Eur J Nutr (2014) 53:617–626
DOI 10.1007/s00394-013-0570-2

ORIGINAL CONTRIBUTION

Dietary fat differentially influences the lipids storage on the adipose tissue in metabolic syndrome patients

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Received: 19 June 2012 / Accepted: 26 July 2013 / Published online: 7 August 2013

Abstract
Purpose Adipose tissue is now recognized as a highly active metabolic and endocrine organ. Our aim was to investigate the effect of the dietary fat on the two main adipose tissue functions, endocrine and lipid store, by analyzing the adipose tissue gene expression from metabolic syndrome patients.
Methods A randomized, controlled trial conducted within the LIPGENE study assigned 39 metabolic syndrome patients to 1 of 4 isoenergetic diets: (1) high-saturated fatty acid (HSFA), (2) high-monounsaturated fatty acid (HMUFA), (3) low-fat, high-complex carbohydrate diet supplemented with long-chain n-3 fatty acids (LFHCC n-3), and (4) low-fat, high-complex carbohydrate diet supplemented with placebo (LFHCC), for 12 weeks each. A fat challenge reflecting the fatty acid composition as the original diets was conducted post-intervention.
Results The long-term consumption of HSFA, LFHCC, and LFHCC n-3 diets, but not HMUFA diet, decreased the perilipin fasting mRNA levels. LFHCC diet consumption increased fasting FABP4 expression, while it was reduced by the consumption of LFHCC n-3 diet. LFHCC meal reduced, while LFHCC n-3 meal intake increased post-prandial CAV1 expression.
Conclusion The quantity and quality of dietary fat induce differential lipid storage and processing related gene expression, which may interact with the expression of adipokines through common regulatory mechanisms.

Keywords Adipokines · Lipid metabolism · Adipose tissue · Diet · Metabolic syndrome

Introduction

Adipose tissue (AT) is essential in the regulation of lipid metabolism, as it is responsible for triglyceride storage and the mobilization of fatty acids (FA) during periods of positive and negative energy balance, respectively. In addition, AT secretes a wide variety of signaling molecules

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Electronic supplementary material The online version of this article (doi:10.1007/s00394-013-0570-2) contains supplementary material, which is available to authorized users.

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(e.g., hormones, growth factors, cytokines, or chemokines), and adipokines [1], which act as paracrine, autocrine, or endocrine effectors regulating AT function, as well as that of cells located in the brain, liver, muscle, or pancreas [2]. Thus, AT is now recognized as a highly active metabolic and endocrine organ controlling energy homeostasis [3].

Lifestyle factors, such as overeating and physical inactivity, induce visceral fat accumulation, which results in the dysfunction of adipocytes. The oversecretion of some adipokines, such as *PAI-1*, *TNF-α*, or visfatin, and the hyposecretion of others, such as adiponectin, might prove to be major mechanisms in lifestyle-related diseases, including diabetes mellitus, hyperlipidemia, hypertension, and atherosclerosis, which make up the so-called metabolic syndrome (MetS) [4]. Hence, the etiology of MetS is the result of a complex interaction between genetic, metabolic, and environmental factors including dietary habits and, particularly, the quality of dietary fat [5].

Adipokine secretion may be regulated dynamically by the nutritional state [6]. The experimental evidence available supports the view that specific diet composition may directly influence the molecular events that govern gene expression in adipocytes, adipokine production, and adipocyte lipid and glucose metabolism [7, 8]. Nevertheless, most of these data have been obtained in animal models, whereas similar studies in humans are scarce. In particular, a previous work has shown that the consumption of a Mediterranean diet, rich in oleic acid, increases postprandial adiponectin (*ADIPOQ*) gene expression in peripheral AT when compared with a low-fat, carbohydrate-rich diet in insulin-resistant patients [9].

Humans spend most of the day in the postprandial state. Each time we eat, depending on what we ingest in terms of fat quality and quantity, changes in AT may be occurring, and therefore, it is very important to investigate and establish what the most suitable diet is for correcting adipocyte endocrine dysfunction, since some of the adipokines have been related to the development of cardiovascular diseases, which is especially important in MetS patients [10, 11].

The aim of this study was to evaluate the effect of four diets differing in quantity and quality of fat on the two main AT functions, endocrine and lipid storage, by analyzing the adipose tissue gene expression of *ADIPOQ*, leptin (*LEP*), resistin (*RETN*), retinol binding protein-4 (*RBP4*), and plasminogen activator inhibitor-1 (PAI-1) in MetS patients. Additionally, we studied the expression of

Table 1 Baseline characteristics of subjects with MetS assigned to each diet

Baseline characteristics	HSFA ($n = 8$)	HMUFA ($n = 9$)	LFHCC ($n = 12$)	LFHCC n-3 ($n = 10$)	*P* value
Age (years)	57.8 ± 3.1	57.1 ± 2.3	56.5 ± 2.0	54.8 ± 2.1	0.839
BMI (kg/m^2)	36.0 ± 1.2	34.5 ± 1.2	35.7 ± 1.0	35.0 ± 1.2	0.817
Waist circumference	111.7 ± 3.1	104.0 ± 2.0	109.0 ± 3.1	108.3 ± 3.3	0.420
TC (mg/dl)	204.0 ± 19.0	192.2 ± 11.1	206.8 ± 14.9	196.9 ± 10.0	0.877
TG total (mg/dl)	226.7 ± 65.2	159.1 ± 20.9	161.5 ± 17.5	158.1 ± 20.3	0.422
c-LDL (mg/dl)	129.7 ± 13	135.9 ± 9.6	148.2 ± 12.1	140.4 ± 8.8	0.693
c-HDL (mg/dl)	41.0 ± 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
Adiponectin (mRNA)	113.1 ± 24.69	68.24 ± 23.28	114 ± 20.16	92.55 ± 22.08	0.452
Resistin (mRNA)	0.058 ± 0.026	0.044 ± 0.020	0.040 ± 0.014	0.110 ± 0.045	0.275
Leptin (mRNA)	0.013 ± 0.007	0.004 ± 0.001	0.004 ± 0.001	0.005 ± 0.002	0.186
RBP4 (mRNA)	154.7 ± 36.55	120.7 ± 34.46	187.2 ± 29.84	167.35 ± 32.69	0.539
Perilipin (mRNA)	102.04 ± 20.02	67.53 ± 18.87	116.8 ± 16.35	96.17 ± 17.91	0.283
Vimentin (mRNA)	139.47 ± 22.49	116.4 ± 19.17	151.2 ± 23.23	167.01 ± 17.64	0.442
Cav1 (mRNA)	0.190 ± 0.444	0.195 ± 0.311	0.167 ± 0.234	0.162 ± 0.290	0.844
FABP4 (mRNA)	9.00 ± 2.453	6.88 ± 0.990	6.71 ± 1.134	8.99 ± 1.769	0.586
LPL (mRNA)	0.058 ± 010	0.044 ± 0.006	0.061 ± 0.011	0.041 ± 0.012	0.536
UCP2 (mRNA)	0.183 ± 0.037	0.140 ± 0.034	0.202 ± 0.030	0.214 ± 0.033	0.435
ACOX 1 (mRNA)	0.012 ± 0.003	0.010 ± 0.001	0.015 ± 0.003	0.014 ± 0.003	0.588
CES1 (mRNA)	0.090 ± 0.017	0.058 ± 0.016	0.097 ± 0.024	0.081 ± 0.024	0.610

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 with 1.24 g/d LC n-3 PUFA diet, *BMI* body mass index, *TC* total cholesterol, *TG* triglycerides, *c-HDL* high-density lipoprotein-cholesterol, *c-LDL* low-density lipoprotein-cholesterol *P* value correspond to ANOVA statistical analysis

genes related to lipid storage, perilipin (*PLIN*), vimentin (*VIM*), caveolin (*CAV1*) and fatty acid binding protein 4 (*FABP4*), and genes related to lipid processing, lipoprotein lipase (*LPL*), acyl-coenzyme A oxidase 1 (*ACOX1*), carboxylesterase 1 (*CES1*), and energy dissipation uncoupling protein 2 (*UCP-2*).

Materials and methods

Participants and recruitment

This study was conducted within the framework of the LIPGENE study (diet, genomics, and metabolic syndrome: an integrated nutrition, agro-food, social, and economic analysis), a Framework 6 Integrated Projected funded by the European Union. A total of 39 patients with MetS (25 females and 14 males) (Table 1) 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. This study was carried out in the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital (Cordoba, Spain), from February 2005 to April 2006. The experimental protocol was approved by the local ethic committee according to the Helsinki Declaration. Clinical Trial Registration number: Study identifier at ClinicalTrials.gov was NCT00429195.

Design

Patients were randomly stratified to 1 of 4 dietary interventions for 12 weeks. MetS was defined by published criteria [12], which conformed to the LIPGENE inclusion and exclusion criteria [13]. Post-intervention 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 provide information about pre-, mid-, and post-intervention food consumption and dietary compliance, have been described in detail by Shaw et al. [13]. Briefly, dietary intake and compliance were assessed by a 3 days (2 weekdays and 1 weekend day) weighed food intake assessments at baseline, week 6, and week 12. Weighed food intake over 2 weekdays and 1 weekend day was obtained using scales provided by the nutritionist. Volunteers were asked to include the food packaging and homemade recipes where possible. Dietary analysis program reflective of the food choices was used (Dietsource version 2.0).

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 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 carbohydrates-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) 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) (Supplemental Table 1). The intervention center performed a pre- (wk 0) and post-intervention (wk 12) postprandial challenge reflecting the fatty acid composition as that consumed on the assigned dietary period. Of note, in this work, we analyzed the postprandial state post-intervention. Patients arrived at the clinical center at 8 h 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 was prepared in the center and reflected fatty acid composition of each subject chronic dietary intervention. The meal composition was determined by using weighing and the food composition tables from the Dietsource 2.0 software (Supplemental Table 2). 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 for 9 h but were allowed to drink water. The composition of the breakfasts was as follows: HSFA, 38 % E from SFA; HMUFA, 43 % E from MUFA; LFHCC with placebo capsules, 16 % E as PUFA; LFHCC with LC (n-3) PUFA, 16 % E as PUFA [1.24 g/d of LC (n-3) PUFA (ratio 1.4 EPA:1 DHA)].

Monitoring for adverse effects

Volunteers were visited each 2 weeks 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.

Subcutaneous adipose tissue sample collection

Subcutaneous adipose tissue samples were obtained from the superficial abdominal subcutaneous adipose tissue lateral to the umbilicus with instrument Bard® Magnum (MG1522), needles Bard® Magnum Core (MN1410) (M & I Medical Sales, Inc., Miami, FL, USA). For the study of postprandial adipocyte function, samples were collected at time 0 and 4 h after administration of the fatty meal and stored at −80 °C.

RNA isolation from adipose tissue

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

qRT-PCR analysis

RT-reaction was performed using the commercial kit message BOOSTER cDNA synthesis kit for qPCR (Epicentre, Madison, WI, USA), according to the manufacturer's instructions. Briefly, it was amplified 500 pg of total RNA and then converts the amplified RNA to cDNA, which was stored at −20 °C. The expression of *LEP*, *CAV1*, *FABP4*, *LPL*, *ACOX1*, and *CES1* genes was measured by using the OpenArray™ NT Cycler system (Applied Biosystems, Carlsbad, CA, USA) and RPLP0 as housekeeping gene, according to the manufacturer's instructions. We used the following Taqman assays: *LEP* (Hs00174877_m1), *CAV1* (Hs00971716_m1), *FABP4* (Hs01086177_m1), *LPL* (Hs00173425_m1), *ACOX1* (Hs01074241_m1), *CES1* (Hs00275607_m1), and *RPLP0* (Hs99999902_m1). The PCR experiment performed in this platform consisted in a larger set of Taqman assays able to analyze the expression 53 target + 3 housekeeping genes, corresponding to different pathways. Thus, 47 from 53 target genes have not been included in this work. The expression of *ADIPOQ*, *RETN*, *RBP-4*, *PAI-1*, *PLIN*, *VIM*, and *UCP-2* genes was measured by using an iQ5 iCycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) by using specific primer pairs and iQ SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) commercial kit in a final volume of 20 µl with 10 pmol of each primer and CLN3 as housekeeping gene. Each reaction was performed on 1 µl of 1:20 (v/v) dilution of the first cDNA strand. The reaction was incubated at 96 °C for 3 min, followed by 40 cycles of 30 s at 96 °C, 30 s at 6 °C, and 20 s at 72 °C when fluorescence was measured. 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) and analyzed by electrophoresis on a 1.6 % agarose gel, TBE 1x. Primers used are shown in Supplemental Table 4. 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) and analyzed by electrophoresis on a 1.6 % agarose gel, TBE 1x.

Statistical analyses

SPSS statistical software, version 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The normal distribution of variables to characterize the postprandial response was assessed by using the Kolmogorov–Smirnov. We performed ANOVA for repeated measured to determine the 12-week dietary intervention effect of the diet and the postprandial effect of the fat meal composition, with dietary intervention as the inter-subject factor. Global *P* values indicate the following: *P1*: the effect of the diet and fat meal composition ingested (between-subject effect); *P2*: the time effect (within-subject effect); and *P3*: the interaction of both factors, indicative of the degree of the response in each group of subjects with each fat meal (diet by time interaction). Post hoc statistical analysis was completed by using the Bonferroni's multiple comparison tests. In the case of *PAI-1* (only postprandial time point), we performed a one-way ANOVA in order to analyze statistical differences between diets. A probability of less than 0.05 was considered significant. A study of the relationship among parameters was also carried out by using Pearson's linear correlation coefficient. All data presented are expressed as mean ± SEM.

Results

Baseline characteristics

No significant differences were observed in the baseline characteristics of the 39 subjects with MetS (Table 1) participating in the dietary intervention (Fig. 1).

Diet and adipokine gene expression

To evaluate whether the intake of the four diets differing in fat quantity and quality could regulate the endocrine function of AT, we analyzed by qRT-PCR the gene

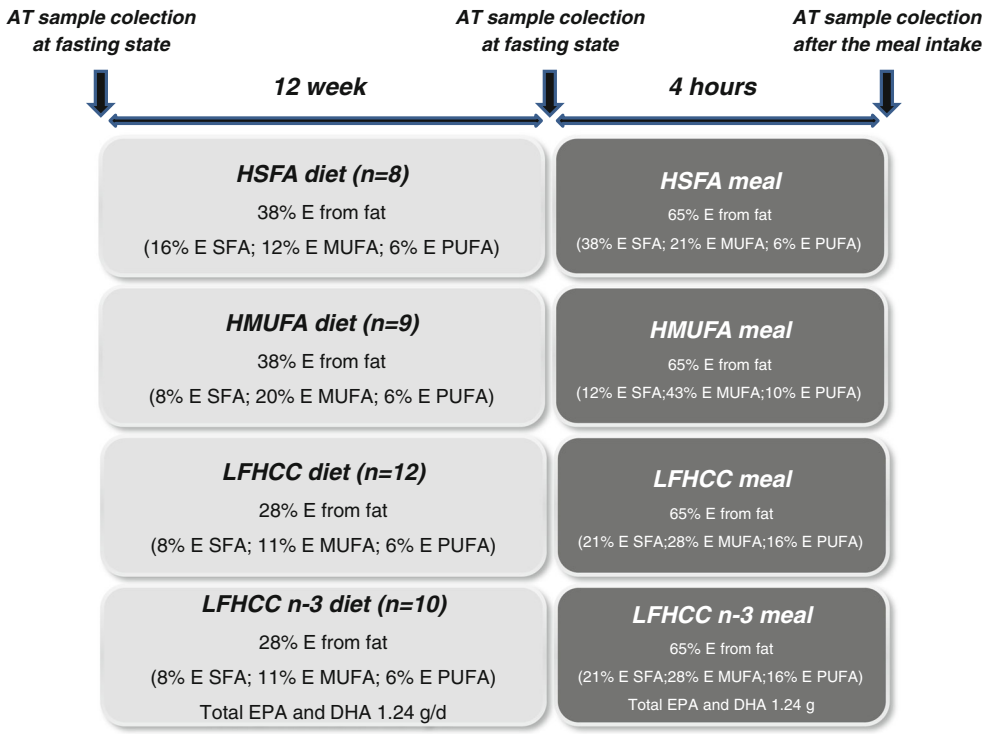

Fig. 1 Design of the study. A randomized, controlled trial conducted within the LIPGENE study assigned 39 MetS patients to 1 of 4 isoenergetic diets: (**a**) high-saturated fatty acid (HSFA), (**b**) high-monounsaturated fatty acid (HMUFA), (**c**) low-fat, high-complex carbohydrate diet supplemented with n-3 polyunsaturated fatty acids (PUFA) (LFHCC n-3), and (**d**) low-fat, high-complex carbohydrate diet supplemented with placebo (LFHCC), for 12 weeks each. A fat challenge (test meal) reflecting the fatty acid composition as the original diets was conducted post-intervention

expression changes of several adipokines at baseline (pre-intervention) and post-intervention (12 week) in the fasting state (Table 2), and post-intervention during the postprandial state, 4 h after the administration of a meal reflecting the fatty acid composition as the original diets that consumed during the intervention period (Table 3).

The long-term consumption of the four diets induced a decrease in fasting *ADIPOQ* and *RBP4* mRNA level as compared to baseline pre-intervention (both $P = 0.006$). No changes were observed in *LEP* and *RETN* mRNA levels after the consumption for any of the diets tested.

We also found that *ADIPOQ* mRNA level decreased in the postprandial state ($P = 0.027$), irrespective of the diet consumed. Additionally, no changes were observed in *RBP4*, *LEP*, and *RETN* mRNA levels in the postprandial state for any of the diets tested as compared to the fasting state.

Interestingly, *PAI-1* transcripts were only detectable in the postprandial state for all the groups, 4 h after the meal ingestion, although no significant differences were found between diets.

Diet and lipid storage and processing

We also studied the gene expression of 3 proteins related to lipid dopplets, perilipin (*PLIN1*), caveolin (*CAV1*) and vimentin (*VIM*) [14, 15], and the intracellular fatty acid binding protein (*FABP4*). The long-term consumption of HSFA, LFHCC, and LFHCC n-3 diets induced the decrease in fasting *PLIN1* mRNA level as compared to baseline ($P = 0.010$, $P = 0.002$, and $P = 0.002$, respectively), while it remained unchanged after HMUFA diet consumption. In addition, fasting *CAV1* mRNA level decreased after the consumption during 12 weeks of the four diets ($P < 0.001$) as compared to the baseline (pre-intervention).

We found an interaction between dietary fat and fasting *FABP4* mRNA level ($P = 0.019$). Post hoc statistical analysis showed that *FABP4* mRNA level statistically increased after the intake of LFHCC diet ($P = 0.042$) and decreased after the intake of LFHCC n-3 diet ($P = 0.036$) (Table 2).

We observed that *PLIN1* mRNA level decreased in the postprandial state ($P = 0.047$), irrespective of the diet consumed. We found an interaction between dietary fat and postprandial *CAV1* mRNA level ($P = 0.021$). Post hoc statistical analysis showed that *CAV1* mRNA level statistically decreased after the intake of LFHCC meal ($P = 0.042$) and increased after the intake of LFHCC n-3 meal ($P = 0.036$) (Table 3). However, when we studied *VIM* gene expression, no significant mRNA level changes were found.

Table 2 Diet and expression of adipokines and lipid storage-related genes at fasting state

	HSFA	HMUFA	LFHCC	LFHCC n-3	Diet effect	Time effect	Diet × time effect
ADIPOQ							
Pre-intervention	113.1 ± 24.69	68.24 ± 23.28	114.0 ± 20.16	92.55 ± 22.08	0.832	0.006	0.079
Post-intervention	51.99 ± 8.75	85.90 ± 25.50	66.02 ± 17.95	45.96 ± 8.53			
LEP							
Pre-intervention	0.013 ± 0.007	0.004 ± 0.001	0.004 ± 0.001	0.005 ± 0.002	0.067	0.373	0.416
Post-intervention	0.007 ± 0.003	0.002 ± 0.002	0.007 ± 0.003	0.002 ± 0.0006			
RETN							
Pre-intervention	0.058 ± 0.026	0.044 ± 0.020	0.040 ± 0.014	0.110 ± 0.045	0.258	0.905	0.801
Post-intervention	0.101 ± 0.056	0.067 ± 0.024	0.064 ± 0.020	0.105 ± 0.044			
RBP4							
Pre-intervention	154.7 ± 36.55	120.7 ± 34.46	187.2 ± 29.84	167.3 ± 32.69	0.711	0.006	0.283
Post-intervention	93.7 ± 10.50	142.8 ± 31.87	108.2 ± 23.26	88.57 ± 14.63			
PAI-1							
Pre-intervention	Not detected	Not detected	Not detected	Not detected	–	–	–
Post-intervention	Not detected	Not detected	Not detected	Not detected			
PLIN1							
Pre-intervention	102.0 ± 20.02	67.53 ± 18.87	116.8 ± 16.35	96.17 ± 17.91	0.631	<0.001	0.019
Post-intervention	51.84 ± 5.81*	83.39 ± 23.49	59.58 ± 14.03*	33.85 ± 6.83*			
CAV1							
Pre-intervention	0.190 ± 0.444	0.195 ± 0.31	0.167 ± 0.234	0.162 ± 0.290	0.513	<0.001	0.319
Post-intervention	0.120 ± 0.03	0.110 ± 0.02	0.140 ± 0.030	0.060 ± 0.010			
VIM							
Pre-intervention	139.4 ± 22.49	116.4 ± 19.17	151.2 ± 23.23	167.0 ± 17.64	0.619	0.378	0.842
Post-intervention	164.7 ± 23.55	157.3 ± 33.52	163.3 ± 22.56	157.0 ± 22.64			
FABP4							
Pre-intervention	09.00 ± 2.453	6.880 ± 0.990	6.710 ± 1.134	8.99 ± 1.769	0.035	0.386	0.019
Post-intervention	14.04 ± 2.440	6.660 ± 2.010	11.92 ± 2.260*	3.56 ± 0.880*			

Mean (±SEM) of adipose tissue gene expression at fasting state pre-intervention and at fasting state after the intervention period (post-intervention). *ADIPOQ* adiponectin, *LEP* leptin, *RETN* resistin, *RBP4* retinol binding protein-4, *PAI-1* plasminogen activator inhibitor, *PLIN1* perilipin, *CAV1* caveolin and *VIM* vimentin, and *FABP4* fatty acid binding protein-4, *HSFA* high-saturated fatty acid diet. *HMUFA* high-monounsaturated fatty acid diet, *LFHCC* low-fat, high-carbohydrate diet, *LFHCC n-3* low-fat, high-carbohydrate diet supplemented with long-chain n-3 polyunsaturated fatty acid. ANOVA for repeated measured: *P1* diet effect, *P2* time effect, *P3* diet by time interaction

* $P < 0.05$ versus fasting state pre-intervention in the post hoc Bonferroni's multiple comparison tests

We have also studied the expression of genes involved in lipid uptake (lipoprotein lipase, *LPL*), fatty acid beta-oxidation (acyl-coenzyme A oxidase 1, *ACOX1*), metabolism of fatty acids and cholesterol (carboxylesterase 1, *CES1*), and energy dissipation (uncoupling protein 2, *UCP-2*) (Supplemental Table 3). We found an interaction between dietary fat and fasting *CES1* mRNA level ($P = 0.004$). Post hoc statistical analysis showed that *CES1* mRNA level statistically increased after the consumption of HMUFA diet ($P = 0.022$) and decreased after the consumption of both LFHCC and LFHCC n-3 diets ($P = 0.022$ and $P = 0.019$, respectively).

No changes were observed in *LPL*, *UCP-2*, and *ACOX1* mRNA levels neither at the fasting nor the postprandial state for any of the diets tested.

Discussion

Our study showed that the diet may differentially modulate AT endocrine function through common regulatory mechanism that control both lipid metabolism and adipokines gene expression as shown in this work to *ADIPOQ* and the lipid droplet-related protein, *PLIN,* which underwent a similar gene expression changes profile by dietary fat.

The transcription regulatory regions, located upstream of the gene coding region, frequently contain several DNA binding sites to different transcription factors that respond to different stimuli. These stimuli can occur independently or in parallel, and the transcription of a gene is promoted by the action of only one or by several transcription factors,

Table 3 Diet and expression of adipokines and lipid storage-related genes at postprandial state

	HSFA	HMUFA	LFHCC	LFHCC n-3	Diet effect	Time effect	Diet × time effect
ADIPOQ							
Fasting	51.99 ± 8.75	85.90 ± 25.50	66.02 ± 17.95	45.96 ± 8.53	0.412	0.027	0.297
Postprandial	36.14 ± 6.21	29.08 ± 4.81	53.13 ± 13.62	31.82 ± 7.42			
LEP							
Fasting	0.0075 ± 0.0025	0.0020 ± 0.0002	0.0072 ± 0.0030	0.0023 ± 0.0006	0.037	0.898	0.781
Postprandial	0.0048 ± 0.0014	0.0025 ± 0.0006	0.0074 ± 0.0023	0.0036 ± 0.0016			
RETN							
Fasting	0.101 ± 0.056	0.067 ± 0.024	0.064 ± 0.020	0.105 ± 0.044	0.962	0.065	0.901
Postprandial	0.144 ± 0.060	0.136 ± 0.037	0.151 ± 0.050	0.157 ± 0.023			
RBP4							
Fasting	93.7 ± 10.50	142.75 ± 31.87	108.21 ± 23.26	88.57 ± 14.63	0.346	0.101	0.676
Postprandial	79.35 ± 21.28	69.02 ± 11.28	101.27 ± 17.12	68.13 ± 11.16			
PAI-1							
Fasting	Not detected	Not detected	Not detected	Not detected	0.691		
Postprandial	0.37 ± 0.12	0.56 ± 0.09	0.53 ± 0.13	0.82 ± 0.20			
PLIN1							
Fasting	51.84 ± 5.81	83.39 ± 23.49	59.58 ± 14.03	33.85 ± 6.83	0.097	0.047	0.320
Postprandial	34.75 ± 6.37	35.27 ± 7.80	46.48 ± 8.83	29.76 ± 5.62			
CAV1							
Fasting	0.12 ± 0.03	0.11 ± 0.02	0.14 ± 0.03	0.06 ± 0.01	0.759	0.238	0.021
Postprandial	0.07 ± 0.01	0.10 ± 0.01	0.09 ± 0.01*	0.12 ± 0.03*			
VIM							
Fasting	164.77 ± 23.55	157.26 ± 33.52	163.25 ± 22.56	157.03 ± 22.64	0.405	0.058	0.460
Postprandial	115.58 ± 13.50	117.65 ± 15.37	205.75 ± 26.39	148.04 ± 20.77			
FABP4							
Fasting	14.04 ± 2.44	6.66 ± 2.01	11.92 ± 2.26	3.56 ± 0.88	0.010	0.365	0.595
Postprandial	10.31 ± 2.29	5.07 ± 1.61	9.64 ± 3.24	5.63 ± 1.29			

Mean (±SEM) of adipose tissue gene expression at fasting state post-intervention and 4 h after the administration of a meal reflecting the fatty acid composition as the original diets that consumed during the intervention period. *ADIPOQ* adiponectin, *LEP* leptin, *RETN* resistin, *RBP4* retinol binding protein-4, *PAI-1* plasminogen activator inhibitor, *PLIN1* perilipin, *CAV1* caveolin and *VIM* vimentin, and *FABP4* fatty acid binding protein-4, *HSFA* high-saturated fatty acid diet, *HMUFA* high-monounsaturated fatty acid diet, *LFHCC* low-fat, high-carbohydrate diet, *LFHCC n-3* low-fat, high-carbohydrate diet supplemented with long-chain n-3 polyunsaturated fatty acid. ANOVA for repeated measured: *P1* diet effect, *P2* time effect, *P3* diet × time interaction

* $P < 0.05$ versus fasting state post-intervention in the post hoc Bonferroni's multiple comparison tests

responding to different stimuli and acting simultaneously [16].

In line with this notion, our study showed that the long-term consumption of HSFA, LFHCC, and LFHCC n-3 diets decrease the fasting expression of *PLIN1*, while it remained unchanged after the consumption of HMUFA diet. In parallel, the expression of *ADIPOQ* underwent the same expression profile, and it decreased after the long-term consumption of the four diets as compared to the pre-intervention, but it tended to remain unchanged after the consumption of HMUFA diet, although it did not reach the statistical significance. Additionally, we also observed a reduction in the postprandial *ADIPOQ* and *PLIN1* gene expression after meals intake. Moreover, the gene expression of both *ADIPOQ* and *PLIN1* positively correlates in fasting state at pre-intervention and post-intervention, and in postprandial state at post-intervention (data not shown). Gene expression is determined by a combination of transcriptional and post-transcriptional regulatory events, and mRNA levels are the balance between transcription rate and the post-transcriptional mRNA degradation [17]. Several transcription factors have been shown to regulate the transcription of both *ADIPOQ* and *PLIN1* genes such as the transcription activator PPARG and PPARA and the transcription repressor FOXC2 [18, 19]. Thus, consequently, the reduction in the *ADIPOQ* and *PLIN1* transcription rate may be caused by a reduction in PPARG and PPARA activation, an activation of the repressor FOXC2, or both events together.

Consequently, ADIPOQ, a key regulator of insulin sensitivity and metabolism [20], underwent the same gene

expression profile as that of *PLIN1*, a lipid droplet-related protein involved in lipolysis, adipocyte size, and lipid accumulation in adipocytes [21]. In addition, despite the gene expression changes, we always observed a trend of both *ADIPOQ* and *PLIN1* mRNA level, to be higher on HMUFA group in both fasting and postprandial state post-intervention. This observation suggest a decrease in the lipid storage capacity in adipose tissue after the long-term consumption of HSFA, LFHCC, and LFHCC n-3 diets, which could be related to an impairment of the insulin sensitivity as recently proposed [22]. Thus, a reduction in adipose tissue lipid store capacity after the long-term consumption of HSFA, LFHCC, and LFHCC n-3 diets may occur in parallel to the reduction in the adipose *ADIPOQ* release.

Moreover, no n-3 PUFA effect was observed on LFHCC n-3 diet as compared to LFHCC on the adipose tissue gene expression of these insulin sensitivity biomarkers *ADIPOQ* and *RBP4*, which supports the idea that n-3 PUFA has no effect on insulin sensitivity improvement [23, 24]. In addition, from the 13 genes which gene expression has been analyzed in our study, only two, *FABP4*, a protein involved in fatty acid metabolism [25, 26], and *CAV1,* a component of the lipid-droplets in lipolytically stimulated cells on in vitro studies [15], underwent a different gene expression as consequence of the supplementation of the LFHCC with n-3 as compared to LFHCC diet without n-3. The observed decrease in the fasting *FABP4* gene expression, which function has been associated with insulin sensitivity [27], after the long–term consumption of LFHCC n-3 diet, also supports the idea that n-3 PUFA has no effect on insulin sensitivity improvement. Moreover, the addition of n-3 PUFA to the LFHCC diet also seems to modify the lipid droplet biogenesis [28] as suggested by the decrease in the *CAV1* gene expression after the long-term consumption of LFHCC and the observed increase when this diet is supplemented with n-3 PUFA.

On the other hand, *PAI-1* gene expression regulation seems to be independent of the transcription factor stated above as shown by IPA transcription factor analysis. *PAI-1* activity shows a circadian change in humans that is high in the morning and low in the evening [29, 30]. *PAI-1* plays a major role in inhibiting fibrinolysis, and high-circulating plasma *PAI-1* concentration is a high-risk factor of thrombotic disease [31]. Interestingly, our study showed that *PAI-1* transcripts were only detectable in the postprandial state, irrespective of the diet consumed in MetS patients. Taken into account that test meals were administered around 8:30 on the morning, the increase from not detectable (fasting state) to detectable (postprandial state) seems to be caused by meal intake as at test meal intake time, *PAI-1* gene expression should be already high because the circadian increase in the morning, although it was not detectable under our experimental conditions.

Thus, the risk or high frequency of acute atherothrombotic events in the morning still seems to be a factor that may be augmented in the postprandial state. In addition, the exacerbated postprandial triacylglyceride response observed in MetS patient as compared to healthy subjects [32, 33] could be also responsible for the postprandial increase in *PAI-1* gene expression. In fact, in vitro studies have shown that several triglyceride-rich lipoproteins such as VLDL [34] and oxidized LDL-c increase PAI-1 production [35], and postprandial lipemia has been associated with a procoagulant state in hypertensive patients [36] and MetS patients [37]. Additionally, VLDL production is suppressed by insulin at the postprandial state, but in insulin resistance individuals as our MetS population, insulin failures to suppress VLDL production [38, 39]. Our results suggest that postprandial VLDL could be the responsible, at least partially of PAI-1 adipose tissue gene expression increase as a VLDL response element is located in the promoter region of the PAI-1 gene [40].

In conclusion, the quantity and quality of dietary fat induce differential lipid storage and processing gene expression, which may interact with the expression of adipokines through common regulatory mechanisms. Further research is needed to extend the knowledge about the effect of the quantity and quality of dietary fat in adipose tissue endocrine function, and this could lead to a useful treatment for correcting the disorders in adipokines release present in metabolic syndrome patients and prevent the cardiovascular risk derived from this condition.

Acknowledgments The CIBEROBN is an initiative of the Instituto de Salud Carlos III, Madrid, Spain. This study was supported partly by research grants from the European community (LIPGENE European proyect-505944), the Spanish Ministry of Health (CB06/03/0047 (CIBER Fisiopatología de la Obesidad y Nutrición)) and the Ministerio de Ciencia e Innovación (AGL2006-01979/ALI, AGL2009-12270 to JL-M and SAF2007-62005 to FP-J), Consejeria de Innovación, Ciencia y Empresa, Proyectos de Investigación de Excelencia Junta de Andalucia (CTS-03039 to MMM, AGR 05/00922, CT5015 to FP-J and P06-CTS-01425 to JL-M), Consejería de Salud, Junta de Andalucía (06/128, 07/43, PI 0193/09 to JL-M, 06/129 to FP-J, PI-0252/2009 to JD-L), Centro de Excelencia Investigadora Aceite de Oliva y Salud (CEAS). FEDER, Fondo Social Europeo. We thank M[a] Jose Gomez-Luna for technical support.

Conflict of interest None.

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