Effects of n−6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial[1–4]

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ABSTRACT

Background: Replacing SFAs with vegetable PUFAs has cardiometabolic benefits, but the effects on liver fat are unknown. Increased dietary n−6 PUFAs have, however, also been proposed to promote inflammation—a yet unproven theory.

Objective: We investigated the effects of PUFAs on liver fat, systemic inflammation, and metabolic disorders.

Design: We randomly assigned 67 abdominally obese subjects (15% had type 2 diabetes) to a 10-wk isocaloric diet high in vegetable n−6 PUFA (PUFA diet) or SFA mainly from butter (SFA diet), without altering the macronutrient intake. Liver fat was assessed by MRI and magnetic resonance proton (1H) spectroscopy (MRS). Proprotein convertase subtilisin/kexin type-9 (PCSK9, a hepatic LDL-receptor regulator), inflammation, and adipose tissue expression of inflammatory and lipogenic genes were determined.

Results: A total of 61 subjects completed the study. Body weight modestly increased but was not different between groups. Liver fat was lower during the PUFA diet than during the SFA diet [between-group difference in relative change from baseline; 16% (MRI; $P < 0.001$), 34% (MRS; $P = 0.02$)]. PCSK9 ($P = 0.001$), TNF receptor-2 ($P < 0.01$), and IL-1 receptor antagonist ($P = 0.02$) concentrations were lower during the PUFA diet, whereas insulin ($P = 0.06$) tended to be higher during the SFA diet. In compliant subjects (defined as change in serum linoleic acid), insulin, total/HDL-cholesterol ratio, LDL cholesterol, and triglycerides were lower during the PUFA diet than during the SFA diet ($P < 0.05$). Adipose tissue gene expression was unchanged.

Conclusions: Compared with SFA intake, n−6 PUFAs reduce liver fat and modestly improve metabolic status, without weight loss. A high n−6 PUFA intake does not cause any signs of inflammation or oxidative stress. Downregulation of PCSK9 could be a novel mechanism behind the cholesterol-lowering effects of PUFAs. This trial was registered at clinicaltrials.gov as NCT01038102. *Am J Clin Nutr* 2012;95:1003–12.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD)[5] affects 25% of the adult population (1) and is strongly associated with metabolic disorders and type 2 diabetes, even independently of abdominal obesity (2, 3). Reduction of liver fat is thus an interesting target for preventing and treating obesity-related metabolic diseases. Besides physical activity and weight loss (4), there is no effective and safe treatment to reduce liver fat. Interestingly, dietary fatty acids may influence the accumulation of hepatic (5) and abdominal (6, 7) fat. A diet high in vegetable n−6 PUFAs decreases abdominal fat content and peripheral insulin resistance compared with a diet high in SFAs (6). A high fat intake may promote liver fat accumulation (8), but observational data also suggest that dietary fat composition could play a role (9, 10). SFAs have been positively related to liver fat (9, 10), whereas the essential n−6 PUFA linoleic acid (18:2n−6) has been inversely related to plasma alanine aminotransferase (ALT) concentrations (10).

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[5] Abbreviations used: ALT, alanine aminotransferase; CAD, coronary artery disease; Ct, comparative threshold cycle; IL-1RA, IL-1 receptor antagonist; MRS, magnetic resonance proton (1H) spectroscopy; NAFLD, nonalcoholic fatty liver disease; OGTT, oral-glucose-tolerance test; PCSK9, proprotein convertase subtilisin/kexin type-9; SCD-1, stearoyl-CoA desaturase 1; TNF-R2, TNF receptor-2.

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Linoleic acid is the main dietary n−6 PUFA and is abundant in plant oils, seeds, and nuts. Solid evidence indicates that replacing SFAs with PUFAs reduces coronary artery disease (CAD) events (11, 12) and possibly prevents diabetes (13). The mechanism for the CAD risk reduction involves the LDL-cholesterol lowering of n−6 PUFAs (14), but other potential metabolic effects need further study. The mechanism behind SFA-induced increases in serum cholesterol may involve both lowered LDL-receptor numbers and PGC-1β induction (15, 16) but the LDL-cholesterol lowering of PUFAs is unclear. Proprotein convertase subtilisin/kexin type 9 (PCSK9) has been identified as a key regulator of plasma LDL cholesterol by modulation of the degradation of LDL receptors (17, 18). PCSK9 inhibition is a drug target to reduce serum cholesterol, particularly in combination with statins, because statins increase PCSK9 (19). Subjects with a certain loss of function variants of the PCSK9 gene have 15–28% lower serum cholesterol and a 47–88% lower CAD risk (18). The effect of PUFA or SFA on circulating PCSK9 concentrations is to our knowledge unknown.

In earlier literature it has been argued that n−6 PUFAs may promote inflammation, mainly by increasing circulating linoleic acid and consequently arachidonic acid and proinflammatory metabolites (20)—a theoretical reasoning that remains to be established in controlled studies. Whereas most lipid research has focused on n−3 PUFA, few controlled studies have investigated the effects of n−6 in humans. On the basis of animal (5) and observational (9, 10) data, we hypothesized that dietary fat modification could influence liver fat, even in the absence of weight loss or caloric restriction. In the randomized HEPFAT trial, we examined the effects of n−6 PUFA and SFA on liver fat content, serum PCSK9, blood lipid concentrations, glucose metabolism, lipid peroxidation, and inflammation in abdominally obese subjects.

SUBJECTS AND METHODS

Study design

The HEPFAT trial was a randomized, 10-wk, parallel-group study conducted in Uppsala, Sweden, between February 2009 and April 2010. Subjects were recruited by advertisements in local newspapers, supermarkets, and primary health care centers. Inclusion criteria assessed by screening were age 30–65 y, sagittal abdominal diameter >25 cm, or waist circumference >88 cm (women) or >102 cm (men). Exclusion criteria were diagnosed liver disease, type 1 diabetes, history of a serious cardiovascular event, BMI (in kg/m^2) >40, excessive alcohol intake, and internal metal or electronic device. After being screened, 67 individuals were eligible (**Figure 1**). All participants gave written informed consent before entering the study. The study was approved by the regional ethical committee in Uppsala.

Intervention

Participants were randomly assigned to either a PUFA diet or an SFA diet. The randomization was stratified according to sex and performed in blocks of 4 (allocation ratio 1:1). The participants were instructed (unblinded) to change the quality of their dietary fat without altering their intakes of total fat and the type and amount of carbohydrates and protein. The participants were encouraged not to change their physical activity or their fish and alcohol intakes during the study. Some key food items were provided: the PUFA group received foods rich in n−6

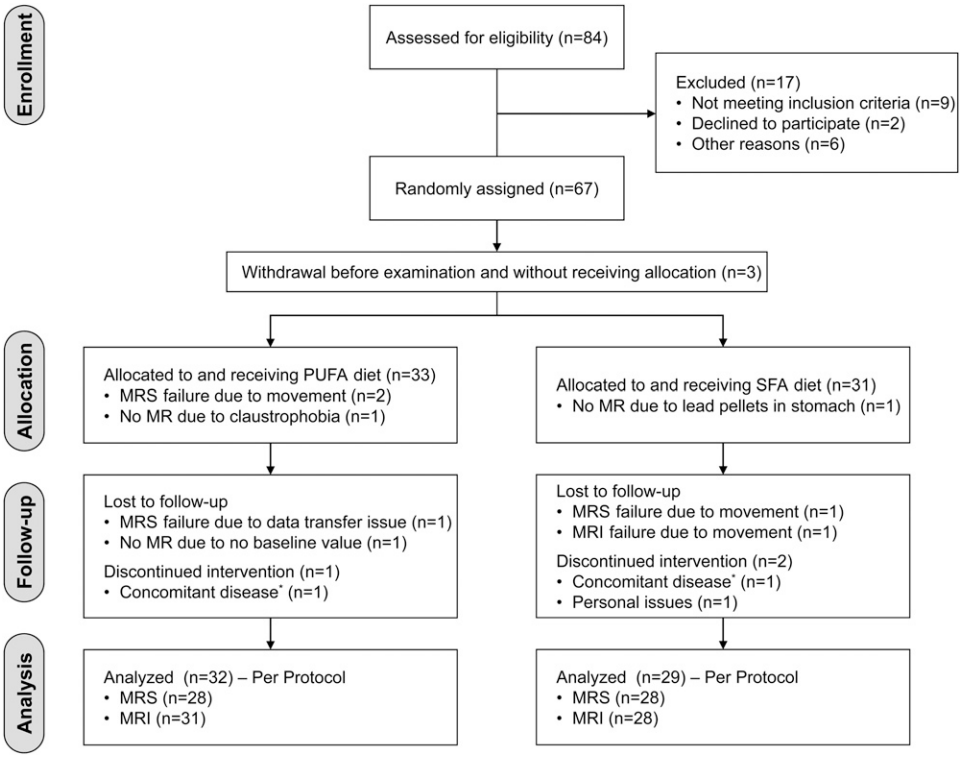

FIGURE 1. Flow diagram. *The diseases were known before enrollment (heart valve disorder and chronic obstructive pulmonary disease). MRI, magnetic resonance imaging; MRS, magnetic resonance proton (1H) spectroscopy.

linoleic acid, ie, scones (baked-on sunflower oil), margarine, sunflower oil, and sunflower seeds, and the SFA group received scones (baked-on butter) and butter. On the basis of weight and sex, participants consuming the PUFA diet were instructed to consume the given food items corresponding to ~15% of energy as linoleic acid. To avoid weight loss during the intervention, all participants were urged to weigh themselves weekly.

Study outcomes

The primary outcome was change in liver fat content measured by MRI and by magnetic resonance proton (1H) spectroscopy (MRS). Secondary outcomes were abdominal and total body fat, serum lipid and PCSK9 concentrations, insulin sensitivity, systemic inflammation, oxidative stress (lipid peroxidation), and adipose tissue gene expression.

Clinical assessments

The examinations were performed at baseline (visit 1), after 5 wk (visit 2), and after 10 wk (visit 3). All visits took place in the morning after an overnight fast. Visits 1 and 3 started with an MR investigation, followed by anthropometric and blood pressure measurements, blood sampling, an oral-glucose-tolerance test (OGTT), and adipose tissue biopsy collection. Morning urine was collected at visits 1 and 3. Visit 2 included anthropometric measurements and blood sampling.

Subcutaneous and visceral adipose tissue volumes were assessed by MRI, and liver fat content was assessed by both MRI and MRS. MR measurements were performed by using a 1.5T clinical scanner (Gyroscan NT; Philips Health Care). Collection and analyses of the MR data were performed at one center under blinded conditions and is described in detail elsewhere (*see* supplemental material under "Supplemental data" in the online issue). Total body fat mass was determined by air-displacement plethysmography (BOD POD; LmiTech).

Body weight was measured while the subjects were in underwear to the nearest 0.1 kg. Blood was drawn from an antecubital vein into evacuated tubes. For the OGTT, 75 g glucose and 350 mL water was consumed, and blood samples were collected at 30, 60, 90, and 120 min.

Biopsy samples were collected from the subcutaneous adipose tissue fat pad below and lateral to the umbilicus by needle aspiration under local anesthesia (1% lidocaine), washed with physiologic saline, frozen on dry ice covered with ethanol, and stored at −70°C. Dietary intake was assessed from 3-d weighed food records before randomization and between visits 2 and 3. The Dietist XP software package (version 3.0, 2007) was used to calculate dietary intake.

Biochemical analyses

Plasma concentrations of glucose, triglycerides, cholesterol, LDL cholesterol, HDL cholesterol, apolipoproteins, ALT, γ-glutamyltransferase, C-reactive protein, and serum insulin concentrations were measured by routine laboratory methods at Uppsala University Hospital. AUCs for insulin and glucose concentrations during the OGTT were calculated according to the trapezoid rule. Serum fatty acid composition was measured in cholesterol esters by gas chromatography as previously described (21), with the following divergences: use of a 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation); an Agilent Technologies system consisting of model GLC 6890N, autosampler 7683, and Agilent ChemStation; and a programmed temperature of 150°C to 260°C. The stearoyl-coA desaturase-1 (SCD-1) index was estimated by serum 16:1/16:0. ELISA was used to determine serum fibroblast growth factor 21 (R&D Systems), PCSK9 (CycLex), proinsulin (Mercodia), and fetuin-A (Mercodia) as well as plasma concentrations of high-molecular-weight adiponectin (Millipore), IL-1β, IL-6, IL-10, IL-1 receptor antagonist (IL-1RA), and soluble TNF receptor-2 (TNF-R2; all R&D Systems). Serum unesterified lathosterol was determined by isotope dilution mass spectrometry and corrected for total cholesterol concentration (22).

Urinary 8-iso-prostaglandin $F_{2\alpha}$ and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ were assessed by radioimmunoassay as indicators of oxidative stress and lipid peroxidation, ie, free radical–induced and enzymatic induced oxidation of arachidonic acid, respectively (23, 24). Data were adjusted for urinary creatinine; 15-keto-dihydro-$PGF_{2\alpha}$ was analyzed only in compliant participants (definition described below).

Adipose tissue mRNA expression of target genes was assessed in compliant subjects by quantitative real-time polymerase chain reaction (iCycler IQ; Bio-Rad Laboratories) by using a comparative threshold cycle (Ct) method. Ct values were normalized to the reference gene LDL receptor–related protein 10 or 18S, according to the following formula:

$$2^{\Delta Ct-\text{target gene}}/2^{\Delta Ct-\text{reference gene}} = \text{arbitrary units} \qquad (1)$$

Statistical analyses

Variables are presented as means ± SDs or medians (quartiles 1–3). In power analyses, 31 individuals per group would be needed to observe a 5% difference in liver fat with an SD for liver fat of 7% (8) ($\alpha = 0.05$, $\beta = 0.20$). Non-normally distributed variables were logarithmically transformed, and nonparametric tests were used if normality was not attained. Statistical analyses were based on per-protocol instead of intention-to-treat principles because we believe the latter analyses are not always optimal in dietary interventions with a limited number of subjects. Also, in contrast with drug therapy with clinical endpoints, the primary aim was academic, ie, to investigate the role of dietary fat quality on liver fat content rather than to evaluate a clinical treatment and its adverse events in patients. We a priori defined compliance using serum fatty acid biomarkers and conducted post hoc analyses, because the change in dietary SFAs and PUFAs will be dependent on the baseline (ie, habitual) intake of the individual. Compliance was defined according to changes in serum linoleic acid, ie, 18:2n−6 >0.0% during the PUFA diet (n = 27) and 18:2n−6 <0.0% during the SFA diet (n = 19). The rationale for using 18:2n−6 is that changes in serum cholesterol esters are strongly associated with changes in dietary intake (25), ie, if no change in serum 18:2n−6 can be observed in an individual, then little actual change from baseline in fatty acid intake has taken place. Hemolytic samples were excluded in sensitive analyses (fasting insulin, n = 3; $AUC_{insulin}$, n = 7; γ-glutamyltransferase, n = 1; cytokines, n = 1; adiponectin, n = 1). Baseline values and changes during the intervention [$\text{measurement}_{(visit3)} - \text{measurement}_{(visit1)}$] were compared between

the groups by 2-sided *t* test or Wilcoxon's rank-sum test. Baseline adjustments were made by ANCOVA or the residual method. *P* values represent the differences in change between the groups when adjusted for baseline. The covariates in secondary analyses were as follows: baseline value for the variable tested, weight change, change in energy intake, and change in total fat intake. The primary statistical analyses were performed blinded. $P \leq 0.05$ was considered statistically significant. The software package STATA version 11 (STATA Corporation) was used.

RESULTS

Study population

Of the 67 included subjects, 61 completed the study (Figure 1). Baseline characteristics and changes during the trial are presented in **Table 1**. The results were similar (all significant differences remained) regardless of whether intention-to-treat or per-protocol analyses were used. None of the variables differed between the groups at baseline ($P > 0.05$), and 66% of both groups were women. Subjects in the PUFA diet were aged 57 (51–63) y and in the SFA diet were aged 56 (50–64) y, and 15% of the subjects had diabetes (previous diagnosis, fasting glucose ≥7.0 mmol/L, or glucose ≥11.0 mmol/L 2 h after an OGTT). Use of antihypertensive drugs and lipid-lowering drugs was 31% and 16%, respectively. Two subjects in the PUFA-diet group reported loose stool shortly after study initiation; no other adverse events were detected.

Dietary changes

Energy intakes were 2052 ± 514 kcal (PUFA diet) and 1945 ± 493 kcal (SFA diet) at baseline and increased by 138 ± 451 kcal (PUFA diet) and 225 ± 509 kcal (SFA diet) during the intervention, but did not differ significantly between the groups ($P = 0.59$). Changes in nutrient composition are presented in **Figure 2**A. During the PUFA diet, the n−6/n−3 ratio increased from 4 (3–5) to 14 (10–19). The intervention had considerable effects on serum fatty acid composition (Figure 2B; *see* supplemental material under "Supplemental data" in the online issue). The proportion of linoleic acid increased from baseline by 11% in the PUFA group. Those n−6 PUFAs that are mainly endogenously synthesized (18:3n−6, 20:3n−6, and 20:4n−6) did not differ between the diets ($P > 0.36$). The SCD-1 index was lower during the PUFA diet than during the SFA diet ($P < 0.001$).

TABLE 1
Baseline characteristics and changes in body composition and metabolic factors during the intervention[1]

	Baseline[2]		Change[3]		
Body composition	PUFA diet	SFA diet	PUFA diet	SFA diet	*P*[4]
Weight (kg)	85.7 ± 10.6[5]	90.8 ± 14.4	0.4 ± 1.4	0.8 ± 1.6	0.41
BMI (kg/m^2)	30.3 ± 3.7	31.3 ± 3.9	0.1 ± 0.5	0.3 ± 0.6	0.41
Waist circumference (cm)	104 (99–111)[6]	108 (101–120)	−1 (−2 to 1)	1 (−1 to 2)	0.86
Visceral AT (L)	5.1 ± 1.7	6.2 ± 2.8	0.0 ± 0.3	0.1 ± 0.3	0.39
Subcutaneous AT (L)	10.0 ± 3.3	10.8 ± 3.5	0.3 ± 0.5	0.1 ± 0.5	0.17
Visceral AT/subcutaneous AT	0.46 (0.31–0.77)	0.49 (0.33–0.92)	−0.01 (−0.02 to 0.01)	0.00 (−0.01 to 0.01)	0.03
Total fat mass (%)	42.9 ± 7.5	43.2 ± 6.6	−0.1 ± 1.4	0.6 ± 1.4	0.09
Lipids and cholesterol					
Serum PCSK9 (μg/L)	273 ± 70	278 ± 54	−36 ± 69	15 ± 78	0.001
Fasting plasma triglycerides (mmol/L)	1.51 ± 0.70	1.44 ± 0.66	−0.07 ± 0.53	0.06 ± 0.39	0.19
Plasma cholesterol (mmol/L)	5.5 ± 0.9	5.5 ± 1.0	−0.2 ± 0.4	0.0 ± 0.5	0.01
Plasma HDL cholesterol (mmol/L)	1.3 (1.1–1.5)	1.4 (1.2–1.6)	0.0 (−0.2 to 0.1)	0.0 (−0.1 to 0.1)	0.54
Plasma LDL cholesterol (mmol/L)	3.4 ± 0.9	3.3 ± 0.7	−0.1 ± 0.3	0.0 ± 0.4	0.09
Plasma apolipoprotein A-I (g/L)	1.4 ± 0.2	1.5 ± 0.3	0.0 ± 0.1	0.1 ± 0.2	0.05
Plasma apolipoprotein B (g/L)	1.0 ± 0.2	1.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.1	0.96
Serum lathosterol/cholesterol (mg/mol)	327 ± 86	350 ± 99	−44 ± 82	34 ± 83	<0.001
Glucose metabolism					
Fasting plasma glucose (mmol/L)	5.3 (4.7–5.8)	5.4 (5.2–6.3)	0.0 (−0.2 to 0.2)	0.0 (−0.4 to 0.2)	0.70
Fasting serum insulin (pmol/L)	62 (47–91)	67 (45–114)	2.2 (−12 to 15)	12 (0–22)	0.06
HOMA-IR	2.0 (1.5–3.4)	2.6 (1.5–5.0)	0.1 (−0.5 to 0.6)	0.3 (0.0–0.7)	0.09
Proinsulin (pmol/L)	6.3 (3.8–11.5)	6.7 (4.2–14.3)	0.6 (−1.0 to 1.7)	0.7 (−0.2 to 4.3)	0.47
Other biochemical markers					
Plasma HMW adiponectin (mg/L)	2.62 (1.69–3.60)	3.56 (2.07–5.78)	−0.02 (−0.36 to 0.36)	−0.03 (−0.74 to 0.68)	0.92
Plasma ALT (μkat/L)	0.43 (0.31–0.63)	0.43 (0.34–0.67)	−0.04 (−0.15 to 0.09)	−0.01 (−0.04 to 0.10)	0.14
Serum FGF21 (ng/L)	206.1 (143.4–292.7)	204.3 (167.2–280.7)	−16.0 (−48.5 to 27.5)	−23.2 (−38.1 to 23.4)	0.91
Serum fetuin-A (μg/mL)	240.4 ± 38.6	251.0 ± 28.6	−0.2 ± 22.1	2.2 ± 23.5	0.56

[1] $n = 32$ (PUFA diet) and $n = 29$ (SFA diet). ALT, alanine aminotransferase; AT, adipose tissue; FGF21, fibroblast growth factor 21; HMW, high-molecular-weight; PCSK9, proprotein convertase subtilisin/kexin type 9.
[2] The variables did not differ significantly between the groups at baseline.
[3] Change denotes measure at follow-up − measure at baseline.
[4] *P* values were derived by ANCOVA or the residual method and were adjusted for baseline values.
[5] Mean ± SD (all such values).
[6] Median (quartiles 1–3) (all such values).

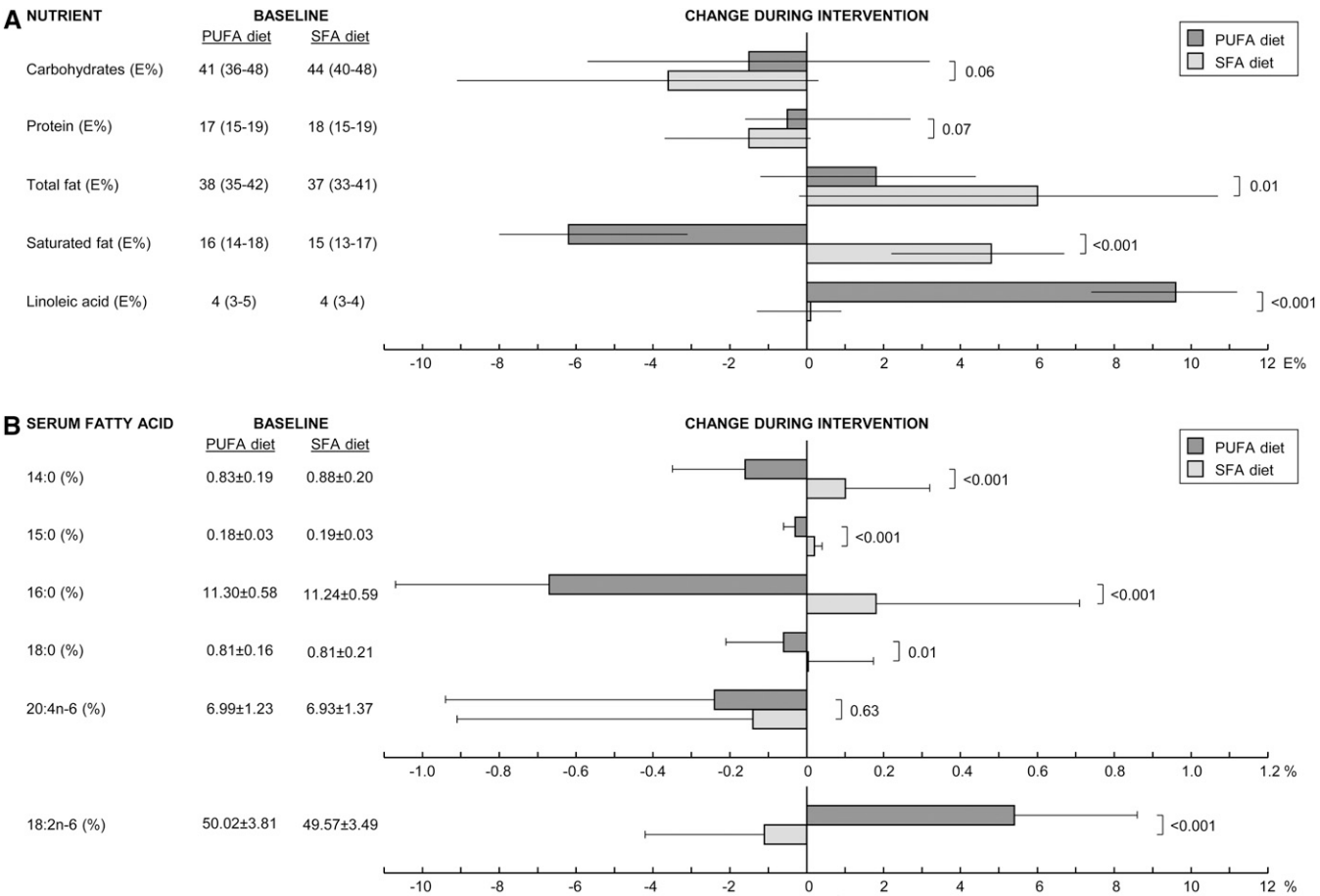

FIGURE 2. Dietary intake and serum fatty acid composition at baseline and changes during the intervention. n = 30 (PUFA diet) and n = 29 (SFA diet). Data are presented as medians (quartiles 1–3) in panel A and as means (±SDs) in panel B. A 2-sided t test was used in the statistical analyses. Change denotes measure at follow-up − measure at baseline. E%, percentage of energy.

Liver and body fat

Body weight modestly increased during both diets without between-groups difference (Table 1). Liver fat values at baseline were as follows: PUFA diet [6.8%, 4.1–10.2 (MRI); 3.2%, 1.0–6.6 (MRS)] and SFA diet [7.5%, 5.5–15.7 (MRI); 3.2%, 1.3–7.7 (MRS)]. Liver fat values at baseline did not differ between the groups. Liver fat was significantly lower during the PUFA diet than during the SFA diet. Changes during the trial were −0.5% (−2.3 to 0.2; MRI) and −0.9% (−1.7 to 0.0; MRS) with the PUFA diet and 0.7% (−0.2 to 2.1; MRI) and 0.3% (−0.6 to 1.8; MRS) for the SFA diet (**Figure 3**). The results were unaltered after adjustment for weight change and total fat intake or after the exclusion of subjects with liver fat ≤1.0% (MRS) at baseline (n = 12), ie, when the assessment is less sensitive. Change in liver fat was inversely related to change in serum linoleic acid concentrations and was positively associated with change in serum SFAs (**Figure 4**). No differences were observed for subcutaneous or visceral adipose tissue. However, a small but significant difference in the visceral-to-subcutaneous adipose tissue ratio was found (Table 1). The changes in percentage total body fat were −0.1 ± 1.4% (PUFA diet) and 0.6 ± 1.4% (SFA diet) (P = 0.09).

PCSK9 and blood lipids

Serum PCSK9 and the lathosterol/cholesterol ratio were lower during the PUFA diet than during the SFA diet. Plasma cholesterol slightly decreased during the PUFA diet (P = 0.01), whereas no significant effects on other blood lipids were found unless compliance with the diets was taken into account (Table 1).

Glucose metabolism

Fasting insulin concentrations tended to be higher during the SFA diet than during the PUFA diet (P = 0.06) and was significant in compliant subjects (**Figure 5**). Fasting glucose, HOMA, $AUC_{glucose}$, $AUC_{insulin}$, and proinsulin did not differ between the diet groups (Table 1).

Liver enzymes and adiponectin

No differences between the diets were observed for ALT, fibroblast growth factor 21, or γ-glutamyltransferase concentrations (Table 1). Change in ALT was related to change in liver fat [r = 0.33, P = 0.01 (MRI) and r = 0.29, P = 0.03 (MRS)], which indicated a stronger correlation in subjects with MRS liver fat ≥1.0% (r = 0.59, $P < 0.001$; n = 43). All 3 liver markers correlated with MRS liver fat at baseline ($P < 0.04$). Plasma high-molecular-weight adiponectin was unchanged by the intervention (Table 1).

Inflammation and oxidative stress

Plasma IL-1RA and TNF-R2 were lower during the PUFA diet than during the SFA diet (**Table 2**). No effects were observed for the other markers of inflammation or oxidative stress.

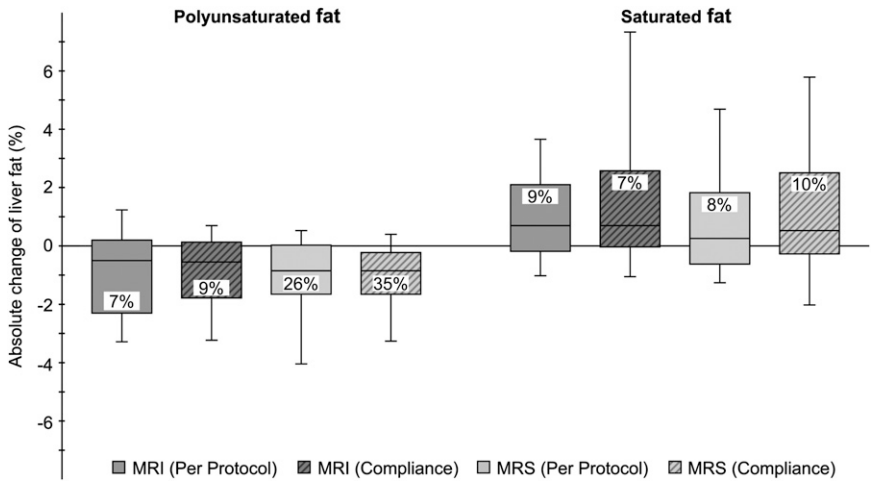

FIGURE 3. Changes in absolute and relative liver fat content during the intervention. The boxes represent the 25th, 50th, and 75th percentiles and reflect absolute changes (percentage units) in liver fat. The lower lines indicate the 10th percentile and the upper lines the 90th percentile. Higher and lower values are not presented in the figure. Numbers within boxes refer to relative changes from baseline. $P \leq 0.03$ for the difference between groups. *P* values were derived from ANCOVA and adjusted for baseline values. Compliant participants were defined according to changes in serum linoleic acid, ie, 18:2n−6 >0.0% during the PUFA diet [n = 26 (MRI), n = 24 (MRS)] and 18:2n−6 <0.0% during the SFA diet [n = 18 (MRI and MRS)]. MRI, magnetic resonance imaging; MRS, magnetic resonance proton (1H) spectroscopy.

Compliance analyses

Results similar to those from the per-protocol analyses were observed for most variables in the compliance analyses (data not shown). However, changes in fasting insulin differed significantly (P = 0.04) between groups [PUFA diet: −0.7 (−14 to 7.2) pmol/L; SFA diet: 14 (−0.7 to 23) pmol/L], whereas other measures of glucose metabolism were nonsignificant ($P \geq 0.08$). Moreover, triglycerides, cholesterol, LDL cholesterol, and the total cholesterol/HDL-cholesterol ratio were significantly lower during the PUFA diet than during the SFA diet (Figure 5).

Adipose tissue gene expression

No effects on adipose tissue mRNA expression were observed for the investigated preselected target genes involved in lipid

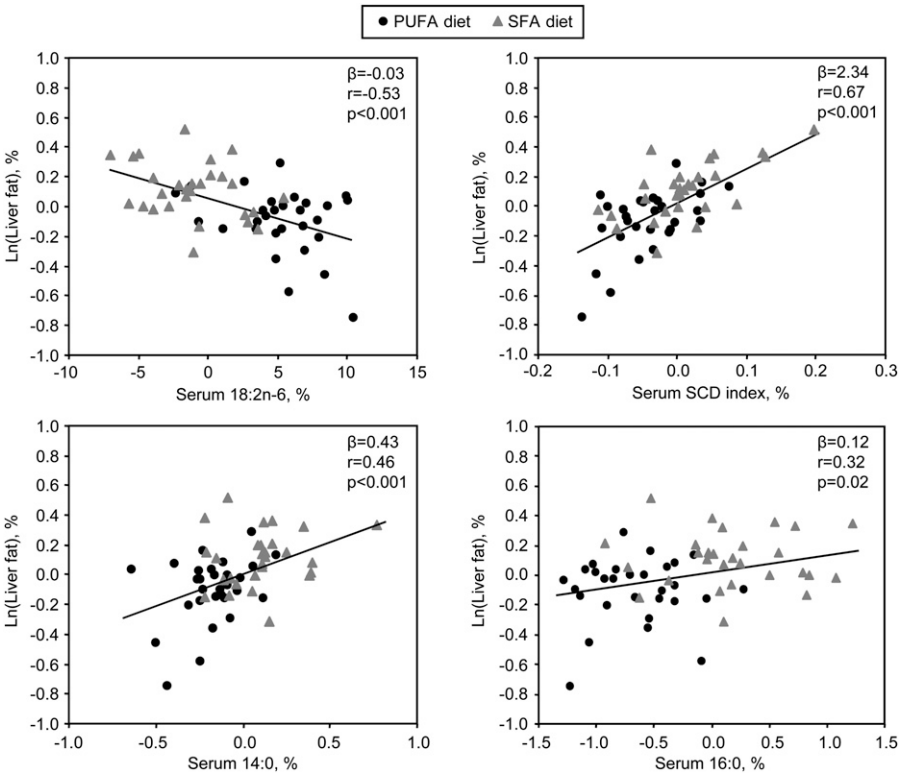

FIGURE 4. Linear regressions showing relations between changes in serum fatty acid composition and changes in logarithmized liver fat measured by magnetic resonance imaging. n = 29 (PUFA diet) and n = 28 (SFA diet). Linear regression and Pearson's correlation were used for the statistical analyses. Fatty acids were measured in serum cholesterol esters. Change denotes measure at follow-up − measure at baseline. SCD, stearoyl-CoA desaturase.

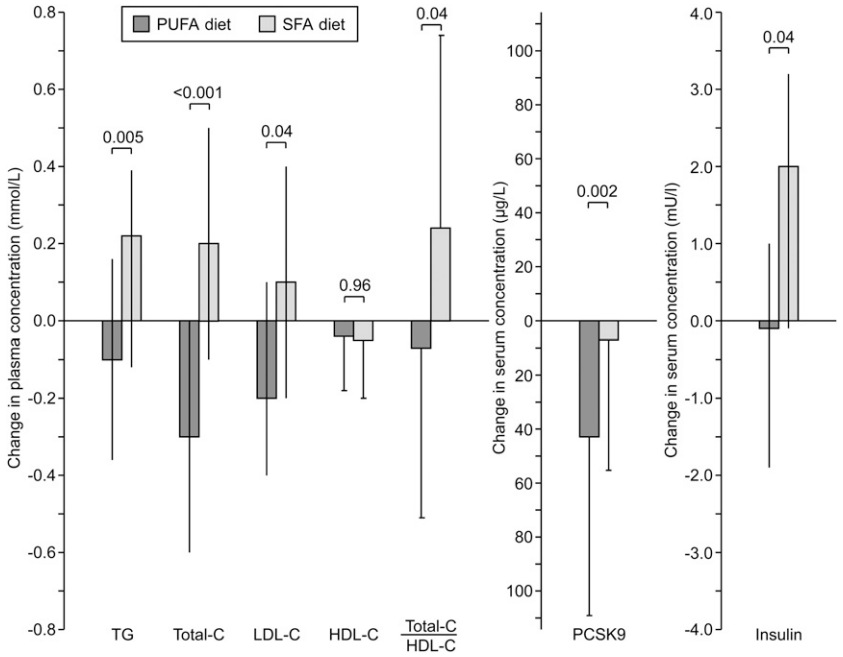

FIGURE 5. Changes in blood lipids and insulin during the intervention in compliant participants. Compliant participants were defined according to changes in serum linoleic acid, ie, 18:2n−6 >0.0% during the PUFA diet (n = 27) and 18:2n−6 <0.0% during the SFA diet (n = 19). P values were derived by ANCOVA and adjusted for baseline values. TG, Total-C, LDL-C, and insulin are skewed and presented as medians (quartiles 1–3). HDL-C, total-C/HDL-C ratio, and PCSK9 are presented as means ± SDs. Change denotes measure at follow-up − measure at baseline. C, cholesterol; PCSK9, protein convertase subtilisin/kexin type 9; TG, triglycerides.

metabolism and inflammation (*see* supplemental material under "Supplemental data" in the online issue).

DISCUSSION

This study provides several new insights concerning the role of n−6 PUFAs in liver fat accumulation, lipoprotein metabolism, and inflammation. First, in the absence of weight loss, a diet high in PUFAs reduced liver fat compared with an SFA-rich diet. Second, this effect was accompanied by a moderate improvement in blood lipids and fasting insulin, which was more evident in compliant subjects. Third, PUFAs decreased serum PCSK9 concentrations—a mechanism potentially explaining the PUFA-induced lowering of plasma LDL cholesterol. Fourth, PUFA had no adverse effects on oxidative stress or inflammation; instead, n−6 PUFA may act as an anti-inflammatory, as evidenced by the reduced TNF-R2 and IL-1RA concentrations.

The reduction in liver fat during the PUFA diet as compared with the SFA diet was observed despite a liver fat content of <5%

TABLE 2
Baseline characteristics and changes in markers of inflammation and oxidative stress during the intervention[1]

	Baseline[2]		Change[3]		
	PUFA diet	SFA diet	PUFA diet	SFA diet	P^4
Plasma C-reactive protein (mg/L)	1.7 (1.3–3.5)[5]	1.7 (0.9–3.4)	−0.2 (−0.6 to 0.5)	0 (−0.5 to 0.7)	0.87
Plasma IL-1β (ng/L)	0.12 (0.12–0.16)	0.14 (0.12–0.28)	0.00 (0.00–0.02)	0.00 (−0.08 to 0.03)	0.36
Plasma IL-6 (ng/L)	1.78 (1.16–2.25)	1.60 (0.99–2.26)	0.00 (−0.36 to 0.45)	0.05 (−0.30 to 0.45)	0.88
Plasma IL-10 (ng/L)	0.77 (0.77–1.41)	0.88 (0.77–1.72)	0.00 (−0.02 to 0.21)	0.00 (0.00–0.21)	0.95
Plasma IL-1RA (ng/L)	289.3 (208.0–497.1)	263.6 (210.1–350.4)	−7.0 (−52.8 to 19.2)	23.0 (−23.7 to 101.8)	0.02
Plasma TNF-R2 (ng/L)	2116 (2009–2501)	2107 (1898–2554)	−108 (−204 to 0)	94 (−22 to 261)	0.005
Urinary 8-iso-$PGF_{2\alpha}$ (nmol/mmol creatinine)	0.56 ± 0.20[6]	0.60 ± 0.18	0.01 ± 0.18	−0.04 ± 0.18	0.46
Urinary 15-keto-dihydro-$PGF_{2\alpha}$ (nmol/mmol creatinine)[7]	0.15 (0.11–0.18)	0.15 (0.12–0.18)	0.00 (−0.03 to 0.02)	−0.01 (−0.04 to 0.02)	0.48

[1] n = 32 (PUFA diet) and n = 29 (SFA diet). IL-1RA, IL-1 receptor antagonist; TNF-R2, TNF receptor-2; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin 2α; 15-keto-dihydro-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-prostaglandin 2α.
[2] The variables did not differ between the groups at baseline.
[3] Change denotes measure at follow-up − measure at baseline.
[4] P values were derived by ANCOVA or the residual method and were adjusted for baseline values.
[5] Median (quartiles 1–3) (all such values).
[6] Mean ± SD (all such values).
[7] The analysis was performed only in compliant participants. Compliance was defined according to changes in serum linoleic acid, ie, 18:2n−6 >0.0% during the PUFA diet (n = 27) and 18:2n−6 <0.0% during the SFA diet (n = 19).

in most participants. Possibly, the effect might have been even larger in subjects with fatty livers (26). The magnitude of the reduction is comparable with the effects of a low-fat or hypocaloric diet (8), exercise (26), and moderate weight loss (4). According to the food records, slight increases in total fat intake were observed with both diets, especially with the SFA diet. Still, effects on liver fat content were diverse during the diets, and adjustment for changes in both total fat and energy intake did not influence the results. Another possible confounder is dietary cholesterol, although there is yet no human data to suggest a direct effect of cholesterol on liver fat content (27).

Clinical trials examining the effects of dietary fats on body fat content are sparse (27). In one study, n−3 PUFA decreased liver fat but, in contrast with our study, that effect may have been caused by weight loss (28). Diets rich in n−6 PUFAs were previously shown to reduce subcutaneous adipose tissue (6) and trunk adipose tissue (7), whereas we observed only a small but significant decrease in the visceral-to-subcutaneous adipose tissue ratio. It has been proposed that n−6 PUFAs may promote adiposity (29)—a view that is apparently not supported by the current or other controlled studies (6, 7).

Circulating PCSK9 and lathosterol, the latter a serum marker for cholesterol synthesis (22), were both reduced during PUFA feeding, which indicates a novel pathway to explain the plasma LDL-cholesterol lowering effect of PUFAs. This finding is of high interest because PCSK9 is a novel drug target and lowering PCSK9 reduces plasma LDL cholesterol (18). Gene activities of both PCSK9 and HMG CoA reductase are regulated by sterol regulatory element-binding protein-2 and, thus, the hepatic cholesterol concentration (30, 31). Therefore, the finding of reduced concentrations of circulating PCSK9 and lathosterol are in line with the notion that PUFA increases hepatic cholesterol, as has also been shown in rats (32), resulting in decreased activity of sterol regulatory element-binding protein-2 and subsequently reduced serum concentrations of PCSK9 and lathosterol. In compliant subjects, the PUFA diet caused an overall improvement in the blood lipid profile, in line with several previous trials in which PUFA was substituted for SFA (14, 33–35). However, the overall effects on LDL cholesterol, and the total/HDL-cholesterol ratio in particular were less pronounced as compared with previous data (14). As judged from the current reported dietary linoleic acid intake and, to some extent, changes in serum linoleic acid, somewhat greater effects would have been expected (14, 25). Because we did not provide meals to the subjects, compliance was probably lower than that in shorter strictly controlled feeding trials (14, 25, 35).

Fasting insulin concentrations tended to increase during the SFA diet as compared with the PUFA diet, which indicated impaired insulin sensitivity. This agrees with a recent 16-wk intervention in which n−6 PUFA improved insulin sensitivity (36). Furthermore, in a 5-wk crossover study, an n−6 PUFA diet improved insulin sensitivity (euglycemic clamp) compared with an SFA diet (6). Note that the current relatively high intake of total fat (~39% of energy) may counteract an insulin-sensitizing effect of unsaturated fats (37). On the other hand, recent epidemiologic data showed that a diet rich in total fat (~40% of energy) was associated with a decreased risk of type 2 diabetes as long as the fat was predominantly plant-based and rich in n−6 PUFAs (38). Whether a PUFA-induced reduction of liver fat could mediate such an association warrants further investigation.

Dietary n−6 PUFA or a high n−6/n−3 ratio has been suggested to increase inflammation and lipid peroxidation through its conversion to arachidonic acid (20). We found no support for such a hypothesis. Despite the marked increase in linoleic acid intake (14% of energy) and the 3.5-fold increase in the dietary n−6/n−3 ratio, serum arachidonic acid concentrations were not elevated. Moreover, neither systemic proinflammatory effects nor signs of free radical–mediated or cyclooxygenase-2–mediated lipid peroxidation were observed. In contrast, IL-1RA and TNF-R2 decreased during the PUFA diet compared with the SFA diet, possibly suggesting antiinflammatory effects of PUFA and/or proinflammatory properties of SFA (20). Notably, these markers are elevated in individuals long before the onset of type 2 diabetes (39, 40). An annual increase in IL-1RA concentrations of 11 ng/L was observed during the 6 y before diabetes onset (39), which suggests that the current changes in IL-1RA (PUFA diet: −7.0 ng/L; SFA diet: +23.0 ng/L) could be clinically relevant. Insufficient statistical power may have been a factor in the lack of effect on other inflammation markers or gene expression in adipose tissue. Possible chance findings due to multiple testing also need to be considered.

The mechanism behind a PUFA-induced reduction in liver fat is unclear. However, long-chain PUFAs are preferentially β-oxidized compared with long-chain SFAs (41). Furthermore, PUFA in contrast with SFA inhibits de novo hepatic fatty acid synthesis and lipogenic gene expression (41–43). The reduction in liver fat was strongly associated with changes in the SCD-1 index, in accordance with observational data (10, 44). The SCD-1 index is associated with hepatic SCD-1 expression in humans (45) and with SCD-1 activity in animals (46, 47); however, this index should still be regarded as a marker of SCD-1 activity from which we cannot draw conclusions on enzyme activity (48). This finding may, however, imply that the SCD-1 index may be a useful serum marker of liver fat content. A decreased serum SCD-1 index during the PUFA diet was not reflected by reduced adipose tissue SCD-1 expression, which indicates that it may mainly reflect hepatic SCD-1 activity, in accordance with previous data (45–47).

This study was limited by a nonblinded design, which was not feasible because of the diets the subjects were advised to eat. The MR analyses and primary statistical analyses were, however, blinded. Nonstandardized diets may increase bias but may also underestimate the effects and reflect realistic changes that can be achieved in clinical practice. Strengths of the study included the assessment of liver fat by both MRI and MRS. Although MRS is considered the gold standard, MRI analysis includes the whole liver and may better represent total liver fat. A limitation was that the imaging and spectroscopy methods used did not include full characterization of all lipid resonances of the liver spectra and therefore did not allow for a more detailed analysis of changes in liver lipid saturation, for example. We therefore cannot completely exclude the possibility that our liver fat measures were biased by changes in lipid saturation levels. The significant correlation between changes in liver fat and changes in plasma ALT concentrations does not, however, support such a possibility, similarly to morphologic and histologic data in rodents that showed a reduction in liver fat content after PUFA feeding (49, 50). The low dropout rate strengthens the data, and the inclusion of free-living subjects with diabetes, hypertension, and dyslipidemia increases the generalizability of the data. Also, this study

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In conclusion, compared with SFAs, dietary n−6 PUFAs reduce liver fat in overweight individuals in the absence of weight loss. This difference was observed even though both diets were rather high in total fat. These results have potential implications for public health, considering the high prevalence of NAFLD; however, the findings need to be confirmed in other populations and in NAFLD patients. A reduced plasma PCSK9 concentration could be a novel mechanism behind the plasma total and LDL-cholesterol-lowering effects of PUFAs.

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