RESEARCH ARTICLE

Postprandial inflammatory response in adipose tissue of patients with metabolic syndrome after the intake of different dietary models

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Scope: Dysfunctional adipose tissue may be an important trigger of molecular inflammatory pathways that cause cardiovascular diseases. Our aim was to determine whether the specific quality and quantity of dietary fat produce differential postprandial inflammatory responses in adipose tissue from metabolic syndrome (MetS) patients.

Methods and results: A randomized, controlled trial conducted within the LIPGENE study assigned MetS patients to 1 of 4 diets: (i) high-saturated fatty acid (HSFA), (ii) high-monounsaturated fatty acid (HMUFA), (iii) low-fat, high-complex carbohydrate diet supplemented with $n-3$ polyunsaturated fatty acids (PUFA) (LFHCC $n-3$), and (iv) low-fat, high-complex carbohydrate diet supplemented with placebo (LFHCC), for 12 wk each. A fat challenge reflecting the fatty acid composition as the original diets was conducted post-intervention. We found that p65 gene expression is induced in adipose tissue ($p = 0.003$) at the postprandial state. In addition, IκBα ($p < 0.001$), MCP-1 ($p < 0.001$) and IL-1β ($p < 0.001$) gene expression was equally induced in the postprandial state, regardless of the quality and quantity of the dietary fat. Notably, IL-6 transcripts were only detected in the postprandial state.

Conclusions: Our results indicate that individuals with MetS typically exhibit exacerbated adipose tissue postprandial inflammatory responses, which seem to be independent of the quality and quantity of dietary fat.

Keywords:
Adipose tissue / Diet / Inflammation / Metabolic syndrome / Postprandial state

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Abbreviations: CVD, cardiovascular diseases; **HMUFA,** high-monounsaturated fatty acid; **HSFA,** high-saturated fatty acid; **LFHCC,** low-fat, high-complex carbohydrate diet; **MetS,** metabolic syndrome; **MCP-1,** monocyte chemoattractant protein-1; **PUFA,** $n-3$ polyunsaturated fatty acids; **SFA,** saturated fatty acids

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1 Introduction

Metabolic syndrome (MetS) is a multi-component disorder characterized by abdominal obesity, dyslipidaemia, hypertension, and impaired insulin sensitivity, which is associated with an increased risk of type 2 diabetes (T2DM) and cardiovascular diseases (CVD) [1].] The etiology of MetS is the result of a complex interaction between genetic, metabolic, and environmental factors, including dietary habits and, probably, the quality of dietary fat [2].

It has been suggested that the harmful effects associated with obesity depend on the dysfunctionality and failure of the adipose tissue. In fact, adipose tissue's expansion and enlargement of adipocytes, typically associated with obesity, has been shown to cause, or at least be associated with, hypoxia, oxidative stress, and even mechanical damage, due to hypertrophy of the adipocyte [3]. In fact, the excess of nutrients has been associated with toxic cellular reactions resulting in stress of the endoplasmic reticulum (ER) and activation of the unfolded protein response (UPR), a process which is linked to the activation of major inflammatory signaling pathways, such as IκB kinase-nuclear factor κB (Ikk-NFκB) pathways, increased production of reactive oxygen species (ROS), and qualitative and quantitative altered adipose secretome, including insulin resistance-promoting cytokines [4, 5].

A low-grade systemic chronic inflammation with an abnormal cytokine production from adipose tissue, increased acute-phase reactants and the activation of inflammatory signaling pathways, often acting over a period of many years constitutes a potential link to the development of atherosclerosis in obese people [6]. This state of low-grade inflammation is also a feature of the complex proatherogenic phenotype occurring during the postprandial state [7], which represents a stressful situation of the homeostasis by its increase in lipid pro-inflammatory particles, a rise in oxidative stress and a transient increase in pro-inflammatory molecules released by human white blood cells and endothelial cells [8]. It is important to note that patients with MetS maintain a postprandial-like state during long periods of the day.

Diet can modulate the postprandial inflammatory response [9–11]. In addition, van Oostrom et al. [8] provided evidence that postprandial triglyceridemia may be related to the pro-inflammatory state of the dysfunctional adipose tissue, typically characterized by an increased expression of activated monocyte markers. MetS patients are particularly vulnerable, since they show an exacerbated hypertriglyceridemia response [12] and abnormalities in the postprandial metabolism of lipoproteins [13]. In fact, there is evidence that postprandial plasma cytokine concentration is higher in obese than lean subjects, and that excessive adipose tissue mass may correlate with high circulating levels of plasma cytokines in obesity [14].

We and others have proposed that adipose tissue failure results in increased lipid leakage from the adipocytes, further facilitating the recruitment and activation of macrophages, thus creating a vicious pro-inflammatory cycle. It is not clear whether this lipid-induced pro-inflammatory effect is dependent on the type of lipids, but there are several studies suggesting that saturated fatty acids (SFA) may exert a particularly strong effect, promoting the inflammatory response within the adipose tissue typically observed in patients affected by MetS [15, 16].

It has been shown that long-term dietary treatments modify the expression of inflammatory genes in adipose tissue in patients with MetS [10, 17], but there is no evidence that this beneficial effect can also affect the postprandial inflammatory activation typically observed in the adipose tissue of MetS patients. Since this postprandial state can be considered the normal metabolic state in which humans with MetS spend most of the day, it is important to know whether dietary treatment can reduce this inflammatory response, particularly given the implications of the postprandial inflammatory response in the development of MetS and CVD [18].

Few postprandial studies have been carried out in MetS subjects, and therefore, research needs to be carried out into the importance of fat quantity and quality as potential factors which modulate the inflammatory state in MetS patients. Thus, the aim of this study was to evaluate the postprandial inflammatory response in adipose tissue after the consumption of four diets with different quantities and qualities of fat, by analyzing the expression of the inflammatory genes p65, IκBα, IκBβ2, IL-6, MCP-1 and IL-1β, in adipose tissue of patients with MetS.

2 Materials and methods

2.1 Participants and recruitment

This study was conducted within the framework of the LIPGENE study (Diet, genomics and MetS: an integrated nutrition, agro-food, social, and economic analysis), a Framework 6 Integrated Project funded by the European Union. A total of 39 patients with MetS (25 females and 14 males) from the LIPGENE cohort were accepted to participate in the postprandial study and successfully concluded the dietary intervention and the post-intervention studies. All participants gave written informed consent and underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrollment. Clinical Trial Registration Number: NCT00429195. 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.

2.1.1 Design

Patients were randomly stratified to 1 of 4 dietary interventions for 12 wk. MetS was defined by published criteria

[19], which conformed to the LIPGENE inclusion and exclusion criteria [20]. Post-intervention high-fat meal was administered providing the same amount of fat (0.7 g/kg body weight), wherein the fatty acid composition reflected that consumed within the intervention period. The intervention study design and intervention protocol, which also provides information about pre, mid, and post-intervention food consumption and dietary compliance have been described in detail by Shaw et al. [20]. Briefly, dietary intake and compliance was assessed by a 3-day (2 weekdays and 1 weekend day) weighed food intake assessments at baseline, wk 6, and wk 12. Dietary analysis program reflective of the food choices were used (Dietsource version 2.0).

2.1.2 Randomization and intervention

Randomization was completed centrally according to age, gender, and fasting plasma glucose concentration using the Minimization Program for Allocating Patients to Clinical Trials (Department of Clinical Epidemiology, London Hospital Medical College, UK) randomization program. The diets differed in the fat quantity and quality while remaining isoenergetic. Two diets were designed to provide 38% energy (E) from fat: a high-fat, saturated fatty acid-rich diet (HSFA), which was designed to provide 16% E as SFA, and a high monounsaturated fatty acid-rich diet (HMUFA) designed to provide 20% E from MUFA. The other 2 diets were low-fat, high-complex carbohydrate-rich diet (LFHCC and LFHCC ($n-3$); 28% E from fat); the LFHCC ($n-3$) diet included a 1.24 g/day supplement of long-chain ($n-3$) polyunsaturated fatty acids (PUFA) (ratio of 1.4 eicosapentaenoic acid (EPA):1 docosahexaenoic acid (DHA)) and the LFHCC diet included a 1.2 g/day supplement of control high-oleic sunflower seed oil capsules (placebo) (Table 1). The intervention center performed a post-intervention (wk 12) postprandial challenge reflecting the fatty acid composition as that consumed on the assigned dietary period. Patients arrived at the clinical center 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 within 20 min under supervision. The test meal, which represents a fat overload providing the same amount of fat (65%), allowed us to study the postprandial responses after a fat challenge that may be influenced by the previous dietary intervention because adaptive effect after a long-term dietary intervention [21]. These test meals were prepared in the center, reflected fatty acid composition of each subject's chronic dietary intervention (Table 2). Subsequent blood samples were drawn at 4 h. Test meals provided an equal amount of fat (0.7 g/kg body weight), E content (40.2 kJ/kg body weight), cholesterol (5 mg/kg of body weight), fiber, and vitamin A (62.9 mmol vitamin A (retinol)/m^2 body surface area). The test meal provided 65% of E as fat, 10% as protein, and 25% as carbohydrates. During the postprandial assessment, participants rested and did not consume any other food but were allowed to drink water. The composition of the breakfasts was as follows: HSFA, 38% E from SFA, based on butter, whole milk, white bread, and egg intake; HMUFA, 43% E from MUFA, based on olive oil, skimmed

Table 1. Composition of diet at the end of intervention period, alongside dietary targets

	HSFA Mean ± SEM	HMUFA Mean ± SEM	LFHCC Mean ± SEM	LFHCC $n-3$ Mean ± SEM
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	8	9	12	10
Energy (MJ/day)	8.33 ± 0.57	7.19 ± 0.61	9.95 ± 0.94	9.95 ± 0.91
% E from fat	40.99 ± 0.91^{a}	40.80 ± 1.14^{a}	26.72 ± 0.69^{b}	26.15 ± 0.75^{b}
% E from SFA	17.69 ± 0.46^{a}	9.37 ± 0.68^{b}	6.62 ± 0.35^{c}	6.87 ± 0.25^{c}
% E from MUFA	13.27 ± 0.63^{c}	21.26 ± 0.56^{b}	11.12 ± 0.26^{a}	10.43 ± 0.34^{a}
% E from PUFA	6.70 ± 0.39^{a}	5.71 ± 0.28^{abc}	5.21 ± 0.31^{bc}	4.71 ± 0.34^{bc}
% E from CHO	37.21 ± 1.77^{a}	40.07 ± 1.38^{a}	49.94 ± 1.26^{b}	53.95 ± 1.54^{b}
% E from complex CHO	36.11 ± 0.49^{a}	38.91 ± 0.82^{a}	48.15 ± 0.94^{b}	52.79 ± 0.52^{b}
% E from protein	20.03 ± 1.28	19.12 ± 0.81	22.74 ± 1.10	19.08 ± 0.97
Total EPA and DHA (g/day)	0.38 ± 0.12^{a}	0.47 ± 0.22^{a}	0.58 ± 0.08^{a}	2.08 ± 0.16^{c}

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 supplemented with 1.24 g/day long-chain $n-3$ PUFA; % E, percentage energy; CHO, carbohydrate. Differences between diets groups were assessed by ANOVA for repeated measured. Values are mean ± SEM. Mean in a row with superscripts without a common letter differ significantly, $p < 0.05$.

Table 2. Meal composition of the fat challenge conducted post-intervention

	HSFA meal	HMUFA meal	LFHCC meal	LFHCC *n*−3 meal
Objective				
% E from fat	65	65	65	65
% E from SFA	38	12	21	21
% E from MUFA	21	43	28	28
% E from PUFA	6	10	16	16
% E from CHO	25	25	25	25
% E from complex CHO	25	25	25	25
% E from protein	10	10	10	10
Energy (kJ/kg body weight)	40.2	40.2	40.2	40.2
Meal composition of the fat challenge conducted post-intervention				
N	8	9	12	10
% E from fat	66.13	64.83	66.44	66.49
% E from SFA	40.31	12.83	18.79	18.79
% E from MUFA	21.86	43.72	28.47	26.77
% E from oleic acid (18:1)	19.85	42.60	27.37[b)]	25.67
% E from PUFA	4.46	8.28	19.18	20.93
% E from LA (18:2)	3.27	7.53	16.35	16.35
% E from ALA (18:3)	1.01	0.48	2.62	2.62
% E from EPA	0.02	0.00	0.01	1.15[a)]
% E from DHA	0.00	0.00	0.00	0.61[a)]
% E from CHO	24.20	24.07	22.76	22.76
% E from complex CHO	24.20	24.07	22.76	22.76
% E from protein	9.64	11.06	12.32	12.32
Energy (kJ/kg body weight)	42.27	42.72	42.67	42.69

% E, percentage energy; CHO, carbohydrate; ALA, α-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
a) These values include the amount of EPA and DHA administered as diet supplement (1.24 g/day of LC (*n*−3) PUFA (ratio 1.4 EPA:1 DHA)).
b) This value include the amount of oleic acid administered as placebo (1.2 g of high-oleic sunflower seed oil).

milk, white bread, eggs, yolk eggs, and tomatoe intake; LFHCC with placebo capsules, 16% E as PUFA; LFHCC with LC (*n*−3) PUFA, 16% E as PUFA (1.24 g/day of LC (*n*−3) PUFA (ratio 1.4 EPA:1 DHA)). Meals after LFHCC diets were based on butter, olive oil, skimmed milk, white bread, eggs, yolk eggs, and walnuts.

2.2 Measurements

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA. In each center, plasma was separated from red cells by centrifugation at $1500 \times g$ for 15 min at 4°C. Analytes determined in frozen samples were analyzed centrally by laboratory investigators of the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital who were unaware of the interventions. Lipid variables were assessed with a DDPPII Hitachi modular analyzer (Roche) using specific reagents (Boehringer-Mannheim). Plasma TG and cholesterol concentrations were assayed by enzymatic procedures [22, 23]. HDL-c was measured by precipitation of a plasma aliquot with dextran sulphate-Mg^{2+}, as described by Warnick et al. [24]. LDL-c was calculated by using the following formula; plasma cholesterol–(HDL-C+large TRL-C+small TRL-C). Plasma glucose was measured by the glucose oxidase method. Plasma insulin concentrations were measured by microparticle enzyme immunoassay (Abbott Diagnostics, Matsudo-shi, Japan) (CV: 2.5–6%).

Monitoring for adverse effects. Volunteers were visited each 2 wk for study. Clinical investigators assessed adverse events by using physical examinations and administering a checklist with diet-related symptoms and gave advice on how to remediate them.

2.3 Plasma molecule immunoassay

Plasma concentration of MCP-1 and IL-6 were determined in duplicate with commercially available enzyme-linked immunosorbent assay kits (R&D Systems) according to the manufacturer's guidelines. The average values of intra-assay CV for these plasma molecular immunoassay were 0.079 for IL-6, and 0.057 for MCP-1.

2.4 Subcutaneous adipose tissue sample collection

Subcutaneous adipose tissue samples were obtained from the superficial abdominal subcutaneous adipose tissue

lateral to the navel with instrument Bard® Magnum (ref. MG1522), needles Bard® Magnum Core (ref. MN1410). To the study of postprandial adipocyte function the samples were timed at 0 and 4 h of administration of the fatty meal. Immediately after extraction, samples were stored in Eppendorf with RNA later until RNA extraction.

2.5 RNA isolation from adipose tissue

Adipose tissue was homogenized by Ultra-Turrax T25 (IKA Labortechnik). After removal of lipids from the top of the tube, RNA was isolated with a commercial kit RiboPure kit (Ambion) that is designed for rapid purification of RNA and high quality. RNA is collected from the aqueous phase by binding to a glass fiber filter. The quantification of RNA is made using the spectrophotometer v3.5.2 Nanodrop ND-1000 spectrophotometer (Nanodrop Technology®, Cambridge, UK).

2.6 Gene expression by reverse transcription polymerase chain reaction

The reverse transcription polymerase chain reaction (RT-PCR) was conducted in two steps. The reverse transcription was performed using the commercial kit Message BOOSTER cDNA Synthesis Kit for qPCR (Epicentre), according to the manufacturer' instructions. Briefly, it was amplified to 500 pg of total RNA and then converted into cDNA that is ready for quantitative PCR, which was stored at −20°C until real-time PCR performing. The PCR reactions were carried out using the iQ SYBR Green kit (BioRad) and the Mx3005 (Stratagene)/iQ5 iCycler (Bio-RAD thermal cycler system). Each reaction was performed on 1 μL of 1:20 v/v dilution of the first cDNA strand, synthesized in the previous step. The reaction was incubated at 96°C for 3 min, followed by 40 cycles of 30 s at 96°C, 30 s at 62°C, 20 s at 72°C and 10 s at 80°C where fluorescence was measured to avoid primer–dimer and background signals. Primers used were commercial kit in a final volume of 20 μL with 10 pmol of each primer. Primers used were:

P65-FWD (5′-CCGGGATGGCTTCTATGAGG-3′), P65-REV (5′-GGGGTTGTTGTTGGTCTGGATG-3′); IκBα–FWD (5′-CACTCCATCCTGAAGGCTACCAAC-3′), IκBα-REV (5′-ACACTTCAACAGGAGTGACACCAG-3′); IκBβ 2-FWD (5′-GCCAGGAGCAGGGGAACTTG-3′), IκBβ2-REV (5′-GC GGCTGCTGTGAACCACAA-3′); IL-6-FWD (5′-CACCTC TTCAGAACGAATTGACAAAC-3′), IL-6-REV (5′-CTCATT-GAATCCAGATTGGAAAC-3′); MCP-1 FWD (5′-CAGCA GCAAGTGTCCCAAAGAAG-3′), MCP1-REV (5′-GGAAA GCTAGGGGAAAATAAGT-3′); IL1β-FWD (5′-CAGGG ACAGGATATGGAGCAA-3′), IL1β-REV (5′-GCAGACTC AAATTCCAGCTTGTTA-3′); CLN3-FWD (5′-CGGCCACC TGCATCTCTGA-3′), CLN3-REV (5′-GGGGTGGGCCTGG GTGTCT-3′).

In order to select suitable housekeeping genes for the accurate calibration of experimental variations in a qRT-PCR analysis, we evaluated several reference genes usually used in our group in other tissues (β-actin and RPL13a) and two more found in expression studies of human adipose tissue (CLN3 and COBRA1) [25]. We analyzed the expression of these four potential housekeeping genes on 24 subjects (6 subjects per diet) in the fasting and postprandial states. geNorm analysis showed an *M* value of 0.071 for RPL13a, 0.062 for CLN3, 0.08 for COBRA1 and 0.076 to β-actin. We selected CLN3 as a housekeeping gene to carry out our gene expression study on the basis of its lower *M* value [26].

Primer efficiencies were: P65 (93.2), IκBα (87.1), IκBβ2 (111.6), IL-6 (112.4), MCP-1 (93.8), IL-1β (96.0), CLN3 (101.6). Specificity of PCR amplifications was verified by a melting curve program (60–95°C with a heating rate of 0.5°C/s and a continuous fluorescence measurement). Expression values were obtained as relative expression of the target gene versus the constitutively expressed CLN3 gene (relative expression $= 2^{-(Ct,\ \text{Target gene} - Ct,\ \text{Reference gene})}$).

2.7 Statistical analyses

SPSS statistical software, version 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. The normal distribution of variables to characterize the postprandial response was assessed using the Kolmogorov–Smirnov test. We performed ANOVA for repeated measurements to determine the postprandial effect of the fat meal composition, with dietary intervention as the inter-subject factor. The global *p*-values indicate: P1: the effect of the diet and fat meal composition ingested (between-subject effect); P2: the time effect (within-subject effect) and P3: the interaction of both factors (diet-by-time interaction). Posthoc statistical analysis was completed using Bonferroni's multiple comparisons test, as previously published [27]. In the case of IL-6 (only one time point), we performed a one-way ANOVA in order to analyze statistical differences between diets, with a probability of <0.05 being considered significant. Minimal effect of diet intervention on gene expression able to be detected was: p65, 0.055; IκBα, 2.2; IκBβ2, 0.3; IL-6, 2.9; MCP-1, 65; IL-1β, 3.7. A study of the relationship among parameters was also carried out using Pearson's linear correlation coefficient. All the data presented are expressed as mean ± SEM.

3 Results

3.1 Baseline characteristics

No significant differences were observed in the baseline characteristics of the 39 subjects with MetS participating in the dietary intervention (Table 3). However, the total

Table 3. Baseline characteristics of subjects with the MetS assigned to each diet

	HSFA ($n=8$)	HMUFA ($n=9$)	LFHCC ($n=12$)	LFHCC $n-3$ ($n=10$)	p
Age (years)	57.8±3.1	57.1±2.3	56.5±2	54.8±2.1	0.839
BMI (kg/m^2)	36±1.2	34.5±1.2	35.7±1	35±1.2	0.817
Waist circumference	111.7±3.1	104±2	109±3.1	108.3±3.3	0.420
TC (mg/dL)	204±19	192.2±11.1	206.8±14.9	196.9±10	0.877
TG total (mg/dL)	226.7±65.2	159.1±20.9	161.5±17.5	158.1±20.3	0.422
LDL-c (mg/dL)	129.7±13	135.9±9.6	148.2±12.1	140.4±8.8	0.693
HDL-c (mg/dL)	41±4.5	44.4±3.3	43.4±3.6	41.4±2.9	0.906
Glucose (mg/dL)	117.7±6.2	120.3±7.1	106.1±3.2	125.1±13.5	0.371
Insulin (mU/mL)	15.3±1.3	11.5±1.3	12.6±1.4	13.3±1.7	0.400

Values presented are the mean±SEM of each diet group. 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/day long-chain $n-3$ PUFA diet; BMI, body mass index; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein-cholesterol. p-Values correspond to ANOVA statistical analyses.

Table 4. Preintervention (0 wk) and post-intervention (12 wk) gene expression of inflammatory genes at fasting state

		HSFA ($n=8$)	HMUFA ($n=9$)	LFHCC ($n=12$)	LFHCC $n-3$ ($n=10$)	P1	P2	P3
p65	0 wk	0.014±0.005	0.013±0.005	0.044±0.017	0.022±0.007	0.361	0.063	0.239
	12 wk	0.018±0.009	0.010±0.001	0.008±0.002	0.016±0.001			
IκBα	0 wk	0.866±0.128	0.960±0.301	0.777±0.131	0.935±0.153	0.385	0.922	0.964
	12 wk	0.643±0.142	1.138±0.354	0.806±0.079	0.913±0.124			
IκBβ2	0 wk	0.275±0.043	0.284±0.051	0.182±0.063	0.345±0.101	0.288	0.752	0.786
	12 wk	0.226±0.027	0.289±0.081	0.228±0.056	0.260±0.066			
MCP1	0 wk	0.977±0.206	1.036±0.185	1.361±0.287	1.413±0.320	0.438	0.124	0.168
	12 wk	1.262±0.237	2.751±0.833	1.228±0.167	1.482±0.423			
IL-1β	0 wk	0.063±0.024	0.187±0.049	0.111±0.036	0.136±0.055	0.036	0.045	0.855
	12 wk	0.163±0.061	0.224±0.074	0.140±0.034	0.194±0.067			

Mean±SEM of relative expression in adipose tissue. 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/day long-chain $n-3$ PUFA diet. p-Value correspond to ANOVA for repeated measured: P1: diet effect; P2: time effect; P3: diet-by-time interaction. *P<0.05.

triglyceride level was higher in the HSFA group than the other three groups, although the difference did not reach statistical significance.

3.2 Diet intake and adipose tissue inflammation

To evaluate the postprandial inflammatory response in adipose tissue after the administration of the four diets (pre and post-intervention gene expression in the fasting state, is shown in Table 4), we administered a meal after the dietary intervention reflecting the fatty acid composition of the original diet to study the effect of the diet, as studied in the postprandial period.

We studied the expression of the inflammation regulatory gene, p65 (sub-unit of the NF-κB transcription factor), and two sub-units of the NF-κB inhibitor (IκBα and IκBβ2 genes) in the fasting state and 4 h after the meal. We found that p65 gene expression is induced in the postprandial state ($p=0.003$). However, we also found that the increase did not differ as a function of the treatment. Posthoc statistical analysis showed that p65 gene expression statistically increased after the intake of low-fat high-carbohydrate LFHCC ($p=0.044$) and LFHCC $n-3$ ($p=0.038$) diets, and tended to increase after the intake of an HMUFA diet ($p=0.072$), as studied in the postprandial period.

When we analyzed the postprandial expression of the NF-κB inhibitors, we showed that IκBα gene expression was significantly induced in the postprandial state ($p<0.001$), irrespective of the diet consumed. In contrast, no significant changes were observed in IκBβ2 mRNA levels in the postprandial state, although there was a trend towards a postprandial increase after consumption of the four diets (Fig. 1).

We have also studied the gene expression of three pro-inflammatory cytokines (interleukin (IL)-6, MCP-1 (monocyte chemoattractant protein-1), and IL-1β). MCP-1 plays a critical role at multiple stages in atherosclerosis, and is associated with the initiation of the fatty streak and the promotion of plaque instability. Similarly, IL-6 and IL-1β have also been implicated in the atherosclerotic process and other CVD risk factors, such as obesity, dyslipidemia, and insulin resistance [28, 29]. In our study, the gene expression of MCP-1 and IL1β in adipose tissue was induced

($p < 0.001$), irrespective of the diet consumed, as studied in the postprandial period.

In the case of IL-6, transcripts were only detected in the postprandial period and no significant differences among diets were found. Similarly, MCP-1 and IL1β gene expression was extremely low in the fasting compared with the postprandial state.

We also measured IL-6 plasma levels before and after a meal. We found a significant postprandial increase in the plasma IL-6 level after consumption of the four meals ($p < 0.001$); this postprandial increase did not differ between diets. Posthoc statistical analysis showed that the circulating IL-6 levels increased after the intake of low-fat high-carbohydrate LFHCC ($p = 0.001$) and LFHCC $n-3$ ($p = 0.015$) diets, and tended to increase after the intake of HSFA ($p = 0.066$) and HMUFA ($p = 0.059$) diets, as studied in the postprandial period. However, no changes existed in MCP-1 plasma levels, despite the induction of MCP-1 gene expression observed in adipose tissue in the postprandial state (Fig. 2).

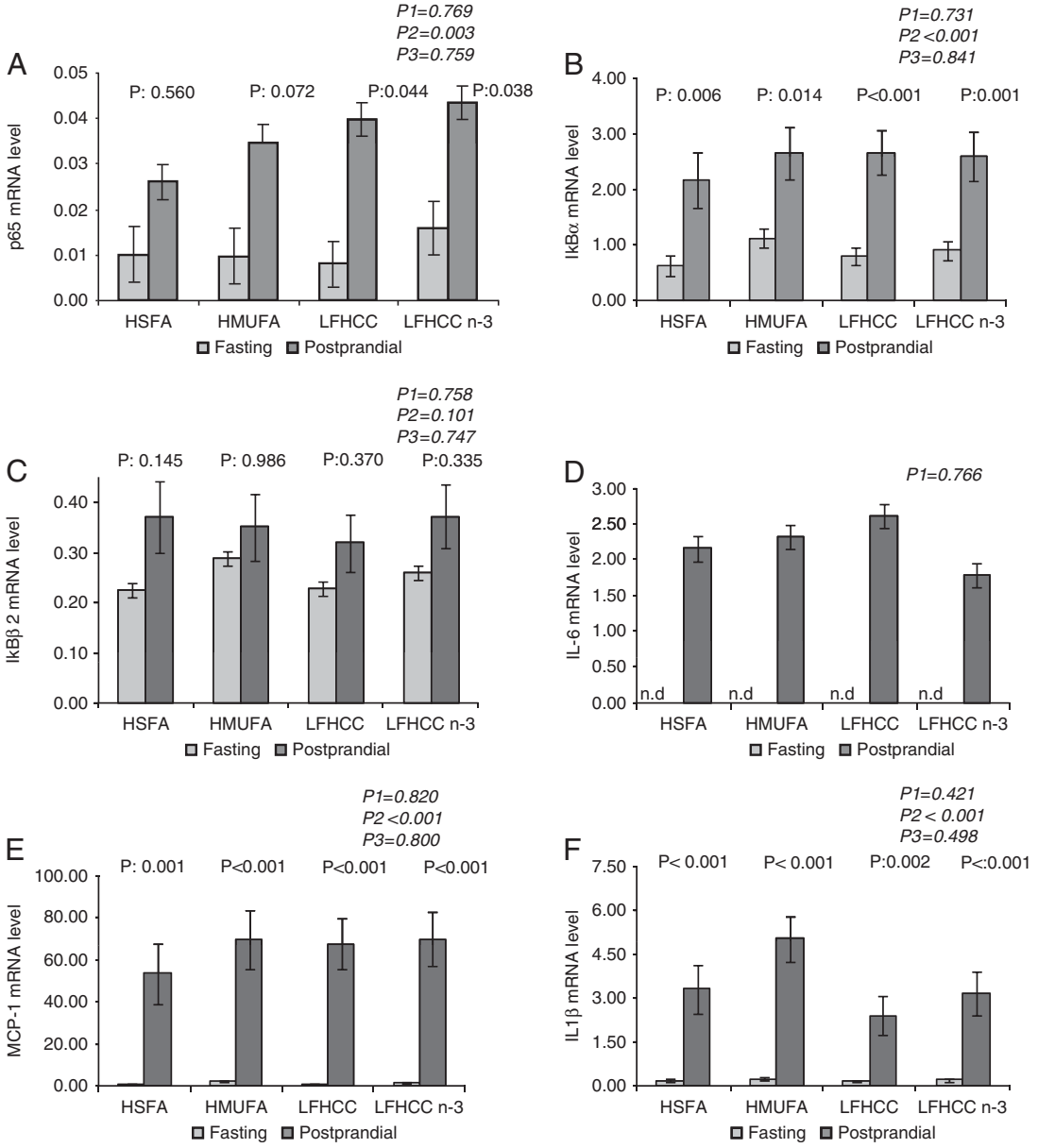

Figure 1. Postprandial p65 (A), IκBα (B), IκBβ2 (C), IL-6 (D), MCP-1 (E) and IL1β (F) gene expression changes in adipose tissue in MetS patients after dietary intervention. Mean (±SEM) of relative expression in AT, $n = 39$. Fasting: Post-intervention, 12-h fasting. Postprandial: Post-intervention, 4 h after the administration of the fatty meal with a fat composition similar to that consumed in each of the HSFA, HMUFA, LFHCC, or LFHCC ($n-3$) diets. ANOVA for repeated measured, global *p*-values: P1: diet effect; P2: time effect; P3: diet-by-time interaction. P: *p*-values from Bonferroni's multiple comparison test.

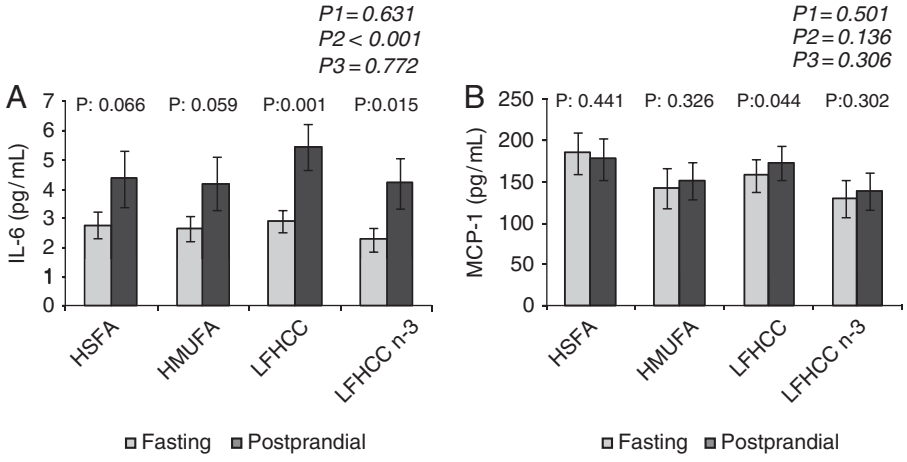

Figure 2. Postprandial (A) IL-6 and (B) MCP-1 plasma concentration changes in MetS patients after the dietary intervention. Mean (±SEM) of (A) IL-6 and (B) MCP-1 plasma levels, $n = 39$. Fasting: Post-intervention, 12-h fasting. Postprandial: Post-intervention, 4 h after the administration of the fatty meal with a fat composition similar to that consumed in each of the HSFA, HMUFA, LFHCC, or LFHCC ($n-3$) diets. ANOVA for repeated measured, global *p*-values: P1: diet effect; P2: time effect; P3: diet-by-time interaction. P: *p*-values from Bonferroni's multiple comparison test.

Table 5. Lipid concentrations in postprandial state

	TC (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	Glucose (mg/dL)	Insulin (mU/mL)
0 h						
HSFA	198.66±15.65	182.01±45.00	41.28±3.94	126.92±7.70	108.96±4.26	13.33±2.23
HMUFA	188.71±10.24	135.00±14.37	43.28±2.93	126.83±8.70	113.40±4.64	12.42±1.07
LFHCC	185.92±12.16	128.56±17.55	41.28±3.01	127.96±10.17	106.06±4.52	12.13±1.85
LFHCC-*n*−3	188.88±9.96	145.96±13.48	39.08±2.21	130.80±9.17	109.96±4.50	14.52±1.81
4 h						
HSFA	203.08±12.40	349.61±79.14	41.31±3.87	123.26±6.41	98.90±2.90	14.70±1.77
HMUFA	190.20±11.15	259.64±44.63	41.52±2.55	123.71±9.83	91.46±3.11	15.41±2.89
LFHCC	193.34±15.03	236.13±31.134	39.85±3.12	132.05±12.54	91.07±7.45	14.63±2.85
LFHCC-*n*−3	194.09±11.82	235.14±26.14	37.60±2.75	130.75±9.83	87.20±3.14	12.73±2.24
Global analysis *p*-values						
Diet effect	0.860	0.259	0.816	0.970	0.715	0.996
Time effect	0.003[a)]	<0.001[a)]	0.007[a)]	0.976	<0.001[a)]	0.196
Diet-by-time effect	0.175	0.112	0.835	0.156	0.412	0.288

Values presented are the mean±SEM of each diet group. 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 supplemented with 1.24 g/day long-chain $n-3$ PUFA; TC, total cholesterol; TG, triglycerides; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol. *p*-Value correspond to ANOVA for repeated measured.
a) $p < 0.05$.

3.3 Correlations between postprandial gene expression in adipose tissue and lipid, lipoprotein parameters, and the HOMA index

We analyzed the relationship between the expression of the inflammatory genes (mRNA levels for p65, IκBα, IκBβ2, IL-6, MCP-1, and IL-1β), and lipid concentrations in the postprandial state and the HOMA index (Table 5). There was a negative correlation between the postprandial mRNA levels of p65 (4 h after a meal) with the plasma HDL-c concentration 4 h after a meal (r: −0.377; $p = 0.018$; r^2: 0.142), and a positive correlation with the TG level 4 h after a meal (r: 0.406; $p = 0.010$; r^2: 0.164) and the HOMA index (r:0.353; $p = 0.028$; r^2:0.125; Fig. 3).

Additionally, there was a positive correlation between the p65 gene expression increment (postprandial value minus fasting value) with the HOMA index (r:0.327; $p = 0.042$; r^2: 0.107). There was also a positive correlation between the p65 gene expression AUC and the TG AUC (r: 0.394; $p = 0.013$; r^2: 0.155), total cholesterol AUC (r: 0.334; $p = 0.038$; r^2: 0.111), and LDL-c AUC (r: 0.344; $p = 0.034$; r^2: 0.118).

4 Discussion

We did not observe any relevant dietary effect between the pre and post-intervention inflammatory status in the fasting

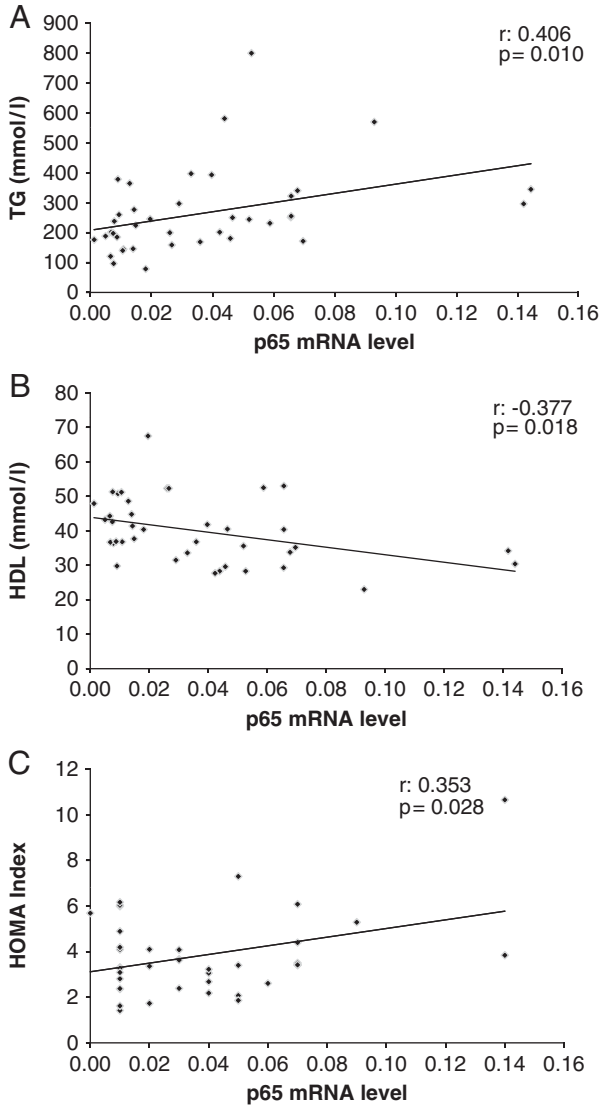

Figure 3. Correlations between postprandial p65 mRNA expression and (A) triglycerides (mmol/L) ($r = 0.406$, $p < 0.010$) and (B) HDL-c (mmol/L) ($r = -0.377$, $p < 0.018$) and (C) HOMA index ($r = 0.353$, $p < 0.028$) in the MetS patients, 4 h after the administration of the fatty meal with a fat composition similar to that consumed in each of the HSFA, HMUFA, LFHCC, or LFHCC ($n-3$) diets. $n = 39$, r: Pearson's linear correlation coefficient.

state. Post-intervention, we administered four high-fat meals providing the same amount of fat (65%); the fatty acid composition reflected that consumed within the intervention period in terms of fat quality. After a high-fat meal intake, which represents a fat challenge, we studied the postprandial responses on a model of the postprandial phase that may be influenced by the previous dietary intervention because of the adaptive effect after a long-term dietary intervention.

Our study showed that in the postprandial state there was an induction of adipose tissue inflammatory genes (p65, MCP-1, IL-6, and IL-1β) in patients with MetS and when this pattern was established, the induction of adipose tissue inflammatory genes was not affected by the quantity and quality of the fat included in the diet consumed, as studied during the postprandial period.

Obesity is associated with different degrees of adipose tissue dysfunction and chronic low-grade inflammation characterized by macrophage infiltration in adipose tissue and the expression of inflammatory genes [30]. However, this inflammatory state of the adipose tissue can be modulated by diet. More specifically, there is evidence that consumption of an SFA-rich diet induces a pro-inflammatory gene expression profile in obese people, whereas consumption of a HMUFA diet leads to an anti-inflammatory profile, which is evident in biological samples taken during the fasting state [17].

Several studies have shown differences between consumption of SFA- and MUFA-rich diets in terms of the activation of peripheral blood mononuclear cells (PBMC), and the degree of low-grade inflammation in obesity [6]. In some studies, it has been shown that MUFA-rich diets reduce the postprandial inflammatory response when compared with SFA-rich diets [31–33].

However, our study did not document such an anti-inflammatory effect by an HMUFA diet in the expression of several inflammatory genes in adipose tissues during the postprandial period.

A HSFA diet tended to raise plasma IL-6 levels, and an HMUFA diet tended to raise both NF-κB p65 sub-unit gene expression and plasma IL-6 levels, as studied during the postprandial period. In this same area, a previous study has shown a similar effect of fat-rich diets (HMUFA and SFA-rich diets) on adipose tissue gene expression of adiponectin when compared with a CHO-rich diet. That study showed that postprandial adiponectin gene expression was lower after a CHO-rich breakfast than an MUFA- or SFA-rich breakfast in insulin-resistant patients [34].

Blackburn et al. [35] showed that IL-6 plasma levels increased 8 h after the consumption of a high-fat meal in people with abdominal obesity. Other study have shown that IL-6 plasma levels increased 6 h after consuming a high-fat test meal containing a high or low ratio of saturated:unsaturated fatty acids in slim people [36]. Thus, the discrepancies between the IL-6 data observed between these studies and the current study could be explained by the timing of the blood sampling; specifically, the earlier sampling (4 h after the ingestion of a meal) may not have allowed sufficient time to observe the increase in the IL-6 level after the consumption of HSFA and HMUFA diets.

The current study has shown a postprandial increase in the expression of the inflammatory cytokines, MCP-1 and IL1β, in adipose tissue irrespective of the quantity and quality of dietary fat. Moreover, the expression of the inflammatory cytokine, MCP-1 gene, increased during the postprandial phase in adipose tissue, but not in plasma.

This observation could be explained given that the synthesis and secretion processes do not occur concomitantly and/or to the occurrence of additional regulatory mechanisms acting on the secretory pathway [37]. This is relevant from a pathophysiologic point of view because postprandial MCP-1 release in adipose tissues may facilitate macrophage recruitment and activation, which promotes macrophage-dependent inflammatory responses and the development of atherosclerosis [38].

We observed an inverse relationship between the expression of the NF-κB transcription factor, sub-unit p65, in adipose tissue and the HDL-c plasma concentration. Given that HDL-c has been shown to inhibit monocyte chemotaxis/adhesion, as well as to reduce vascular macrophage content in inflammatory conditions [39], the negative correlation found between HDL-c and NF-κB observed in the current study strongly supports the view that an increase in the inflammatory response in adipose tissue occurs when the protective effect of HDL-c diminishes. In line with this notion, it has been shown in vitro that HDL-c mediates suppression of NF-κB activity in endothelial cells [40]. Our study has also shown that the expression of NF-κB transcription factor sub-unit p65 in adipose tissue is also related to other lipid parameters. Specifically, a positive correlation was found between the p65 and TG plasma concentration. Taken together, these findings suggest that NF-κB transcription factor sub-unit p65 gene expression in adipose tissue is related to the lipid plasma composition.

Growing evidence links inflammation to the pathogenesis of insulin resistance [41] and there is evidence that the over-expression of NF-κB is enough to impair insulin sensitivity in skeletal muscle cells [42]. Thus, the fact that our study showed a positive relationship between the gene expression of NF-κB transcription factor sub-unit p65 in adipose tissue and the HOMA index supports the link between inflammation and insulin resistance.

One of the limitations of our study was that the proportion of fat between diet on dietary intervention and test meals was not the same. This mismatch is due to the fact that the percentage of fat is different between long-term diets, but the same in test meals. To study the postprandial state after the dietary intervention, we decided administer a fat challenge with the same amount of fat, which did not correspond to the previous diets (two high-fat and two low-fat diets). To perform postprandial studies requires a fat overload standardized the among fat administered by body weight [21, 43]. Thus, we administered a high-fat meal enriched in SFA after a HSFA diet, a high-fat meal enriched in MUFA after an HMUFA diet, and we administered a high-fat meal with the same fatty acid proportion consumed within the low-fat diet intervention period. In addition, different amounts of fat on the fat challenge could affect the results in the following two ways: fat amount determines the postprandial response and the possible differences observed could have been due to fat quantity, but not to fat quality; and we were studying adipose tissue, an organ that functions as a fat store. However, the fat quantity effect (low-fat long-term dietary intervention) was studied comparing the pre and post-intervention inflammatory status in the fasting state, and this could have an effect on the postprandial response because of the adaptive effect after the long-term intervention. In addition, in future studies, it will be interesting to analyze the effect of meals with the same macronutrient composition to that consumed in the habitual diet on the postprandial adipose tissue response. Another limitation, concerning the sample size, is that our study was able to detect gene expression changes of double or one half, which is sufficient to discern a relevant biological effect, but a smaller effect, could not have been detected. Of note, our study represents the first approach to the diet effect on adipose tissue during the postprandial state in MetS patients and represents a starting point on elucidating diet interaction on adipocyte function.

In conclusion, our results suggest that an exacerbated adipose tissue postprandial inflammatory response occurs in individuals with MetS, which seems to be independent of the quality and quantity of the dietary fat. This finding is important because the postprandial state is probably the most common metabolic state that humans experience during the day, and thus makes a substantial contribution to the environmental factors which increases cardiometabolic risk.

Further research is needed to extend our knowledge about the effect of the type of diet on postprandial inflammatory events in adipose tissue, the specific cellular and molecular alterations, and the elucidation of nutritionally based therapeutic strategies for the MetS.

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