHCC [low-fat (28 % energy)/high-complex-carbohydrate diet (8 % SFA, 11 % MUFA and 6 % PUFA), with 1 g/day of sunflower oil high in oleic acid (placebo)]; and (iv) LFHCC \(n-3\) [low-fat (28 % energy)/high-complex-carbohydrate diet (8 % SFA, 11 % PUFA)].
MUFA and 6 % PUFA), with 1.24 g/day of LC (long-chain n−3 PUFA]).

Post-intervention (week 12), we performed a postprandial challenge with the same fat composition as consumed during the assigned dietary period (Figure 1). Patients presented to the clinical centre at 08:00 hours 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 was then ingested under supervision within 20 min. The test meal reflected the fatty acid composition of each subject’s dietary intervention. Subsequent blood samples were drawn at 2 and 4 h. Test meals provided an equal amount of fat (0.7 g/kg of body weight), cholesterol (5 mg/kg of body weight) and vitamin A (60 000 units/m² of 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 and did not consume any other food for 9 h, but were allowed to drink water. The composition of the breakfasts was as follows: HSFA breakfast (38 % SFA, 21 % MUFA and 6 % PUFA); HMUFA breakfast (12 % SFA, 43 % MUFA and 10 % PUFA); LFHCC breakfast with placebo capsule (21 % SFA, 28 % MUFA and 16 % PUFA); LFHCC with LC n−3 PUFA (21 % SFA, 28 % MUFA and 16 % PUFA, with 1.24 g/day of LC n−3 PUFA).

**Measurements**

Blood was collected in tubes containing 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 °C.

Biomarkers were determined in frozen samples by laboratory investigators who were blinded to the interventions. Lipid parameters were assessed with a DDPTII Hitachi modular analyser (Roche), using specific reagents (Boehringer-Mannheim). Plasma TG [triacylglycerol (triglyceride)] and cholesterol concentrations were assayed using enzymatic procedures [20,21]. Apo (apolipoprotein) A-I and apoB were determined by turbidimetry [22]. HDL-C [HDL (high-density lipoprotein)-cholesterol] was measured by precipitation of an aliquot of plasma with dextran sulphate-Mg²⁺, as described by Warnick et al. [23]. LDL-C [LDL (low-density lipoprotein)-cholesterol] was calculated using the following formula:

\[ \text{LDL-C} = \text{plasma cholesterol} - \text{HDL-C} + \text{large TRL-C} + \text{small TRL-C} \]
where TRL-C is TG-rich lipoprotein-cholesterol. Plasma glucose concentrations were measured using an Architect-CG16000 analyzer (Abbott Diagnostics) by the exoquinase method, and plasma insulin concentrations were measured by chemoluminescence with an Architect-I2000SR analyser (Abbott Diagnostics). Plasma fatty acid composition was determined using the enzymatic colorimetric assay for the determination of NEFAs (non-esterified ‘free’ fatty acids) using the Half-micro test (Roche Diagnostics).

**Determinant of oxidative stress biomarkers**

LPOs (lipid peroxides) in plasma were estimated using the method described by Eldermeier et al. [24]. This method uses a chromatogenic reagent which reacts with the lipid peroxidation products malondialdehyde and 4-hydroxyalkenals at 45 ± 1 °C, yielding a stable chromophore with a maximum absorbance at 586 nm. Protein carbonyl content was carried out in plasma samples using the method described by Levine et al. [25]. Samples were incubated with 500 ml of a 10 mM solution of 2,4-dinitrophenylhydrazine in 2 M HCl for 60 min. Subsequently, the proteins were precipitated from the solutions using 500 ml of 20 % (v/v) trichloroacetic acid. The proteins were then washed three times with a solution of ethanol and ethylacetate (1:1, v/v) and dissolved in 1 ml of 6 M guanidine hydrochloride (containing 20 mM phosphate buffer, pH 2.3, in trifluoroacetic acid) at 37 °C. The carbonyls were evaluated in a spectrophotometer (UV-1603; Shimadzu) at a wavelength of 360 nm [26].

Antioxidant enzyme activity

Total SOD (superoxide dismutase; E.C.1.15.1.1) activity was determined using a colorimetric assay in plasma at a wavelength of 525 nm, according to the laboratory method described by McCord and Friedovich [31] and Nebot et al. [32]. GPx (glutathione peroxidase; E.C.1.11.1.9) activity was evaluated in plasma using the Glutathione Peroxidase assay kits (Cayman Chemical). This assay is based on the oxidation of NADPH to NADP+, catalysed by a limiting concentration of glutathione reductase, with a maximum absorbance at 340 nm. GPx activity was measured based on the formation of GSSG from the GPx-catalysed oxidation of GSH by \( \text{H}_2\text{O}_2 \), coupled with NADPH consumption in the presence of exogenously added glutathione reductase, with a maximum absorbance at 340 nm. This assay is based on the method described by Flohé and Gunzler [33].

Although the GSH/GSSG ratio and antioxidant enzyme activities may be measured in red blood cells, their measurement in plasma provides an approximation of the blood state, reflecting the similar change at the intracellular level [34].

**Statistical analyses**

All results are means ± S.E.M. SPSS 17 for Windows was used for statistical analysis. The data were analysed using repeated-measures ANOVA and Student’s t test for paired data analysis and ANOVA for repeated measures. In this analysis we studied: (i) the postprandial time points; (ii) the effect of the type of fat meal ingested, independent of time (represented by dietary fat); and (iii) the interaction of both factors, indicative of the degree of the postprandial response in each group of subjects with each breakfast (represented by a 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 Huynh–Feldt contrast statistic was used when the sphericity assumption was not satisfied. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

Dietary compliance was good, with a 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 with the HMUFA diet group, who consumed 21 % as mono-unsaturated fat, whereas 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 group to 26.5 %,
Dietary intake at baseline and at the end of the intervention period, alongside postprandial plasma TG, glucose, insulin, GSH, the MetS randomized to each dietary intervention. During treatment with the HSFA diet we observed an increase in plasma palmitic acid (C16:0; 11 %), stearic acid (C18:0; 20 %) and myristic acid (C14:0; 90 %) compared with the baseline state. Finally, in our typical Mediterranean diet population, induced an increase in DHA (96 %) and EPA (206 %). Table 2 shows the age, baseline BMI (body mass index) and lipid-related risk factors in the 75 subjects with the MetS randomized to each dietary intervention.

The effect of dietary fat quality and quantity on postprandial plasma TG, glucose, insulin, GSH, the GSH/GSSG ratio, LPOs, protein carbonyls, \( \text{H}_2\text{O}_2 \), SOD, total antioxidant capacity and GPx were assessed post-intervention. Postprandial glucose and insulin levels after the intervention were not significantly different between diets (results not shown). We observed a significant increment in the AUC (area under the curve) for postprandial TG during the HSFA diet (5231.87 mmol \( \cdot \text{min}^{-1} \cdot \text{l}^{-1} \)) compared with the other three diets. Differences in TG values were accompanied by a similar pattern for postprandial glucose, insulin, LPOs, protein carbonyls, \( \text{H}_2\text{O}_2 \), SOD, total antioxidant capacity and GPx.

Table 2 shows the age, baseline BMI (body mass index) and lipid-related risk factors in the 75 subjects with the MetS randomized to each dietary intervention.

<table>
<thead>
<tr>
<th>Diet</th>
<th>HSFA (n = 17)</th>
<th>HMUFA (n = 18)</th>
<th>LFHCC (n = 20)</th>
<th>LFHCC n = 3 (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (MJ/day)</td>
<td>8.81 ± 0.5^a</td>
<td>8.12 ± 0.4^a</td>
<td>8.48 ± 0.4^a</td>
<td>8.84 ± 0.4^a</td>
</tr>
<tr>
<td>%E from fat</td>
<td>43.1 ± 1.3^a</td>
<td>42.8 ± 1.2^a</td>
<td>41.4 ± 1.1^a</td>
<td>45.5 ± 1.4^a</td>
</tr>
<tr>
<td>%E from SFA</td>
<td>11.6 ± 0.5^a</td>
<td>10.7 ± 0.5^a</td>
<td>10.2 ± 0.4^a</td>
<td>12.0 ± 0.4^a</td>
</tr>
<tr>
<td>%E from MUFA</td>
<td>20.9 ± 0.9^a</td>
<td>21.6 ± 0.7^a</td>
<td>20.4 ± 0.7^a</td>
<td>22.7 ± 0.9^a</td>
</tr>
<tr>
<td>%E from PUFA</td>
<td>4.45 ± 0.2^a</td>
<td>4.84 ± 0.2^a</td>
<td>4.39 ± 0.2^a</td>
<td>4.80 ± 0.2^a</td>
</tr>
<tr>
<td>%E from CHO</td>
<td>37.5 ± 1.3^a</td>
<td>38.9 ± 1.0^a</td>
<td>40.9 ± 1.3^a</td>
<td>36.5 ± 1.7^a</td>
</tr>
<tr>
<td>%E from protein</td>
<td>17.2 ± 0.5^a</td>
<td>17.6 ± 0.7^a</td>
<td>16.6 ± 0.6^a</td>
<td>16.8 ± 0.5^a</td>
</tr>
<tr>
<td>Total EPA and DHA (g/day)</td>
<td>0.39 ± 0.06^a</td>
<td>0.42 ± 0.1^a</td>
<td>0.36 ± 0.07^a</td>
<td>0.43 ± 0.06^a</td>
</tr>
</tbody>
</table>

| 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/day) | 1.24          |               |               |                     |

| End of intervention |               |               |               |                     |
| Energy (MJ/day) | 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^a | 26.5 ± 0.5^a       |
| %E from SFA | 17.9 ± 0.3^a | 9.1 ± 0.4^a  | 6.6 ± 0.3^a  | 6.4 ± 0.3^a        |
| %E from MUFA | 12.8 ± 0.3^a | 21.1 ± 0.4^a | 11.5 ± 0.3^a | 11.1 ± 0.3^a       |
| %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^a | 54.1 ± 0.9^a       |
| %E from protein | 19.2 ± 0.7^a | 19.2 ± 0.7^a | 21.2 ± 1.0^a | 18.8 ± 0.6^a       |
| Total EPA and DHA (g/day) | 0.42 ± 0.1^a | 0.41 ± 0.1^a | 0.47 ± 0.08^a | 1.83 ± 0.1^b       |

Whereas carbohydrate consumption rose to 51.2 and 54 % respectively. The LFHCC n = 3 diet group attained their target intake of EPA (eicosapentaenoic acid; \( \text{C}_{22.5-6} \)) and DHA (docosahexaenoic acid; \( \text{C}_{22.6,6-3} \)) at 1.8 g/day. In addition, analysis of plasma fatty acids obtained after each dietary period showed good adherence in the different intervention stages. During treatment with the HSFA diet, we observed an increase in plasma palmitic acid (C16:0; 11 %), stearic acid (C18:0; 20 %) and myristic acid (C14:0; 90 %) compared with the baseline state. Consistently, the chronic intake of the LFHCC n = 3 diet induced an increase in DHA (96 %) and EPA (206 %). Finally, in our typical Mediterranean diet population, subjects consumed a high intake of oleic acid (C18:1) at baseline (21.6 % energy from MUFA) and no significant increase was observed at the end of the HMUFA diet period (21.1 % energy from MUFA).

Table 2 shows the age, baseline BMI (body mass index) and lipid-related risk factors in the 75 subjects with the MetS randomized to each dietary intervention.

The effect of dietary fat quality and quantity on postprandial plasma TG, glucose, insulin, GSH, the GSH/GSSG ratio, LPOs, protein carbonyls, \( \text{H}_2\text{O}_2 \), SOD, total antioxidant capacity and GPx were assessed post-intervention. Postprandial glucose and insulin levels after the intervention were not significantly different between diets (results not shown). We observed a significant increment in the AUC (area under the curve) for postprandial TG during the HSFA diet (5231.87 mmol \( \cdot \text{min}^{-1} \cdot \text{l}^{-1} \)) compared with the HMUFA diet (2095.32 mmol \( \cdot \text{min}^{-1} \cdot \text{l}^{-1} \)) and LFHCC n = 3 diet (2354.65 mmol \( \cdot \text{min}^{-1} \cdot \text{l}^{-1} \)) (\( P < 0.018 \) when HSFA compared with HMUFA, \( P < 0.018 \) when HSFA compared with LFHCC n = 3).

Figure 2(A) shows that postprandial plasma GSH levels were higher 2 h after the intake of the HMUFA diet (\( P < 0.05 \)) compared with 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 with the LFHCC and LFHCC n = 3 diets. As an indicator of redox status, we measured the GSH/GSSG ratio. Interestingly, a consistent postprandial
increase in the GSH/GSSG ratio was observed 2 h after the HMUFA diet \((P < 0.001)\) compared with the other three diets (Figure 2B). The GSH/GSSG ratio 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 three 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 \(n=3\) diet \((P < 0.005)\). Given the important potential effect of fat composition, we also measured lipid peroxidation. Interestingly, plasma LPO levels were significantly higher 2 h after the HSFA diet compared with subjects adhering to the HMUFA and LFHCC \(n=3\) diets \((P < 0.05; \text{Figure 3A})\). Moreover, plasma LPO levels remained lower later in the postprandial phase 4 h after the HMUFA diet compared with the other diets (Figure 3A). In the case of the postprandial concentration of protein carbonyls, a significant time–diet interaction \((P < 0.02)\) confirmed that levels were significantly higher 2 h after the HSFA diet, but were lower 2 h after the HMUFA diet (Figure 3B). Consistent with this effect, postprandial SOD was significantly lower 2 and 4 h after the HMUFA diet compared with the HSFA and LFHCC diets \((P < 0.001; \text{Figure 3C})\). Again, an intermediate effect was found after the intake of the two LFHCC diets compared with the HSFA diet (Figure 3C). Postprandial plasma \(H_2O_2\) levels were significantly 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 the 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, the GSH/GSSG ratio, GSSG, LPOs, protein carbonyls, \(H_2O_2\), SOD, total antioxidant capacity and GPx at baseline (i.e. before the test meal). In this context, we found significant 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 GSH plasma levels compared with the HSFA, LFHCC and LFHCC \(n=3\) diets \((P < 0.002; \text{Figure 2A})\). Moreover GSH levels remained significantly lower after the HSFA diet compared with the LFHCC diet \((P < 0.05; \text{Figure 2A})\). Protein carbonyl concentrations were significantly lower after the HMUFA diet compared with the other three diets \((P < 0.001; \text{Figure 3B})\). Finally, SOD was significantly higher after the HSFA diet compared with the HMUFA \((P < 0.002)\) and LFHCC \(n=3\) \((P < 0.043)\) diets (Figure 3C).

**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 aetiology of the syndrome is poorly understood. Our present study supports the notion that the HMUFA diet improves postprandial oxidative stress parameters as measured by GSH and the GSH/GSSG ratio. In addition, the HMUFA diet induced lower postprandial plasma levels of LPOs, protein carbonyl concentrations and SOD compared with subjects adhering to the other three diets. Furthermore, postprandial plasma \(H_2O_2\) levels were unfavourably increased during the HSFA diet compared with the other three diets.

Fasting is not the typical physiological state of the modern human being, who spends most the time in the postprandial state. Therefore an assessment of the postprandial lipaemic 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

| Table 2  Baseline characteristics of the subjects with the MetS assigned to each diet |
|----------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic | Diet                | HSFA \((n = 17)\) | HMUFA \((n = 18)\) | LFHCC \((n = 20)\) | LFHCC \(n=3\) \((n = 20)\) | \(P\) value |
| Age (years)     | 50.58 ± 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           |
| Apolipoprotein B (mg/dl) | 92.1 ± 4.1 | 89.06 ± 3.8      | 101 ± 5.6        | 90.7 ± 5.05      | 0.291           |
| Apoa-I (mg/dl)  | 133.5 ± 4.02        | 135.03 ± 5.7     | 135.1 ± 5.3      | 130.2 ± 4.3      | 0.876           |

Values are means ± S.E.M.
Postprandial oxidative stress and dietary fat

Figure 2  GSH (A), the GSH/GSSG ratio (B) and GSSG (C) in the postprandial state at the end of each dietary period. Results are means ± S.E.M., n = 75. Results were analysed using ANOVA for repeated measures. * P < 0.05 for the HMUFA diet compared with the HSFA, LFHCC and LFHCC n−3 diets; † P < 0.05 for the HSFA diet compared with HMUFA, LFHCC and LFHCC n−3 diets; # P < 0.05 for the HSFA diet compared with LFHCC diet; ¶ P < 0.05 for the HMUFA diet compared with HSFA and LFHCC diets.

with the MetS have greater levels of oxidative stress after a fat overload [14,15], the present well-controlled intervention study is, to 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 measure 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 present study, the HMUFA diet produced a postprandial increase in GSH plasma levels and the GSH/GSSG ratio compared with the other three diets (HSFA and LFHCC diets). Thus our present results obtained in the postprandial state support previous evidence in the fasting state suggesting that dietary patterns similar to those of the Mediterranean-style diet exert positive effects on the GSH/GSSG ratio [11]. Additionally, Fitó et al. [39] found that olive oil caused increases in plasma glutathione reductase levels. These findings support the idea that the HMUFA diet may trigger an increase in GSH and the GSH/GSSG ratio, together with a reduction in GSSG by means of stimulating glutathione reductase activity. The major levels of GSH may help to regenerate the most important antioxidants such as ascorbic acid, α-tocopherol and others [40]. This situation may explain the highest antioxidant effect of the HMUFA diet compared with the LFHCC and LFHCC n−3 diets on oxidative stress characterized by a reduction in lipid peroxidation, carbonylated protein and H2O2 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, as is suggested by our present results. Initially, GSH is oxidized into GSSG by the enzyme GPx; in this process, GSH quenches peroxides. GSSG reverts to GSH via glutathione reductase with concomitant oxidation of NADPH [41]. Diverse nutrients and biofactors in foods characteristic of a Mediterranean diet may provide higher NADPH [42] and up-regulate glutathione reductase activity [43], which may lead to a decrease in GSSG and a resulting higher GSH/GSSG ratio. Interestingly the effects observed in 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 the potential effects of the background diet on the oxidative stress status. Consistent with this, we have demonstrated that the long-term consumption of an HMUFA diet increased GSH plasma levels and decreased protein carbonyl 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
Figure 3  LPOs (A), protein carbonyls (B), SOD (C), \(H_2O_2\) (D), total antioxidant capacity (E) and GPx (F) in the postprandial state at the end of each dietary period

Results are means ± S.E.M., \(n = 75\). Results were analysed using ANOVA for repeated measures. *\(P < 0.05\) for the HMUFA diet compared with the HSFA, LFHCC and LFHCC \(n−3\) diets; †\(P < 0.05\) for the HSFA diet compared with the HMUFA, LFHCC and LFHCC \(n−3\) diets; ‡\(P < 0.05\) for the HSFA diets compared with the LFHCC \(n−3\) diet; §\(P < 0.05\) for the MUFA diet compared with the HSFA diet; ¶\(P < 0.05\) for the HMUFA diet compared with HSFA and LFHCC diets.

oxidative stress. Our results are important, particularly in view of the lack of randomized controlled trials assessing the effects of diets rich in MUFAs typical of the traditional Mediterranean diet on glutathione redox pathways in the general population and in patients with the MetS.

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 LPOs, where NEFAs can modulate the signalling functions of ROS [46]. In the present study, the HMUFA diet induced lower postprandial plasma levels of LPO and protein carbonyl concentrations compared with subjects adhering to the other three diets. Although not explored, and beyond the remit of our present study, a probable mechanistic explanation for the observed effects lies in the high intake of MUFAs, together with natural antioxidants present in the Mediterranean diet that could prevent the accumulation of LPOs. In accordance with our present results, a PREDIMED substudy [10] demonstrated that...
adherence to a Mediterranean diet was associated with reduced oxidative status, as shown by lower serum concentrations of oxidized LDL compared with a low-fat diet [10]. On the other hand, our present findings 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].

Over the past few years, several studies have shown that n−3 PUFAs have potentially cardioprotective effects, especially in high-risk patients with dyslipidaemia, and might therefore be expected to be of benefit in T2DM. In our present study, when compared with the HSFA diet, the effect of the two LFHCC diets was a favourable intermediate. Consistent with this, a previous study has shown that daily supplementation of EPA and DHA for 3 months reduced levels of 8-iso-PGF_{2α} (prostaglandin F2α) measured in plasma, but not 15-keto-dihydro-PGF_{2α}, compared with controls among healthy subjects in the KANWU study [47]. An isoprostane-reducing effect of n−3 PUFAs has also been observed in treated hypertensive patients with T2DM [48]. In contrast with these previous studies, PUFAs have been suggested to be more prone to oxidation than SFAs and MUFAs [49,50]. Although only suggestive, these results need to be confirmed and extended.

In response to oxidative stress, cells attempt to fortify their 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 inactivate ROS. Superoxide is converted into H_{2}O_{2} by a group of enzymes known as SOD. H_{2}O_{2}, in turn, is converted into water and molecular oxygen by either catalase or GPx. In addition, GPx can reduce lipid hydroperoxides and other organic hydroperoxides. A previous study [51] has shown that the expression of SOD is up-regulated by superoxide, so the decrease in SOD after consumption of the HMUFA diet could be explained by 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 element sequences in their promoters that bind to the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2) in response to increased ROS, promoting an increase in the expression of these enzymes [52]. In our present study, the long-term consumption of the HSFA diet also increased 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 an increased activation of Nrf2, which we believe could be a direct consequence of a higher production of superoxide in this situation.

In the present 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 significant differences between these parameters, a trend was observed between the HSFA diet compared with the HMUFA diet. Interestingly, our results also expand our recent finding in this same population of an inverse association between the HMUFA diet and inflammation [18], as 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].

Hyperlipidaemia and hyperglycaemia have been associated with increased oxidative damage affecting lipoproteins and the antioxidant status [53]. Previous evidence suggests that prolonged increases in TRL, associated with decreased chylomicron clearance, result in a restriction in vitamin E transfer to LDL and HDL along with a greater susceptibility to oxidation [53]. In this context, we have demonstrated in a recent study that an HMUFA diet can lower postprandial lipoprotein abnormalities associated with the MetS (Y. Jimenez-Gomez, C. Marin, P. Perez-Martinez, J. Hartwich, M. Malczewska-Malec, I. Golabek, B. Kiec-Wilk, C. Cruz-Teno, P. Gomez, M.J. Gomez-Luna, C. Defoort, M.J. Gibney, F. Perez-Jimenez, H.M. Roche and J. Lopez-Miranda, unpublished work). In addition, 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 in the postprandial oxidative stress observed in these patients. However, the detailed relationships among hyperlipidaemia, hyperglycaemia, hyperinsulinaemia and oxidative stress are still under investigation.

Our present study does have some limitations. Ensuring adherence to dietary instructions is difficult in a feeding trial. However, adherence to the recommended dietary patterns was good, as judged by our 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 present 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 cardioprotective effects associated with the Mediterranean diet, particularly in subjects with the MetS.
these findings support recommendations to consume an HMUFA diet as a useful tool to prevent cardiovascular disease in patients with the MetS.

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