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RESEARCH ARTICLE

Dietary fat modifies the postprandial inflammatory state in subjects with metabolic syndrome: the LIPGENE study

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Scope: Our aim was to investigate whether the inflammatory state associated to metabolic syndrome (MetS) patients is affected by diets with different fat quality and quantity.

Methods and results: Seventy-five subjects from LIPGENE cohort were included in this feeding trial and randomly assigned to one of four diets: high saturated fatty acids (HSFA); high monounsaturated fatty acids (HMUFA) and two low-fat, high complex carbohydrate (LFHCC) diets, supplemented with long-chain *n*-3 polyunsaturated fatty acids (LFHCC *n*-3) or placebo (LFHCC), for 12 weeks each. A postprandial fat challenge, reflecting the intervention dietary fat composition, was conducted post-intervention. The HMUFA diet significantly reduced postprandial nuclear transcription factor-kappaB (NF-kB) activity and the nuclear p65 protein levels relative to fasting values ($p < 0.05$). Furthermore, we observed a postprandial decrease in this protein with the HMUFA diet compared with the HSFA and LFHCC diets ($p < 0.05$). The postprandial response of inhibitory molecule from NF-kB mRNA levels increased with the HMUFA diet compared with the HSFA and LFHCC *n*-3 diets ($p < 0.05$). Postprandial tumor necrosis factor-α and Metalloproteinase 9 mRNA levels were also reduced after the HMUFA diet compared with the HSFA diet ($p < 0.05$).

Conclusion: Our results indicate that the long-term consumption of a healthy diet model with HMUFA attenuates the postprandial inflammatory state associated with MetS.

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1 Introduction

The metabolic syndrome (MetS) is a cluster of metabolic abnormalities leading to increased risk for cardiovascular disease and diabetes type 2 [1]. Metabolic, genetic and environmental factors, in particular dietary excess, play an important role in its development and progression [2, 3]. The obese,

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Abbreviations: CHO, carbohydrate; **DHA**, docosahexaenoic acid; **E**, energy; **HMUFA**, High monounsaturated fatty acids diet; **HSFA**, High saturated fatty acids diet; **IkB-α**, inhibitory molecule from NF-kB; **IL-6**, interleukin 6; **IR**, insuline resistance; **LC**, long chain; **LFHCC**, low-fat, high-complex carbohydrate diet; **MCP-1**, monocyte chemoattractant protein-1; **MetS**, metabolic syndrome; **MIF**, macrophage migration inhibitory factor; **MMP-9**, metalloproteinase 9; **NEFA**, non-esterified fatty acids; **NF-kB**, nuclear transcription factor kappa B; ***n*-3 PUFA**, *n*-3 polyunsaturated fatty acids; **PBMC**, peripheral blood mononuclear cells; **TNF-α**, tumor necrosis factor-α.

insulin-resistant (IR), pro-inflammatory state are central to the disease process [1].

Obesity and IR are characterized by increased macrophage infiltration, altered cytokine production and activation of inflammatory signalling pathway in adipose tissue. In this context, previous studies have confirmed an increase in the expression of pro-inflammatory cytokines in peripheral blood mononuclear cells (PBMC) and adipose tissue of the obese subjects [4]. The amount and type of dietary fat, such as high intake of saturated fatty acids (SFA) has been reported to contribute to the development of components of MetS producing pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) via the nuclear transcription factor-kappaB (NF-kB) activation [1].

Inflammation at the cellular level can be described as an increase in the NF-kB (p50/p65) in the nucleus accompanied by a decrease in its inhibitors inhibitory molecule from NF-kB (IkB-α) and/or IkB-β [5]. NF-kB is a pleiotropic transcription factor activated by reactive oxygen species (ROS), among others, and inhibited by antioxidants [6]. This factor regulates the expression of several cytokines, chemokines, cell adhesion molecules, immunoreceptors and inflammatory enzymes [7], molecules that are involved in diseases such as atherosclerosis and IR. Previous studies have confirmed that fat consumption induced the activation of inflammatory markers during the postprandial phase [8, 9]. However, to date no study has determined the effect of a long-term dietary fat intervention on the postprandial inflammation response in subjects with MetS. In this regard, we have previously demonstrated that Mediterranean diet attenuated PBMC NF-kB activation compared with a Western SFA-rich diet in young healthy subjects [10, 11]. Interestingly, a recent study by Weldon et al. [12] demonstrated that docosahexaenoic acid (DHA) suppressed the mRNA expression and production of several pro-inflammatory cytokines, including TNF-α and IL-6, in Lipopolysacharide-stimulated human acute monocytic leukemia cell line (THP-1) monocyte-derived macrophages.

On the other hand, it should be remembered that we spend most of our time in a postprandial state, with a continual fluctuation in the degree of lipemia throughout the day. In addition, previous evidence suggests that postprandial lipemia induces endothelial dysfunction [13], which is accompanied by an acute inflammatory response [14] and a pro-coagulant state [15]. For all these reasons, it is essential to know what changes are produced during the postprandial phase that is influenced by the quantity and quality of the fat ingested [16].

To this end, our aim was to determine whether the long-term consumption of four isoenergetic diets with different fat contents has a selective influence on the postprandial NF-kB activity and the related expression of pro-inflammatory genes in PBMC of MetS patients. In a next step, we explored whether plasma cytokine levels were affected by these diets.

2 Methods and Materials

2.1 Participants and recruitment

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. Seventy-five patients with MetS (28 males and 47 females) from LIPGENE cohort were included in the study. 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 Research Unit at the Reina Sofia University Hospital from February 2005 to April 2006. The experimental protocol was approved by the local ethic committee at each of the intervention centre, according to the Helsinki Declaration. The study was registered with The US National Library of Medicine Clinical Trials registry (NCT00429195).

2.2 Design

Each volunteer was randomly stratified to one of four dietary interventions for 12 weeks (Supporting Information Fig. 1). MetS was defined by published criteria [17] that conformed to the LIPGENE inclusion and exclusion criteria [18]. The MetS patients of our study were not taking medications for lipids and diabetes; however, 53% patients were taking medications for blood pressure. Post-intervention was administered a breakfast or test meal with the same fat composition that is consumed in each of the diets. The intervention study design and the dietary strategy protocol have been previously described in detail by Shaw et al. [18], which also provides information about the control of food consumption during intervention, adherence to the dietary advices and details of diet composition at baseline and end of intervention period. Briefly, to realize the dietary assessment volunteers completed 3-day weighed food diaries at baseline, week 6 and week 12. Weighed food intake over 2 weekdays and 1 weekend day was obtained using scales provided by the investigators. The dietary analysis program used for our centre was the 'Dietsource version 2.0'.

2.3 Randomisation and intervention

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 isoenergetic diets (Table 1) was as follows:

Table 1. Composition of diets pre- and post-intervention period, alongside dietary targets

Completers	HSFA Mean ± SEM	HMUFA Mean ± SEM	LFHCC Mean ± SEM	LFHCC *n*-3 Mean ± SEM
Pre-intervention				
N	17	18	20	20
Energy (MJ/day)	8.8 ± 0.5	8.1 ± 0.4	8.5 ± 0.4	8.8 ± 0.4
%E from fat	43.3 ± 1.3	42.8 ± 1.2	41.4 ± 1.1	45.5 ± 1.4
%E from SFA	11.6 ± 0.5	10.7 ± 0.5	10.2 ± 0.4	12.0 ± 0.4
%E from MUFA	20.9 ± 0.9	21.7 ± 0.7	20.4 ± 0.7	22.7 ± 0.9
%E from PUFA	4.4 ± 0.2	4.8 ± 0.2	4. 4 ± 0.2	4.8 ± 0.2
%E from CHO	37.6 ± 1.3	38.9 ± 1.0	40.9 ± 1.3	36.6 ± 1.7
%E from protein	17.2 ± 0.5	17.7 ± 0.7	16.6 ± 0.6	16.8 ± 0.5
Total EPA and DHA (g/day)	0.4 ± 0.06	0.4 ± 0.1	0.4 ± 0.07	0.4 ± 0.06
Objective				
%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
Post-intervention				
N	17	18	20	20
Energy (MJ/day)	8.2 ± 0.4	7.7 ± 0.4	7.7 ± 0.4	9.2 ± 0.5
%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 ± 0.2[c)]	6.4 ± 0.3[c)]
%E from MUFA	12.8 ± 0.3[c)]	21.1 ± 0.3[b)]	11.5 ± 0.2[a)]	11. 1 ± 0.3[a)]
%E from PUFA	6.1 ± 0.3	5.7 ± 0.1	5.3 ± 0.2	5.0 ± 0.2
%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 ± 0.9[b)]	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)]

Values are presented as the mean ± SEM of each diet group. Differences ($p < 0.05$) between diet groups were assessed by one-way ANOVA. [a)], [b)] and [c)] Mean values within a row with unlike superscript letters were significantly different.
HSFA = High saturated fatty acids diet, HMUFA = High monounsaturated fatty acids diet, LFHCC = Low-fat, high-complex carbohydrate diet, LFHCC *n*-3 = Low-fat, high-complex carbohydrate diet with *n*-3 polyunsaturated fatty acids.

(i) High saturated fatty acids (HSFA) diet (38% energy (E): 16% SFA, 12% MUFA 6% PUFA).
(ii) High monounsaturated fatty acids (HMUFA) diet (38% E: 8% SFA, 20% MUFA, 6% PUFA).
(iii) Low-fat (28% E), high-complex carbohydrate (LFHCC) diet (8% SFA, 11% MUFA, 6% PUFA) with 1 g/day high-oleic sunflower oil (placebo).
(iv) Low-fat (28%E), high-complex carbohydrate diet (8%SFA, 11% MUFA, 6% PUFA) with 1.24 g/day long-chain *n*-3 polyunsaturated fatty acids (LC *n*-3 PUFA), (LFHCC *n*-3).

Post-intervention period (week 12), we performed a postprandial challenge with the same fat composition as consumed during the dietary period. 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 cannulation 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 h. Test meals provided an equal amount of fat (0.7 g/kg body weight), cholesterol (5 mg/kg of body weight) and vitamin A (62.9 μmol vitamin A (retinol)/m^2 body surface area). The test meal provided 65% of E as fat, 10% as protein and 25% as carbohydrates (CHO). During the postprandial assessment, subjects rested, 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, 6% PUFA); HMUFA breakfast (12% SFA, 43% MUFA, 10% PUFA); LFHCC breakfast with placebo (21% SFA, 28% MUFA, 16% PUFA); LFHCC n-3 breakfast with 1.24 g of LC n-3 PUFA (21% SFA, 28% MUFA, 16% PUFA).

2.4 Measurements

2.4.1 Lipid analysis

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 a 4°C.

Total cholesterol and triglycerides in plasma and lipoprotein fractions were assayed by enzymatic procedures. Apolipoprotein (apo) A-I and apo B were determined by turbidimetry [19]. High-density lipoprotein (HDL-C) was measured by precipitation of a plasma aliquot with dextran

sulphate-Mg^{2+}. Low-density lipoprotein (LDL-C) was calculated using the Friedewald formula [20].

2.4.2 Plasma-soluble inflammatory markers

Plasma concentrations of IL-6, TNF-α and monocyte chemoattractant protein-1 (MCP-1) were determined in duplicate with commercially available enzyme-linked inmunosorbent assay (ELISA) kits (R&D Systems, Mineapolis, MN, USA). The quantitative determination of non-esterified fatty acids (NEFA) was measured using the Free Fatty Acids Half-Micro Colorimetric kit (Roche Diagnostic, Indianapolis, IN, USA).

2.4.3 Isolation of PBMC

Blood was collected in tubes containing EDTA. The blood samples were diluted 1:1 in PBS, and cells were separated in Ficoll gradient by centrifugation at 800 × *g* for 25 min at 20°C. PBMC were collected, washed with PBS and resuspended in Trizol (Tri Reagent®, Sigma Aldrich, St Louis, MO, USA).

2.4.4 NF-kB (p65) DNA binding activity

PBMC nuclear extracts were prepared as described by Hernández-Presa et al. [21]. Nuclear NF-kB activity was determined using the NF-kB (p65) Transcription Factor Assay kit (Cayman Chemical Co, Ann Arbor, MI, USA).

2.4.5 Western blotting

Cytoplasmic and nuclear lysates were prepared from PBMC as described previously [21]. Protein concentrations were determined using the two-dimensional Quant kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) with bovine serum albumin as a standard. Cytoplasmic and nuclear proteins (40 μg) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The following proteins were detected using their corresponding antibodies: IkB-α (C-21, 36 kDa, Santa Cruz Biotechnology, CA), β-Actin (clone AC-15, 42 kDa, Sigma Aldrich), p65 (C-20, 65 kDa, Santa Cruz Biotechnology) and Transcription factor IIB (TFIIB; C-18, 30 kDa, Santa Cruz Biotechnology). Immunocomplexes were detected with appropriate peroxidase-conjugated secondary antibody (Sigma Aldrich) and detected by enhanced chemiluminescence (ECL advance; GE Healthcare). Values for cytoplasmic IkB-α were normalized with the signal for β-Actin and values for nuclear p65 were normalized with the signal for TFIIB. Protein levels were quantified using the image analysis software Quantity One, version 4.4.0 (BIO-RAD, Barcelona, Spain). Results were expressed in arbitrary units (AU).

2.4.6 Total RNA isolation and real-time (RT)-PCR

Total cellular RNA from PBMC was extracted using the Trizol method and quantified by NanoDrop 1000A Spectrophotometer. RNA integrity was verified on agarose gel electrophoresis. Next, since PCR can detect even a single molecule of DNA, RNA samples were digested in DNAse I (AMPD-1, Sigma Aldrich) before RT-PCR. Total RNA was reverse transcribed into cDNA using the iScript cDNA Synthesis kit (BIORAD, Hercules, CA, USA). The expression levels of the TNF-α, IL-6, IkB-α, p65, metalloproteinase 9 (MMP-9), macrophage migration inhibitory factor (MIF) and MCP-1 genes were measured by RT PCR with the use of iQ5-BIORAD thermal cycler system. All measurements were performed in duplicate. The specificity and the size of the PCR products were tested through a melt curve and resolved on a 2% agarose gel. The expression of each target gene was normalized with the signal for the *Homo sapiens ribosomal protein L13a* gene (RPL13a). Results were expressed in AU.

2.5 Statistical analysis

Statistical analysis used SPSS statistical software, version 17.0 (SPSS, Inc., Chicago, IL). All data in the text and tables are expressed as mean ± SEM. The normal distribution of variables to characterize the postprandial response was assessed using the Kolmogorov–Smirnov test and log transformed if appropriate. PBMC IkB-α, MMP-9, IL-6, MCP-1, p65 and plasma IL-6, TNF-α were log transformed before statistical analyses. The data were analysed using one-way ANOVA, Student *t*-test for paired data analysis and repeated measures ANOVA (RM-ANOVA). In this analysis, we studied the statistical effects of time independently of diet. We also studied the statistical effects of diet, independently of the postprandial time points. Furthermore, we also studied the effect of the interaction of both factors – diet and time – which is indicative of the magnitude of the postprandial response in each meal. In this analysis, we adjusted for the covariates of gender and smoking status. Post hoc statistical analysis was completed by using the Bonferroni's test to identify significant differences between dietary treatments. A study of the relationship among parameters was also carried out using Pearson's linear correlation coefficient. $p < 0.05$ was considered statistically significant.

3 Results

3.1 Achievement of dietary targets

Table 1 shows the composition of diets pre- and post-intervention wherein dietary targets were largely achieved. Total eicosapentaenoic (EPA) and DHA, percentage of E (%E) from SFA, MUFA and fat was log transformed before statistical analysis. There were no significant differences in dietary

composition at pre-intervention period between the four diet groups. However, during the post-intervention, %E from fat was significantly lower in the LFHCC and LFHCC *n*-3 diets compared with the HSFA and HMUFA diets. Also dietary SFA (%E) was significantly greater in HSFA diet compared to all other diets. MUFA levels were also greater in the HMUFA diet compared with the other diets, MUFA levels were also greater in the HSFA diet compared with the LFHCC and LFHCC *n*-3. %E as CHO intake was significantly greater in LFHCC and LFHCC n-3 diets compared with the HSFA and HMUFA diets. Total EPA and DHA acid (g/d) was consistently greater in the LFHCC n-3 than the other diets.

3.2 Diet intake and pro-inflammatory cytokines

Table 2 shows the characteristics pre- and post-intervention of 75 subjects with MetS, randomised to each dietary intervention. With the aim of identifying the postprandial effects of long-term consumption of four diets, on the expression of a number of inflammatory parameters in PBMC, we measured the nuclear NF-kB activity, nuclear p65 and cytoplasmic IkB-α protein levels. Furthermore, we analysed the transcription of pro-inflammatory genes regulated by NF-kB at the end of dietary intervention. After 12 weeks of dietary intervention and 4 h after the fat overload with the HMUFA diet, we observed a postprandial decrease in nuclear NF-kB activity relative to fasting values ($p = 0.027$). By contrast, this effect was not observed after the consumption of the other three diets (Figure 1A). Moreover, the postprandial decrease in nuclear NF-kB activity was associated with a postprandial decrease in the nuclear levels of p65 ($p = 0.002$) following consumption of HMUFA diet (Figure 1B). Consistently, we observed a postprandial decrease in this protein with the HMUFA diet compared with the HSFA ($p = 0.009$) and LFHCC diets ($p = 0.003$). Moreover, we analysed cytoplasmic IkB-α protein levels in the post-intervention period. No significant differences were found, after the ingestion of the different diets in cytoplasmic levels of IkB-α ($p > 0.05$; data not show).

Interestingly, after long-term consumption of the HMUFA diet and 4 h after the fat overload, we observed a postprandial increase in the transcription of PBMC IkB-α gene (Figure 2A) compared with HSFA ($p = 0.017$) and LFHCC *n*-3 ($p = 0.043$) diets. On the other hand, after 12 weeks of dietary intervention with the LFHCC *n*-3 diet and fasting (0 h), we observed the higher IKB-α mRNA expression with this diet (Figure 2A) than did the HSFA ($p = 0.015$) and HMUFA ($p = 0.023$) diets.

Postprandial PBMC TNF-α (Figure 2B) and MMP-9 (Figure 2C) mRNA levels were reduced after intake of HMUFA diet compared with HSFA diet ($p = 0.022$ and $p = 0.05$, respectively). Moreover, the intake of the four breakfasts induced an increase in the expression of PBMC TNF-α, MMP-9 and IL-6 genes independently of the diet consumed, reflecting an acute inflammatory response during the postprandial period (Table 3). There were no significant differences in the postprandial PBMC p65, MIF and MCP-1 mRNA levels among the four diets (Table 3).

In the next step, we analysed plasma concentrations of IL-6, TNF-α, MCP-1 and NEFA during the post-intervention period. Postprandial plasma levels of MCP-1 (Figure 3) were reduced after intake of HMUFA ($p = 0.046$) and LFHCC *n*-3 ($p = 0.026$) diets compared to HSFA diet. Furthermore, 4 h after the intake of the breakfasts, we observed an increase in the postprandial plasma levels of IL-6 independently of the diet consumed (Table 3). No significant differences were found, after the ingestion of the different diets in plasma levels of TNF-α and NEFA (Table 3).

On the other hand, we have analysed the influence of gender and smoking status in our findings. In this context, we did not observe any significant differences compared with the previous analysis according these confounding factors.

3.3 Correlation analysis

We observed a positive correlation between nuclear NF-kB (p65) activity with TNF-α gene at postprandial period (4 h; $p < 0.040$; Figure 4A). There was also a positive correlation between TNF-α mRNA expression with IL-6 gene at fasting (0 h; $p = 0.037$), and postprandial period (4 h; $p = 0.018$; Figure 4B). In addition, we found a positive correlation between TNF-α mRNA levels with MCP-1 mRNA expression at postprandial state (4 h; $p = 0.022$; Figure 4C). Furthermore, we observed a positive correlation between TNF-α mRNA levels with MMP-9 mRNA expression at fasting (0 h; $p < 0.01$; Figure 4D), and postprandial state (4 h; $p = 0.017$).

4 Discussion

The results of our study show that after long-term consumption of the HMUFA diet and 4 h after the fat overload, we observed a postprandial decrease in NF-kB activation and in the nuclear p65 protein levels in MetS patients. Moreover, we observed a postprandial increased in the transcription of PBMC IkB-α gene. The reduced transcriptional activity of PBMC TNF-α and MMP-9 after HMUFA diet, as reflected in decreased mRNA levels, is consistent with decreased NF-kB binding and also with an improvement in the pro-inflammatory state of MetS patients.

The MetS is associated with unhealthy lifestyles, and its most important feature is IR. Inflammation interferes with insulin signalling and thus plays a role in the pathogenesis of MetS. Furthermore, treatment of MetS aims at ameliorating IR through lifestyle changes, including exercise and diet [1]. Williams et al. [22] showed that dietary patterns close to the MUFA-enriched Mediterranean diet could serve as an anti-inflammatory dietary pattern, which could protect from or even treat diseases that are related to chronic inflammation, including the MetS. Nevertheless, although we have observed

Table 2. Characteristics pre- and post-intervention of subjects with the MetS assigned to each diet

Characteristics	HSFA (n = 17)	HMUFA (n = 18)	LFHCC (n = 20)	LFHCC *n*-3 (n = 20)	*p* Value
Pre-intervention					
Age, years	58.5 ± 1.9	54.6 ± 1.8	56.3 ± 1.8	55.3 ± 1.4	0.449
BMI, Kg/m^2	35.2 ± 0.9	34.4 ± 0.8	35.4 ± 0.7	35.1 ± 0.8	0.798
Systolic blood pressure (mmHg)	151.8 ± 4.2	145.1 ± 3.9	149.7 ± 3.2	149.8 ± 3.1	0.706
Diastolic blood pressure (mmHg)	89.3 ± 2.5	93.5 ± 2.4	91.3 ± 2.1	93.6 ± 1.9	0.767
Total cholesterol, mmol/L	5.2 ± 0.2	4.9 ± 0.2	5.3 ± 0.2	4.9 ± 0.2	0.405
Total triglycerides, mmol/L	1.9 ± 0.3	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.2	0.654
LDL-cholesterol, mmol/L	3.5 ± 0.2	3.4 ± 0.1	3.8 ± 0.2	3.4 ± 0.2	0.374
HDL-cholesterol, mmol/L	1.1 ± 0.06	1.1 ± 0.06	1.1 ± 0.06	1.1 ± 0.05	0.834
Apo B, g/L	0.92 ± 0.04	0.89 ± 0.04	1.01 ± 0.05	0.90 ± 0.05	0.291
Apo A-1, g/L	1.33 ± 0.04	1.35 ± 0.05	1.35 ± 0.05	1.30 ± 0.04	0.876
Post-intervention					
Age, years	58.5 ± 1.9	54.6 ± 1.8	56.3 ± 1.8	55.3 ± 1.4	0.449
BMI, Kg/m^2	35.2 ± 0.9	34.8 ± 0.8	35.0 ± 0.7	34.7 ± 0.8	0.904
Systolic blood pressure (mmHg)	148.0 ± 3.6	133.7 ± 3.8	140.9 ± 3.2	143.9 ± 3.1	0.628
Diastolic blood pressure (mmHg)	87.2 ± 2.4	89.7 ± 2.2	90.3 ± 1.8	87.8 ± 2.4	0.961
Total cholesterol, mmol/L	5.1 ± 0.2	4.8 ± 0.1	4.9 ± 0.2	4.6 ± 0.2	0.362
Total triglycerides, mmol/L	1.7 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	0.614
LDL-cholesterol, mmol/L	3.4 ± 0.1	3.3 ± 0.1	3.4 ± 0.2	3.1 ± 0.2	0.520
HDL-cholesterol, mmol/L	1.05 ± 0.05	1.09 ± 0.06	1.05 ± 0.05	1.01 ± 0.04	0.728
Apo B, g/L	0.88 ± 0.02	0.87 ± 0.03	0.93 ± 0.04	0.85 ± 0.05	0.574
Apo A-1, g/L	1.32 ± 0.04	1.30 ± 0.05	1.24 ± 0.03	1.19 ± 0.04	0.167

Values are presented as the mean ± SEM of each diet group. The data were analysed using one-way ANOVA.
HSFA = High saturated fatty acids diet, HMUFA = High monounsaturated fatty acids diet, LFHCC = Low-fat, high-complex carbohydrate diet, LFHCC *n*-3 = Low-fat, high-complex carbohydrate diet with *n*-3 polyunsaturated fatty acids.

an increase in the postprandial inflammatory response, the ingestion of a HMUFA diet, as compared to the other diets, reduced the postprandial increase of PBMC TNF-α and MMP-9 mRNA levels associated to a decrease in NF-kB activation.

In most cells, NF-kB (p50/p65) is present in an inactive form in the cytoplasm, bound to an inhibitor IkB. Certain stimuli result in the phosphorylation, ubiquitination and subsequent degradation of IkB proteins thereby enabling

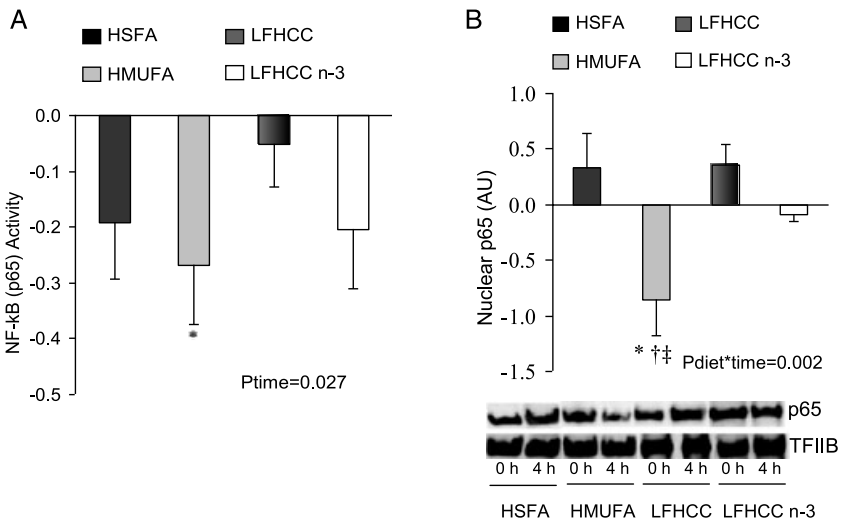

Figure 1. Postprandial changes of nuclear NF-kB (p65) activity (A) and p65 protein levels (B) in PBMC. n = 75. Data were analysed using Student *t*-test (A) and one-way ANOVA (B). Results are expressed as mean ± SEM. $^{*}p < 0.05$: HMUFA diet, postprandial changes (time 4 h versus fasting). †HMUFA versus HSFA and ‡HMUFA versus LFHCC diet. HSFA = High saturated fatty acids diet, HMUFA = High monounsaturated fatty acids diet, LFHCC = Low-fat, high-complex carbohydrate diet, LFHCC *n*-3 = Low-fat, high-complex carbohydrate diet with *n*-3 polyunsaturated fatty acids.

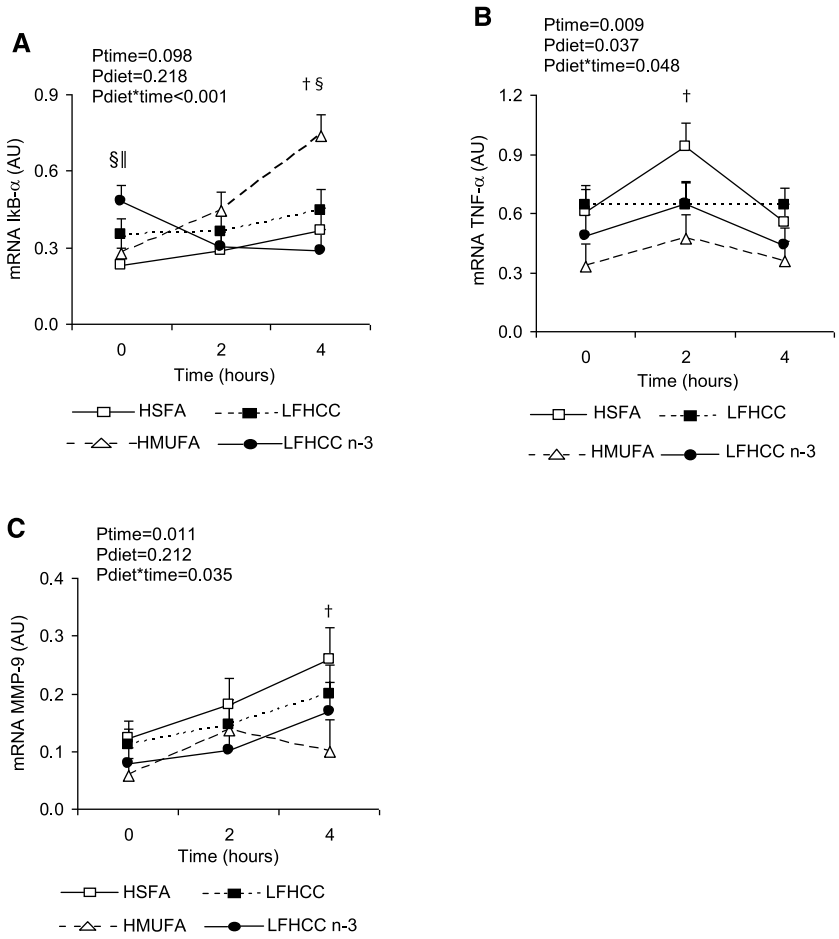

Figure 2. mRNA expression of IκB-α (A), TNF-α (B) and MMP-9 (C) measured by RT-PCR from total RNA. n = 75. Data were analysed using RM-ANOVA. Results are expressed in AU. $p < 0.05$: †HMUFA versus HSFA, §HMUFA versus LFHCC *n*-3 and ‖LFHCC *n*-3 versus HSFA diet. HSFA = High saturated fatty acids diet, HMUFA = High monounsaturated fatty acids diet, LFHCC = Low-fat, high-complex carbohydrate diet, LFHCC *n*-3 = Low-fat, high-complex carbohydrate diet with *n*-3 polyunsaturated fatty acids.

translocation of this transcription factor into the nucleus. NF-kB bind to DNA sequences thereby regulating expression of target genes [23]. Aljada et al. [24] have demonstrated that consumption of a hyper-caloric breakfast increased the nuclear NF-kB activity, accompanied by a reduction in the cytoplasmic IkB-α expression in healthy subjects. In a similar young healthy population, we previously demonstrated a postprandial decrease in the nuclear activity of NF-kB after consumption of HMUFA rather than HSFA breakfast [10, 11]. However, at present no studies have been performed regarding the effect of long-term dietary fat in the typical pro-inflammatory environment of MetS patients. In the current study, we observed a postprandial decrease in the nuclear activity of NF-kB 4 h after consumption of the HMUFA diet. Consistently, we observed a postprandial decrease in nuclear p65 protein levels with the HMUFA diet relative to fasting values. Furthermore, we observed a postprandial decreased in this protein following consumption of HMUFA diet compared with the HSFA and LFHCC diets. These data support previous studies suggesting that this type of fat had beneficial effect on the inflammatory profile. The duration of this pro-inflammatory effect is important because the time for another meal is generally 4–5 h after a meal, with the potential for further NF-kB activation, ROS load and pro-inflammatory changes. It is possible that chronic postprandial state may result in permanent increases in NF-kB binding activity and in total expression as a protein in the cell [25].

Moreover, after 12 weeks of dietary intervention and 4 h after the different fat overload, we analysed cytoplasmic IkB-α protein levels in the post-intervention period and no significant differences were found. In contrast, after long-term consumption of the HMUFA diet and 4 h after the fat overload, we observed a postprandial increase in the transcription of PBMC IkB-α gene. This observation could be explained given that the transcription and translation processes do not occur concomitantly. On the other hand, it is possible that in a low-grade chronic inflammatory state, such as obesity or MetS, IkB-β might play a major role in the decrease of nuclear activity NF-kB.

Interestingly, we observed a postprandial increased in the transcription of PBMC IkB-α gene 4 h after the fat overload with the HMUFA diet, in comparison with the HSFA and LFHCC n-3 diets. This finding could be explained by the fact that other transcription factors could be involved as modulator of IkB genes expression. Previous data suggest that different transcription factors bind to the promoters of

Table 3. Postprandial changes of inflammatory parameters in PBMC and plasma in subjects with MetS

	HSFA (n = 17)		HMUFA (n = 18)		LFHCC (n = 20)		LFHCC n-3 (n = 20)		*p* Values		
	Δ 2 h	Δ 4 h	Δ 2 h	Δ 4 h	Δ 2 h	Δ 4 h	Δ 2 h	Δ 4 h	time	diet	diet × time
PBMC IL-6 mRNA (AU)	−0.1 ± 0.4	1.7 ± 0.8[a)]	0.9 ± 0.4	2.8 ± 0.8[a)]	0.2 ± 0.4	1.6 ± 0.7[a)]	−0.2 ± 0.4	1.1 ± 0.7[a)]	<0.001	0.281	0.918
PBMC MCP-1 mRNA (AU)	0.2 ± 0.7	0. 6 ± 0.8	−0.1 ± 0.7	−0.2 ± 0.8	−0.2 ± 0.7	0.5 ± 0.7	−1.2 ± 0.7	−0.3 ± 0.7	0.106	0.631	0.526
PBMC p65 mRNA (AU)	−0.1 ± 0.3	0.2 ± 0.4	0.4 ± 0.3	0.7 ± 0.4	0.4 ± 0.2	0.4 ± 0.3	−0.03 ± 0.2	0.6 ± 0.3	0.509	0.711	0.574
PBMC MIF mRNA (AU)	0.1 ± 0.2	−0.04 ± 0.2	−0. 3 ± 0.2	−0.3 ± 0.2	−0.05 ± 0.2	0.001 ± 0.2	−0.2 ± 0.2	−0.3 ± 0.2	0.414	0.479	0.572
plasma IL-6 (pg/mL)	0.5 ± 0.5	2.1 ± 0.7[a)]	1.3 ± 0.4	2.0 ± 0.6[a)]	0.6 ± 0.4	2.5 ± 0.6[a)]	1.01 ± 0.4	2.8 ± 0.6[a)]	<0.001	0.831	0.350
plasma TNF-α (pg/mL)	−0.02 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	−0.2 ± 0.1	−0.02 ± 0.1	−0.005 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	0.899	0.356	0.922
plasma NEFA (mM)	−0.3 ± 0.04[a)]	−0.05 ± 0.06	−0.2 ± 0.04[a)]	−0.02 ± 0.06	−0.3 ± 0.04[a)]	0.02 ± 0.05	−0.3 ± 0.04[a)]	−0.07 ± 0.05	<0.001	0.752	0.193

Values are presented as the mean ± SEM of postprandial changes (Δ) relative to fasting values in each diet group. The data were analysed using RM-ANOVA.
a) $p < 0.05$: Postprandial changes (Δ) relative to fasting values in each diet group.
AU = Arbitrary units, HSFA = High saturated fatty acids diet, HMUFA = High monounsaturated fatty acids diet, LFHCC = Low-fat, high-complex carbohydrate diet, LFHCC *n*-3 = Low-fat, high-complex carbohydrate diet with *n*-3 polyunsaturated fatty acids.

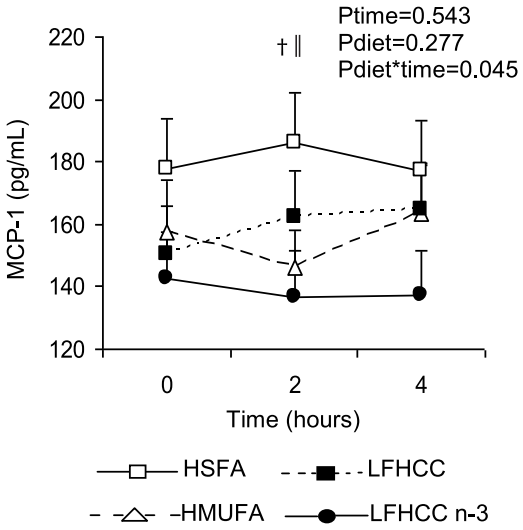

Figure 3. Plasma levels of MCP-1 measured by ELISA. n = 75. Data were analysed using RM-ANOVA. Result is expressed in pg/mL. $p < 0.05$: †HMUFA versus HSFA and ‖LFHCC *n*-3 versus HSFA diet. HSFA = High saturated fatty acids diet, HMUFA = High monounsaturated fatty acids diet, LFHCC = Low-fat, high-complex carbohydrate diet, LFHCC *n*-3 = Low-fat, high-complex carbohydrate diet with *n*-3 polyunsaturated fatty acids.

genes belong to the IkB family up-regulating IkB expression [26–28]. We speculated that these transcription factors would be modulated by the long-term consumption of MUFA. One of these transcription factors could be members of the forkhead box transcription factor O (FoxO) family, although these aspects need further investigation [26–28]. The newly synthesized IkB-α can release p50/p65 heterodimers from kB sites and then induce transport of these bound heterodimers from the nucleus into the cytoplasm, thereby restoring its inactive state and in consequence, inhibiting the inflammatory response [29].

Emerging knowledge of key pathogenic mechanisms suggests that TNF-α is related to MetS [30]. In endothelial cells, TNF-α induces a decrease in the expression of the insulin receptor itself, in addition to causing a reduction in tyrosine phosphorylation of the insulin receptor [31] as well as a reduction in the expression of glucose–insulin-sensitive (GLUT-4) receptors. We observed that long-term consumption of HMUFA diet reduced the postprandial TNF-α mRNA levels in PBMC relative to HSFA diet. Elevated postprandial TNF-α mRNA expression has been observed in healthy subjects following a HSFA diet [32]. Previous studies have confirmed an increase in the expression of pro-inflammatory cytokines in PBMC and adipose tissue of the obese subjects [4]. PBMC are known to migrate to the arterial wall to form foam cells in atherosclerotic lesions and into adipose tissue to activate adipocytes into producing pro-inflammatory cytokines [4]. The infiltration of adipose tissue by macrophages is strongly correlated with BMI in humans. Adipose tissue itself or the infiltrating macrophages are able to secrete pro-

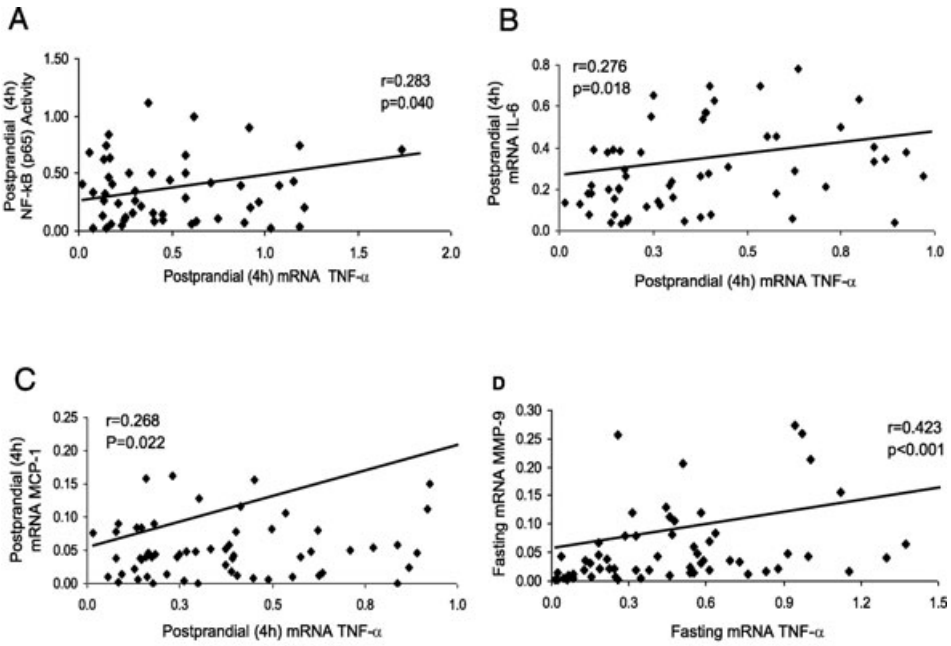

Figure 4. Correlation between nuclear NF-kB (p65) activity and TNF-α mRNA expression at 4 h of postprandial period (A). Correlation between TNF-α mRNA levels with IL-6 (B) and MCP-1 (C) genes at postprandial state (4 h). Correlation between TNF-α mRNA expression with MMP-9 gene at fasting (D).

inflammatory cytokines such as TNF-α, which promote production of adipokines [33]. Furthermore, TNF-α stimulates lypolysis that in turn promotes the development of the IR, hyperinsulinemia and high blood pressure [34]. Thus, it became evident that the overproduction of TNF-α in adipose tissue could be one link between obesity and IR and ultimately playing a major role in the development of MetS [35]. Some evidence in humans indicates that obesity and IR are associated with activation of PBMC and changing in the expression of a variety of cytokines genes related to inflammation and the immune response [5, 36, 37]. In our study, we observed a beneficial effect of the HMUFA diet compared with HSFA diet on PBMC TNF-α expression, however, no significant differences were found in plasma levels. This fact could be explained given that the plasma half-life of TNF-α is short [38]. On the other hand, there exist different time course for the synthesis and secretion of this protein [32, 39] and/or different additional regulatory mechanisms acting on the secretory pathway. Recent studies have revealed that newly synthesized cytokines can be routed via compartments such as recycling endosomes prior to their secretion [40].

Another interesting factor is MMP-9, which is involved in several stages of atherosclerosis through remodelling of the extracellular matrix. Foam cells segregate a greater amount of MMP-9 in response to oxidised lipoproteins and cytokines [41]. The expression of MMP-9 in atherosclerotic plaques coincides with the production of free radicals [42], which are found in greater quantity in MetS patients. Moreover, MMP-9 might be a potential mediator of the nicotinamide adenine dinucleotide phospathe (NADPH) oxidase dependent ROS production in the atherosclerotic process and previous studies suggested that NADPH oxidase activity increase significantly after consumption of a hypercaloric breakfast [24]. Robert et al. [43] observed a lower level of plasma MMP-9 in MetS patients after they had consumed a healthy diet and taken exercise for 21 days. In our study, we observed a reduction in postprandial MMP-9 mRNA levels following long-term consumption of HMUFA diet, and a postprandial increase after HSFA diet in PBMC. The mechanism responsible for rises in the levels of TNF-α and MMP-9 with HSFA diet could be the increase in ROS production inducing the activation of the inflammatory response when this type of fat is consumed. Furthermore, TNF-α has been shown to induce MMP-9 expression, however, the inductive mechanisms of MMP-9 by TNF-α remain unclear [44].

We have also studied the gene expression and plasma levels of MCP-1. MCP-1 is involved in several stages of atherosclerosis and regulates the transmigration of monocytes and other mononuclear cells on inflammatory sites [45]. In our study, we observed a postprandial decrease in plasma levels of MCP-1 following long-term consumption of HMUFA and LFHCC n-3 diets compared to HSFA diet. However, when we analysed the MCP-1 mRNA levels, we did not observe any significant differences between diets. This observation could be explained as MCP-1 can be expressed by PBMC and/or by macrophages infiltrated in adipose tissue, among other cell types. The increased levels of this chemokine could be due to the expression of different cell types, especially macrophages infiltrated in adipose tissue, since the patients in our study have abdominal obesity and MetS [46].

On the other hand, we observed a postprandial increased of TNF-α, MMP-9 and IL-6 mRNA levels, in addition to IL-6 plasma levels, independently of the type of diet consumed.

We also observed a positive relationship between the expression of TNF-α with MMP-9, IL-6 and MCP-1 mRNA expressions, in addition to nuclear NF-kB (p65) activity in the postprandial period. These results are consistent with the induction of an inflammatory response during the postprandial period and raise fundamental issues about the relation between food intake, oxidative damage, inflammation and atherosclerosis [24].

This study suggest that PBMC may provide a representative view of the inflammatory status in terms of activation of transcription factor, inflammatory markers in plasma and expression of pro-inflammatory genes in patients with MetS. Macronutrient intake induce a pro-oxidant state that is accompanied by an increase in biomarkers inflammation, cell adhesion molecules and endothelial dysfunction, while all these factors involved in the genesis of atherosclerosis. These effects are magnified in subjects with abdominal obesity and IR as patients with MetS. Thus, PBMC may be used as a model to evaluate systemic inflammation and its relationship to IR in obese and MetS patients [5].

Chronic or long-term diet ingestion thus results in a more faithful translation of the effects of the different dietary models, in that meal consumption is not an isolated phenomenon. This study does have certain limitations. Ensuring adherence to dietary instructions is difficult in a feeding trial. In this context, in our study the adherence to recommended dietary patterns was good, as judged by measurements of compliance. On the other hand, this design has the strength of reproducing real-life conditions with home-prepared foods, reflecting the subjects' usual practice. Another limitation in our study is concerning the sample size. Our cohort is very well characterized in terms of our metabolic phenotyping. However, our results belong to a single study and new more evidences are needed in order to confirm our findings.

In conclusion, this study has demonstrated that dietary fat modulates the inflammatory response in MetS patients. The long-term consumption of a healthy diet model with HMUFA attenuates the postprandial inflammatory state associated with MetS. These novel findings support recommendations to consume this dietary pattern as a useful preventive measure against the chronic inflammation that underlies in MetS patients.

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