Long-chain n−3 PUFAs reduce adipose tissue and systemic inflammation in severely obese nondiabetic patients: a randomized controlled trial[1–3]

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ABSTRACT

Background: Chronic adipose tissue inflammation is a hallmark of obesity, triggering the development of associated pathologies, particularly type 2 diabetes. Long-chain n−3 PUFAs reduce cardiovascular events and exert well-established antiinflammatory effects, but their effects on human adipose tissue inflammation are unknown.

Objective: We investigated whether n−3 PUFAs reduce adipose tissue inflammation in severely obese nondiabetic patients.

Design: We treated 55 severely obese nondiabetic patients, scheduled to undergo elective bariatric surgery, with 3.36 g long-chain n−3 PUFAs/d (EPA, DHA) or an equivalent amount of butterfat as control, for 8 wk, in a randomized open-label controlled clinical trial. The primary efficacy measure was inflammatory gene expression in visceral and subcutaneous adipose tissue samples (subcutaneous adipose tissue and visceral adipose tissue), collected during surgery after the intervention. Secondary efficacy variables were adipose tissue production of antiinflammatory n−3 PUFA–derived eicosanoids, plasma concentrations of inflammatory markers, metabolic control, and the effect of the Pro12Ala *PPARG* polymorphism on the treatment response.

Results: Treatment with n−3 PUFAs, which was well tolerated, decreased the gene expression of most analyzed inflammatory genes in subcutaneous adipose tissue ($P < 0.05$) and increased production of antiinflammatory eicosanoids in visceral adipose tissue and subcutaneous adipose tissue ($P < 0.05$). In comparison with control subjects who received butterfat, circulating interleukin-6 and triglyceride concentrations decreased significantly in the n−3 PUFA group ($P = 0.04$ and $P = 0.03$, respectively). The Pro12Ala polymorphism affected the serum cholesterol response to n−3 PUFA treatment.

Conclusions: Treatment with long-chain n−3 PUFAs favorably modulated adipose tissue and systemic inflammation in severely obese nondiabetic patients and improved lipid metabolism. These effects may be beneficial in the long-term treatment of obesity. This trial was registered at clinicaltrials.gov as NCT00760760. *Am J Clin Nutr* 2012;96:1137–49.

INTRODUCTION

The dramatic extent of the obesity epidemic has become a public health issue of worldwide importance, by substantially raising metabolic and cardiovascular morbidity (1). The risk of developing obesity-related complications is proportional to the degree of obesity (2) and is tightly correlated with chronic low-grade adipose tissue and systemic inflammation (3). Two hallmarks of adipose tissue inflammation are increased inflammatory gene expression (4) and macrophage infiltration (5, 6). Adipose tissue macrophages differentiate into inflammatory M1 or regulatory M2 macrophages (6). The transition of M2 to M1 macrophage polarization may be facilitated by relative adipose tissue hypoxia (7) and is characterized by increased expression of transcription factor hypoxia inducible factor 1α. Hypoxia is hypothesized to upregulate inflammatory adipokines, cytokines, and chemokines (8) secreted and released by adipocytes and macrophages (9); to sustain inflammation; and to initiate adipose tissue fibrotic remodeling (10). Elevated concentrations of proinflammatory cytokines such as IL-6 and acute phase reactants such as C-reactive protein are in turn independent risk factors for the development of type 2 diabetes (11) and cardiovascular disease (12).

Dietary factors substantially modulate obesity-related inflammation (13) and cardiovascular risk (14). An increased ratio of dietary n−6 to n−3 PUFAs has been linked to the risk of chronic inflammatory diseases (15). Despite a lower dietary PUFA intake, obese individuals have higher proportions

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[2] Supported by the Austrian National Bank Project no. 12735, the European Community's 7th Framework Programme (FP7/2007-2013) under grant agreement no. 201608, and the "Christian Doppler Gesellschaft," Federal Ministry of Economy, Family and Youth, and the National Foundation for Research, Technology and Development (all to TMS). The Omacor capsules were generously provided by Solvay Pharma, Austria.

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Received February 20, 2012. Accepted for publication August 14, 2012.

First published online October 3, 2012; doi: 10.3945/ajcn.112.037432.

of arachidonic acid (AA)[4] in their adipose tissue than do their nonobese twins, which renders their adipocytes more vulnerable to inflammation (16). Long-chain n−3 PUFAs are natural opponents of many n−6 PUFA actions: they reduce inflammation (17) and confer cardiovascular protection (18, 19). In addition to modulating inflammatory gene expression in immune cells (20), antiinflammatory effects of n−3 PUFAs are mediated to a large extent through eicosanoids (21). Recent studies have identified EPA and DHA as precursors of new classes of potent anti-inflammatory- and inflammation-resolving eicosanoids, known as resolvins and protectins, which actively help terminate an inflammatory response (22). The implication of n−3 PUFAs in general and, specifically, resolvins and protectins in human adipose tissue inflammation has not been elucidated to date. On the basis of the broad published evidence on the antiinflammatory effects of n−3 PUFAs and our own finding that n−3 PUFAs block high-fat-diet–induced adipose tissue inflammation in a murine model of obesity and type 2 diabetes (23, 24), we hypothesized that treatment with n−3 PUFAs interferes with adipose tissue and systemic inflammation and improves metabolic control in severely obese patients. We examined the effect of an 8-wk treatment with EPA and DHA on adipose tissue inflammation, adipose tissue production of antiinflammatory-resolving lipid mediators, plasma fatty acid profiles (such as the n−3 to n−6 fatty acid ratio), circulating inflammatory marker molecule and adipokine concentrations, and finally metabolic control in obese patients in a randomized controlled clinical trial. Because the Pro12Ala polymorphism in the *PPARG* gene is a potent modifier of n−3 PUFA effects with respect to plasma lipids (25), we investigated a potential dependence on this polymorphism.

SUBJECTS AND METHODS

Study population

Eligible participants were severely obese [BMI (in kg/m^2) ≥40) and nondiabetic patients [fasting plasma glucose <126 mg/dL and 2-h plasma glucose after a 75-g oral-glucose-tolerance test (OGTT) <200 mg/dL] between 20 and 65 y of age who were scheduled to undergo elective bariatric surgery at the Department of Surgery of the Medical University of Vienna. Patients were excluded in cases of acute illness within the past 2 wk; known diabetes mellitus or use of antidiabetic medication; acquired immunodeficiency (HIV infection, AIDS); hepatitis or other significant liver disease; severe or untreated cardiovascular, renal, or pulmonary disease; untreated or inadequately treated clinically significant thyroid disease; anemia; active malignant disease; inborn or acquired bleeding disorder, including warfarin treatment; pregnancy or breastfeeding; or documented intolerability to n−3 PUFA.

Study design and intervention

This clinical trial was randomized, open-label, controlled, and single center, performed in compliance with the Helsinki Declaration of 1975 as revised in 1983 and with the Good Clinical Practice guidelines. The trial had been approved by the Ethics Committee of the Medical University of Vienna (EK-Nr. 488/2006) and was conducted at the Clinical Research Unit of the Division of Endocrinology and Metabolism, Department of Medicine III, Medical University of Vienna. All recruited patients were randomly assigned to n−3 PUFA or control treatment by using minimization software balanced for age and sex and made available by the local Department of Medical Statistics and Informatics. Patients were either assigned to treatment with 3.36 g long-chain n−3 PUFAs/d or an equivalent amount of butterfat matched for caloric content, which served as the control. Long chain n−3 PUFAs were supplied in 1-g gelatin capsules (Omacor; Solvay Pharma) containing 90% ethyl esters of long-chain n−3 PUFAs, including 460 mg EPA and 380 mg DHA. Butter was supplied in 20-g portions as a control, containing 82% (wt:wt) fat. Generally, butter is very popular in Austria and is widely used in the habitual diets of the resident population, with an average person ingesting ∼10 g/d (26) Therefore, butterfat was selected as a control fat—in an amount meant to match the caloric content of the n−3 PUFA capsules. We did not restrict butter consumption for the n−3 PUFA group; therefore, patients from both groups were likely to include butter in their diets throughout the trial. Participants were advised to consume 4 capsules or 5 g butter/d, respectively, in addition to an isocaloric diet consisting of 55% carbohydrates, 15% protein, and 30% fat throughout the trial. Consumption of marine fish was explicitly discouraged for the entire study period. None of the patients were on intensive exercise training before or during the trial, but each subject was advised to maintain usual physical activity at a constant level.

The intervention lasted for 8 wk, and the participants returned for follow-up visits every other week. At baseline and at the end of the intervention, we performed anthropometric measurements (BMI, hip and waist circumference, and systolic and diastolic blood pressure), blood sampling, and a 75-g 2-h OGTT. We measured systemic inflammatory markers and metabolic variables (secondary efficacy variables). At the end of the intervention, patients underwent elective bariatric surgery. During the operation, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) biopsy samples were collected to assess inflammatory gene expression (primary efficacy measures) and as n−3 PUFA–derived lipid mediator production (secondary efficacy variables). Sample size was calculated to 25 per group to detect a 50% change in the expression of inflammatory genes at a P value <0.05 with a power of 80%. We supposed a reduction of most primary outcome variables (inflammatory gene expression in adipose tissue) by 50% to be clinically significant. This difference corresponds to a change of +1.0 in dCt values. dCt is the logarithmic measure of gene expression analysis by quantitative real-time polymerase chain reaction (PCR) normalized to a control (housekeeping) gene. A dropout rate of 20% was expected, thus we aimed to recruit 62 patients.

Dietary long-chain n−3 PUFA intake and compliance

Usual dietary intake of EPA and DHA as well as overall intake of SFAs was evaluated at baseline by using a food-frequency questionnaire asking for the usual intake and portion size of the main sources for EPA and DHA. EPA and DHA contents were

[4]Abbreviations used: AA, arachidonic acid; AIFAI, antiinflammatory fatty acid index; ddCt, delta delta Ct; HETE, hydroxyeicosatetraenoic acid; hsCRP, high-sensitivity C-reactive protein; OGTT, oral-glucose-tolerance test; PCR, polymerase chain reaction; PD1, protectin D1; PPARG, peroxisome proliferator–activated receptor-γ; RM-ANOVA, repeated-measures ANOVA; RvD1, resolvin D1; RvE1, resolvin E1; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; 17-HDHA, 17-hydroxy-DHA; 18-HEPE, 18-hydroxy-EPA.

taken from the German Federal Food Code (BLS III) and from a standardized food screener validated in the European Prospective Investigation into Cancer and Nutrition (27, 28). Medication compliance was assessed by 2 methods. First, we counted the number of returned capsules at the follow-up visits. Second, we determined n−3 PUFA fatty acyl moieties of plasma phospholipids at baseline and at the end of treatment.

Plasma phospholipid fatty acid profiles

We determined the fatty acid profiles in plasma phospholipids by thin-layer chromatography followed by gas chromatography–mass spectrometry. Lipids were extracted according to a method previously described by Nikolaidis et al (29) with minor modifications. Major lipids were separated by chromatography on silica gel 60W plates (Merck) by using *n*-hexane:diethyl ether:acetic acid (80:30:1; vol:vol:vol) with 50 mg butylated hydroxytoluene/100 mL as a mobile phase and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids Inc) as a standard for phospholipid extraction. Lipid spots were visualized under ultraviolet light after the plate was sprayed with a solution of 100 mg berberine chloride in 100 mL ethanol. Phospholipids were scraped into glass tubes containing methanol:toluene solution (4:1) followed by methanolysis and extraction by using a one-step procedure, as described previously (30). Fatty acid methyl esters were analyzed by gas chromatography and electron-impact ionization mass spectroscopy by using appropriate standards, as described elsewhere (30, 31). Results are expressed as mol% of the sum of all detected fatty acids. The antiinflammatory fatty acid index (AIFAI) (32) was calculated as the ratio of the total mol% of 20:5n−3, 22:6n−3, and 20:3n−6 divided by mol% of 20:4n−6; the antiinflammatory AA/EPA index was calculated as the ratio (mol%) of 20:4n−6 to 20:5n−3.

Plasma concentration of cytokines, adipokines, and metabolic variables

We used commercial enzyme-linked immunosorbent assays (R&D Systems) to measure plasma concentrations of IL-6 and serum concentrations of high-sensitivity C-reactive protein (hsCRP) and a commercial radioimmunoassay for measuring serum leptin and adiponectin concentrations (Millipore) according to the manufacturer's instruction. Free fatty acids were quantitatively determined by an in vitro enzymatic colorimetric method at 546 nm wavelength (Wako Diagnostics). Fasting plasma concentrations of triglycerides, cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein B, glucose, insulin, C-peptide, and glycosylated hemoglobin and red and white blood cell counts were determined by routine laboratory methods. Insulin sensitivity and pancreatic β cell function were assessed by homeostasis model assessment of insulin resistance indexes (33) and established OGTT-derived indexes (34), including the clamp-like index (35).

Analysis of adipose tissue inflammation

Biopsy samples of VAT and SAT (∼3 g each), which we obtained during bariatric surgery, were immediately divided for further analysis and either immersed in RNAlater (Ambion Inc) for isolation of total RNA, immediately snap-frozen in liquid nitrogen and stored at −80°C for immunohistochemistry, or applied to solid-phase extraction for subsequent determination of lipid mediators. Gene expression was analyzed in duplicate by reverse-transcriptase real-time PCR by using commercial TaqMan Gene Expression Assays (Applied Biosystems), normalized to ubiquitin as endogenous control. Gene expression was analyzed according to the delta delta Ct (ddCt) method normalized to ubiquitin C, and expression levels were calculated as 2^{-ddCt} as described (36). The arithmetic mean expression levels for a given target gene in SAT in the control group was arbitrarily set to 100%.

Lipid mediator analysis of adipose tissue samples

Lipid mediators were extracted by using solid-phase extraction and analyzed by HPLC-tandem mass spectrometry as described in detail elsewhere (A Neuhofer et al, unpublished observations, 2012). Briefly, VAT and SAT (∼400 mg) were homogenized in 1 mL methanol after the addition of deuterated prostaglandin E2 (Cayman Chemical) as internal standard. Cleared supernatant fluid was acidified to pH 3.0, loaded onto Oasis HLB Extraction Cartridges (Waters), and eluted with ethyl acetate/methanol (1:99, vol:vol). The extracts were analyzed by HPLC tandem mass spectrometry by using a triple quadrupole mass spectrometer (API 5000; AB SCIEX) equipped with a reversed-phase column (ACE 3 C18-AR, 150 × 2.1 mm; ACT). Tandem mass spectrometric analysis was conducted in electrospray negative ionization mode, and lipid mediators were identified by multiple reaction monitoring and by using specific transitions. HPLC retention times were established and optimized by using synthetic standards (kindly provided by Serhan CN or purchased from Cayman Chemical). Calibration curves were calculated, and recovery was checked for each compound.

Immunohistochemistry

Frozen VAT samples from each group were sectioned in 20-μm thin slices and stained for macrophages by using a monoclonal mouse CD68 antibody (Dako Clone KP1) followed by biotinylated goat anti-mouse second step (Vector Laboratories) and detected with streptavidine Alexa Fluor 488 (Invitrogen, Molecular Probes) and nuclei counterstaining with DAPI.

DNA extraction and genotyping

Genomic DNA was isolated from whole blood by using a commercial kit (Qiagen Inc). The *PPARG* Pro12Ala polymorphism (rs1801282) was genotyped by using a commercial real-time PCR TaqMan SNP genotyping assay (Applied Biosystems) on an ABI real-time PCR system (Applied Biosystems).

Statistical analysis

The statistical analysis included all patients who completed the trial and from which appropriate materials were obtained ($n = 49$ for adipose tissue and 55 for blood variables, except as otherwise indicated). Normally distributed data are presented as means ± SDs or means ± SEMs, otherwise as medians (IQRs). Group differences between ddCt values in VAT and SAT were analyzed by Student's *t* test. Treatment effectiveness was

considered achieved if statistical significance was demonstrated at the prespecified nominal α-level (0.05) for most of the primary endpoints. No adjustment for multiple endpoints is necessary under this scenario (37). Continuous variables determined before and after the treatment were log transformed if required and analyzed by repeated-measures ANOVA (RM-ANOVA), in a full factorial design, considering the time $\times$ treatment interaction, where time stands for the within-subject and within-treatment of the between-subject effect, respectively. The group $\times$ time (before and after intervention) interaction was used to test the null hypothesis that the changes in values of the variables over the period of treatment were parallel for both groups. An ANCOVA was conducted in case of significant baseline differences between treatment groups. A secondary analysis was performed for those variables significantly changed in RM-ANOVA by paired *t* tests for normally distributed data, otherwise Wilcoxon's test, to identify treatment-induced differences between individual groups. Correlations were explored by using the Pearson product-moment correlation coefficient (*r*), with the use of a 2-tailed test for significance or Spearman's method as relevant. To test the effect of the Pro12Ala *PPARG* polymorphism on the treatment effects, we calculated a 2-factor factorial ANOVA, with polymorphism (Pro12 compared with Ala12 allele carriers) and group (control compared with n−3 PUFA group) as the 2 independent variables and the ddCt values, resolving lipid mediator concentration, and treatment-induced differences of the analyzed serum and plasma variables as dependent variables. All analyses were performed with PASW Statistics 18 (SPSS Inc). The differences were considered statistically significant at 2-sided values of $P < 0.05$.

RESULTS

Study population and intervention

This trial was aimed to evaluate the effect of long chain n−3 PUFAs on adipose tissue and systemic inflammation and on metabolic control in severely obese nondiabetic patients. The recruitment of study participants began in August 2008 and was completed in July 2010. Sixty-two of 148 screened patients were enrolled in the study and randomly assigned to n−3 PUFA or control treatment; the progression of patients throughout the study is shown in **Figure 1**. Fifty-five patients completed the study (n = 27 patients in the intervention group and 28 in the control group). Adipose tissue samples were collected for analysis of adipose tissue inflammation, the primary efficacy variable, from only 49 patients (n = 23 in the intervention group, 26 in the control group) because of technical reasons. No patients were lost to follow-up. After randomization, we excluded 4 patients (3 from the control and 1 from the n−3 PUFA group) whom had newly diagnosed type 2 diabetes mellitus during the 2-h OGTT and 1 other patient from the n−3 PUFA group in

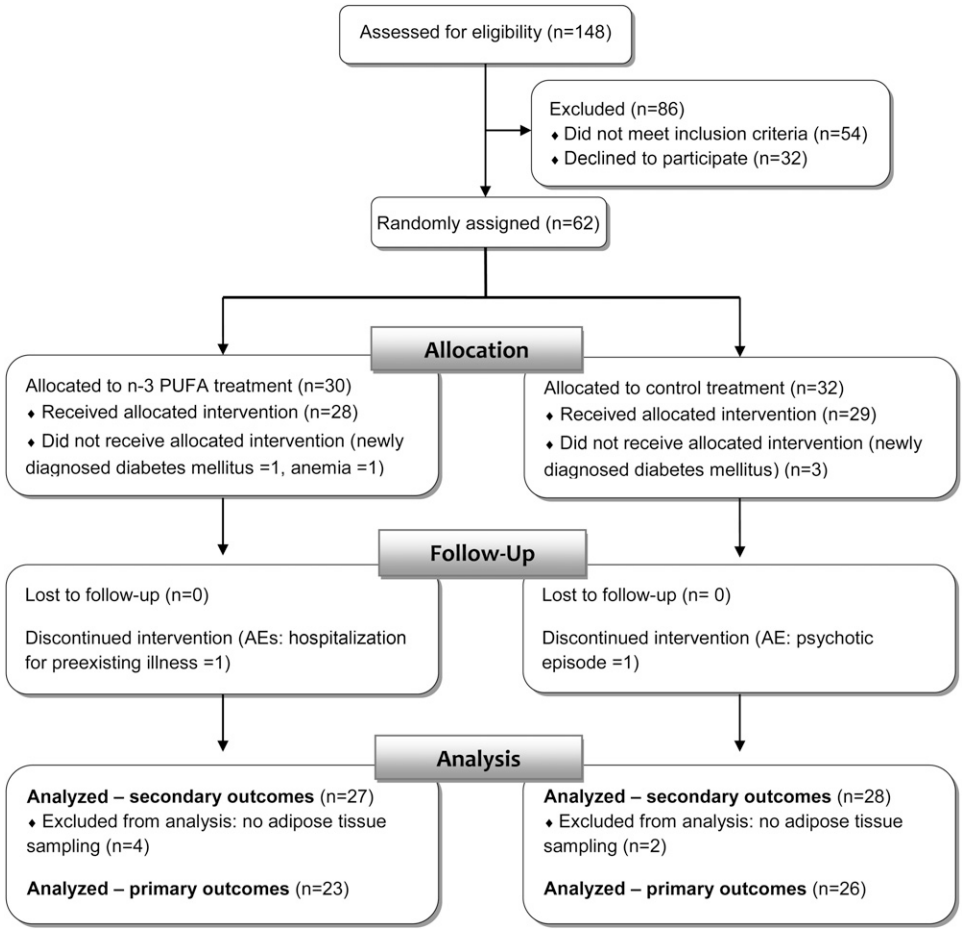

FIGURE 1. CONSORT flow diagram. AE, adverse event.

whom we diagnosed anemia. During the follow-up period, we further excluded 2 patients: 1 patient from the control group developed a psychotic episode that required hospitalization, and 1 patient from the n−3 PUFA group required hospitalization for decompensation of preexisting heart failure. Baseline demographic, anthropometric, and clinical characteristics of the patients were comparable in both groups (**Table 1**), with the exception of slightly elevated baseline glycosylated hemoglobin concentration and leukocyte count in the n−3 PUFA group (P = 0.03 and 0.001, respectively). Compared with baseline values, we did not detect significant changes in weight, BMI, waist-to-hip ratio, and blood pressure during the intervention period. The median (IQR) daily intake of EPA and DHA [$\sum$(EPA,DHA)/d] before the intervention was 0.25 (0.00–0.69) g/d, which is much lower than recommended in the Technical Committee on Dietary Lipids of the International Life Sciences Institute North America (38). The overall dietary intake of SFAs, evaluated at baseline, was either moderate (17–24 g/d) or high (>24 g/d) for most patients. The use of supplementary medication was documented in 30 patients and did not differ between groups (16 in the control group, 14 in the n−3 PUFA group). Most commonly used drugs were proton pump inhibitors (20% of all patients) and antihypertensive drugs (16.4% angiotensin-converting enzyme inhibitors, 14.5% diuretics, and 12.7% β-blockers).

The n−3 PUFA capsules were safe, with only 2 dropouts as a result of adverse events: 1 in the n−3 PUFA and 1 in the control group (Figure 1). n−3 PUFAs were well tolerated; a similar number of patients reported any adverse event (12 of 27 and 12 of 28 in the n−3 PUFA and control groups, respectively). Participants reported nausea, diarrhea, or change in bowel habits and fatigue, never exceeding moderate intensity, as the most

TABLE 1
Anthropometric, inflammatory, and metabolic variables at baseline and at the end of the study in both groups[1]

	Baseline		After treatment		
	Control (n = 28)	n−3 PUFA (n = 27)	Control (n = 28)	n−3 PUFA (n = 27)	P[2]
Age (y)	38 ± 2[3]	39 ± 2			
Sex, female (n)	23	23			
$\sum$EPA+DHA intake (g/d)[4]	0.18 (0.01, 0.67)[5]	0.26 (0.00, 0.73)			
SFA intake >17 g/d (%)[6]	94.4	100			
Anthropometric measurements					
Weight (kg)	132 (111.3, 147.8)	130 (116, 142.8)	133 (112.9, 152.6)	130 (115.5, 147.9)	0.10
BMI (kg/m^2)	44.6 (40.1, 48.3)	48.7 (44.0, 51.7)	45.1 (40.8, 48.1)	48.9 (44.7, 51)	0.07
Waist-to-hip ratio	0.92 (0.86, 0.95)	0.91 (0.86, 0.96)	0.92 (0.86, 0.96)	0.91 (0.85, 0.94)	0.08
Systolic BP (mm Hg)	126 ± 3	125 ± 3	119 ± 3	118 ± 3	0.94
Diastolic BP (mm Hg)	79 ± 2	81 ± 2	78 ± 2	80 ± 2	0.36
Inflammatory markers and adipokines					
IL-6 (pg/mL)	4.17 (3.10, 6.08)	4.78 (3.85, 8.86)	4.72 (3.10, 6.30)	4.40 (3.48, 6.25)*	0.04
hsCRP (mg/dL)	0.80 (0.39, 1.06)	0.65 (0.34, 2.00)	0.75 (0.37, 0.98)	0.84 (0.37, 1.61)	0.51
Leukocytes (mg/dL)[7]	6.45 ± 0.29	8.18 ± 0.29	7.19 ± 0.30	7.87 ± 0.36#	0.32
Adiponectin (μg/mL)	8.9 ± 0.6	8.4 ± 0.7	8.3 ± 0.6	8.3 ± 0.6	0.12
Leptin (ng/mL)	66.0 ± 4.6	69.7 ± 3.8	70.3 ± 4.1	69.0 ± 3.6	0.10
Metabolic variables					
Triglycerides (mg/dL)	154 ± 10	153 ± 8	164 ± 15	130 ± 6*	0.03
Total cholesterol (mg/dL)	198 ± 7	210 ± 7	206 ± 8	213 ± 7	0.46
HDL cholesterol (mg/dL)	44 ± 2	49 ± 2	46 ± 2	49 ± 3	0.22
LDL cholesterol (mg/dL)	123.1 ± 5.7	130.8 ± 6	128.7 ± 6.7	138.4 ± 6.1	0.78
Apolipoprotein B (mg/dL)	101.8 ± 4.5	108.4 ± 4.7	101.9 ± 4.4	109.3 ± 4.5	0.87
Hb A_{1c} (%)[7]	5.4 ± 0.1	5.7 ± 0.1	5.4 ± 0.1	5.6 ± 0.1#	0.70
Fasting glucose (mg/dL)	93 ± 2	94 ± 2	91 ± 2	93 ± 2	0.11
Fasting insulin (μg/mL)	17.1 (11.4, 28.4)	17.6 (8.5, 29.4)	17.2 (10.5, 26.2)	19.2 (10.3, 24.0)	0.98
Fasting C-peptide (ng/mL)	4.1 ± 0.4	4.1 ± 0.4	3.4 ± 0.3	4.0 ± 0.3	0.24
AUC glucose	135.2 ± 3.7	140.1 ± 5.1	135.0 ± 3.6	141.8 ± 5.0	0.40
AUC insulin	80.7 (49.7, 123.3)	95.7 (51.7, 146.2)	85.2 (56.6, 123.2)	112.0 (59.8, 150.7)	0.96
AUC C-peptide	10.8 (8.0, 13.5)	11.3 (8.2, 13.5)	10.4 (8.92, 12.18)	11.99 (8.7, 15.1)	0.31
HOMA-IR	3.79 (2.60, 6.84)	4.17 (1.81, 7.62)	3.96 (2.37, 5.64)	4.50 (2.22, 6.03)	0.88
CLIX	3.64 (2.94, 4.83)	3.24 (2.7, 4.98)	3.76 (3.36, 4.92)	3.48 (3.00, 4.30)	0.20

[1] *Significantly different from baseline (within group), $P < 0.05$ (paired t test or Wilcoxon's test for nonnormally distributed variables). #Significantly different at baseline, $P < 0.05$ (Student's t test or Mann-Whitney U test for nonnormally distributed variables). BP, blood pressure; CLIX, clamp-like index; Hb A_{1c}, glycosylated hemoglobin; hsCRP, high-sensitivity C-reactive protein.

[2] P values for treatment-induced changes were calculated by group × time interaction (repeated-measures ANOVA) unless indicated otherwise.

[3] Mean ± SEM (all such values).

[4] Information was missing for 10 cases and 9 controls.

[5] Median; IQR in parentheses (all such values).

[6] Information is missing for 10 cases and 10 controls.

[7] P values derived from ANCOVA because of significant baseline differences.

commonly occurring adverse events in both groups. A small but clinically insignificant elevation of alanine aminotransferase was detected in the n−3 PUFA group as compared with the control group (mean change from baseline: 7.3 ± 3.5 U/L in the n−3 PUFA group and −2.0 ±2.3 U/L in the control group; $P = 0.02$); no differences were found during the subsequent follow-up visit ($P = 0.7$; data not shown).

Dietary n−3 PUFAs lead to an antiinflammatory shift in plasma fatty acids

An average compliance of 94% with the n−3 PUFA treatment was confirmed by analysis of plasma phospholipid fatty acids (**Table 2**), which showed a significant increase in long-chain n−3 PUFAs (20:5, 22:5, and 22:6) at the expense of n−6 PUFAs in the intervention group. Consequently, the mean n−6 to n−3 PUFA ratio decreased by −10.58 ± 1.04 in the n−3 PUFA group compared with −0.15 ± 0.87 in the control group; $P < 0.0001$). Similarly, the mean ratio of AA to EPA was dramatically reduced in the n−3 PUFA group at the end of the treatment period (−36.18 ± 4.69 in the n−3 PUFA group compared with −4.38 ± 4.27 in the control group; $P < 0.0001$). The AIFAI was consistently increased after the treatment (0.26 ± 0.03 in the n−3 PUFA group compared with 0.00 ± 0.02 in the control group; $P < 0.0001$).

Reduction of adipose tissue inflammatory gene expression by n−3 PUFAs

In this trial, we detected a significant reduction in gene expression of a panel of classic inflammatory genes in the n−3 PUFA group. Expression of both *CCL2* [chemokine (C-C motif) ligand 2 or "MCP-1"] and *CCL3* [chemokine (C-C motif) ligand 3 or MIP-1-α] in SAT was downregulated by n−3 PUFA treatment compared with the control treatment (**Figure 2**, A and B). In addition, n−3 PUFA treatment also tended to reduce expression of *IL6* and tended to increase expression of the antiinflammatory gene *ADIPOQ* (adiponectin) in SAT (Figure 2, C and D) with borderline significance ($P < 0.10$). The expression of *HIF1A* decreased after n−3 PUFA treatment in SAT ($P = 0.04$). In addition gene expression of the established profibrotic marker *TGFB* (transforming growth factor β1) was significantly downregulated in SAT after n−3 PUFA treatment (Figure 2, E and F). Expression of overall (*CD68*) and M2 macrophage markers *CD163* and *MRC1* (mannose receptor C type 1) was not significantly changed between treatment groups and

TABLE 2
Plasma phospholipid fatty acid profile in control subjects and n−3 PUFA–treated patients before and after the intervention[1]

	Baseline		After treatment		
Fatty acid	Control ($n = 28$)	n−3 PUFA ($n = 27$)	Control ($n = 28$)	n−3 PUFA ($n = 27$)	P^2
14:0 (mol% of total fatty acids)	0.7 ± 0.3	0.8 ± 0.2	0.9 ± 0.3	0.9 ± 0.2	0.94
15:0 (mol% of total fatty acids)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.30
16:0 (mol% of total fatty acids)	53.8 ± 3.1	53.9 ± 2.8	53.8 ± 5.2	55.4 ± 4.7	0.23
16:1n−7 (mol% of total fatty acids)	0.08 ± 0.03	0.08 ± 0.02	0.08 ± 0.03	0.08 ± 0.03	0.67
18:0 (mol% of total fatty acids)	30.9 ± 3.0	30.7 ± 2.6	30.7 ± 5.8	29.0 ± 5.5	0.35
18:1n−9 (mol% of total fatty acids)	2.1 ± 0.3	2.2 ± 0.3	2.2 ± 0.4	2.3 ± 0.3	0.43
18:2n−6 (mol% of total fatty acids)	5.1 ± 0.5	5.2 ± 0.8	5.2 ± 0.6	4.7 ± 0.9*	0.002
18:3n−6 (mol% of total fatty acids)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01*	0.01
18:3n−3 (mol% of total fatty acids)	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.03	0.05 ± 0.05	0.21
20:0 (mol% of total fatty acids)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.26
20:1n−9 (mol% of total fatty acids)	0.04 ± 0.01	0.04 ± 0.01	0.13 ± 0.48	0.04 ± 0.01	0.33
20:2n−6 (mol% of total fatty acids)	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.08 ± 0.02*	0.001
20:3n−6 (mol% of total fatty acids)	0.4 ± 0.4	0.3 ± 0.1	0.4 ± 0.4	0.2 ± 0.1*	<0.0001
20:4n−6 (mol% of total fatty acids)	1.7 ± 0.5	1.6 ± 0.4	1.7 ± 0.4	1.5 ± 0.4	0.07
20:5n−3 (mol% of total fatty acids)	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.03	0.22 ± 0.1*	<0.0001
22:0 (mol% of total fatty acids)	2.2 ± 0.4	2.2 ± 0.5	2.1 ± 0.4	2.3 ± 0.7	0.52
22:4n−6 (mol% of total fatty acids)	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.04 ± 0.02*	0.01
22:5n−3 (mol% of total fatty acids)	0.11 ± 0.04	0.09 ± 0.03	0.12 ± 0.03	0.13 ± 0.03*	<0.0001
22:6n−3 (mol% of total fatty acids)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1*	<0.0001
24:0 (mol% of total fatty acids)	1.3 ± 0.3	1.3 ± 0.4	1.3 ± 0.3	1.4 ± 0.3	0.24
24:1n−9 (mol% of total fatty acids)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.89
SFA (mol% of total fatty acids)	89.5 ± 0.8	89.5 ± 0.7	89.4 ± 1.1	89.7 ± 1.1	0.20
UFA (mol% of total fatty acids)	10.6 ± 0.8	10.6 ± 0.7	10.8 ± 1.2	10.4 ± 1.1	0.12
n−3 PUFA (mol% of total fatty acids)	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.9 ± 0.3*	<0.0001
n−6 PUFA (mol% of total fatty acids)	7.4 ± 0.2	7.3 ± 0.2	7.5 ± 0.2	6.6 ± 0.2*	0.001
n−6/n−3 ratio	17.3 ± 4.7	19.1 ± 5.1	17.2 ± 5.1	8.9 ± 3.1*	<0.0001
AA/EPA	44.7 ± 21.0	45.6 ± 21.1	40.3 ± 20.2	9.5 ± 9.2*	<0.0001
AIFAI	0.5 ± 0.3	0.4 ± 0.1	0.4 ± 0.3	0.6 ± 0.1*	<0.0001

[1] All values are means ± SDs. *Significantly different from baseline (within group), $P < 0.05$ (paired t test). There were no significant differences between the fatty acid profiles of the 2 groups at baseline. AA, arachidonic acid (20:4); AIFAI, antiinflammatory fatty acid index; UFA, unsaturated fatty acid.
[2] P values for treatment-induced changes were calculated by group × time interaction (repeated-measures ANOVA).

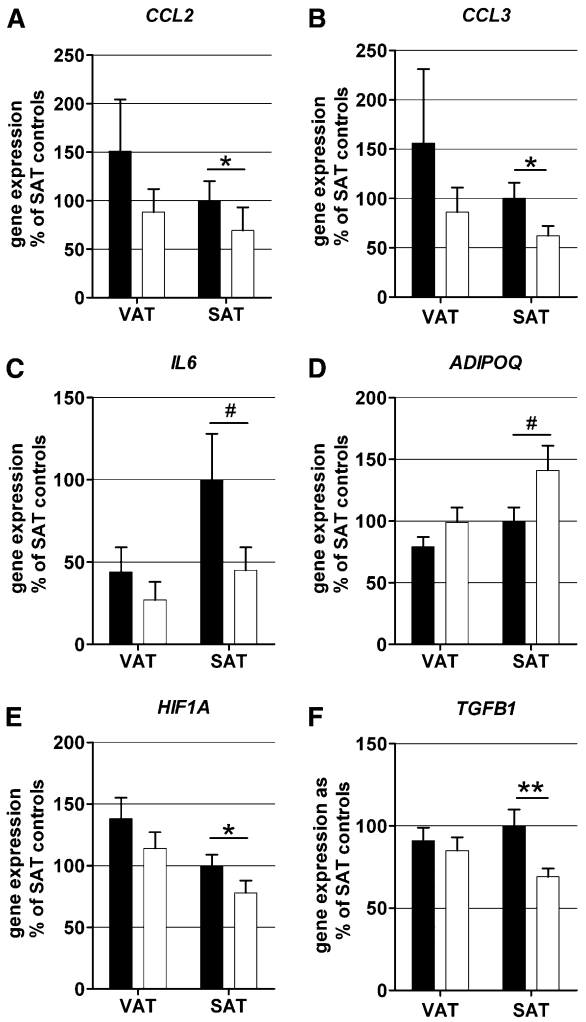

FIGURE 2. Gene expression in the VAT and SAT of n−3 PUFA–treated patients (white bars; $n = 23$) and control subjects (black bars; $n = 26$): *CCL2* or MCP-1 (A), *CCL3* or MIP1-α (B), *IL6* (C), *ADIPOQ* (D), *HIF1A* (E), and *TGFB1* (F). Adipose tissue expression of inflammatory genes was lower in patients treated with n−3 PUFAs. Gene expression was normalized to ubiquitin C and is presented as the mean (±SEM) of SAT from control subjects (the mean of the SAT 2^{-ddCt} was set to 100%). Statistical analyses of the mean differences in ddCt values in each group were performed by unpaired Student's *t* test, separately for VAT and SAT. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{\#}P < 0.1$. ddCt, delta delta Ct; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

immunohistochemistry did not show differences in macrophage numbers (**Figure 3**, A and B). However, expression of *CD40,* an M1 macrophage marker, was significantly reduced in the SAT of n−3 PUFA–treated patients. Interestingly, we found a significant positive correlation between expression of *HIF1A* and *CD40* in VAT of the control and the n−3 PUFA group, which indicated an association between macrophage action and hypoxia (Figure 3C).

Increased abundance of antiinflammatory-resolving lipid mediators by n−3 PUFA treatment

The resolving lipid mediators 18-hydroxy-EPA (18-HEPE) and its derivative resolvin E1 (RvE1) are enzymatically generated from EPA, whereas 17-hydroxy-DHA (17-HDHA), resolvin D1 (RvD1), and protectin D1 (PD1) are derived from DHA. AA is metabolized to eicosanoid precursors and eicosanoids such as prostaglandin E2, 15-hydroxyeicosatetraenoic acid (15-HETE), and 12-HETE. Abundance of these n−3 and n−6 PUFA−derived lipid mediators was analyzed in 42 patients (18 n−3 PUFA−treated and 24 control subjects), from which we collected sufficient amounts of adipose tissue. In general, these substances were more abundant in VAT than in SAT, with the exception of 18-HEPE and 12-HETE, which showed similar concentrations at both sites (**Figure 4**). n−3 PUFA significantly increased the production of RvE1, 17-HDHA, PD1, and RvD1 in the VAT of n−3 PUFA–treated patients compared with controls (Figure 4, B, C, D, and E). Abundance of 17-HDHA and PD1 in the n−3 PUFA group was more than double that in the control-treated patients ($P = 0.02$ and $P = 0.03$, respectively). We did not detect PD1 in the SAT of either group. RvD1 and RvE1 were only detected in adipose tissue from n−3 PUFA–treated patients, with RvD1 limited to VAT. Production of AA-derived eicosanoids was not different between groups.

n−3 PUFA modulates the concentration of circulating systemic inflammatory markers and adipokines

Treatment of obese patients with n−3 PUFA or control differentially affected mean plasma IL-6 concentrations ($P = 0.04$ for group × time interaction in RM-ANOVA), but secondary analysis showed that this change was confined to a significant reduction in the n−3 PUFA group only ($P = 0.045$). We found significant positive correlations between both plasma IL-6 and serum hsCRP concentrations with AA plasma phospholipid concentrations at the end of n−3 PUFA treatment (**Figure 5**, A and B). The serum hsCRP concentration did not differ significantly between treatments.

Metabolic changes by n−3 PUFA treatment

Treatment with n−3 PUFA decreased the mean serum triglyceride concentration compared with the control treatment ($P = 0.03$ for group × time interaction in RM-ANOVA) because of a significant reduction in the n−3 PUFA group ($P = 0.0002$; paired *t* test). Notably, at the end of treatment, there was a highly significant negative correlation between the plasma phospholipid EPA content and serum triglyceride concentration (Figure 5C). No changes in other lipid fractions were identified between the control and the n−3 PUFA groups (Table 1). Fasting and OGTT-derived serum glucose, insulin, and C-peptide concentrations were not affected by the treatment. Moreover, we found no significant changes in the analyzed variables of glucose homeostasis and insulin resistance (clamp-like index), as shown in Table 1. Hence, changes in important energy substrates induced by n−3 PUFA were confined to reduced triglyceride concentrations.

Effect of *PPARG* Pro12Ala polymorphism

In our study population we detected 36 of 55 (65.5%) Pro12Pro (Pro12), 16 of 55 (29%) Pro12Ala, and 3 of 55 (5.5%) Ala12Ala genotypes. Ala12 carriers were pooled for all analyses. No statistically significant differences were found in any of the analyzed variables between genotypes at baseline,

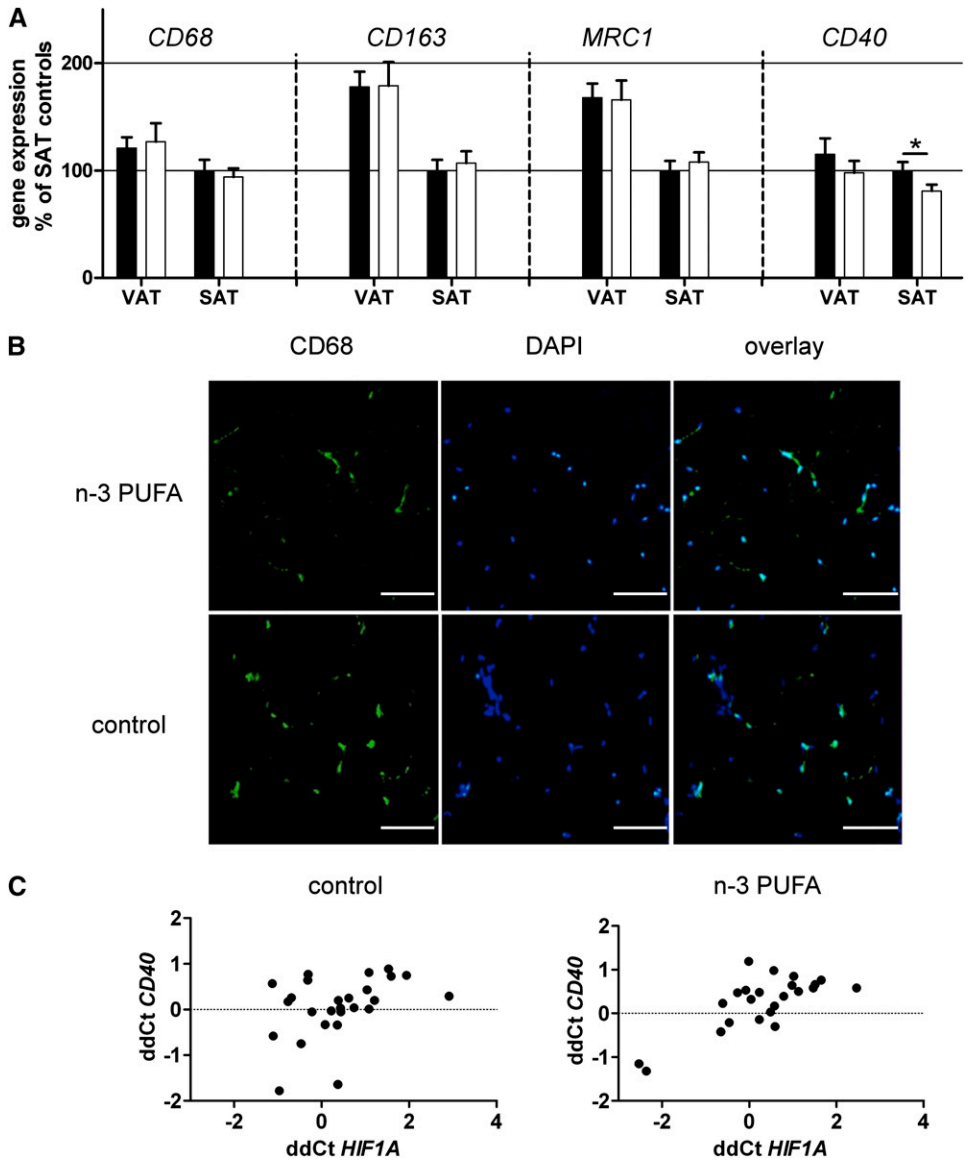

FIGURE 3. Gene expression of the macrophage markers *CD68, MRC1, CD163*, and *CD40* in the VAT and SAT of obese n−3 PUFA–treated patients (white bars; $n = 23$) and control subjects (black bars; $n = 26$). M1 macrophage polarization after n−3 PUFAs is lower than that after control treatment and correlates with the transcription factor *HIF1A*. (A) Data are shown as the means (±SEMs) of SAT from control subjects (mean of the SAT 2^{-ddCt} was set to 100%). Statistical analyses of mean differences of ddCt values in each group were performed by unpaired Student's *t* test, separately for VAT and SAT. (B) Representative histologic sections showing macrophages in the VAT of an n−3 PUFA–treated patient and one control subject, performed by CD68 immunofluorescence staining (green). Images were captured at 20× magnification. Bars represent 20 μm. (C) Expression of the M1 macrophage marker *CD40* correlated with the hypoxia marker *HIF1A* in the VAT of the control and n−3 PUFA groups. Statistical analysis was performed with a Spearman's rank correlation test. Control group: Spearman's $\rho = 0.44$, $P = 0.03$. n−3 PUFA group: $\rho = 0.62$, $P = 0.002$. *$P \leq 0.05$. ddCt, delta delta Ct; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

except for a lower serum γ-glutamyltransferase concentration in Ala12 carriers than in Pro12 homozygotes (30.0 ± 3.7 compared with 42.9 ± 3.8 U/L; $P = 0.03$). The *PPARG* genotype had no effect on adipose tissue gene expression of inflammatory markers (primary outcome variable), adipose tissue–resolving lipid mediator concentration, systemic inflammatory marker serum concentration, and glucose metabolism. However, serum concentrations of total and non-HDL cholesterol and apolipoprotein B increased significantly with n−3 PUFA treatment in Ala12 carriers as compared with Pro12 carriers (**Figure 6**). The same trend was observed for LDL cholesterol and inversely for HDL cholesterol (both $P <$ 0.10). Triglycerides and free fatty acids concentrations remained unaffected by genotype.

DISCUSSION

Adipose tissue inflammation is the basis of obesity-related systemic inflammation, which predisposes patients to the development of metabolic and cardiovascular disease (39). This is the first randomized controlled clinical study, which shows that treatment with long-chain n−3 PUFAs alleviates obesity-associated chronic inflammation of SAT and VAT in severely obese nondiabetic patients, as quantified by local gene expression and

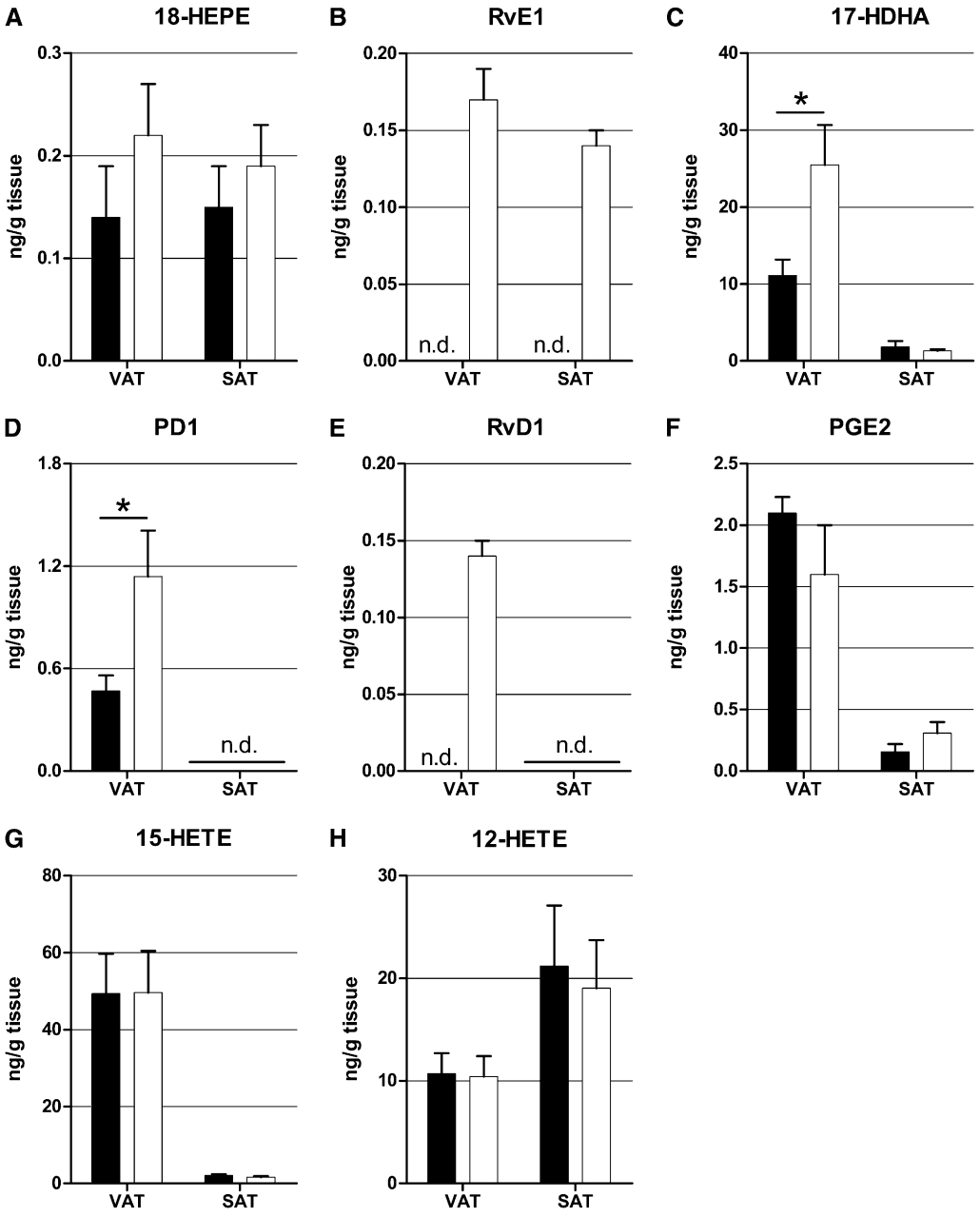

FIGURE 4. Mean (±SEM) concentration of lipid mediators derived from EPA [ie, 18-HEPE (A) and RvE1 (B)] and DHA [ie, 17-HDHA (C), PD1 (D), and RvD1 (E)] and from AA [ie, PGE2 (F), 15-HETE (G), and 12-HETE (H)] in VAT and SAT from patients treated with n−3 PUFAs (white bars; $n = 18$) and control subjects (black bars; $n = 24$). Adipose tissue production of antiinflammatory-resolving lipid mediators is enhanced after n−3 PUFA treatment. Statistically significant differences between groups were calculated by using unpaired Student's t test. $^*P < 0.05$. n.d., nondetectable; PD1, protectin D1; PGE2, prostaglandin E2; RvD1, resolvin D1; RvE1, resolvin E1; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; 12-HETE, 12-hydroxyeicosatetraenoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; 17-HDHA, 17-hydroxy-DHA; 18-HEPE, 18-hydroxy-EPA.

systemic levels. As proinflammatory fatty acid indexes decreased, the synthesis of antiinflammatory n−3 PUFA–derived resolvins and protectins increased, which indicated that altered eicosanoid formation could be a crucial molecular mechanism underlying the effects of n−3 PUFAs on obesity-driven inflammation.

We and others previously showed that inflammatory gene expression is increased in morbidly obese patients, including *CCL2* and *CCL3* (40), and is associated with metabolic and cardiovascular disorders (41). In this study, we showed that, compared with control treatment, n−3 PUFAs generally downregulated SAT and VAT gene expression of most proinflammatory marker genes, which we analyzed, namely *CCL2, CCL3, IL6, HIF1A*, and *TGFB1,* even though not all changes were statistically significant. Supplementation with long-chain n−3 PUFAs for 26 wk has been shown to decrease the expression of genes involved in inflammation and atherogenesis, but also in hypoxia signaling (42), as confirmed here by a reduced expression of *HIF1A*. A reduction in hypoxia could underlie the mitigation of inflammatory gene expression in adipose tissue by n−3 PUFAs (8). In adipose tissue, hypoxia directly induced proinflammatory M1 macrophage marker molecule

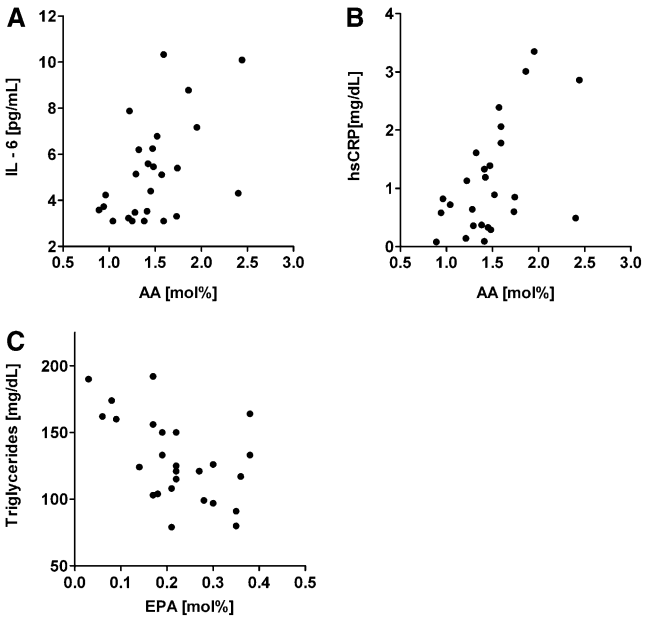

FIGURE 5. Correlation of plasma PUFA concentrations with inflammatory and metabolic variables in the n−3 PUFA group. The proportion of plasma phospholipid AA correlated with plasma IL-6 (A) and serum hsCRP (B) concentrations in the n−3 PUFA group (n = 27) at the end of the treatment. IL-6: Spearman's ρ = 0.46, P = 0.02; hsCRP: ρ = 0.43, P = 0.006. (C) The proportion of plasma phospholipid EPA negatively correlated with serum triglycerides in the n−3 PUFA group at the end of the treatment (n = 26; Spearman's ρ = −0.46, P = 0.02). Statistical analysis was performed by using a Spearman's rank correlation test. AA, arachidonic acid; hsCRP, high-sensitivity C-reactive protein.

CD40 concentrations in severe chronic obstructive pulmonary disease (43), which produce high concentrations of chemokines, leading to the further recruitment of macrophages to the tissue and to the development of insulin resistance in humans (7, 44). We found that the expression of *CD40* was downregulated by n−3 PUFA treatment, whereas expression of typical overall macrophage marker CD68 and M2 macrophage markers *MRC1* and *CD163* remained unchanged (Figure 3A), as was the total number of macrophages (data not shown). Furthermore, the truly interesting correlation between hypoxia and macrophage polarization shown in Figure 3C substantially corroborates the hypothesis that, by ameliorating hypoxia, n−3 PUFAs cause an antiinflammatory shift in adipose tissue macrophage phenotype. Hypoxia could initiate pathological fibrotic remodeling in adipose tissue (10). We showed that n−3 PUFAs reduced the expression of the well-known fibrosis marker *TGFB1* (transforming growth factor β1), which validates our previous data showing that n−3 PUFAs can prevent adipose tissue remodeling in murine models of obesity (24).

The formulation and dosage of the long-chain n−3 PUFA capsules were generally well tolerated. The plasma phospholipid fatty acid profiles showed that most patients adhered to the study medication, but also that long-chain n−3 PUFAs reduced important inflammatory fatty acid indexes, such as AIFAI, the AA to EPA ratio, or the n−6 to n−3 ratio in severely obese patients. Specifically, the striking reduction of the n−6 to n−3 PUFA ratio, a valuable surrogate marker for the level of chronic obesity-associated low-grade inflammation (45), is of great prognostic relevance, because it is likely that a high intake of widespread n−6 PUFAs without counteracting n−3 PUFAs increases the risk of coronary heart disease and death (19). In the n−3 PUFA group, treatment significantly decreased plasma concentrations of circulating inflammatory marker IL-6 but not hsCRP. At baseline, IL-6 and hsCRP did not correlate with the proinflammatory AA (data not shown). The positive correlation between concentrations of both IL-6 and hsCRP with the relative proportion of AA in plasma phospholipids was only detected in the n−3 PUFA group at treatment end and might indicate that *1*) systemic inflammatory markers are crucially based on the systemic availability of proinflammatory n−6 PUFAs, and *2*) only those patients who succeed in reducing AA respond with lower systemic inflammation, as EPA antagonizes AA metabolism and thereby reduces the generation of inflammatory AA-derived eicosanoids (17). The fact that the negative correlation of AA phospholipid concentration with inflammatory measures was evident only at the end of n−3 PUFA treatment suggests that a certain threshold of EPA concentration is required to successfully counteract the metabolism of AA to proinflammatory mediators, keeping in mind that plasma phospholipid concentrations are only a proxy marker for fatty acid composition in other tissues. Thus, long-chain n−3 PUFAs favorably affected the inflammatory status because of their crucial function as substrates for eicosanoid synthesis. We detected the potent antiinflammatory eicosanoids resolvins and protectins (also known as resolving lipid mediators) in human adipose tissue and showed that treatment with their precursors, EPA and DHA, leads to a substantial increase in resolving lipid mediator concentrations. After n−3 PUFA treatment, EPA-derived 18-HEPE and RvE1 comparably increased in both VAT and SAT. On the other hand, DHA-derived lipid mediators were more increased in VAT than in SAT for unknown reasons—an observation that is highly interesting, but not yet understood. Because resolution of chronic adipose tissue inflammation could be hindered by a lack of resolving lipid mediators (22), increased substrate availability for synthesis of antiinflammatory resolvins and protectins adds to the beneficial and complex antiinflammatory actions of n−3 PUFAs.

Studies evaluating the effect of n−3 PUFAs on glucose homeostasis in humans have contradictory outcomes (46, 47), which largely depend on trial design and characteristics of the study patients. Similar to many of these trials (48, 49), our results show that an 8-wk treatment with n−3 PUFAs has no influence on glucose homeostasis, nor did it affect adipose tissue gene expression or serum concentrations of the insulin-sensitizing adiponectin.

Long-chain n−3 PUFAs significantly reduced serum triglyceride concentrations, as was also seen in other trials (49). The main hypolipidemic constituent of the treatment appears to be EPA rather than DHA, because plasma phospholipid EPA concentrations significantly correlated with serum triglyceride concentrations in the n−3 PUFA group at treatment end. The n−3 PUFA effects on lipid metabolism and cardiovascular disease risk could be influenced by the Pro12Ala polymorphism in the *PPARG* gene (25, 50). The PPARG genotype affected the treatment response on serum concentrations of total and non-HDL cholesterol and apolipoprotein B, which was significantly greater in Ala12 carriers than in Pro12 carriers with n−3 PUFA treatment. Hence, the cardioprotective effect of n−3 PUFAs could be mitigated in Ala12 carriers by unfavorable changes in serum cholesterol (51, 52). However, the *PPARG* gene-nutrient

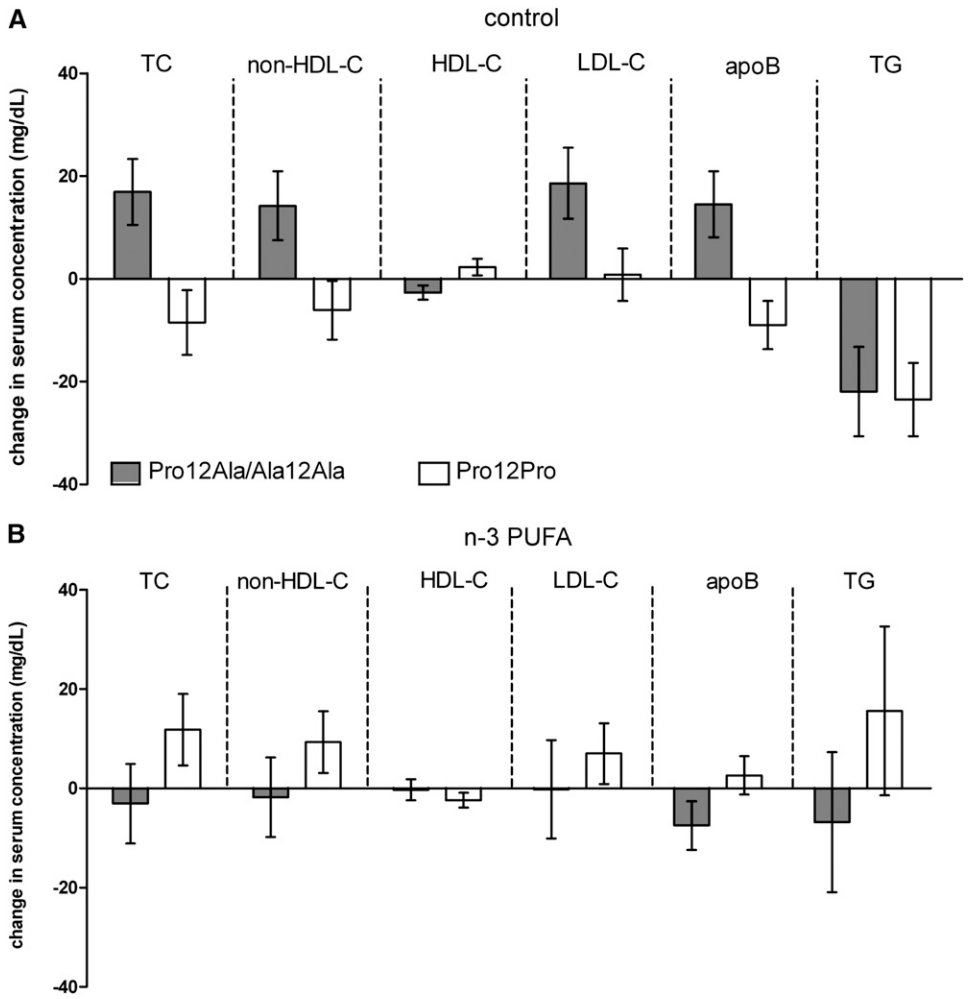

FIGURE 6. Changes in serum concentrations of TC, non-HDL-C, HDL-C, LDL-C, apoB, and TG in control (A) and n−3 PUFA–treated patients (B) according to *PPARG* genotypes (gray columns, Ala12 carriers; white columns, Pro12 homozygotes; $n = 6$ and 21 for control, and 11 and 15 for n−3 PUFA-treated groups). *PPARG* polymorphism affected n−3 PUFA's effects on serum lipids. The effects of treatment and genotype on the analyzed variables were estimated by 2-factor ANOVA. Significance of the group × polymorphism effect (2-factor ANOVA): TC, $P = 0.02$; non-HDL-C, $P = 0.04$; HDL-C, $P = 0.06$; LDL-C, $P = 0.08$; apoB, $P = 0.003$; and TG, $P = 0.46$. apoB, apolipoprotein B; C, cholesterol; TC, total cholesterol; TG, triglycerides.

interaction did not affect our primary analysis variable—adipose tissue inflammation.

One limitation of our study was that changes in gene expression from baseline are not known, because adipose tissue biopsies were obtainable only at treatment end. The unblinded treatment allocation is not regarded as a relevant issue because primary efficacy measures were not based on subjectivity-related items. For laboratory analysis, the study personnel were blinded to the patient's identities, and the samples were numbered and processed in random order. Our choice of placebo (ie, butterfat) might raise the question of whether the differences observed between the 2 groups were due to the "antiinflammatory" effects of n−3 PUFAs or to the "proinflammatory" effects of butterfat (or a combination). Although it cannot be completely ruled out by the data collected here, the latter interpretation is rather unlikely. First, butter is very popular in local diets, and the general intake is 10 g/d in Austria (26). Second, proinflammatory SFAs comprise 51% butter fat; hence, the control treatment added only ∼2 g SFA to the diet, which already includes >17g SFA/d at baseline in virtually all patients. Third, no treatment-induced changes were found in the concentrations of the main fatty acids found in butter (14:0, 16:0, 18:0, and 18:1) specific to the control group. A slight discrepancy between gene expression was found between SAT and VAT, which was attributed to the fact that treatment with n−3 PUFAs repeatedly affected inflammatory gene expression in VAT with merely borderline significance. This was probably to the result of a larger heterogeneity of VAT sampling, as indicated by a higher variance in VAT than in SAT rather than a reduced biological response to n−3 PUFAs, as evidenced by the highly increased production of n−3 PUFA–derived mediators. We and others have shown that relevant inflammatory genes such as *CCL2, CCL3, CD40*, and *TNFA* are expressed at comparable levels in SAT and VAT, whereas *IL6* mRNA levels are even higher in SAT (40, 53). Therefore, our findings limited to SAT are equally relevant in the context of adipose tissue inflammation and obesity.

Among the strengths of our study were the randomized design, the availability of tissue biopsy samples, and the analysis of fatty acid profiles in plasma phospholipids as a measure of treatment

adherence. In addition, we investigated adipose tissue concentrations of n−3 PUFA–derived resolving lipid mediators—a highly exciting field that has recently emerged in chronic inflammation research. Hence, this was the first randomized clinical trial to show an increase in resolving lipid mediators after treatment with their n−3 PUFA precursors.

In conclusion, n−3 PUFA treatment improved adipose tissue and systemic inflammation in severely obese patients compared with control treatment. Whereas the beneficial effects of long-chain n−3 PUFAs are evident, the metabolic effects beyond reduced serum triglyceride concentration need to be studied in more detail.

We thank all participants of the study; Erika Nowotny, Astrid Hofer, Patrick O'Brien, and Liliana Ionasz for excellent technical assistance; Christian Göbl for helpful discussions; and Alexander Jürets for help with manuscript preparation.

The authors' responsibilities are as follows—TMS: designed research, raised grants, and coordinated the complete project; BKI: conducted the trial; BKI, EEH, AN, and MS: collected the data; BKI and MZ: analyzed the data and performed the statistical analyses; BKI: wrote the manuscript; GP and AB: provided adipose tissue samples; DM: analyzed lipid mediator production; KS: analyzed the nutritional data; TMS, MZ, AN, KS, and EEH: critically reviewed the manuscript; MK and MGB: provided clinical support and help with implementation of the research plan; and BKI and TMS: had primary responsibility for the final content. All authors approved the final manuscript and took part in the discussion of the results. None of the authors declared a conflict of interest related to this article. Solvay Pharma had no responsibility in this study.

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