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# Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans[1–3]

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## ABSTRACT

**Background:** Cross-sectional studies have identified a high intake of simple sugars as an important dietary factor predicting nonalcoholic fatty liver disease (NAFLD).

**Objective:** We examined whether overfeeding overweight subjects with simple sugars increases liver fat and de novo lipogenesis (DNL) and whether this is reversible by weight loss.

**Design:** Sixteen subjects [BMI (kg/m$^2$): 30.6 ± 1.2] were placed on a hypercaloric diet (>1000 kcal simple carbohydrates/d) for 3 wk and, thereafter, on a hypocaloric diet for 6 mo. The subjects were genotyped for rs739409 in the *PNPLA3* gene. Before and after overfeeding and after hypocaloric diet, metabolic variables and liver fat (measured by proton magnetic resonance spectroscopy) were measured. The ratio of palmitate (16:0) to linoleate (18:2n−6) in serum and VLDL triglycerides was used as an index of DNL.

**Results:** Carbohydrate overfeeding increased weight (±SEM) by 2% (1.8 ± 0.3 kg; $P < 0.0001$) and liver fat by 27% from 9.2 ± 1.9% to 11.7 ± 1.9% ($P = 0.005$). DNL increased in proportion to the increase in liver fat and serum triglycerides in subjects with *PNPLA3-148II* but not *PNPLA3-148MM*. During the hypocaloric diet, the subjects lost 4% of their weight (3.2 ± 0.6 kg; $P < 0.0001$) and 25% of their liver fat content (from 11.7 ± 1.9% to 8.8 ± 1.8%; $P < 0.05$).

**Conclusions:** Carbohydrate overfeeding for 3 wk induced a >10-fold greater relative change in liver fat (27%) than in body weight (2%). The increase in liver fat was proportional to that in DNL. Weight loss restores liver fat to normal. These data indicate that the human fatty liver avidly accumulates fat during carbohydrate overfeeding and support a role for DNL in the pathogenesis of NAFLD. This trial was registered at www.hus.fi as 235780. *Am J Clin Nutr* 2012;96:727–34.

## INTRODUCTION

The intake of simple sugars has increased in recent years (1, 2). In cross-sectional epidemiologic studies, simple sugar intake, especially that of fructose, has been found to be related to increased liver fat content and to the severity of histologic changes in subjects with nonalcoholic fatty liver disease (NAFLD)[4] independent of age, sex, obesity, and total caloric intake (3–6). Simple sugars are converted in the liver into SFAs through a process called de novo lipogenesis (DNL) (7–9). DNL is greater in subjects with than in those without NAFLD (10, 11). The amount of triglycerides in the liver is closely correlated with the amount of saturated triglyceride fatty acids in human liver biopsies (12) and with their splanchnic production in patients with NAFLD (13). These data suggest that DNL contributes to human NAFLD.

Only a few studies have directly measured the effect of excessive carbohydrate consumption on liver fat content. The first study examined the effects of 4 wk of fructose overfeeding in 7 lean healthy volunteers and found no change in liver fat (14), whereas later 1-wk studies by the same group did report increases in liver fat content in healthy lean volunteers (15–17). Another 4-wk study in

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[4] Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DNL, de novo lipogenesis; FFA, free fatty acid; NAFLD, nonalcoholic fatty liver disease; *PNPLA3*, patatin-like phospholipase domain–containing 3; $^1$H-MRS, proton magnetic resonance spectroscopy; γGT, γ-glutamyl transferase.

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20 lean healthy subjects found no effect on liver fat content (18). The reasons for these discrepant results are unclear but could be due to the study subjects being mostly lean and healthy with a normal baseline liver fat content. No study has determined whether overfeeding with simple sugars increases liver fat content in overweight subjects with increased baseline liver fat content or whether this increase is associated with an increase in DNL.

A recent meta-analysis showed that the I148M variant (rs738409 C>G) in the patatin-like phospholipase domain–containing protein 3 (*PNPLA3*) gene is associated with increased liver fat (19). The mutant PNPLA3 is unable to hydrolyze intrahepatocytic triglycerides (20, 21). Liver fat is closely correlated with serum triglycerides and VLDL production (22, 23). Subjects homozygous for the I148M variant at rs738409 in the *PNPLA3* gene (*PNPLA3-148MM*), however, have normal serum triglycerides despite increased liver fat content (19). Because triglyceride hydrolysis is required for incorporation of triglycerides into VLDL particles (24, 25), one could predict that stimulation of DNL by carbohydrate feeding does not increase serum triglyceride concentration in subjects with the *PNPLA3-148MM* genotype as compared with those with the wild-type genotype (*PNPLA3-148II*). The *PNPLA3* genotype might, thus, modify the metabolic response to carbohydrate overfeeding.

In the current study, the primary aim was to determine the effects of 3 wk of overfeeding with simple carbohydrates on liver fat and DNL in overweight subjects. We also investigated whether the observed changes were reversible by a 6-mo weight-loss period, which restored body weight. The latter was done because, otherwise, the study would not have been considered ethically acceptable. Because the *PNPLA3* genotype may influence the response of serum triglycerides to stimulation of DNL, we genotyped the study subjects with respect to rs738409 of the *PNPLA3* gene and recruited subjects with the *PNPLA3-148MM* or the *PNPLA3-148II* homozygous genotype. Analyses of response to carbohydrate overfeeding in subjects differing with respect to the *PNPLA3* genotype were, however, merely exploratory and hypothesis-generating and not the primary objective of the study.

## SUBJECTS AND METHODS

### Study subjects

The study subjects were recruited among the nondiabetic individuals who had been previously genotyped at rs738409 in the *PNPLA3* gene in our laboratory [studies listed in Kotronen et al (26)] or in the population-based Finnish Cardiovascular Risk Factor Study 2007 (27). Inclusion criteria included *1*) age >18 y and *2*) information available on the *PNPLA3* genotype at rs738409. Exclusion criteria included *1*) the *PNPLA3-148IM* genotype; *2*) type 1 or 2 diabetes; *3*) a preexisting liver condition other than NAFLD or alcoholic fatty liver disease (eg, autoimmune, viral, or drug-induced liver disease) or significant other disease based on history, clinical examination, and laboratory tests (vide infra); *4*) alcoholic fatty liver disease as defined by excessive use of alcohol (>20 g/d); *5*) use of antihypertensive and lipid-lowering agents; and *6*) pregnancy or lactation. The potentially eligible study subjects were contacted by mail to inquire about their interest in the study and whether they would consent to be contacted by phone to explain more about the planned research project. The study protocol was approved by the Ethics Committee of the Helsinki University Central Hospital.

### Study design

*Screening (visit 1)*

All eligible subjects for the study based on a telephone interview were invited for a screening visit after a 12-h fast. At this visit, the purpose and nature of the study were explained to the subjects once more before their written informed consent was obtained. A history and physical examination was performed, and blood samples were collected for the measurement of complete blood count and concentrations of creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γGT), HDL and LDL cholesterol, glucose, glycosylated hemoglobin $A_{1c}$, insulin, and C-peptide; antimitochondrial, antinuclear, and anti–smooth muscle antibodies; and antibodies against hepatitis A, B (core), and C. A pregnancy test was performed in women of child-bearing potential. The patients suitable for the study based on the screening visit met a dietitian who gave instructions for keeping a 3-d food diary before the baseline visit. These food diary data served as the basis for giving dietary advice for the weight-loss period after the carbohydrate overfeeding.

*Baseline measurements (visits 2 and 3)*

At the baseline visit, weight, height, and waist and hip circumferences were recorded, and baseline blood samples were collected for the measurement of complete blood count and HDL- and LDL-cholesterol and free fatty acid (FFA) concentrations and to perform liver-function tests. Thereafter, a 2-h oral-glucose-tolerance test was performed and the subjects met the dietitian (visit 2). Baseline proton magnetic resonance spectroscopy ($^1$H-MRS) for measurement of liver fat content and magnetic resonance imaging for measurement of abdominal subcutaneous and intraabdominal adipose tissue were conducted within a day of the baseline measurements (visit 3).

After the baseline visit, the subjects started a 3-wk high-carbohydrate hypercaloric diet. The subjects were instructed to continue their normal diet and in addition to consume an extra 1000 kcal/d with 98% of energy from carbohydrates. The extra diet consisted of candy (Oy Karl Fazer Ab), pineapple juice (Tuko Logistics Oy), sugar-sweetened soft drinks (Oy Hartwall Ab), and/or carbohydrate-loading drink (Squeezy Sports Nutrition GmbH) and was provided free of charge from the research unit to the study participants.

*Measurements during carbohydrate overfeeding (visits 4 and 5)*

The subjects came for a clinical visit once a week during the intervention. At these visits, body weight, waist and hip circumferences, and blood pressure were measured. The subjects also met the dietitian on every visit.

*Measurements after carbohydrate overfeeding and during weight loss (visits 6–11)*

After 3 wk of the high-carbohydrate hypercaloric diet, the measurements described for visits 2 and 3 were repeated. The $^1$H-MRS and magnetic resonance imaging studies were conducted within a day of visit 7. All subjects met the dietitian and were given dietary advice based on the food diary that the study subject had kept before the carbohydrate overfeeding (eg, to include more vegetables and full-grain products and less

simple sugars, white flour, and alcoholic drinks in the diet, to moderate portion size) to promote weight loss over the next 6 mo. During the 6-mo weight-loss period, the subjects visited the research unit every 3 wk. At these visits, body weight, waist and hip circumferences, and blood pressure were measured. The subject also met the dietitian on every visit.

*Measurements after the weight-loss intervention (visits 12 and 13)*

After 6 mo, the measurements described for visits 2 and 3 were repeated. All subjects met the dietitian, who went through the food diary that the subjects were instructed to keep for 3 d during the weight-loss intervention to give the study participants diet and lifestyle advice for future weight maintenance.

### Liver fat content ($^1$H-MRS)

Liver fat content was measured by using $^1$H-MRS with a 1.5-tesla Magnetom Avanto scanner (Siemens). A point-resolved spectroscopy localization technique with time of repetition of 3000 ms, echo time of 30 ms, and 16 acquisitions was used to obtain nonsuppressed liver spectra. Otherwise, measurements and data analysis were performed as previously described (28, 29). Liver fat content was expressed as a mass fraction (28). The $^1$H-MRS measurement has been validated against the histologically determined lipid content (30, 31) and against estimates of fatty infiltration by computed tomography (29) and magnetic resonance imaging (32) and has proven to be precise.

### Measurements of adipose tissue volume

Volumes of abdominal subcutaneous and intraabdominal adipose tissue were quantified from 16 T1-weighed axial magnetic resonance images acquired by using a 1.5-tesla Magnetom Avanto scanner (Siemens) with selective fat excitation reaching from 8 cm above to 8 cm below the L4/5 lumbar intervertebral disk, as previously described (29). Abdominal subcutaneous and intraabdominal adipose tissue volumes were measured by a single investigator using image analysis software (SliceOmatic version 4.3; Tomovision).

### Anthropometric measurements

Body weight was recorded to the nearest 0.1 kg by using a calibrated digital scale (Soehnle) while the subjects were barefoot and wearing light indoor clothing. Height was recorded to the nearest 0.5 cm by using a nonstretchable tape. BMI was defined as weight (kg)/height (m)$^2$. Body circumferences were measured with a nonstretchable band for the waist, midway between the lower rib margin and the iliac crest, and, for hip circumference, over the greater trochanters and recorded to the nearest 0.5 cm.

### Genotyping

Genotyping at rs738409 in the *PNPLA3* gene in the subjects who had previously participated in the metabolic studies in our laboratory was performed by using a TaqMan polymerase chain reaction method as previously described (26). For the Finnish Cardiovascular Risk Factor Study 2007 participants, the rs738409 of the *PNPLA3* was provisionally genotyped with the MassARRAY System (Sequenom) as previously described (33), and the genotype was verified by using a direct Sanger sequencing with standard purification and sequencing reaction protocols with the use of an ABI 3130 XL analyzer (PE Applied Biosystems). The forward primer used was 5′ CCCTGCTCACTTGGAGAAAG 3′ and the reverse primer used was 5′ CTGCAGGCAGGAGATGTGT 3′.

### DNL in vivo

DNL primarily produces SFAs in humans (7). This increases the palmitate (16:0) to linoleate (18:2n−6) ratio (16:0/18:2n−6 ratio, ie, the lipogenic index) in VLDL triglyceride fatty acids (7, 34). The increase in this index is closely correlated with the rate of hepatic palmitate synthesis as measured by mass isotopomer distribution analysis of palmitate during infusion of [$^{13}$C]acetate (35). To verify that carbohydrate overfeeding in the current study indeed increases the lipogenic index in VLDL, which are synthesized in the liver, we isolated VLDL by ultracentrifugation (36) in a subgroup of 6 subjects before and after the overfeeding diet and analyzed their serum fatty acid composition. In all subjects, the index was measured in serum total triglycerides before and after the overfeeding diet. The concentrations of fatty acid methyl esters were determined in serum total triglycerides and VLDL triglycerides as previously described (37). The 16:0/18:2n−6 ratios in serum and VLDL triglycerides were determined by gas chromatography (38).

### Other measurements

Fasting plasma glucose, insulin, C-peptide, liver enzyme, LDL- and HDL-cholesterol, triglyceride, and FFA concentrations were measured as previously described (39). An oral-glucose-tolerance test was performed by using a 75-g glucose solution and measuring plasma glucose, insulin, and C-peptide concentrations at 0, 30, 60, and 120 min. The HOMA-IR index was calculated by using the following formula: fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5 (40). Plasma sodium and potassium concentrations were measured with indirect ion-selective electrodes and plasma creatinine with an enzymatic kit from Roche Diagnostics by using an autoanalyzer (Roche Diagnostics Hitachi Modulator, Hitachi Ltd). Blood counts were performed by using a flow cytometric method on a Sysmex apparatus (TOA Medical Electronics Co Ltd).

### Statistical methods

All data were tested for normality of distribution by using a Kolmogorov-Smirnov test. Normally distributed data are shown as means ± SEMs, and nonnormally distributed data are shown as medians (25th–75th percentiles). A paired *t* test and Wilcoxon signed-rank test were used to compare the differences in the variables studied before and after the dietary interventions for normally and nonnormally distributed data, respectively. The Holm-Bonferroni method was used to adjust for double comparison against the postoverfeeding values. An unpaired *t* test and Mann-Whitney *U* test were used to compare the changes in the variables studied between the *PNPLA3* rs738409 genotype groups for normally and nonnormally distributed data, respectively. Pearson product-moment correlation coefficients were used

for correlation calculations after the entered data had passed the normality test. Spearman rank correlation coefficients were used to analyze the relation between the nonnormally distributed variables. Two-tailed $P$ values $<0.05$ were considered statistically significant. The calculations were performed by using GraphPad Prism version 4.03 for Windows (GraphPad Software Inc) and Microsoft Office Excel 2007 for Windows (Microsoft).

## RESULTS

### Baseline characteristics

Physical and biochemical baseline characteristics of study subjects are shown in **Table 1**. Baseline liver fat averaged 9.2 ± 1.9%. A total of 56% of the subjects had NAFLD (41).

### Body composition

*Carbohydrate overfeeding*

During the 3-wk carbohydrate overfeeding period, body weight increased by 2% (1.8 ± 0.3 kg; $P < 0.0001$) compared with baseline. BMI, waist and hip circumferences, and abdominal subcutaneous (by 3%) and intraabdominal (by 5%) adipose tissue volumes also increased significantly during the overfeeding period as depicted in Table 1.

*Weight loss*

During the 6-mo weight-loss period, the subjects lost 4% (3.2 ± 0.6 kg; $P < 0.0001$) of their body weight. BMI, waist and hip circumferences, and abdominal subcutaneous (by 13% compared with the end of carbohydrate overfeeding) and intraabdominal (by 13%) adipose tissue volumes also decreased significantly during the weight-loss period (Table 1).

### Liver fat and liver-function tests

*Carbohydrate overfeeding*

Liver fat increased by 27% from 9.2 ± 1.9% to 11.7 ± 1.9% ($P < 0.05$) during the carbohydrate overfeeding (**Figure 1**A). Serum ALT increased significantly by 28% from 50 ± 11 to 64 ± 16 U/L ($P < 0.005$; Figure 1B), serum γGT from 39 ± 6 to 58 ± 14 U/L ($P < 0.005$), and serum AST from 36 ± 4 to 43 ± 6 U/L ($P < 0.05$).

*Weight loss*

Weight loss decreased liver fat content by 25% from 11.7 ± 1.9% to 8.8 ± 1.8% ($P < 0.05$; Figure 1A). Serum ALT decreased by 23% from 64 ± 16 to 49 ± 8 U/L ($P = 0.06$; Figure 1B) and serum γGT from 58 ± 14 to 36 ± 6 U/L ($P < 0.005$). Serum AST remained unchanged (43 ± 6 compared with 39 ± 3 U/L; $P = 0.39$).

The absolute change in liver fat content was positively correlated with the absolute change in body weight during both the

**TABLE 1**
Clinical characteristics of the study subjects with a median age of 54 y (25th–75th percentile: 40–59 y) before and after carbohydrate overfeeding and weight loss[1]

| | Baseline ($n$ = 5 M, 11 F) | After overfeeding | After weight loss |
|---|---|---|---|
| Body composition | | | |
| Weight (kg) | 88.7 ± 4.1[2] | 90.5 ± 4.1*** | 87.3 ± 4.1‡‡‡ |
| BMI (kg/m$^2$) | 30.6 ± 1.2 | 31.2 ± 1.3*** | 30.0 ± 1.3‡‡‡ |
| Waist (cm) | 102 ± 3 | 104 ± 4* | 99 ± 4‡‡ |
| Hip (cm) | 105 (102–119)[3] | 107 (105–124)** | 105 (101–119)‡‡ |
| Subcutaneous adipose tissue (cm$^3$) | 4440 (3700–6210) | 4570 (4000–6280)** | 3970 (3590–6160)‡‡ |
| Intraabdominal adipose tissue (cm$^3$) | 2180 ± 300 | 2290 ± 310** | 1990 ± 260‡ |
| Lipids | | | |
| fS-Triglyceride (mmol/L) | 0.99 (0.77–1.23) | 1.47 (1.03–1.76)* | 0.93 (0.80–1.24)‡‡ |
| fS-HDL cholesterol (mmol/L) | 1.36 (1.14–1.68) | 1.33 (1.11–1.54)* | 1.51 (1.22–1.79)‡‡ |
| fS-LDL cholesterol (mmol/L) | 3.2 ± 0.2 | 3.4 ± 0.3 | 3.3 ± 0.3 |
| fS-FFA (μmol/L) | 424 ± 31 | 416 ± 38 | 439 ± 38 |
| Lipogenic index | | | |
| 16:0/18:2n−6 ratio in fS-TG | 2.1 (1.9–2.3) | 2.6 (2.4–4.1)*** | NA |
| 16:0/18:2n−6 ratio in fS-VLDL-TG | 2.1 ± 0.3 | 3.2 ± 0.5* | NA |
| Glucose, insulin, and C-peptide | | | |
| fP-glucose (mmol/L) | 5.1 (4.8–5.6) | 5.3 (5.1–5.4) | 5.3 (5.0–5.7) |
| 2-h glucose (mmol/L) | 6.0 (5.4–6.7) | 5.9 (4.7–8.0) | 5.9 (5.1–7.7) |
| HOMA-IR (mU · mmol/L$^2$) | 1.7 ± 0.3 | 1.8 ± 0.2 | 2.0 ± 0.4 |
| fS-insulin (mU/L) | 5.6 (4.0–12.6) | 7.5 (4.7–9.7) | 6.2 (3.9–12.7) |
| 2-h insulin (mU/L) | 59.4 (27.2–81.7) | 45.0 (29.2–94.9) | 34.0 (24.6–127.0) |
| fS-C-peptide (nmol/L) | 0.82 ± 0.08 | 0.90 ± 0.08 | 0.84 ± 0.1 |
| 2-h C-peptide (nmol/L) | 3.42 ± 0.38 | 3.35 ± 0.34 | 3.45 ± 0.43 |

[1] Significantly different from baseline (paired $t$ test or Wilcoxon signed-rank test followed by Holm-Bonferroni correction): *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$. Significantly different from after overfeeding (paired $t$ test or Wilcoxon signed-rank test followed by Holm-Bonferroni correction): ‡$P < 0.05$, ‡‡$P < 0.005$, ‡‡‡$P < 0.0005$. FFA, free fatty acid; fP, fasting plasma; fS, fasting serum; NA, not applicable; TG, triglyceride.

[2] Mean ± SEM (all such values).

[3] Median; 25th–75th percentiles in parentheses (all such values).

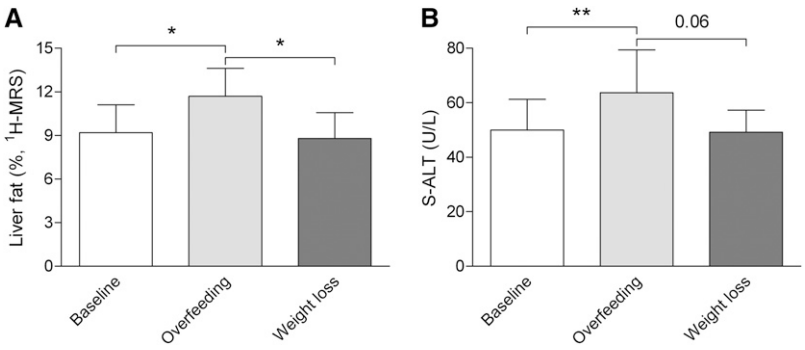


**FIGURE 1.** Mean (±SEM) liver fat content (A) and S-ALT concentrations (B) before and after carbohydrate overfeeding and weight loss. $*P < 0.05$, $**P < 0.005$ (paired $t$ test, Holm-Bonferroni–adjusted $P$ values shown). S-ALT, serum alanine aminotransferase; $^1$H-MRS, proton magnetic resonance spectroscopy.

carbohydrate overfeeding and weight loss (**Figure 2**). The percentage change in liver fat correlated with that in abdominal subcutaneous adipose tissue volume ($\rho = 0.64$, $P < 0.01$ during overfeeding; $\rho = 0.52$, $P < 0.05$ during weight loss; and $\rho = 0.69$, $P < 0.0001$ for both interventions combined) and intraabdominal adipose tissue volume ($\rho = 0.20$, $P = 0.45$ during overfeeding; $\rho = 0.32$, $P = 0.23$ during weight loss; and $\rho = 0.59$, $P < 0.0005$ for both interventions combined).

## Metabolic variables

### *Carbohydrate overfeeding*

Fasting serum concentrations of triglycerides increased, whereas those of HDL cholesterol decreased significantly during carbohydrate overfeeding (Table 1). The lipogenic index (16:0/18:2n−6 ratio) increased significantly during the intervention [2.1 ± 0.3 compared with 3.2 ± 0.5; $P < 0.05$ in VLDL triglycerides; 2.1 (1.9–2.3) compared with 2.6 (2.4–4.1), $P < 0.005$ in total serum triglycerides]. The molar percentage of 16:0 fatty acids in total serum triglycerides increased (30.4 ± 0.2% compared with 33.3 ± 0.2%; $P < 0.01$), whereas that of 18:2n−6 decreased (13.2 ± 0.2% compared with 10.4 ± 0.1%; $P < 0.005$) significantly. A significant positive correlation was found between the absolute change in the lipogenic index in VLDL triglycerides and that of total serum triglycerides ($\rho = 1.00$, $P < 0.005$). No statistically significant changes in fasting FFAs or in glucose, LDL cholesterol, insulin, and C-peptide concentrations were found in the fasting state or in response to oral-glucose-tolerance testing (Table 1).

In all study subjects, the absolute change in the lipogenic index (16:0/18:2n−6 ratio) in total serum triglycerides was significantly correlated with the absolute change in liver fat content during carbohydrate overfeeding ($\rho = 0.56$, $P < 0.05$; **Figure 3**A) and almost significantly with serum triglycerides ($\rho = 0.49$, $P = 0.06$; Figure 3B). The absolute change in the lipogenic index did not correlate with that in body weight ($\rho = -0.15$, $P = 0.59$). The absolute change in liver fat content also did not correlate with that in fasting serum FFAs ($\rho = 0.27$, $P = 0.31$).

### *Weight loss*

After weight loss, fasting serum concentrations of triglycerides decreased, whereas those of HDL cholesterol increased significantly (Table 1). Fasting FFA and glucose, LDL-cholesterol, insulin, and C-peptide concentrations remained unchanged (Table 1).

## Effect of *PNPLA3* genotype at rs738409

No significant differences were found in baseline characteristics; in changes in body weight, liver fat content, or serum glucose, insulin, triglyceride, and HDL-cholesterol concentrations; or in abdominal subcutaneous and intraabdominal adipose tissue volumes between the *PNPLA3-148II* and *PNPLA3-148MM* genotypes (data not shown). In the *PNPLA3-148II* subjects ($n = 7$), the increase in the lipogenic index in total serum triglycerides was significantly correlated with the increase in liver fat content ($r = 0.89$, $P < 0.01$; Figure 3A) and that in serum triglyceride concentrations ($r = 0.86$, $P < 0.05$; Figure 3B) during carbohydrate overfeeding. Such relations were not observed in 9 subjects with the *PNPLA3-148MM* genotype ($r = -0.28$, $P = 0.47$ and $r = 0.10$, $P = 0.81$, respectively; Figure 3, A and B).

## DISCUSSION

In the current study, we quantitated the effects of overfeeding with a "candy diet" containing 1000 extra carbohydrate kcal/d on liver fat content and DNL in overweight subjects. During the 3-wk period of carbohydrate overfeeding, the study subjects gained 1.8 kg, which represented 2% of their body weight. If all excess calories were stored as fat (assuming 9 kcal corresponds to 1 g

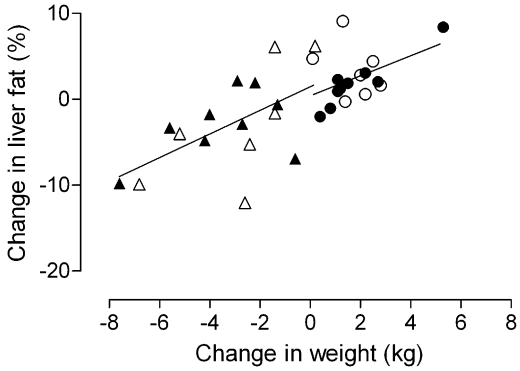


**FIGURE 2.** Relation between the absolute change in body weight and that in liver fat content during carbohydrate overfeeding (circles) and weight-loss (triangles) interventions. Open symbols (○, △) denote carriers of the *PNPLA3-148II* genotype, and closed symbols (●, ▲) denote carriers of the *PNPLA3-148MM* genotype. Pearson product-moment correlation coefficients for the relation between the absolute change in body weight and that in liver fat content are $r = 0.47$ ($P = 0.06$) during overfeeding and $r = 0.57$ ($P < 0.05$) during weight loss.

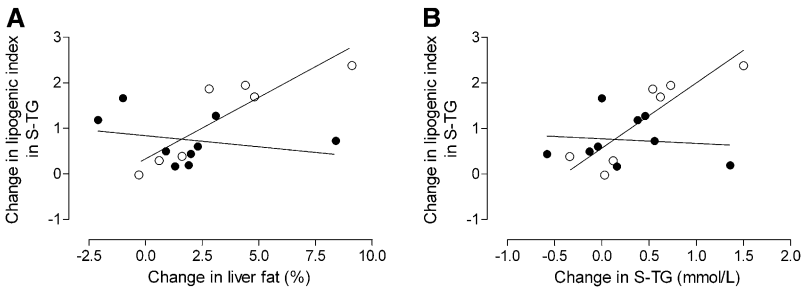


**FIGURE 3.** Relation between the absolute change in the lipogenic index (16:0/18:2n−6 ratio) in S-TG and the absolute change in liver fat content (A) and S-TG concentrations (B) during carbohydrate overfeeding in carriers of the *PNPLA3-148II* genotype (○) and carriers of the *PNPLA3-148MM* genotype (●). Slopes are significantly different between the genotypes: $P < 0.005$ (A) and $P < 0.01$ (B). Pearson product-moment correlation coefficients are $r = 0.89$ ($P < 0.01$) for the *PNPLA3-148II* genotype and $r = -0.28$ ($P = 0.47$) for the *PNPLA3-148MM* genotype (A) and $r = 0.86$ ($P < 0.05$) for the *PNPLA3-148II* genotype and $r = 0.10$ ($P = 0.81$) for the *PNPLA3-148MM* genotype (B). S-TG, serum triglyceride.

fat), one would predict a weight gain of 2.3 kg over a 3-wk period. This shows that the subjects were, on average, compliant with the diet, although there was interindividual variation in weight gain (Figure 2). The increase in body weight was accompanied by a 27% increase in liver fat above the baseline value of 9.2%. The change in body weight was significantly positively correlated with that in liver fat (Figure 2). The ratio of SFAs to essential fatty acids in serum and VLDL triglycerides, an index of DNL, also increased significantly. The increase in DNL was significantly correlated with that in liver fat content (Figure 3A).

In human NAFLD, excess intrahepatocytic triglycerides originate, based on studies using stable isotopes, from peripheral lipolysis and from DNL (10, 42). Evidence for the latter is based on the analysis of human liver biopsy samples (12) and on hepatic venous catheterization studies (13). In human liver biopsy samples, the amount of liver fat is directly proportional to the absolute and relative amount of SFAs and SFA-containing triglycerides (12). Direct measurement of triglyceride composition across the splanchnic bed has shown that splanchnic production of SFA-containing triglycerides is increased in proportion to liver fat content (13). In the current study, we used the lipogenic index, ie, the ratio of SFAs (16:0) to unsaturated fatty acids (18:2n−6), as a surrogate measure of DNL. This method was first used by Hudgins et al (43), who showed in humans that VLDL triglycerides become markedly enriched with palmitate (16:0) and deficient in linoleate (18:2n−6) within 10 d of feeding an isocaloric high-carbohydrate diet. Recently, the same research group showed that this, indeed, is a consequence of increased palmitate synthesis as measured directly by using mass isotopomer distribution analysis of palmitate during infusion of [$^{13}$C]acetate (35). We also previously showed that 3 d of high-carbohydrate feeding in healthy normal-weight to overweight subjects stimulates DNL as measured by using the 16:0/18:2n−6 ratio in serum and VLDL triglycerides (34).

Whereas in the current study the 16:0/18:2n−6 ratio increased significantly in response to carbohydrate overfeeding, fasting serum FFA concentrations remained unchanged. In the overfeeding studies measuring liver fat content, fasting serum FFAs have been reported to remain unchanged in a 4-wk glucose/fructose overfeeding study (18) or significantly decrease in response to 1-wk (15–17) and 4-wk (14) carbohydrate overfeeding. These data suggest that peripheral lipolysis does not provide fatty acids for hepatic triglycerides synthesis during carbohydrate overfeeding. We found the absolute change in the 16:0/18:2n−6 ratio in serum triglycerides, but not that in body weight or serum FFAs, to be significantly positively correlated ($P < 0.05$; Figure 3) with the increase in liver fat content during carbohydrate overfeeding. DNL rather than lipolysis, therefore, seems to be the mechanism underlying hepatic triglyceride synthesis during carbohydrate overfeeding.

During the 6-mo weight-loss period, the study subjects lost 3.2 kg, or 3.6%, of their body weight. Concurrently, the liver fat content decreased by 25%, ie, the relative decrease in liver fat far exceeded that in body weight. As during overfeeding, the change in body weight correlated with the percentage change in liver fat (Figure 2). Previous weight-loss studies addressing the effects of weight loss on liver fat have shown that even small decreases in body weight result in considerable decreases in liver fat. Loss of 3% (44), 9% (45), 7% (46), 10% (47), 10% (47), and 14% (47) of body weight has decreased liver fat content as measured by using $^{1}$H-MRS by 31% (44), 46% (45), 41% (46), 37% (47), 29% (47), and 40% (47), respectively. The time course of changes in liver fat and body weight appear to differ. In a 12-wk weight-loss study that used a very-low-energy diet in 32 obese individuals, 80% of the total decrease in liver fat occurred during the first 2 wk of the diet when weight loss was still statistically non-significant (48). The human liver, thus, seems to be a depot that rapidly and markedly responds to changes in caloric excess or deficiency.

The current study design does not allow distinction between the effect of caloric excess and that of simple sugars on liver fat content. To our knowledge, only one study has compared the effects of overfeeding with fat, carbohydrate, or a combination thereof on liver fat content in humans (17). This study found no significant differences in changes in liver fat content in response to the different overfeeding diets, but the number of subjects was low. A differential response was observed in serum VLDL triglycerides, which significantly increased during fructose and decreased during the fat overfeeding period (17), demonstrating that fructose but not fat overconsumption stimulates hepatic DNL and VLDL triglyceride production (49). Consistent with these data, an increase in serum triglycerides during carbohydrate overfeeding was also noted in the current study.

Previous carbohydrate overfeeding studies have given contradictory results regarding the diet's effect on fasting plasma glucose and serum insulin concentrations. One study reported an

increase in both glucose and insulin (18), some an increase in only glucose (14) or insulin (15, 17), and one no change in either variable (16). In the current study, whereas fasting plasma glucose concentrations remained unchanged, there was a tendency for fasting serum insulin concentrations to increase in response to carbohydrate overfeeding, but this was not statistically significant, perhaps because of a limited sample size.

Hydrolysis of triglycerides is required for VLDL synthesis in hepatocytes as inhibition of triglyceride hydrolysis prevents VLDL secretion in primary rat hepatocytes (50). The *PNPLA3-148MM* variant impairs the hydrolysis of especially monounsaturated triglycerides formed by DNL (21). This increases liver fat content but does not cause hypertriglyceridemia in serum (19). We therefore reasoned that stimulation of DNL in carriers of the *PNPLA3-148MM* genotype might not promote VLDL synthesis as the *PNPLA3-148II* carriers. Lack of a positive correlation between change in DNL and serum triglycerides in carriers of the *PNPLA3-148MM* genotype, who have defective triglyceride hydrolysis, are consistent with cross-sectional data showing that *PNPLA3-148M* carriers are not hypertriglyceridemic despite having increased liver fat. However, the number of subjects was not high enough to observe significant differences in serum triglycerides and, thus, this observation should be interpreted with caution and regarded as hypothesis-generating.

We conclude that that short-term overfeeding with simple carbohydrates markedly increases liver fat and stimulates DNL in overweight subjects. These changes are reversible by weight loss. These data support a role for excess simple sugar intake in the pathogenesis of NAFLD.

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