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A Low-Fat, High-Complex Carbohydrate Diet Supplemented with Long-Chain (n-3) Fatty Acids Alters the Postprandial Lipoprotein Profile in Patients with Metabolic Syndrome[1–4]

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Abstract

Dietary fat intake plays a critical role in the development of metabolic syndrome (MetS). This study addressed the hypothesis that dietary fat quantity and quality may differentially modulate postprandial lipoprotein metabolism in MetS patients. A multicenter, parallel, randomized, controlled trial conducted within the LIPGENE study randomly assigned MetS patients to 1 of 4 diets: high-SFA [HSFA; 38% energy (E) from fat, 16% E as SFA], high-monounsaturated fatty acid [HMUFA; 38% E from fat, 20% E as MUFA], and 2 low-fat, high-complex carbohydrate [LFHCC; 28% E from fat] diets supplemented with 1.24 g/d of long-chain (LC) (n-3) PUFA (ratio 1.4 eicosapentaenoic acid:1 docosahexaenoic acid) or placebo (1.24 g/d of high-oleic sunflower-seed oil) for 12 wk each. A fat challenge with the same fat composition as the diets was conducted pre- and postintervention. Postprandial total cholesterol, triglycerides (TG), apolipoprotein (apo) B, apo B-48, apo A-I, LDL-cholesterol, HDL-cholesterol and cholesterol, TG, retinyl palmitate, and apo B in TG-rich lipoproteins (TRL; large and small) were determined pre- and postintervention. Postintervention, postprandial TG ($P < 0.001$) and large TRL-TG ($P = 0.009$) clearance began earlier and was faster in the HMUFA group compared with the HSFA and LFHCC groups. The LFHCC (n-3) group had a lower postprandial TG concentration ($P < 0.001$) than the other diet groups. Consuming the LFHCC diet increased the TG ($P = 0.04$), large TRL-TG ($P = 0.01$), TRL-cholesterol ($P < 0.001$), TRL-retinyl palmitate ($P = 0.001$), and TRL-apo B ($P = 0.002$) area under the curve compared with preintervention values. In contrast, long-term ingestion of the LFHCC (n-3) diet did not augment postprandial TG and TRL metabolism. In conclusion, postprandial abnormalities associated with MetS can be attenuated with LFHCC (n-3) and HMUFA diets. The adverse postprandial TG-raising effects of long-term LFHCC diets may be avoided by concomitant LC (n-3) PUFA supplementation to weight-stable MetS patients. J. Nutr. 140: 1595–1601, 2010.

Introduction

The insulin resistance syndrome was initially described by Reaven in 1988 (1) and was recently redefined as metabolic syndrome (MetS)[10] by the National Cholesterol Education Program's Adult Treatment Panel III report (2,3). MetS is a

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[3] This trial was registered at clinicaltrials.gov as NCT00429195.

[4] Supplemental Figure 1 and Supplemental Tables 1–3 are available with the online posting of this paper at jn.nutrition.org.

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[10] Abbreviations used: apo, apolipoprotein; AUC, area under the curve; DHA, docosahexaenoic acid; E, energy; EPA, eicosapentaenoic acid; HDL-C, HDL cholesterol; HMUFA, high-monounsaturated fatty acid diet; HSFA, high-SFA diet; LC, long-chain; LDL-C, LDL-cholesterol; LFHCC, low-fat, high-complex carbohydrate diet; LPL, lipoprotein lipase; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; RM-ANOVA, repeated-measures ANOVA; RP, retinyl palmitate; TG, triglyceride; TRL, triglyceride-rich lipoprotein; TRL-C, triglyceride-rich lipoprotein cholesterol.

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very common cause of type 2 diabetes, the incidence of which is increasing rapidly. MetS is also strongly associated with coronary artery disease (3). MetS refers to the aggregation of atherosclerotic risk factors, including insulin resistance, dyslipidemia, and hypertension (4). This syndrome is also characterized by additional abnormalities, such as central obesity, endothelial dysfunction, low HDL-cholesterol (HDL-C), and a pro-coagulant state (5).

The etiology of this syndrome is ill-defined, but there is no doubt that genetic, metabolic, and environmental factors, including diet, play an important role in its development (6). In particular, the amount and type of dietary fat are reported to contribute to the development of MetS, as suggested by observational studies (7,8). Thus, dietary modifications should be a primary intervention to attenuate metabolic risk factors that lead to MetS. However, a key question is whether to reduce SFA intakes via low-fat, high-complex carbohydrate (LFHCC) diets or by moderate-fat diets rich in monounsaturated fat (MUFA). Previous studies showed that consumption of high-MUFA (HMUFA) diets induces lower triglycerides (TG) and higher HDL-C concentrations compared with low-fat, high-carbohydrate diets in healthy participants (9). Also, Roche et al. (10) observed that the adverse effects produced by the intake of low-fat, high-carbohydrate diets on blood lipids can be attenuated by the addition of long-chain (LC) (n-3) PUFA in healthy individuals.

MetS is a postprandial disease, because increased plasma TG concentrations are one of the major abnormalities found in these patients. Every time that we eat a meal there are physiological changes in postprandial lipoproteins. However, modifications in the metabolism of these postprandial lipoproteins could play an important role in the development of cardiovascular complications (11). Postprandial hyperlipidemia is characteristic of MetS, with increased postprandial TG-rich lipoprotein (TRL) concentrations, which tend to be enriched with cholesterol esters in MetS patients (12).

Thus, potential dietary modifications of postprandial lipemia are of major importance, because the abnormal lipoprotein profile presented in MetS greatly increases the risk of diabetes and coronary artery disease (13). To date, few studies have investigated the acute and long-term effects of dietary fat amount on postprandial lipoprotein response in patients with MetS (14). Furthermore, the acute impact of dietary fat quality and the effect of a long-term dietary MUFA, LC (n-3) PUFA, and high-complex carbohydrate intervention on postprandial lipoprotein metabolism have never been investigated in these patients. Therefore, we addressed the hypothesis that the acute and long-term dietary fat intake would differentially modulate postprandial lipoprotein response in MetS patients. To that purpose, the aim of this study was to determinate the effects of the quantity and quality of dietary fat on postprandial lipoprotein metabolism in a well-characterized cohort of MetS patients.

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 164 patients with MetS (104 females and 60 males) from the LIPGENE cohort were accepted to participate in the postprandial study; 130 patients (86 females and 44 males) completed the preintervention postprandial lipemia study and 117 of them (76 females and 41 males) successfully concluded the dietary intervention and the postintervention postprandial lipemia studies (**Supplemental Fig. 1**). All participants gave written informed consent and underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrolment. This study was carried out in the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, Spain, and in the Department of Clinical Biochemistry, Jagiellonian University School of Medicine, Poland, from February 2005 to April 2006. The experimental protocol was approved by the local ethic committee at each of the intervention centers according to the Helsinki Declaration.

Design. Patients were randomly stratified to 1 of 4 dietary interventions for 12 wk. MetS was defined by published criteria (3), which conformed to the LIPGENE inclusion and exclusion criteria (15). Pre- and postintervention, a fat meal was administered providing the same amount of fat (0.7 g/kg body weight), wherein the fat composition reflected that consumed within the intervention period. The intervention study design and intervention protocol, which also provides information about pre-, mid-, and postintervention food consumption and dietary compliance at each center, have been described in detail by Shaw et al. (15). Briefly, dietary intake and compliance was assessed by a 3-d (2 weekdays and 1 weekend day) weighed food intake assessments at baseline, wk 6, and wk 12. Dietary analysis programs reflective of the Spanish and Polish food choices were used at the 2 European centers (Jagiellonian University Medical College, Poland: Dietitia software; Cordoba, Spain: 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 (**Supplemental Table 1**). Two diets were designed to provide 38% energy (E) from fat: a high-fat, SFA-rich diet (HSFA), which was designed to provide ~16% E as SFA, and a HMUFA diet designed to provide ~20% E from MUFA. The other 2 diets were LFHCC diets (LFHCC and LFHCC (n-3); 28% E from fat); the LFHCC (n-3) diet included a 1.24-g/d supplement of LC (n-3) PUFA [ratio of 1.4 eicosapentaenoic acid (EPA):1 docosahexaenoic acid (DHA)] and the LFHCC diet included a 1.24- g/d supplement of control high-oleic sunflower seed oil capsules (placebo) (**Supplemental Table 2**).

Capsules containing LC (n-3) PUFA were used instead of fish for several reasons. LC (n-3) PUFA capsules provide a fixed amount and stable composition of fatty acids that are more convenient to consume than increasing fish consumption, because these can be administered easily as a dietary supplement. Also, fish oil supplements provide equivalent amounts of EPA and DHA as oily fish, which are equally effective at enriching blood lipids as LC (n-3) PUFA (16). Finally, some patients did not eat fish and it was an effective method of ensuring that the population received the recommended dosage of EPA and DHA.

Following the same protocol, each intervention center performed a pre- (wk 0) and postintervention (wk 12) postprandial challenge with the same fat composition as that consumed on the assigned dietary period. Patients arrived at the clinical centers at 0800 h following a 12-h fast refrained from smoking during the fasting period and abstained from alcohol intake during the preceding 7 d. 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 was prepared in each center, reflected fatty acid composition of each subject chronic dietary intervention. Subsequent blood samples were drawn at 2, 4, 6, and 8 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 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. 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)].

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 × *g* for 15 min at 4°C. The large TRL (Sf > 400) were isolated from 4 mL of plasma by a single ultracentrifugal spin (36,200 × *g*, 30 min, 4°C) in a type TY65 rotor (Beckman Instruments). Large TRL, contained in the top layer, were removed by aspiration and the infranatant was centrifuged at a density of 1.019 kg/L for 24 h at 183,000 × *g* in the same rotor. The small TRL (Sf 12–400) were removed from the top of the tube. All operations were conducted in subdued light. Large and small TRL fractions were stored at −70°C until assayed for retinyl palmitate (RP) and biochemical determinations.

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). TG and cholesterol in plasma and lipoprotein fractions were assayed by enzymatic procedures (17,18). Apolipoprotein (apo) A-I and apo B were determined by turbidimetry (19). HDL-C was measured by precipitation of a plasma aliquot with dextran sulfate-Mg2+, as described by Warnick et al. (20). LDL-cholesterol (LDL-C) was calculated using the following formula: plasma cholesterol − [HDL-C + large TRL-cholesterol (TRL-C) + small TRL-C]. Apo B-48 plasma concentrations were determined by ELISA (Biovendor) as previously described (21). The RP content of large and small TRL fractions was assayed using a method previously described by Ruotolo et al. (22).

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.

Statistical analyses. All data presented in the text, figures, and tables are expressed as mean ± SEM. SSPS 15 for Windows was used for the statistical analyses. For a parallel design, statistical power calculations indicated that 24 participants/group would be needed to detect mean differences of 50 (mmol/L)min [SD, 6 (mmol/L)min] in the TG area under the curve (AUC) (0.05; power 0.8). Although we used the TG AUC to set sample size, we were equally interested in changes in the other variables of our study. The AUC, defined as the area between fasting and postprandial concentrations, was calculated by the trapezoidal rule. The normal distribution of variables to characterize the postprandial response was assessed using the Kolmogorov-Smirnov test. Log transformations of data were performed when these variables were not normally distributed. One-way ANOVA was used to test the association among diet groups and anthropometric measures, fasting and postprandial lipid and lipoprotein concentrations, and the composition of diet at pre- (baseline) and postintervention. Repeated-measures ANOVA (RM-ANOVA) was used to compare the effect of diet on postprandial lipoprotein concentrations. In this analysis, we studied: the effect of diet, independently of time (represented as *P* diet); the effect of time alone or the change in the variable, independently of diet (represented as *P* time); and the interaction of both factors, indicative of the magnitude of postprandial response among diet groups (represented as *P* diet × time). Post-hoc statistical analysis was completed by using the protected least significant difference test to identify significant differences between dietary treatments. The contrast statistic used when the sphericity assumption was not satisfied was Huynh-Feldt. $P < 0.05$ was considered significant.

Results

The MetS patients assigned to the 4 isoenergetic diets did not differ in age, BMI, lipids, or apo concentrations pre- (baseline) or postintervention (**Supplemental Table 3**).

Achievement of dietary targets. Dietary composition at preintervention did not differ among the 4 diet groups (Supplemental Table 1). During the intervention period, %E from fat was significantly higher in the HSFA and HMUFA diet groups than in the LFHCC and LFHCC (n-3) groups. The %E from SFA and MUFA was significantly higher in the HSFA and HMUFA diet groups, respectively, compared with the other diet groups. Finally, %E from PUFA was significantly greater in patients who consumed the HSFA diet than the LFHCC (n-3) diet. Intake of EPA and DHA was significantly higher in the LFHCC (n-3) diet group than in the other groups (Supplemental Table 1).

Postprandial lipid and lipoprotein metabolism. The effect of dietary fat quality on acute postprandial lipoprotein metabolism (the preintervention postprandial response) demonstrated that there were no significant differences in the acute postprandial response among the participants ($n = 130$) assigned to the 4 diets (**Table 1**). Postintervention, postprandial plasma TG concentrations were lower in MetS patients who consumed the LFHCC (n-3) diet ($P < 0.001$) (**Fig. 1***A*) compared with the other 3 diet groups. On the other hand, participants in the HMUFA group had an earlier increase and a faster clearance of postprandial plasma TG ($P < 0.001$) (Fig. 1*A*) and large TRL-TG ($P = 0.009$) (Fig. 1*B*) concentrations compared with the HSFA and LFHCC diet groups, indicating a lower postprandial response with consumption of the HMUFA diet compared with the HSFA and LFHCC diets. The small TRL-TG postprandial response did not differ among the 4 diet groups (Fig. 1*C*).

To study the long-term effect of dietary fat modification on postprandial lipoprotein metabolism, we analyzed the significant changes between pre- and postintervention among diet groups (**Table 2** and **Fig. 2***A–F*). Compared with preintervention values, long-term ingestion of the LFHCC diet increased the postintervention postprandial AUC for total plasma TG ($P = 0.04$) (Fig. 2*A*), large TRL-TG ($P = 0.01$) (Fig. 2*C*), large TRL-RP ($P = 0.001$) (Table 2), small TRL-RP ($P = 0.02$) (Table 2), large TRL-C ($P < 0.001$) (Fig. 2*D*), and large TRL-apo B ($P = 0.002$) (Table 2) in MetS patients. In contrast, long-term ingestion of the LFHCC (n-3) diet did not increase the postintervention postprandial AUC for total plasma TG (Fig. 2*A*), large TRL-TG (Fig. 2*C*), large TRL-C (Fig. 2*D*), and large and small TRL-RP (Table 2) compared with the LFHCC diet. In addition, participants in the LFHCC (n-3) group increased the postintervention large TRL-apo B AUC ($P = 0.02$) (Table 2). Furthermore, long-term ingestion of the HMUFA diet did not produce the adverse effects of the LFHCC diet on postprandial lipoprotein metabolism (Table 2 and Fig. 2*A–F*). The HMUFA diet group showed an increase in only postprandial large TRL-RP AUC postintervention ($P = 0.04$) (Table 2). Long-term ingestion of the HSFA diet did not alter the postintervention postprandial response compared with the preintervention phase (Table 2 and Fig. 2*A–F*).

Adverse effects. No serious adverse events occurred. Mild symptoms included 19% of patients in the LFHCC (n-3) diet group who experienced regurgitations when taking the fish oil capsules. Participants allocated to the LFHCC, HMUFA, or HSFA diets did not report any adverse effects.

Discussion

The increasing incidence of MetS has serious implications for human health (23). Because the pathogenesis of MetS is strongly linked to excessive food consumption, in particular fat intake, our aim was to study the effect of 4 diets with different dietary fat quality and quantity on postprandial lipoprotein metabolism in patients with MetS before and after a 12-wk dietary intervention period. Each fat-rich breakfast or test meal

TABLE 1 Acute postprandial plasma lipid and lipoprotein responses in MetS patients assigned to the 4 groups at baseline[1]

Lipid and lipoprotein AUC	HSFA	HMUFA	LFHCC	LFHCC (n-3)
n	32	35	31	32
Total cholesterol, *(mmol/L)min*	2584 ± 91	2395 ± 65	2472 ± 98	2575 ± 91
Total TG, *(mmol/L)min*	1137 ± 125	1139 ± 90	992 ± 66	1073 ± 79
LDL-C,[2] *(mmol/L)min*	1708 ± 66	1553 ± 52	1674 ± 96	1729 ± 72
HDL-C, *(mmol/L)min*	535 ± 26	517 ± 23	538 ± 21	520 ± 27
apo B, *(g/L)min*	444 ± 18	425 ± 15	445 ± 21	458 ± 21
apo B-48, *(g/L)min*	4.6 ± 0.4	5.7 ± 0.5	5.4 ± 0.6	5.1 ± 0.4
Large TRL-C,[2] *(mmol/L)min*	153 ± 19	150 ± 18	114 ± 15	131 ± 19
Large TRL-TG, *(mmol/L)min*	451 ± 53	449 ± 46	355 ± 35	409 ± 45
Large TRL-RP,[2] *(mmol/L)min*	83 ± 18	81 ± 14	66 ± 11	75 ± 10
Large TRL-apo B, *(g/L)min*	9.6 ± 1.4	7.3 ± 0.8	7.6 ± 1.0	8.1 ± 1.0
Small TRL-C,[2] *(mmol/L)min*	200 ± 27	171 ± 15	158 ± 12	193 ± 15
Small TRL-TG, *(mmol/L)min*	301 ± 27	278 ± 37	236 ± 23	298 ± 31
Small TRL- TRL-RP,[2] *(mmol/L)min*	20.3 ± 12.9	19.6 ± 3.0	15.6 ± 2.7	24.1 ± 1.6
Small TRL-apo B, *(g/L)min*	11.8 ± 2.6	8.1 ± 1.3	10.8 ± 1.7	14.5 ± 1.6

[1] Values are mean ± SEM. There were no effects of diet (1-way ANOVA).
[2] Data were log-transformed before statistical analyses.

contained the same fat composition as the diet consumed within the intervention period. This study demonstrated that consumption of an isocaloric LFHCC (n-3) and HMUFA diet improved postprandial blood lipid abnormalities associated with MetS without weight loss compared with the HSFA and LFHCC diets. In addition, we observed for the first time, to our knowledge, that the potentially adverse effects of LFHCC diets on postprandial lipoprotein metabolism could be attenuated by LC (n-3) PUFA supplementation in patients with MetS.

At baseline (preintervention postprandial study), the acute postprandial lipoprotein response of patients with MetS did not differ among the 4 fat-rich meals. In agreement with our findings, Lovine et al. (24) showed no substantial differences in the acute TG response to different type of dietary fat administered to hypertriglyceridemic patients. These results suggest that only long-term dietary interventions could improve the postprandial lipoprotein abnormalities in patients with MetS. It has been observed that intakes of unsaturated fatty acids (25), (n-3) PUFA (26), dairy products (27), and whole grains (28) appeared to influence the prevalence of this syndrome, either positively or negatively, but little emphasis has been placed on the specific therapeutic diets that improve lipoprotein abnormalities present in MetS patients. In this sense, the PREDIMED study (29) has shown that a non-energy–restricted traditional Mediterranean diet enriched with nuts reduced the overall prevalence of MetS. Furthermore, foods that improve insulin sensitivity might also modulate the metabolic abnormalities linked with insulin resistance (25).

Changes in dietary fat composition are clearly associated with significant changes in plasma lipoprotein concentrations. High fat intake, particularly high-saturated fat diets, induce weight gain, insulin resistance, and hyperlipidemia in humans (30). Also, high-carbohydrate diets increase plasma TG and reduce HDL-C concentrations compared with high-fat diets (31). Interestingly, high-carbohydrate diets have 2 effects on TG metabolism. First, a high influx of carbohydrate into the liver

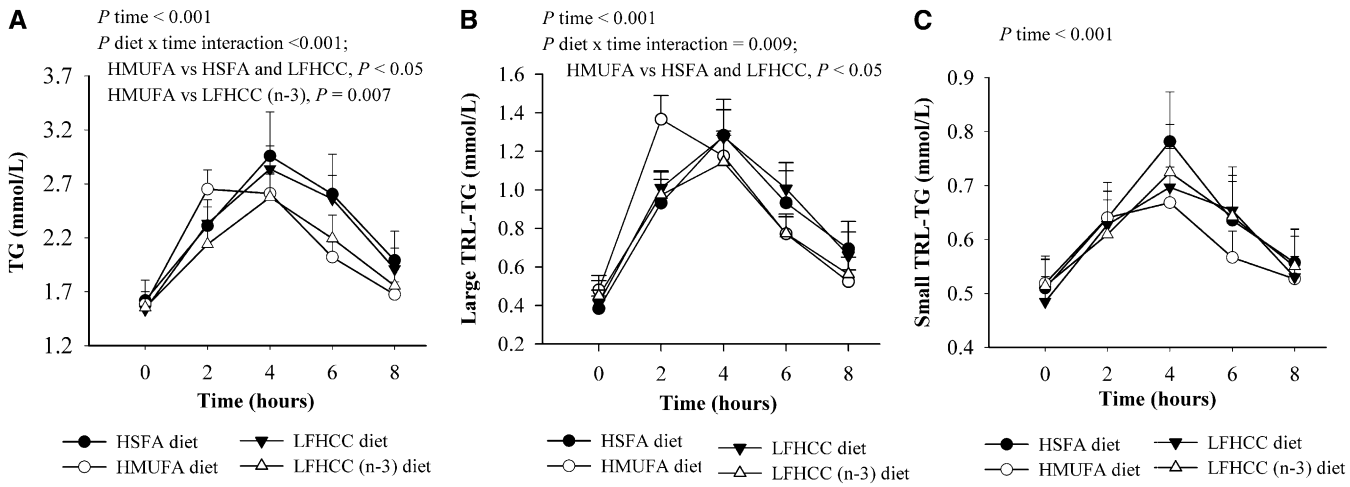

FIGURE 1 Postprandial plasma TG (*A*), large TRL-TG (*B*), and small TRL-TG (*C*) responses in MetS patients who consumed HSFA, HMUFA, LFHCC, or LFHCC (n-3) diets for 12 wk. Results are expressed as mean ± SEM, $n = 26$ (HSFA group); $n = 32$ (HMUFA group); $n = 31$ (LFHCC group); and $n = 28$ [LFHCC (n-3) group]. RM-ANOVA was used to calculate the effects of diet (*P* diet), time (*P* time), and their interaction (*P* diet × time). Large TRL-TG and small TRL-TG concentrations were log transformed before statistical analysis.

TABLE 2 Changes from baseline in postprandial plasma lipid and lipoprotein responses in MetS patients who consumed HSFA, HMUFA, LFHCC, or LFHCC (n-3) diets for 12 wk[1]

Δ Lipid and lipoprotein AUC	HSFA	HMUFA	LFHCC	LFHCC (n-3)	*P*-value[2]		
					D	T	D × T
n	26	32	31	28			
Total cholesterol, *(mmol/L)min*	12.9 ± 44.2 (0.5%)	−43.3 ± 37.4 (−1.8%)	−8.1 ± 70.9 (−0.3%)	−138.6 ± 45.2 (−5.4%)	—[4]	—	—
LDL-C,[3] *(mmol/L)min*	−14.7 ± 54.2 (−0.9%)	−42.5 ± 40.3 (−2.7%)	−39.9 ± 66.1 (−2.3%)	−90.8 ± 50.5 (−5.3%)	—	—	—
HDL-C, *(mmol/L)min*	−12.2 ± 22.5 (−2.3%)	−7.5 ± 19.8 (−1.5%)	−24.2 ± 13.0 (−4.6%)	−33.8 ± 18.0 (−6.4%)	—	0.04	—
apo B-48, *(g/L)min*	−0.03 ± 0.3 (−0.6%)	0.2 ± 0.4 (3.6%)	0.6 ± 0.4 (11.2%)	0.3 ± 0.4 (5.9%)	—	—	—
Large TRL-RP,[3] *(mmol/L)min*	−22.0 ± 15.9 (22.1%)	26.0 ± 17.6* (32.1%)	56.3 ± 17.4* (83.8%)	24.0 ± 14.6 (29.9%)	—	0.007	0.003
Large TRL-apo B, *(g/L)min*	−0.4 ± 1.4 (−4.1%)	1.7 ± 1.1 (22.2%)	3.5 ± 1.1* (45.5%)	2.9 ± 1.2* (36.4%)	—	0.001	—
Small TRL-RP,[3] *(mmol/L)min*	−3.6 ± 3.4 (−16.0%)	6.3 ± 4.4 (32.0%)	10.4 ± 2.0* (65.5%)	2.3 ± 5.2 (10.3%)	—	0.03	—
Small TRL-apo B, *(g/L)min*	2.5 ± 1.9 (17.4%)	3.5 ± 1.6 (40.8%)	−0.2 ± 1.9 (−1.8%)	−2.4 ± 1.9 (−15.1%)	—	—	—

[1] Values are mean ± SEM (percent change from baseline). *Different from baseline, $P < 0.05$.
[2] Calculated using RM-ANOVA. D, Diet effect; T, time effect; D × T, diet × time interaction.
[3] Data were log-transformed before statistical analyses.
[4] –, $P \geq 0.05$.

enhances hepatic lipogenesis (32). Second, high-carbohydrate diets may decrease the synthesis of lipoprotein lipase (LPL) (33). Plasma TG concentrations are decreased when HMUFA diets are substituted for high-carbohydrate diets in healthy individuals (34) and patients with type 2 diabetes (9). Previous research showed that the adverse effects of low-fat, high-carbohydrate diets on blood lipids could be attenuated by the addition of LC (n-3) PUFA in healthy normolipemic individuals (10). This postprandial lipoprotein finding has now been confirmed for the first time, to our knowledge, in patients with MetS, an effect achievable without weight loss.

Epidemiologic and experimental studies have shown that dietary saturated fat and high plasma SFA levels are associated with several dyslipidemic features of MetS (35,36). Very few studies have evaluated the effect of dietary fat composition on postprandial lipoprotein metabolism in individuals with MetS (37). In addition, the effect of a long-term dietary MUFA, LC (n-3) PUFA, and high-complex carbohydrate intervention on

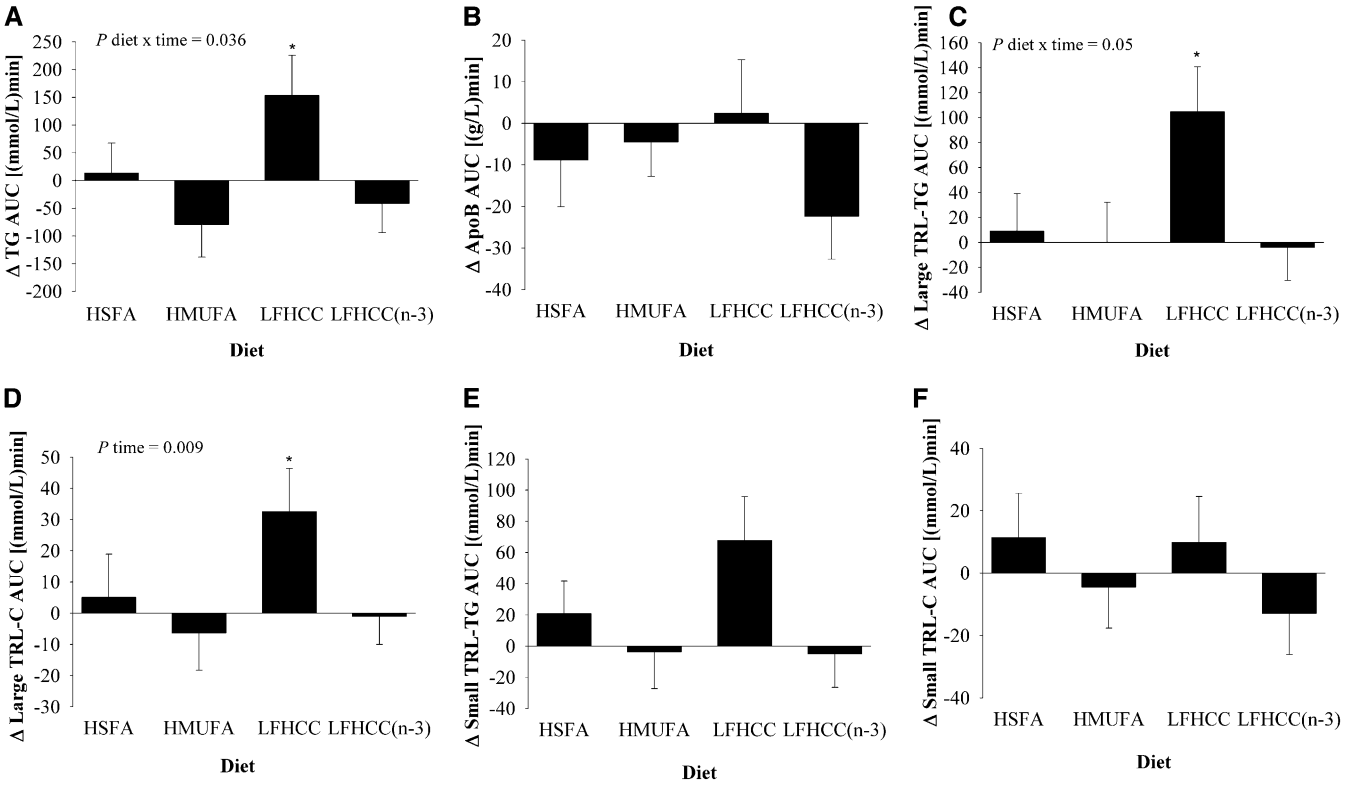

FIGURE 2 Postintervention changes from baseline (preintervention phase) of postprandial plasma TG (*A*), apo B (*B*), large TRL-TG (*C*), large TRL-C (*D*), small TRL-TG (*E*), and small TRL-C AUC (*F*). Results are expressed as mean ± SEM, $n = 26$ (HSFA group); $n = 32$ (HMUFA group); $n = 31$ (LFHCC group); and $n = 28$ [LFHCC (n-3) group]. *Different from baseline, $P < 0.05$. RM-ANOVA was used to calculate the effects of diet (*P* diet), time (pre- vs. postintevention) (*P* time), and their interaction (*P* diet × time). AUC in large and small TRL-C were log transformed before statistical analyses.

postprandial lipoprotein metabolism has never, to our knowledge, been investigated in MetS patients. An interesting study showed different postprandial lipemic patterns following the intake of a standard high-fat test meal in northern and southern European healthy males (38), suggesting that dietary MUFA may influence the nature and extent of postprandial lipemia. In southern Europeans, plasma TG response was much greater during the early postprandial phase and returned to near-fasting concentrations much earlier compared with age- and gender-matched northern Europeans with equivalent fasting TG concentrations (38). In another study, consumption of a MUFA-rich diet was also associated with an earlier postprandial peak in plasma TG and apo B-48 concentrations (39). The present study demonstrates that a long-term MUFA-rich dietary intervention in MetS patients produces an earlier postprandial peak and more rapid clearance of plasma TG and large TRL-TG concentrations compared with isocaloric long-term SFA-rich or LFHCC diets. The mechanisms that might explain our findings are complex and could reflect differences in chylomicron synthesis, secretion, or clearance or may be attributable to differences in the rate of VLDL secretion, reflecting long-term effects of different fatty acid substrates on hepatic lipogenesis. In our study, we suggest that the postprandial TG response in the HMUFA diet group, which was characterized by more rapid gradients to both the rising and declining components of the postprandial TG response curve, could be explained by both faster rates of digestion, absorption, and secretion of chylomicrons and more efficient processing of TRL (40). This could be explained by LPL activity, because there were no significant differences in apo B-48 postprandial response. In addition, the post-HMUFA diet postprandial lipemic profile suggests a faster rate of chylomicron entry into the circulation, reflecting accelerated rates of digestion and absorption or upregulation of chylomicron synthesis and secretion. In this sense, oleic acid has been shown to be preferentially esterified into TG in the enterocyte (41). Contrary to our results, the DELTA study (42) did not observe any significant difference in post-fat load plasma TG between an average American carbohydrate and MUFA diet in participants with and without insulin resistance or MetS. Differences between studies, including duration of intervention period being shorter (7 wk) in the DELTA study compared with 12 wk in the present study, may account for this discrepancy. Moreover, the DELTA study did not administer a LFHCC diet supplemented with LC (n-3) PUFA, nor were the acute effects of dietary fat on postprandial lipoprotein metabolism determined and postprandial TRL fractions were not analyzed. In addition, we found in these patients elevated RP levels in large TRL AUC. MUFA-rich diets have been reported to promote gastrointestinal secretions and to stimulate stomach emptying (43), which would increase the rate of supply of monoacylglycerols and fatty acids to the enterocyte.

This study concurs with others that showed that LC (n-3) PUFA supplementation effectively reduces plasma TG concentrations (44). The long-term effect of the LFHCC (n-3) diet, pre- vs. postintervention phases, showed several beneficial effects of LC (n-3) PUFA supplementation in combination with a LFHCC diet by preventing the increase in postprandial TG and TRL particles induced by long-term LFHCC diets. Fish oil supplements correct many metabolic alterations associated with insulin resistance (45), including reduced postprandial plasma TG concentration (46). LC (n-3) PUFA can reduce hepatic VLDL lipogenesis and/or enhance fatty acid oxidation (47) and may facilitate TRL-TG removal through enhanced LPL activity in plasma (48).

Our study presents some limitations. First, ensuring complete adherence to dietary instructions is difficult in a feeding trial. However, adherence to recommended dietary patterns was good, as judged as per dietary assessment (15). Second, consumption of LC (n-3) PUFA capsules caused some mild adverse effects in some MetS patients. LC (n-3) PUFA capsules were used instead of enhanced fish intake to ensure a stable composition and fixed dose of LC (n-3) PUFA in this study. Oily fish are of course an optimal alternative to enhance LC (n-3) PUFA intake within the context of a low-fat dietary intervention. On the other hand, our design has the strength of reproducing real-life conditions with home-prepared foods, reflecting usual practice. It would be interesting to extend our studies beyond 12 wk to confirm the longer term effects of dietary fat interventions on cardiovascular risk factors in MetS.

In conclusion, our data suggest that long-term intake of an isocaloric, low-fat, high-carbohydrate diet supplemented with LC (n-3) PUFA and MUFA-rich diets have beneficial effects on postprandial lipoprotein response in patients with MetS. On the other hand, the addition of LC (n-3) PUFA to a LFHCC diet may normalize the adverse postprandial lipoprotein effects produced by this diet. Importantly, both diets were effective exclusive of weight loss, which is highly pertinent given the pandemic of obesity-induced MetS that will occur in Europe and North America over the next 20–30 y due to excessive weight gain.

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Literature Cited

1. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37:1595–607.
2. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP). JAMA. 2001;285:2486–97.
3. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; National Heart, Lung, and Blood Institute, American Heart Association. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Arterioscler Thromb Vasc Biol. 2004;24:e13–8.
4. Reaven G. Metabolic syndrome: pathophysiology and implications for management of cardiovascular disease. Circulation. 2002;106:286–8.
5. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005; 112:2735–52.
6. Phillips C, Lopez-Miranda J, Perez-Jimenez F, McManus R, Roche HM. Genetic and nutrient determinants of the metabolic syndrome. Curr Opin Cardiol. 2006;21:185–93.
7. Liese AD, Roach AK, Sparks KC, Marquart L, D'Agostino RB Jr, Mayer-Davis EJ. Whole-grain intake and insulin sensitivity: the Insulin Resistance Atherosclerosis Study. Am J Clin Nutr. 2003;78:965–71.

8. Pereira MA, Jacobs DR Jr, Van Horn L, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. JAMA. 2002;287: 2081–9.
9. Garg A, Bonanome A, Grundy SM, Zhang ZJ, Unger RH. Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. N Engl J Med. 1988;319:829–34.
10. Roche HM, Gibney MJ. Postprandial triacylglycerolaemia: the effect of low-fat dietary treatment with and without fish oil supplementation. Eur J Clin Nutr. 1996;50:617–24.
11. Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr. 2007;98:458–73.
12. Ruotolo G, Howard BV. Dyslipidemia of the metabolic syndrome. Curr Cardiol Rep. 2002;4:494–500.
13. Giugliano D, Ceriello A, Esposito K. Are there specific treatments for the metabolic syndrome? Am J Clin Nutr. 2008;87:8–11.
14. Volek JS, Phinney SD, Forsythe CE, Quann EE, Wood RJ, Puglisi MJ, Kraemer WJ, Bibus DM, Fernandez ML, et al. Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet. Lipids. 2009;44:297–309.
15. Shaw DI, Tierney AC, McCarthy S, Upritchard J, Vermunt S, Gulseth HL, Drevon CA, Blaak EE, Saris WH. LIPGENE food-exchange model for alteration of dietary fat quantity and quality in free-living participants from eight European countries. Br J Nutr. 2009;101:750–9.
16. Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n-3. Am J Clin Nutr. 2007;86:1621–5.
17. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem. 1973;19:476–82.
18. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20:470–5.
19. Riepponen P, Marniemi J, Rautaoja T. Immunoturbidimetric determination of apolipoproteins A-1 and B in serum. Scand J Clin Lab Invest. 1987;47:739–44.
20. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem. 1982;28:1379–88.
21. Kinoshita M, Kojima M, Matsushima T, Teramoto T. Determination of apolipoprotein B-48 in serum by a sandwich ELISA. Clin Chim Acta. 2005;351:115–20.
22. Ruotolo G, Zhang H, Bentsianov V, Le NA. Protocol for the study of the metabolism of retinyl esters in plasma lipoproteins during postprandial lipemia. J Lipid Res. 1992;33:1541–9.
23. Isomaa B, Almgren P, Tuomi T, Forsén B, Lahti K, Nissén M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care. 2001;24:683–9.
24. Iovine C, Gentile A, Hattemer A, Pacioni D, Riccardi G, Rivellese AA. Self- monitoring of plasma triglyceride levels to evaluate postprandial response to different nutrients. Metabolism. 2004;53:620–3.
25. Vessby B. Dietary fat and insulin action in humans. Br J Nutr. 2000; 83 Suppl 1:S91–6.
26. Connor WE. Importance of n-3 fatty acids in health and disease. Am J Clin Nutr. 2000;71 Suppl 1:171S–5S.
27. Azadbakht L, Mirmiran P, Esmaillzadeh A, Azizi F. Dairy consumption is inversely associated with the prevalence of the metabolic syndrome in Tehranian adults. Am J Clin Nutr. 2005;82:523–30.
28. McKeown NM. Whole grain intake and insulin sensitivity: evidence from observational studies. Nutr Rev. 2004;62:286–91.
29. Salas-Salvadó J, Fernández-Ballart J, Ros E, Martínez-González MA, Fitó M, Estruch R, Corella D, Fiol M, Gómez-Gracia E, et al. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. Arch Intern Med. 2008;168:2449–58.
30. Feskens EJ, Virtanen SM, Räsänen L, Tuomilehto J, Stengård J, Pekkanen J, Nissinen A, Kromhout D. Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. Diabetes Care. 1995;18:1104–12.
31. Sacks FM, Katan M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am J Med. 2002;113 Suppl 9B:S13–24.
32. Aarsland A, Chinkes D, Wolfe RR. Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. Am J Clin Nutr. 1997;65:1774–82.
33. McNeel RL, Mersmann HJ. Low- and high-carbohydrate diets: body composition differences in rats. Obes Res. 2005;13:1651–60.
34. Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N Engl J Med. 1986;314: 745–8.
35. Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr. 2004;23:447–56.
36. Vessby B. Dietary fat, fatty acid composition in plasma and the metabolic syndrome. Curr Opin Lipidol. 2003;14:15–9.
37. Kolovou GD, Anagnostopoulou KK, Pavlidis AN, Salpea KD, Iraklianou SA, Tsarpalis K, Damaskos DS, Manolis A, Cokkinos DV. Postprandial lipemia in men with metabolic syndrome, hypertensives and healthy subjects. Lipids Health Dis. 2005;4:21.
38. Zampelas A, Roche H, Knapper JM, Jackson KG, Tornaritis M, Hatzis C, Gibney MJ, Kafatos A, Gould BJ, et al. Differences in postprandial lipaemic response between Northern and Southern Europeans. Atherosclerosis. 1998;139:83–93.
39. Roche HM, Zampelas A, Knapper JM, Webb D, Brooks C, Jackson KG, Wright JW, Gould BJ, Kafatos A, et al. Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. Am J Clin Nutr. 1998;68:552–60.
40. Silva KD, Kelly CN, Jones AE, Smith RD, Wootton SA, Miller GJ, Williams CM. Chylomicron particle size and number, factor VII activation and dietary monounsaturated fatty acids. Atherosclerosis. 2003;166: 73–84.
41. Dashti N, Smith EA, Alaupovic P. Increased production of apolipoprotein B and its lipoproteins by oleic acid in Caco-2 cells. J Lipid Res. 1990;31:113–23.
42. Berglund L, Lefevre M, Ginsberg HN, Kris-Etherton PM, Elmer PJ, Stewart PW, Ershow A, Pearson TA, Dennis BH, et al. Comparison of monounsaturated fat with carbohydrates as a replacement for saturated fat in subjects with a high metabolic risk profile: studies in the fasting and postprandial states. Am J Clin Nutr. 2007;86:1611–20.
43. Kafatos A, Comas GE. Biological effect of olive oil in human health. In: Kiritsakis A, editor. Olive oil. Champaign (IL): American Oil Chemists Society; 1990. p. 157–81.
44. Roche HM, Gibney MJ. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am J Clin Nutr. 2000;71 Suppl 1:S232–7.
45. Collier GR, Sinclair AJ. Role of N-6 and N-3 fatty acids in the dietary treatment of metabolic disorders. Ann N Y Acad Sci. 1993;683:322–30.
46. Harris WS, Connor WE, Alam N, Illingworth DR. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. J Lipid Res. 1988;29:1451–60.
47. Clarke SD, Jump D. Polyunsaturated fatty acids regulate lipogenic and peroxisomal gene expression by independent mechanisms. Prostaglandins Leukot Essent Fatty Acids. 1997;57:65–9.
48. Harris WS, Lu G, Rambjor GS, Walen AI, Ontko JA, Cheng Q, Windsor SL. Influence of n-3 fatty acid supplementation on the endogenous activities of plasma lipases. Am J Clin Nutr. 1997;66:254–60.