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Overfeeding Polyunsaturated and Saturated Fat Causes Distinct Effects on Liver and Visceral Fat Accumulation in Humans

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OBESITY STUDIES

Excess ectopic fat storage is linked to type 2 diabetes. The importance of dietary fat composition for ectopic fat storage in humans is unknown. We investigated liver fat accumulation and body composition during overfeeding saturated fatty acids (SFAs) or polyunsaturated fatty acids (PUFAs). LIPOGAIN was a double-blind, parallel-group, randomized trial. Thirty-nine young and normal-weight individuals were overfed muffins high in SFAs (palm oil) or n-6 PUFAs (sunflower oil) for 7 weeks. Liver fat, visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (SAT), total adipose tissue, pancreatic fat, and lean tissue were assessed by magnetic resonance imaging. Transcriptomics were performed in SAT. Both groups gained similar weight. SFAs, however, markedly increased liver fat compared with PUFAs and caused a twofold larger increase in VAT than PUFAs. Conversely, PUFAs caused a nearly threefold larger increase in lean tissue than SFAs. Increase in liver fat directly correlated with changes in plasma SFAs and inversely with PUFAs. Genes involved in regulating energy dissipation, insulin resistance, body composition, and fat-cell differentiation in SAT were differentially regulated between diets, and associated with increased PUFAs in SAT. In conclusion, overeating SFAs promotes hepatic and visceral fat storage, whereas excess energy from PUFAs may instead promote lean tissue in healthy humans.

Fat accumulation in the liver, pancreas, and abdomen may have long-term, adverse metabolic consequences (1–3). Although obesity is a major health concern, abdominal obesity is of greater clinical relevance. Accumulation of liver fat, including nonalcoholic fatty liver disease (NAFLD), is present in ~25% of adults in Western countries and has been proposed as a causative factor in the development of cardiometabolic disorders and type 2 diabetes (4–8). In obesity, the prevalence of NAFLD is extremely high and may reach 75% (9). Thus, liver fat may be a key target in the prevention and treatment of metabolic diseases. Why certain individuals deposit liver fat to a larger extent than others during weight gain is unknown. High-fat diets have been shown to increase liver fat in both humans and rodents when compared with low-fat diets (10–12). Cross-sectional data suggest that dietary fat composition could play a key role in liver fat accumulation with polyunsaturated fatty acids (PUFAs) inversely (13) and saturated fatty acids (SFAs) directly associated with liver fat and liver fat markers (14,15). In addition, animals fed high-fat diets with PUFAs reduced body and liver fat accumulation compared with SFA diets (16–21). In the recent HEPFAT trial, we showed that an isocaloric diet rich in PUFAs given for 10 weeks reduced liver fat content and tended to reduce insulin resistance compared with a diet rich in SFAs in individuals with abdominal obesity and type 2 diabetes (22).

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Overweight and obesity are mainly results of long-term energy excess. To prevent early excessive adiposity and its metabolic consequences, it is necessary to investigate dietary factors that could initially influence body fat accumulation and ectopic fat storage. We hypothesized that liver fat accumulation during moderate weight gain could be counteracted if the excess energy originates mainly from PUFAs rather than from SFAs. The aim was to investigate the effects of excess intake of the major n-6 PUFAs in the diet, linoleic acid, or the major SFAs in the diet, palmitic acid, on liver fat accumulation, body composition, and adipose tissue gene expression in healthy, normal-weight individuals.

RESEARCH DESIGN AND METHODS

Participants

Healthy, normal-weight men and women were recruited by local advertising. Inclusion criteria were age 20–38 years, BMI 18–27 kg/m^2, and absence of diabetes and liver disease. Exclusion criteria included abnormal clinical chemistry, alcohol or drug abuse, pregnancy, lactation, claustrophobia, intolerance to gluten, egg, or milk protein, use of drugs influencing energy metabolism, use of n-3 supplements, and regular heavy exercise (>3 h/week). Subjects were instructed to maintain their habitual diet and physical activity level throughout the study. Subjects were fasted for 12 h before measurements and discouraged from physical exercise or alcohol intake 48 h before measurements.

Study Design

The LIPOGAIN study was a 7-week, double-blind, randomized, controlled trial with parallel group design in free-living subjects. The study was carried out from August through December 2011 at the Uppsala University Hospital, Uppsala, Sweden. Subjects were randomized by drawing lots, with a fixed block size of 4 and allocation ratio 1:1. Subjects were stratified by sex and were unaware of the block size. The allocation sequence was only known by one of the researchers (F.R.) but concealed from all other investigators and participants. Double-blinding was ensured by labeling, and the code was concealed from all investigators until the study was finalized.

Dietary Intervention

Forty-one participants were randomized to eat muffins containing either sunflower oil (high in the major dietary PUFA linoleic acid, 18:2 n-6) or palm oil (high in the major SFA palmitic acid, 16:0). Both oils were refined. Body weight was measured, and muffins were provided to participants weekly at the clinic. Muffins were baked in large batches under standardized conditions in a metabolic kitchen at Uppsala University. Muffins were added to the habitual diet, and the amount was individually adjusted to achieve a 3% weight gain. The amount of muffins consumed per day was individually adjusted weekly (i.e., altered by ±1 muffin/day depending on the rate of weight gain of the individual). Subjects were allowed to eat the muffins anytime during the day. Except for fat quality, the muffins were identical with regard to energy, fat, protein, carbohydrate, and cholesterol content, as well as taste and structure. The composition of the muffins provided 51% of energy from fat, 5% from protein, and 44% from carbohydrates. The sugar to starch ratio was 55:45. We chose palm oil as the source of SFA for several reasons; it is particularly high in palmitic acid and low in linoleic acid and is widely used in various foods globally. Sunflower oil was chosen as the source of PUFA because it is high in linoleic acid (the major PUFA in Western diet) but low in palmitic acid. Both oils were devoid of cholesterol and n-3 PUFAs, thus avoiding potential confounding of these nutrients.

Outcome Measures

The primary outcome of this study was liver fat content (determined by magnetic resonance imaging [MRI]). Secondary outcomes included other body fat depots (MRI and Bod Pod; COSMED, Fridolfing, Germany), total body fat (MRI and Bod Pod), and lean tissue (MRI and Bod Pod). All outcome measures were measured at two time points: at baseline and at the end of the intervention. MRI was the primary assessment method.

Assessments of Liver Fat, Pancreatic Fat, and Body Composition

Liver fat content, pancreas fat content, and body composition were assessed by MRI using a 1.5T Achieva clinical scanner (Philips Healthcare, Best, the Netherlands) modified to allow arbitrary table speed. Collection and analyses of the MRI data were performed by two operators at one center under blinded conditions. The coefficients of variation between the two operators were 2.14 ± 2.14%, and the results from the two operators did not differ significantly ($P > 0.4$). The average from the two operators was used. Body composition was also measured using whole-body air displacement plethysmography (Bod Pod) according to the manufacturer's instructions. Pancreas fat content was assessed by duplicate measurements (SD 0.36%), and the average was used. The same images were used as from the liver fat measurements. The operator was trained by an experienced radiologist. Total-body water content was measured by bioelectrical impedance analysis (Tanita BC-558; Tanita Corporation, Tokyo, Japan).

Global Transcriptome Analysis of Adipose Tissue

Adipose tissue biopsies were taken subcutaneously, 3 to 4 cm below and lateral to the umbilicus by needle aspiration under local anesthesia (1% lidocaine). The samples were washed with saline, quickly frozen in dry ice covered with ethanol, and stored at −70°C until analysis. Hybridized biotinylated complementary RNA was prepared from total RNA and hybridized to a GeneChip Human Gene 1.1 ST Array (Affymetrix Inc., Santa Clara, CA) using standardized protocols. The microarray data have been submitted to

the Gene Expression Omnibus in a Minimum Information About a Microarray Experiment–compliant format (accession number GSE43642).

Assessment of Fat Oxidation

D-3-hydroxybutyrate was analyzed as a marker of hepatic β-oxidation using a kinetic enzymatic method utilizing Ranbut reagent (RB1008; Randox Laboratories, Crumlin, U.K.) on a Mindray BS-380 chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics, Shenzhen, China). All samples were analyzed in a single batch.

Dietary Assessment, Physical Activity, and Compliance

Dietary intake was assessed by 4-day weighed food records (at baseline and week 7), and processed with Dietist XP version 3.1 dietary assessment software. During these 4-day periods, subjects wore accelerometers (Philips Respironics, Andover, MD) on their right ankle to assess physical activity. Food craving, hunger, and satiety were assessed in the morning (only at week 7) by the Food Craving Inventory and Visual Analog Scales, respectively. Fatty acid composition was measured in the intervention oils as well as in plasma cholesterol esters and adipose tissue triglycerides by gas chromatography as previously described (22,23). Hepatic stearoyl-CoA desaturase-1 (SCD-1) activity was estimated as the 16:1n-7/16:0 ratio in cholesterol esters (22).

Biochemical Measures

Fasting concentrations of plasma glucose and serum insulin were measured as previously described (22), and homeostasis model assessment of insulin resistance was calculated (24). Plasma total adiponectin concentrations were measured by ELISA (10–1193–01; Mercodia, Uppsala, Sweden).

Statistical Analysis

Based on previous data (22), 22 subjects per group were needed to detect a 1.5% difference between groups in liver fat with $\alpha = 0.05$ and $\beta = 0.2$. Differences in changes between groups were analyzed per protocol with the Student *t* test. Nonparametric variables were log-transformed or analyzed nonparametrically (e.g., liver fat) with a Mann-Whitney *U* test if normality was not attained by the Shapiro-Wilk test and Q-Q plots. CIs were, however, obtained using *t* test calculations for all variables. Data are given as mean ± SD or median (interquartile range [IQR]). Correlations between outcome variables and fatty acids are given as Pearson *r* or Spearman ρ. A *P* value <0.05 was considered statistically significant. SPSS version 21 (SPSS Inc.) and JMP version 10.0.0 were used for analyzing data. Significance analysis of microarrays (SAM) was used to compare gene expression between groups.

Ethics

This study was conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent prior to inclusion, and the study was approved by the regional ethics committee.

RESULTS

Of the 55 participants assessed for eligibility, 41 were randomized, but 2 dropped out before the study started, leaving 39 participants with baseline data. All 39 participants completed the study (Fig. 1). One individual from each group was excluded from the primary analyses due to considerable and unexplained weight loss during the intervention (>3 SD below the mean weight gain, more than can be attributed to day-to-day variation). Including those two outliers, however, did not affect the results, except for differences between groups for the Bod Pod analyses, which were no longer statistically significant in the intention-to-treat analysis. Presented data are thus based on 37 participants who were considered compliant with the intervention. The mean age (26.7 ± 4.6 vs. 27.1 ± 3.6 years) and sex distribution (5:13 vs. 6:13 women/men, respectively) were similar between the PUFA and SFA groups. Fatty acid composition of the intervention oils is shown in Table 1. Baseline characteristics regarding body composition are shown in Table 2.

Weight Gain, Body Composition, and Fat Oxidation

Both groups gained 1.6 kg in weight; however, the MRI assessment showed that the SFA group gained more liver fat, total fat, and visceral fat, but less lean tissue compared with subjects in the PUFA group (Table 2). Relative changes are shown in Fig. 2. The ratios of lean/fat tissue gained in the PUFA and SFA groups were ~1:1 and 1:4, respectively. Pancreatic fat decreased by 31% ($P = 0.008$) in both groups combined, but without significant differences between groups ($P = 0.75$, data not shown). D-3-hydroxybutyrate decreased by 0.11 (0.15) mmol/L or −70% and 0.05 (0.09) mmol/L or −45% in the PUFA and SFA groups, respectively, without significant difference between groups ($P = 0.14$). When total-body water content was taken into account by using a three-compartment model for assessment of fat and lean tissue, the results remained and were even strengthened (data not shown).

Dietary Intake and Physical Activity

Both groups consumed on average 3.1 ± 0.5 muffins/day, equaling an additional 750 kcal/day. Both groups increased their energy intake comparably, without any differences in macronutrient intake during the study (Table 3). Food craving, hunger, and satiety showed no differences between groups (data not shown). In both groups combined, energy expenditure due to physical activity was 1,039.7 ± 112.5 kcal at baseline, and the total energy expenditure at baseline was 2,683.9 ± 245.3 kcal, without differences between groups. Physical activity did not change or differ between groups ($P = 0.33$) during the intervention (data not shown).

Plasma and Tissue Fatty Acid Composition

Changes in fatty acid composition in plasma as well as adipose tissue reflected dietary intakes, indicating high compliance (Table 4). In addition to the dietary biomarkers,

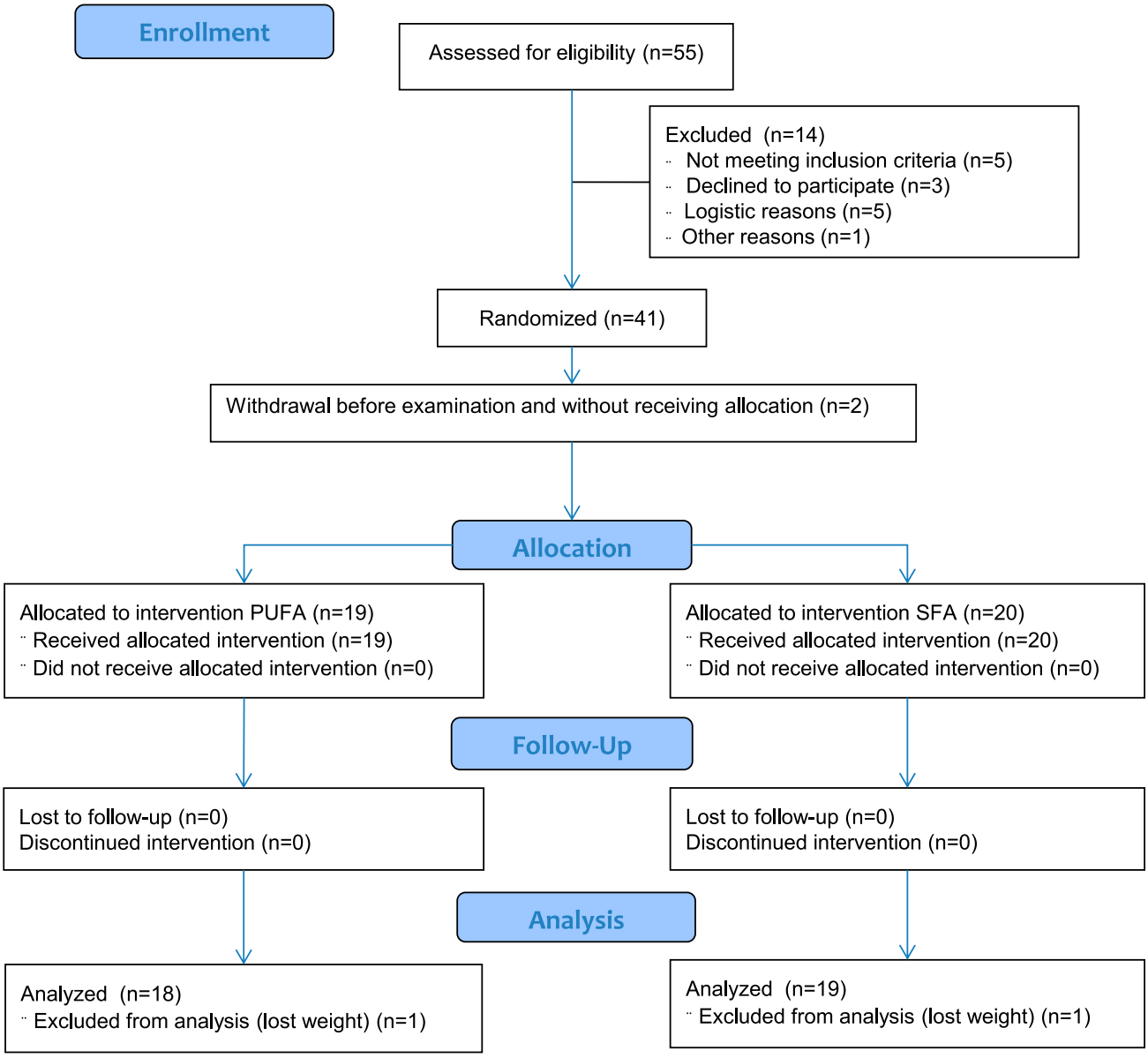

Figure 1—Flow diagram for the LIPOGAIN trial.

the estimated SCD-1 activity in plasma cholesterol esters was decreased by PUFAs (Table 4). Changes in liver fat and visceral fat and total adipose tissue (TAT) were directly associated with changes in plasma palmitic acid, whereas liver fat and TAT were inversely associated with linoleic acid. The SCD-1 index was associated with change in liver fat. Changes in lean tissue were inversely associated with changes in palmitic acid and directly with linoleic acid (Fig. 3).

Transcriptomics

Comparison of adipose tissue gene expression between groups at baseline revealed no significant differences in gene expression (false discovery rate [FDR] 50%). Absolute differences in gene expression were calculated for each gene in each subject, comparing after with before intervention. These absolute differences in gene expression were compared between intervention groups with SAM. Twelve genes were significantly differently expressed with FDR 25% and 8 with FDR 0% (Table 5). These absolute differences in gene expression were next adjusted for weight gain and compared between PUFAs and SFAs. Altogether, 20 genes were differentially regulated between groups PUFA and SFA according to SAM (FDR 25%), including the 12 genes previously discovered (Table 5). Five genes that were most differently expressed between groups were selected for PCR confirmation; three genes were confirmed (carbonic anhydrase 3 [CA3]; connective tissue growth factor [CTGF]; and aldehyde dehydrogenase 1 family member A1 [ALDH1A1]), and one

Table 1—Fatty acid composition of the intervention oils

	Sunflower oil	Palm oil
8:0	0.02	0.02
10:0	0.02	0.04
12:0	0.05	0.31
14:0	0.08	1.1
16:0	6.2	47.5
16:1	0.08	0.16
18:0	4.1	4.2
18:1	23.8	37.2
18:2 n-6	65.3	8.9
18:3 n-3	0.08	0.23
20:0	0.26	0.32
20:3 n-6	—	—
20:4 n-6	—	—
20:5 n-3	—	—
22:6 n-3	—	—
Total SFAs	10.7	53.5
Total MUFAs	23.9	37.4
Total PUFAs	65.4	9.2

Fatty acids are reported as a percentage of all fatty acids assessed by gas chromatography. MUFA, monounsaturated fatty acid.

gene showed a trend of expression in the same direction (phosphodiesterase 8B [PDE8B]; one-sided $P = 0.21$). 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 1 could not be confirmed.

Changes in mRNA expression among several of the genes selected for PCR confirmation were associated with changes in target fatty acids in subcutaneous adipose tissue (SAT). CA3 was inversely associated with SCD-1 index ($r = -0.46$; $P = 0.004$) and directly associated with linoleic acid ($r = 0.45$; $P = 0.006$). PDE8B was directly associated with linoleic acid ($r = 0.51$; $P = 0.002$) and inversely with palmitic acid ($r = -0.35$; $P = 0.035$). CTGF was inversely but not significantly associated with linoleic acid ($\rho = -0.32$; $P = 0.06$) and directly with palmitic acid ($r = 0.34$; $P = 0.04$). ALDH1A1 was inversely associated with linoleic acid ($r = -0.39$; $P = 0.02$) and directly with SCD-1 index ($r = 0.37$; $P = 0.03$).

Glucose, Insulin, and Adiponectin

Fasting plasma glucose was 4.6 (4.4–5.0) mmol/L and 4.5 (4.3–4.9) mmol/L in PUFA and SFA groups at baseline, respectively ($P = 0.69$), and was virtually unchanged during the intervention: 0.06 ± 0.3 mmol/L and −0.06 ± 0.4 mmol/L in PUFA and SFA groups, respectively ($P = 0.53$ for difference between groups). Fasting serum insulin was 5.8 ± 2.7 and 5.0 ± 2.0 mU/L in the PUFA and SFA groups at baseline, respectively ($P = 0.33$), and increased to a similar extent in both groups: 0.92 ± 2.2 and 0.94 ± 1.3 in PUFA and SFA groups, respectively ($P = 0.97$). Homeostasis model assessment of insulin resistance was 1.23 ± 0.63 and 1.04 ± 0.43 in PUFA and SFA groups at baseline, respectively ($P = 0.28$), and increased to a similar extent in both groups during the intervention: 0.22 ± 0.49 and 0.18 ± 0.30 in the PUFA and SFA groups, respectively ($P = 0.79$). Adiponectin was 8.5 (6.1–9.6) and 6.4 (5.4–9.4) in the PUFA and SFA groups at baseline, respectively ($P = 0.24$), and increased by 0.92 ± 1.46 and 0.42 ± 0.94, respectively ($P = 0.34$).

DISCUSSION

Despite comparable weight gain after 49 days, this double-blind trial showed that overeating energy from PUFAs prevented deposition of liver fat and visceral and total fat compared with SFAs. Excess energy from SFAs caused an increase of liver fat compared with PUFAs. Further, the inhibitory effect of PUFAs on ectopic fat was accompanied by an augmented increase in lean tissue and less total body fat deposition compared with SFAs. Thus, the type of fat in the diet seems to be a novel and important determinant of liver fat accumulation, fat distribution, and body composition during moderate weight gain. We also observed fatty acid–dependent differences in adipose tissue gene expression. The significant decrease in pancreatic fat in both groups during weight gain was an unexpected finding that needs confirmation due to the low amounts of pancreatic fat in this lean population.

Cross-sectional studies have shown that patients with higher SFA and lower PUFA intake have increased liver fat content (13,15,25), which is also in accordance with lower PUFA levels in fatty livers (14,26). A previous isocaloric trial in abdominally obese subjects indicated that the present associations may be causal, since replacing SFAs from butter with PUFAs from sunflower oil reduced liver fat (20,22). Thus, together these trials indicate that SFAs (high in 16:0) per se might promote hepatic steatosis, both during isocaloric and hypercaloric conditions. These results also support the current nutritional recommendations in general (i.e., to partly replace SFAs with PUFAs). PUFAs (i.e., linoleic acid) are found in plant-based foods such as nuts, seeds, and nontropical vegetable oils (27). Increased intake of these foods has in general been associated with cardiometabolic benefits including lowering blood lipids and reduced risk of cardiovascular disease and type 2 diabetes (27–29). There are, however, no clear reasons to believe that sunflower oil would be more effective in preventing liver fat accumulation than other PUFA-rich oils and fats.

The mechanisms behind the differential effects on liver fat deposition are unknown, but may involve differences in hepatic lipogenesis and/or fatty acid oxidation and storage (30). In NAFLD patients, increased de novo lipogenesis is a major contributor to liver fat accumulation and steatosis (31,32). In the current study, a fructose–SFA interaction on liver fat is possible since the muffins contained significant amounts of fructose (33). Early animal data showed that carbohydrate-induced lipogenesis

Table 2—Liver fat and body composition before and after 7 weeks of PUFA or SFA overeating

	PUFA (n = 18) baseline	Mean absolute change	SFA (n = 19) baseline	Mean absolute change	Mean difference in change (95% CI)	P value
Body weight, kg	67.4 ± 8.2	1.6 ± 0.85	63.3 ± 6.8	1.6 ± 0.96	−0.02 (−0.63 to 0.58)	0.94
BMI, kg/m^2	20.8 (19.5–23.1)	0.5 ± 0.3	19.9 (18.9–20.7)	0.5 ± 0.3	0.01 (−0.18 to 0.20)	0.98
Waist girth, cm	79.4 ± 5.6	0.97 ± 2.2	76.1 ± 5.1	1.0 ± 2.3	−0.03 (−1.53 to 1.47)	0.97
Liver fat, % (MRI)	0.75 (0.65–1.0)	0.04 ± 0.24	0.96 (0.79–1.1)	0.56 ± 1.0	−0.52 (−1.0 to −0.01)	0.033
Lean tissue, L (MRI)	43.4 ± 8.4	0.86 ± 0.62	41.8 ± 6.9	0.31 ± 0.68	0.55 (0.11–0.98)	0.015
VAT, L (MRI)	0.99 (0.50–1.6)	0.11 ± 0.21	0.81 (0.52–1.0)	0.22 ± 0.16	−0.12 (−0.24 to 0.01)	0.035
VAT/SAT ratio (MRI)*	0.08 ± 0.04	0.00 ± 0.01	0.07 ± 0.03	0.01 ± 0.01	−0.01 (−0.02 to 0.00)	0.073
Abdominal SAT, L (MRI)	2.2 (1.9–3.1)	0.25 ± 0.32	1.8 (1.5–2.8)	0.34 ± 0.23	−0.09 (−0.27 to 0.10)	0.32
Total body fat, L (MRI)	14.4 (12.6–19.6)	0.97 ± 1.0	12.9 (10.4–18.2)	1.5 ± 0.70	−0.57 (−1.2 to 0.01)	0.013
Lean tissue, % (Bod Pod)	81.9 ± 6.3	−0.81 ± 1.2	85.6 ± 7.4	−1.7 ± 1.1	0.93 (0.15–1.70)	0.021
Total body fat, % (Bod Pod)	18.1 ± 6.3	0.81 ± 1.2	14.4 ± 7.4	1.7 ± 1.1	−0.93 (−1.70 to −0.15)	0.021

Data are means ± SD or median (IQR) and 95% CI. For nonnormal variables, P values are obtained from nonparametric analyses. Mean difference in change is calculated as mean absolute change in PUFAs minus mean absolute change in SFAs. *Calculated as VAT/(TAT − VAT).

was inhibited by adding linoleic acid, whereas palmitate had no effect (34), and SFAs have enhanced steatosis and increased hepatic lipogenesis compared with PUFAs (20,21). Hepatic activity of the lipogenic enzyme SCD-1 may be elevated in steatosis (26). Also, SCD-1–deficient mice were protected against hepatic lipogenesis, whereas SCD-1 inhibitors markedly reduced hepatic triglyceride accumulation (35). In humans, a strong association between the change in liver fat and the change in hepatic SCD-1 index was reported in weight-stable subjects (22), a finding currently confirmed during hypercaloric conditions.

PUFAs are more readily oxidized than SFAs (36–38), thereby potentially lowering hepatic exposure to nonesterified fatty acids, a major substrate in triglyceride synthesis. Concentrations of D-3-hydroxybutyrate were, however, if anything, lower with PUFAs than SFAs, thus not supporting a differential effect on hepatic fat oxidation. Animal studies have also indicated that SFAs, compared with PUFAs, lower brown tissue adipose activity and thermogenesis (16–19,39–45).

The increase in lean tissue was nearly threefold higher during PUFA overeating compared with SFA. Although lean tissue was a secondary outcome, this finding is intriguing since obese persons with reduced lean tissue (sarcopenic obesity) are more insulin-resistant and at higher risk for physical disability (46,47). A previous supplementation trial in postmenopausal women reported that a daily dose of 8 g PUFA (safflower oil) increased lean tissue and reduced trunk fat (48). In accordance, rats isocalorically fed with PUFAs (high in linoleic acid) gained more lean tissue and less fat compared with an SFA-rich diet, in line with similar studies (16,17,49,50). The mechanism behind these observations remains to be determined. The differential increase in lean tissue was consistent when assessed by two different methods (MRI and Bod Pod). This difference was unlikely an artifact due to changes in total-body water content since the results were similar in the three-compartment model. Although supported by animal studies, this finding needs to be replicated in additional human studies.

In the current study, n-6 PUFAs were investigated, but it is possible that n-3 PUFAs have similar effects on body fat accumulation (50–52). The amount of sunflower oil used in the current study (~40 g per day) corresponds to about three times the customary intake of linoleic acid in the Swedish population. Given that palm oil was used as the SFA source, the wide use of this oil by the food industry may be of concern. In fact, palm oil is one of the most used oils worldwide, suggesting a potential global impact if it promotes adiposity. The health effects of palm oil, however, remain uncertain and should be further investigated. The effects on ectopic fat deposition observed in this study, however, do not seem to be palm oil–specific, but rather SFA- or palmitate-specific since we previously showed similar results during isocaloric conditions using butter as the source of SFAs (22).

Given the different influence on fat deposition, we expected diet-specific influences on adipose gene expression. Overall, differences in SAT gene expression between diets were modest, which may relate to similar weight gain and little differences in SAT. Although speculative, downregulation of ALDH1A1 by PUFAs might be relevant, as this gene inhibits energy dissipation and promotes fat

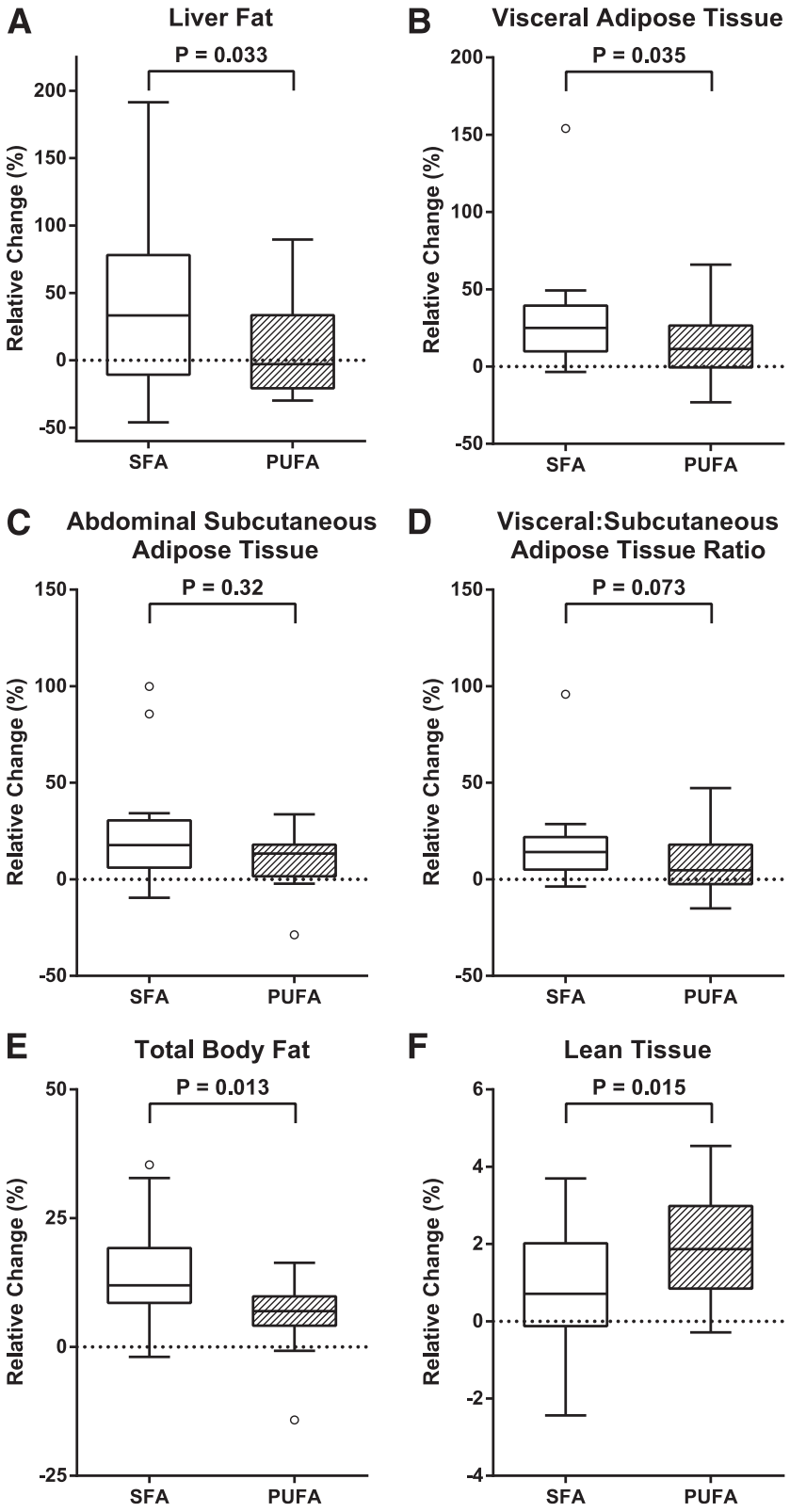

Figure 2—Relative changes in liver fat and body composition by MRI during 7 weeks of overeating SFAs or PUFAs. *A–F*: Relative changes are calculated for each individual as change during the intervention/baseline measurement. Boxes represent medians and IQRs, whiskers represent the most extreme value besides outliers, and circles represent outliers (>1.5 IQRs outside IQR). *P* values represent between-group *t* test or Mann-Whitney *U* test. *A*: Change in liver fat is in percentage. *B*, *C*, *E*, and *F*: Changes are in liters. *D*: VAT/SAT ratio is calculated as VAT/(TAT − VAT).

Table 3—Dietary intake data before and after 7 weeks of overeating PUFA or SFA

	PUFA (n = 18)		SFA (n = 19)			
	Baseline	Mean absolute change	Baseline	Mean absolute change	Mean difference in change (95% CI)	P value
Dietary intake						
Energy, kcal	2,504 ± 525	632 ± 499.5	2,535.1 ± 591	500 ± 550	132.2 (−218.3 to 482.6)	0.45
Carbohydrates, E%	44.7 ± 8.9	−1.4 ± 6.3	50.2 ± 8.8	−2.5 ± 5.2	1.1 (−2.8 to 4.9)	0.57
Protein, E%	14 (13–16)	−2.2 ± 4	14 (14–17)	−2.5 ± 2.8	0.3 (−2.1 to 2.6)	0.83
Fat, E%	35.3 ± 5.1	5 ± 5.8	31.7 ± 6.9	5.1 ± 5.6	−0.1 (−3.9 to 3.8)	0.98
SFA, E%	13.1 ± 2.6	−1.6 ± 2.8	11.5 ± 3.6	4.9 ± 2.8	−6.5 (−8.3 to −4.6)	0.0001
MUFA, E %	11.5 ± 2.5	0.9 ± 2.7	9.9 ± 2.8	3 ± 2.7	−2.1 (−3.9 to −0.3)	0.026
PUFA, E%	5 ± 1.5	7.9 ± 2.1	4.2 ± 1.4	0.3 ± 1.3	7.6 (6.4–8.8)	0.0001
Alcohol, E%	2 (0–8.5)	−1.5 ± 5.5	1 (0–3)	−0.2 ± 2.3	−1.3 (−4.2 to 1.6)	0.37

Data are means ± SD or median (IQR) and 95% CI. For nonnormal variables, P values are obtained from nonparametric analyses. Mean difference in change is calculated as mean absolute change in PUFAs minus mean absolute change in SFAs. E%, energy percent; MUFA, monounsaturated fatty acid.

storage (53). Interestingly, ALDH1A1-deficient mice are protected from diet-induced liver fat accumulation and insulin resistance (53). The observed associations between changes in SAT fatty acids and mRNA expression support a direct influence of the fatty acids consumed on adipose tissue gene expression. For example, ALDH1A1 was inversely associated with changes in linoleic acid, but directly associated with the SCD-1 index. As gene expression

Table 4—Fatty acid composition in CE and SAT before and after 7 weeks of overeating PUFA or SFA

	PUFA (n = 18)		SFA (n = 19)		Mean difference in change (95% CI)	P value
	Baseline	Mean change	Baseline	Mean change		
CE						
14:0	0.61 ± 0.18	−0.04 ± 0.16	0.62 ± 0.13	−0.07 ± 0.09	0.03 (−0.06 to 0.12)	0.5
15:0	0.28 ± 0.06	−0.03 ± 0.04	0.31 ± 0.07	−0.06 ± 0.05	0.03 (−0.004 to 0.06)	0.09
16:0	10.6 ± 0.55	−0.92 ± 0.58	10.6 ± 0.57	0.3 ± 0.59	−1.2 (−1.61 to −0.83)	0.0001
16:1	1.9 ± 0.45	−0.52 ± 0.36	2.0 ± 0.62	0.03 ± 0.46	−0.55 (−0.83 to −0.27)	0.0003
18:0	0.84 ± 0.17	−0.11 ± 0.12	0.74 ± 0.13	−0.06 ± 0.12	−0.05 (−0.13 to 0.03)	0.19
18:1	21.8 ± 1.96	−3.9 ± 1.12	21.6 ± 1.83	−0.02 ± 1.23	−3.9 (−4.7 to −3.13)	0.0001
18:2 n-6	52.5 (51.63–55.08)	6.4 ± 2.12	53.3 (51.32–55.66)	0.59 ± 2.29	5.8 (4.34–7.29)	0.0001
18:3 n-6	0.73 ± 0.28	0.06 ± 0.25	0.71 ± 0.26	0.07 ± 0.28	−0.009 (−0.19 to 0.17)	0.92
18:3 n-3	0.84 ± 0.19	−0.2 ± 0.14	0.97 ± 0.2	−0.12 ± 0.17	−0.07 (−0.17 to 0.03)	0.17
20:3 n-6	0.59 ± 0.11	0.03 ± 0.08	0.66 ± 0.17	0.02 ± 0.12	0.007 (−0.06 to 0.07)	0.84
20:4 n-6	6.3 ± 1.06	−0.02 ± 0.75	6.01 ± 1.38	−0.67 ± 0.6	0.64 (0.19–1.1)	0.007
20:5 n-3	1.3 ± 0.54	−0.56 ± 0.56	1.1 ± 0.44	0.03 ± 0.6	−0.6 (−0.98 to −0.21)	0.004
22:6 n-3	0.74 ± 0.21	−0.16 ± 0.13	0.74 ± 0.19	−0.05 ± 0.2	−0.11 (−0.22 to 0.008)	0.07
SCD-1	0.18 ± 0.04	−0.04 ± 0.03	0.19 ± 0.06	−0.002 ± 0.04	−0.03 (−0.06 to −0.009)	0.008
SAT						
14:0	4.0 ± 0.70	−0.3 ± 0.37	4.0 ± 0.78	−0.16 ± 0.23	−0.14 (−0.35 to 0.07)	0.18
15:0	0.35 ± 0.08	−0.02 ± 0.02	0.35 ± 0.07	−0.02 ± 0.02	−0.006 (−0.02 to 0.008)	0.42
16:0	21.7 ± 1.55	−1.1 ± 0.66	21.7 ± 1.66	1.2 ± 1.18	−2.3 (−2.9 to −1.6)	0.0001
16:1	4.3 ± 1.05	−0.02 ± 0.41	4.4 ± 0.58	0.46 ± 0.52	−0.49 (−0.81 to −0.17)	0.004
17:0	0.32 ± 0.08	−0.03 ± 0.05	0.31 ± 0.05	−0.03 ± 0.02	0 (0.03 to −0.03)	1.0
18:0	5.0 ± 0.96	−0.35 ± 0.49	4.9 ± 0.81	−0.44 ± 0.5	0.1 (−0.24 to 0.43)	0.56
18:1	50.2 ± 2.41	−0.63 ± 0.83	50.4 ± 2.14	−0.88 ± 1.0	0.26 (−0.34 to 0.88)	0.41
18:2 n-6	11.6 (11.15–13.14)	2.4 ± 1.05	11.2 (10.15–12.78)	−0.11 ± 0.28	2.5 (1.97–3.04)	0.0001
18:3 n-6	0.1 (0.09–0.11)	0.009 ± 0.05	0.1 (0.09–0.16)	0.004 ± 0.03	0.006 (−0.02 to 0.03)	0.69
18:3 n-3	1.1 ± 0.18	0.02 ± 0.08	1.2 ± 0.21	0.008 ± 0.09	0.009 (−0.05 to 0.06)	0.75
20:3 n-6	0.13 ± 0.02	0.002 ± 0.009	0.13 ± 0.04	−0.005 ± 0.009	0.007 (0.001–0.01)	0.018
20:4 n-6	0.26 ± 0.06	0.01 ± 0.02	0.24 ± 0.07	0.009 ± 0.03	0.0006 (−0.02 to 0.02)	0.95
20:5 n-3	0.14 ± 0.04	−0.007 ± 0.02	0.12 ± 0.03	0.0006 ± 0.02	−0.007 (−0.02 to 0.006)	0.27
22:5 n-3	0.17 ± 0.04	−0.008 ± 0.01	0.16 ± 0.04	−0.01 ± 0.02	0.004 (−0.006 to 0.02)	0.41
22:6 n-3	0.15 ± 0.07	−0.01 ± 0.02	0.16 ± 0.07	−0.004 ± 0.03	−0.007 (−0.03 to 0.01)	0.41
SCD-1	0.2 ± 0.05	0.009 ± 0.02	0.2 ± 0.03	0.009 ± 0.03	0.0005 (−0.02 to 0.02)	0.96

Data are means ± SD or median (IQR) and 95% CI. For nonnormal variables, P values are obtained from nonparametric analyses. Fatty acids are reported as a percentage of all fatty acids assessed by gas chromatography. CE, cholesterol ester.

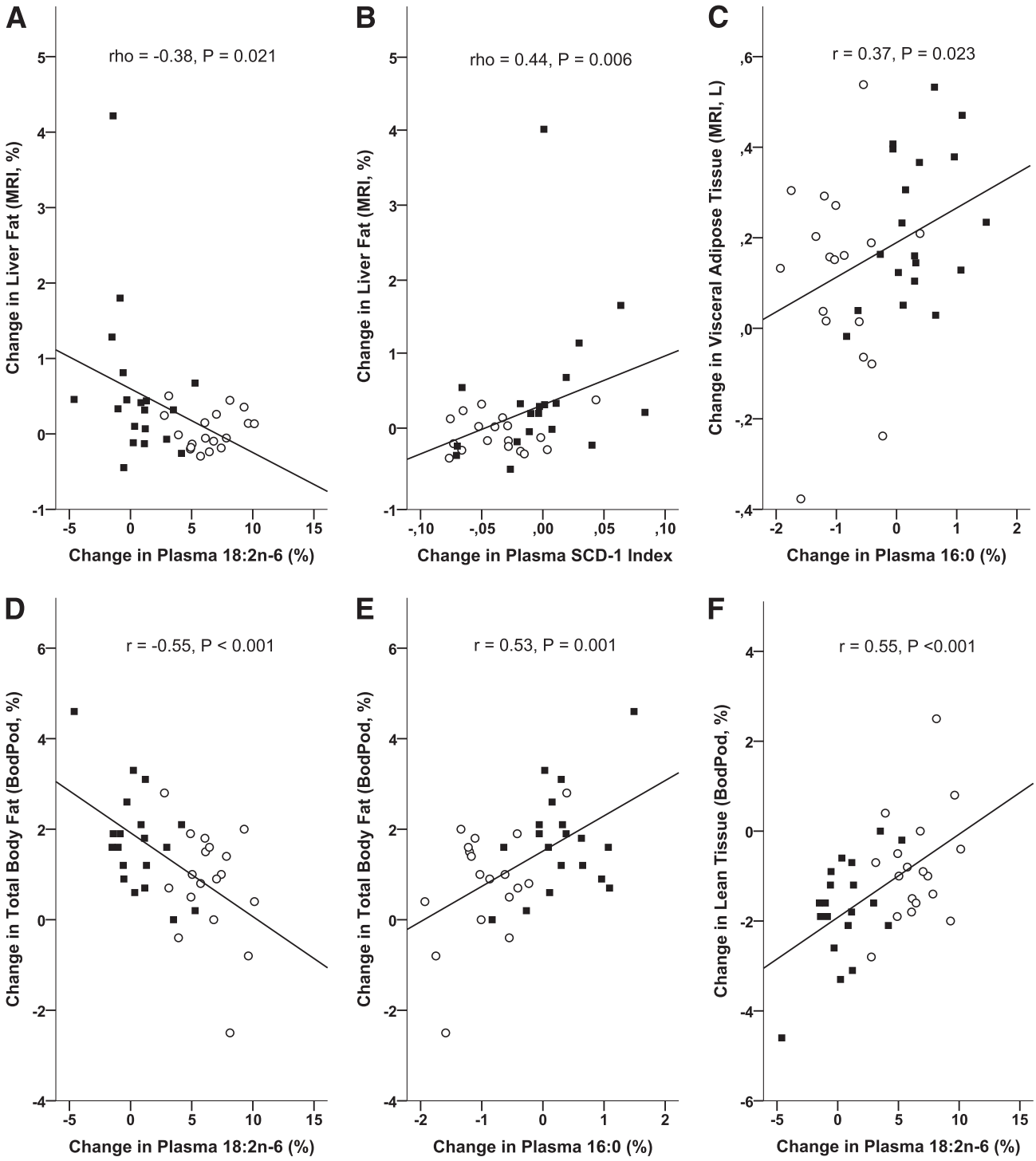

Figure 3—Correlations between changes in outcome measures and changes in plasma cholesterol esters. White circles, PUFA group; black squares, SFA group. *A*, *D*, *F*, and *H*: 18:2 n-6 is linoleic acid (in percentage of all fatty acids by gas chromatography). *B*: SCD-1 index is calculated as palmitoleic/palmitic acid (in percentages of all fatty acids by gas chromatography). *C*: The dependent variable (change in VAT) was log transformed before analysis of Pearson *r*. *C*, *E*, *G*, and *I*: 16:0 is palmitic acid (in percentage of all fatty acids by gas chromatography). *A*–*I*: ρ, Spearman correlation coefficient; *r*, Pearson correlation coefficient.

was measured only in SAT, the gene expression results cannot be directly extrapolated to other depots, such as visceral adipose tissue (VAT) and liver fat. Firm conclusions about the mechanisms of PUFA-induced changes in liver metabolism can therefore not be drawn from the current study. These findings thus need confirmation in VAT and liver, which may not be feasible in humans. However, a recent animal study (54) investigated the

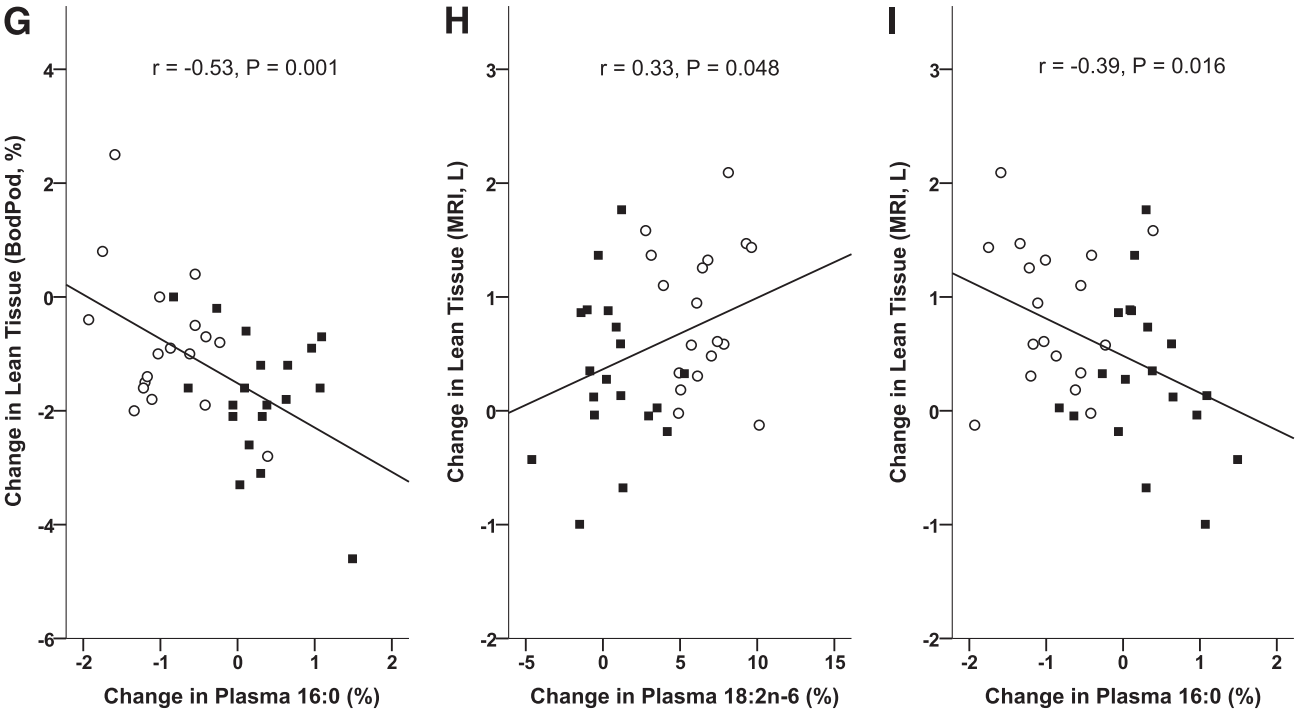

Figure 3—*Continued*

effect of overfeeding rats with different types of fat varying in linoleic acid content. Rats fed a diet higher in PUFAs (linoleic acid) showed lower liver fat accumulation together with lower hepatic gene expression of several fatty acid transporters (FATP-2, FATP-5, and CD36) and lipogenic enzymes (fatty acid synthase, acetyl-CoA carboxylase, and SCD-1) compared with rats fed a diet lower in linoleic acid. Hepatic gene expression of carbohydrate-responsive element–binding protein and sterol regulatory element–binding protein-1c were also lower in rats fed a diet higher in linoleic acid. Accordingly, we observed that the estimated SCD-1 activity in plasma cholesterol esters (reflecting hepatic metabolism) was markedly decreased in the PUFA group (Table 4), implying that the mechanisms may be at least partly similar (i.e., decreased hepatic lipogenesis).

Some strengths of this study should be mentioned. This study was double-blinded, which rarely is feasible in dietary interventions that include foods rather than supplements or capsules. Our body composition data are strengthened by consistent findings using two independent methods (MRI and Bod Pod). All subjects completed the trial. Both groups in the current study consumed vegetable oils without any cholesterol, thus excluding any confounding effect of dietary cholesterol (55) that is abundant in SFAs from animal sources. Assessment of fatty acid composition in plasma lipids and adipose tissue suggested high adherence to the interventions in both groups. Accelerometer monitoring suggested no bias due to differences in physical activity between groups. As we compared two common dietary fatty acids (the major PUFA, linoleic acid, and the major SFA, palmitic acid) in the Western diet, the results of this study could be relevant to many populations.

This study also has several potential limitations. Notably, our results may not apply to obese or insulin-resistant individuals who might show a different response to the diets, both with regard to ectopic fat accumulation and glucose metabolism. Also, the current healthy, young, and overall lean individuals had very low liver and visceral fat content at baseline. Thus, the lack of differences in fasting insulin concentrations were not surprising (i.e., the absolute increase of liver fat during SFA treatment was most likely too small to produce significant metabolic differences between the diets in this healthy study group). It should, however, be noted that the study was not designed or powered to examine differences in insulin sensitivity, and we did not measure hepatic or whole-body insulin sensitivity directly, which lowered the ability to detect any possible differences between groups. The data thus need confirmation in older individuals with NAFLD or type 2 diabetes and in other ethnic groups. The short duration of the study may not resemble long-term effects. However, results on liver fat are strongly supported by similar effects reported in weight-stable obese subjects, in which also modest effects on insulin levels and

Table 5—Comparison of absolute gene expression difference before and after 7 weeks of overeating PUFA and SFA

Gene name	Symbol	PUFA change	SFA change	Footnote
Carbonic anhydrase III, muscle specific	CA3	1.55	1.02	a,b,c
α-Kinase 3	ALPK3	1.20	0.86	a,b,c
Insulin-like growth factor 1	IGF1	1.22	0.99	a,b,c
Phosphodiesterase 8B	PDE8B	1.39	1.02	a,b,c
Storkhead box 1	STOX1	1.22	0.89	a,b,c
MOCO sulphurase COOH-terminal domain containing 1	MOSC1	1.17	1.00	a,b,c
Heat-shock 70-kDa protein 12A	HSPA12A	1.52	1.17	a,b,c
Glycerophosphodiester phosphodiesterase domain containing 5	GDPD5	1.37	0.94	a,b,c
Odz, odd Oz/ten-m homolog 4	ODZ4	1.80	1.16	a,c
Pleckstrin homology domain containing, family H member 2	PLEKHH2	0.82	1.04	a,c
Chloride intracellular channel 1	CLIC1	0.87	1.03	a,c
Connective tissue growth factor	CTGF	0.81	1.15	a,c
Transmembrane protein 120B	TMEM120B	1.46	1.12	c
KIAA0427	KIAA0427	1.34	1.09	c
Quinoid dihydropteridine reductase	QDPR	1.25	1.05	c
Aldehyde dehydrogenase 9 family, member A1	ALDH9A1	1.32	1.08	c
Transmembrane protein 120A	TMEM120A	1.10	0.94	c
FAT tumor suppressor homolog 1	FAT1	1.27	1.08	c
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	PFKFB1	1.21	1.00	c
Aldehyde dehydrogenase 1 family, member A1	ALDH1A1	0.80	1.09	c

Absolute expression difference after vs. before intervention groups is significant with [a]FDR 25% or [b]FDR 0%. [c]Absolute expression differences after vs. before intervention was adjusted for differences in weight gain between individuals, followed by comparison between intervention groups at significance FDR 25%. For genes significant in this group only, weight change–adjusted differences in expression levels after vs. before intervention are shown.

triglycerides were observed (22). The MRI methods used relied on fixed-spectrum models and thus did not allow full characterization of all lipid resonances of the liver spectra to detect changes in liver lipid saturation. However, results from plethysmography were consistent with MRI results regarding body fat deposition. Finally, it should be noted that sunflower oil contains more vitamin E than palm oil, and vitamin E supplementation has decreased steatosis (56). However, the present vitamin E levels were most likely too low to have an effect, and there was no correlation between change in liver fat and change in vitamin E intake (data not shown). Furthermore, the effects of PUFAs were not exclusive to liver fat.

In conclusion, overeating different types of fat seems to have different anabolic effects in the body. The fate of SFAs appears to be ectopic and general fat accumulation, whereas PUFAs instead promote lean tissue in healthy subjects. Given a detrimental role of liver fat and visceral fat in diabetes, the potential of early prevention of ectopic fat and hepatic steatosis by replacing some SFAs with PUFAs in the diet should be further investigated.

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