Genetic variation in \textit{PNPLA3} (adiponutrin) confers sensitivity to weight loss–induced decrease in liver fat in humans$^{1-3}$

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ABSTRACT

Background: The rs738409 C → G single nucleotide polymorphism in the patatin-like phospholipase domain-containing 3 (\textit{PNPLA3}; adiponutrin) leads to a missense mutation (I148M), which is associated with increased liver fat but not insulin resistance. The I148M mutation impedes triglyceride hydrolysis in vitro, and its carriers have an increased risk of developing severe liver disease.

Objective: We explored whether the rs738409 \textit{PNPLA3} G allele influences the ability of weight loss to decrease liver fat or change insulin sensitivity.

Design: We recruited 8 subjects who were homozygous for the rs738409 \textit{PNPLA3} G allele (PNPLA3-148MM) and 10 who were homozygous for the rs738409 \textit{PNPLA3} C allele (PNPLA3-148II). To allow comparison of changes in liver fat, the groups were matched with respect to baseline age, sex, body mass index, and liver fat. The subjects were placed on a hypocaloric low-carbohydrate diet for 6 d. Liver fat content (proton magnetic resonance spectroscopy), whole-body insulin sensitivity of glucose metabolism (euglycemic clamp), and lipolysis ([\textsuperscript{2}H\textsubscript{5}]glycerol infusion) were measured before and after the diet.

Results: At baseline, fasting serum insulin and C-peptide concentrations were significantly lower in the PNPLA3-148MM group than in the PNPLA3-148II group, as predicted by study design. Weight loss was not significantly different between groups (PNPLA3-148MM: −3.1 ± 0.5 kg; PNPLA3-148II: −3.1 ± 0.4 kg). Liver fat decreased by 45% in the PNPLA3-148MM group ($P < 0.001$) and by 18% in the PNPLA3-148II group ($P < 0.01$).

Conclusion: Weight loss is effective in decreasing liver fat in subjects who are homozygous for the rs738409 \textit{PNPLA3} G or C allele. This trial was registered at www.hus.fi as 233775. \textit{Am J Clin Nutr} 2011;94:104–11.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a rapidly increasing liver disorder covering a range of conditions from simple steatosis to nonalcoholic steatohepatitis (1). The latter may progress to cirrhosis (1). Studies in twins have suggested that the heritability of liver enzymes and possibly liver fat in NAFLD is considerable (2). Recently, Romeo et al (3) identified a single nucleotide polymorphism (I148M, rs738409) in the patatin-like phospholipase domain-containing 3 gene (\textit{PNPLA3}; adiponutrin) to be highly significantly associated with NAFLD, independent of obesity. The I148M variant in the adiponutrin gene represents a cytosine (C) to guanine (G) substitution, which leads to an amino acid change from isoleucine (I) to methionine (M) at codon 148 (I148M) in the \textit{PNPLA3} gene. Subjects homozygous for this variant [\textit{PNPLA3} C148M, prevalence ∼5% (4)] have a liver fat content, measured by proton magnetic resonance spectroscopy (1H-MRS) (3, 5, 6) and magnetic resonance imaging (MRI) (7), 1.3–2.4–fold higher than that of weight-matched homozygous carriers of \textit{PNPLA3} C148II. Carriers of \textit{PNPLA3} C148MM also have a greater incidence of steatosis, as determined by ultrasound (8) and liver biopsy analysis (9–13), and more liver fibrosis and inflammation (9–13) than subjects homozygous for \textit{PNPLA3} C148II.

Studies in vitro have suggested that the I148M variant abolishes triglyceride hydrolysis, which suggests that \textit{PNPLA3} I148M is a loss-of-function mutation (14). On the other hand, overexpression of \textit{PNPLA3}-I148M in mice results in steatosis, which would suggest that the variant is a gain-of-function mutation (14). There are no data currently available regarding how and whether the variant influences changes in liver fat in response to interventions such as weight loss or overfeeding. Given that many studies have shown carriers of the rs738409 \textit{PNPLA3} minor G allele to be at increased risk of severe liver disease, and that weight loss is perhaps the best treatment of NAFLD, such a study would seem of clinical relevance (9–13).

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Consistently across all studies, despite higher liver fat content, insulin sensitivity is not altered in carriers of PNPLA3-148MM compared with carriers of PNPLA3-148II (3, 5–7). Thus, if PNPLA3-148MM carriers are matched for liver fat to those with PNPL3-148II, one would predict the former to be more insulin sensitive than the latter.

The primary objective of the present study was to compare the effects of a hypocaloric low-carbohydrate diet on liver fat content between carriers of PNPLA3-148MM and carriers of PNPLA3-148II (15). We aimed to achieve similar weight loss in both groups, which we matched for liver fat at baseline to allow accurate comparison of its changes. We also examined how weight loss affects whole-body insulin sensitivity of glucose metabolism and lipolysis measured with [2H5]glycerol in the PNPLA3-148MM and PNPLA3-148II groups.

SUBJECTS AND METHODS

Subjects and study design

The study subjects were recruited among previously genotyped nondiabetic subjects (6). Exclusion criteria included 1) PNPLA3-148MI variant, 2) type 1 or 2 diabetes, 3) renal insufficiency, 4) preexisting liver or significant other disease other than NAFLD (ie, autoimmune, viral, or drug-induced liver disease), 5) excessive use of alcohol (>20 g/d), and 6) pregnancy or lactation. At baseline, the study groups were matched for age, sex, body mass index (BMI), and liver fat.

Screening visit

All subjects suitable for the study based on a telephone interview were invited to the laboratory for a screening visit. At this visit, medical histories were collected, physical examinations were performed, and blood samples were collected for the measurement of total blood counts, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, total cholesterol, HDL and LDL cholesterol, triglycerides, bilirubin, glucose, glycated hemoglobin, insulin, C-peptide, creatinine, albumin, potassium, sodium, prothrombin time, transferrin saturation, antismoke muscle, antinuclear and antimitochondrial antibodies, and hepatitis A, B, and C serology. A serum pregnancy test was performed in women of childbearing age. Patients suitable for the dietary intervention based on the screening visit were invited for a metabolic study, which consisted of a baseline visit, a 6-d hypocaloric low-carbohydrate intervention period, and a final visit after the diet. Measurements of liver fat content by 1H-MRS were conducted within a day of the clinical visit before and after the dietary intervention.

Baseline and final visit

At the baseline visit, weight and height were recorded, and basal blood samples were collected for the measurement of total blood count, liver function tests, and fasting glucose, insulin, C-peptide, total cholesterol, HDL and LDL cholesterol, and triglyceride concentrations. After the blood tests were performed, a needle aspiration biopsy sample was collected from abdominal subcutaneous adipose tissue (see below). Thereafter, a euglycemic hyperinsulinemic clamp was performed. Blood samples for measurement of glucose, insulin, free fatty acid (FFAs), and glycerol concentrations were withdrawn during the clamp (see below). After the baseline visit, the study subjects were placed on a standardized hypocaloric (1000-kcal deficit/d) low-carbohydrate (<20 g/d) diet for 6 d. The diet was designed by dietitians (AR and LS) and provided entirely by the central kitchen of the Helsinki University Central Hospital. The baseline measurements were repeated after 6 d of the diet. Adherence to the low-carbohydrate diet was controlled for by verifying development of ketosis as measured by plasma β-hydroxybutyrate concentrations before and after the intervention (see below). All measurements were carried out in the clinical research center, where patients arrived in the morning after an overnight fast at home. The purpose, nature, and potential risks of the study were explained to the subjects before their written informed consent was obtained. The study protocol was approved by the ethics committee of the Helsinki University Central Hospital.

Measurement of liver fat content

Liver fat content was measured by using 1H-MRS, as previously described (16). This measurement has been validated against histologically determined lipid content (17) and against estimates of fatty infiltration by computed tomography (18). The reproducibility of repeated measurements of liver fat in nondiabetic subjects as determined on 2 separate occasions in our laboratory is 11% (19).

Whole-body insulin sensitivity of glucose metabolism and of lipolysis

Whole-body insulin sensitivity of glucose metabolism was determined by using the euglycemic hyperinsulinemic clamp technique (20). The duration of the insulin infusion was 120 min, and the continuous infusion rate was 0.4 mU·kg⁻¹·min⁻¹ (20). The low insulin infusion rate was chosen to maximize the likelihood of detecting changes in lipolysis. Of note, this infusion rate is not ideal for measuring the insulin sensitivity of glucose uptake or suppression of glucose production because the latter processes have dose-response characteristics very different from those of insulin compared with lipolysis (21, 22). The rate of whole-body lipolysis and the effect of insulin on lipolysis were determined by infusing [2H5]glycerol, which was infused for 120 min before and for 120 min during the insulin infusion (start of insulin infusion = time point 0). Before the infusions began, two 18-gauge catheters (Venflon; Viggo-Spectramed, Helsingborg, Sweden) were inserted: one in a left antecubital vein and another retrogradely in a heated (+65°C) dorsal hand vein for sampling of arterialized venous blood for insulin (−120, −60, 0, 30, 60, 90, and 120 min), FFAs (−120, −60, −30, 0, 10, 15, 20, 25, 30, 45, 60, and 120 min), β-hydroxybutyrate (−120, −60, 0, 15, 30, 45, 60, and 120 min), and glycerol and its isotopic enrichment (−120, −30, −20, −10, 0, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min). Glycerol, insulin, and glucose were infused into the antecubital vein. The whole-body insulin sensitivity of glucose metabolism was determined from the glucose infusion rate required to maintain euglycemia corrected for glucose pool size (M-value) during the insulin infusion (0–120 min). Measurement of lipolysis (glycerol rate of appearance; Rg) was technically unsuccessful in one patient (patient included in all other analyses).
Indirect calorimetry

Respiratory gas exchange and resting energy expenditure were recorded for 40 min before the insulin infusion (−70 to −30 min) and for 40 min during euglycemic hyperinsulinemia (75–115 min) by indirect calorimetry using a computerized flow-through canopy system (Deltatrac metabolic monitor; Datex, Helsinki, Finland) as described previously (23). Protein oxidation was estimated from urinary urea nitrogen excretion (23). Rates of carbohydrate and lipid oxidation and total energy expenditure were calculated from the gas exchange data as previously described (23, 24). Because ketone body production and utilization after the ketogenic diet can be predicted to influence indirect calorimetry calculations (24), only data on nonprotein respiratory quotient (NPRQ) are given after the diet.

Subcutaneous fat biopsy samples

To determine whether weight loss influences PNPLA3 expression, a needle aspiration biopsy sample of subcutaneous adipose tissue was collected under local anesthesia with lidocaine at baseline and at the completion of the study. The adipose tissue sample was immediately frozen in liquid nitrogen until analyzed.

Quantitative real-time reverse transcriptase polymerase chain reaction analysis of mRNA expression

Total RNA was isolated by using the RNeasy Mini Kit (Qiagen, Valencia, CA). The RNAs (1000 ng) were reverse transcribed by using the SuperScript VILO cDNA synthesis kit (Invitrogen/Life Technologies, Carlsbad, CA). Each sample was amplified in duplicate (in 2 independent experiments) for the gene of interest and the housekeeping marker 36B4 on a 7000 Sequence Detection System (Applied Biosystems/Life Technologies, Carlsbad, CA) by using the manufacturer’s SYBR-Green kit. Relative quantification for the gene of interest was carried out by using normalization to the 36B4 mRNA values. The following primers were used: PNPLA3, 5’-CTGTACCTGCGCTGGAAT-3’ (sense) and 5’-GGGAAGGTGTAATCC-CATGCTCAACATG-3’ (antisense); 36B4, 5’-CATGCTCAACATTCCCCCTTCTCC-3’ (sense) and 5’-GGGAAAGTGTGAATCCTGCTCCACAG-3’ (antisense).

Analytic procedures

Plasma glucose concentrations during the clamp were measured in duplicate with the glucose oxidase method by using Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). For screening purposes, fasting plasma glucose was measured by using a hexokinase method on an autoanalyzer (Roche Diagnostics Hitachi 917; Hitachi Ltd, Tokyo, Japan). Serum insulin and C-peptide concentrations were measured by time-resolved fluoroimmunoassay with the use of Insulin and C-peptide kits (AUTOdelfia; Wallac, Turku, Finland), respectively. Glycated hemoglobin was measured by HPLC with a fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, CA). Serum total and HDL cholesterol and triglyceride concentrations were measured with respective enzymatic kits from Roche Diagnostics by using an autoanalyzer (Roche Diagnostics Hitachi 917). Serum alanine aminotransferase, serum aspartate aminotransferase, and plasma creatinine concentrations were measured as recommended by the European Committee for Clinical Laboratory Standards. Serum C-reactive protein was analyzed by using a commercial kit (Ultrasensitive CRP Kit; Orion Diagnostica, Espoo, Finland). Ketone bodies were measured with the use of a photometric method with an Olympus AU640 analyzer (Olympus Corporation, Shizuoka, Japan). Urea concentrations in urine were measured by using an enzymatic photometric assay (Roche Diagnostics) with a Modular Analyzer (Hitachi Ltd, Tokyo, Japan). FFAs were measured by using an enzymatic colorimetric assay [NEFA-HR (2); Wako Chemicals GmbH, Neuss, Germany] with a Konelab 60i analyzer (Thermo Electron Corporation, Vantaa, Finland). [1H3]Glycerol enrichment was measured by gas chromatography–mass spectrometry (GC-MS) as previously described (25). Briefly, after deproteinization, plasma sample were derivatized with pyridine and acetic anhydride (1:1) and then reconstituted with ethyl acetate (80 μL). Samples were then injected (2 μL) in the GC-MS (Agilent, Palo Alto, CA), and enrichment was evaluated as the peak area ratio between fragments of mass 148 and 145, after correction for baseline values. During the last 20 min of the tracer equilibration period, both plasma glycerol concentrations and [1H3]glycerol enrichments were stable in all subjects. Therefore, the Ra of endogenous glycerol was calculated as the ratio of the tracer infusion rate to the plasma tracer enrichment (mean of 3 determinations). During the clamp, glycerol Ra was calculated from [1H3]glycerol enrichment by using Steele’s equation (25).

Statistical analyses

Demographic and clinical characteristics of the study groups at baseline were compared by using Fisher’s exact test for categorical variables and unpaired t test for continuous variables. Distribution of continuous variables was tested for normality by using a Kolmogorov-Smirnov test, and, because the studied variables were normally distributed, the use of a parametric t test was justified. To analyze the effect of dietary intervention on the study groups with different genotypes and the potential interaction between the 2 factors, we used 2-factor analysis of variance for repeated measures with Bonferroni post hoc tests for the analysis of the effect of time. For variables significantly different between the groups at baseline, we used analysis of covariance to adjust for the baseline values when calculating the final significance of change due to the dietary intervention. Correlations were calculated as exploratory analyses on the data post hoc by using Spearman’s rank correlation coefficient. For the statistical analyses, GraphPad Prism version 4.03 (GraphPad Software Inc, San Diego, CA), SYSTAT version 10 (SPSS Inc, Chicago, IL), and Lotus 1–2–3 (Lotus SmartSuite Release 9.5; Lotus Development Corporation, IBM Corporation, New York, NY) were used. The data are reported as means ± SEMs. Two-tailed P values <0.05 were considered significant.

RESULTS

Baseline characteristics

The clinical and biochemical characteristics of the study groups are summarized in Table 1. At baseline, the groups were comparable with respect to sex, age, weight, BMI, and liver fat content. Fasting serum insulin and C-peptide concentrations at baseline were significantly lower in the PNPLA3-148MM group than in the PNPLA3-148II group (Table 1).
concentrations in either group (data not shown).

Liver fat

In the entire group, liver fat decreased by 30.0 ± 6.1%, from 11.1 ± 1.4% at baseline to 8.4 ± 1.4% after the 6-d diet (P < 0.001). Liver fat content decreased in the PNPLA3-148MM group by 44.6 ± 7.0%, from 10.1 ± 1.8% to 6.5 ± 1.7% (P < 0.001), and in the PNPLA3-148II group by 18.4 ± 7.8%, from 11.9 ± 2.1% to 9.9 ± 2.0% (P < 0.01) (Figure 1B). Also, the absolute change in liver fat was significantly greater in the PNPLA3-148MM group than in the PNPLA3148II group (−3.7 ± 0.5% compared with −2.0 ± 0.6%; P = 0.042; Figure 1C). The percentage change in liver fat was significantly higher in the PNPLA3-148MM group than in the PNPLA3148II group (P = 0.027; Figure 1D). The absolute and percentage decreases in liver fat were also statistically significant if the baseline liver fat content was included in the analysis as a covariate (P = 0.03).

Fasting serum insulin and whole-body insulin sensitivity of glucose metabolism

In all subjects, fasting serum insulin decreased significantly after the diet, from 10.1 ± 1.4 to 6.4 ± 1.1 mU/L (P < 0.001). The decrease was significant in both the PNPLA3-148MM (P < 0.05) and PNPLA3-148II (P < 0.0001) groups (Table 1).

During the insulin infusion, steady state serum insulin concentrations (30–120 min) for all subjects were similar before (24.5 ± 2.1 mU/L) and after (23.9 ± 1.5 mU/L) the diet (P = 0.69), and no differences were observed between the PNPLA3-148MM and PNPLA3-148II groups (data not shown).

In the entire group, whole-body insulin sensitivity of glucose metabolism (M-value) increased from 0.8 ± 0.1 to 1.1 ± 0.2 mg · kg⁻¹ · min⁻¹ (P = 0.02). The effect of time was statistically significant (P = 0.01), whereas the time × genotype interaction was not statistically significant (Figure 2).

Plasma β-hydroxybutyrate and serum FFA concentrations and whole-body insulin sensitivity of lipolysis

In all subjects, fasting plasma β-hydroxybutyrate concentrations increased 5.4-fold, from 0.17 ± 0.02 to 0.93 ± 0.2 mmol/L after the diet (P < 0.0005). After the diet, fasting plasma β-hydroxybutyrate concentrations increased significantly in the PNPLA3-148MM group from 0.16 ± 0.01 to 1.00 ± 0.28 mmol/L (P < 0.01) and in the PNPLA3-148II group from 0.15 ± 0.01 to 0.62 ± 0.18 mmol/L (P < 0.05) (Figure 3, A and B). No significant effect of genotype on β-hydroxybutyrate concentrations was observed, either basally or during hyperinsulinemia or in response to insulin either before or after the diet at any time point (Figure 3, A and B).

In all subjects, fasting serum FFAs increased from 540 ± 35 μmol/L before to 750 ± 35 μmol/L after the diet (P = 0.0001). The changes in serum FFAs by the diet and by insulin were similar between the PNPLA3-148MM and PNPLA3-148II groups (Figure 3, C and D).

The sensitivity of lipolysis to insulin increased in all subjects, because the percentage suppression of glycerol Ra (lipolysis) by insulin was significantly higher after (48.9 ± 2.6%) than before (36.1 ± 4.3%) the diet (P = 0.002). The percentage suppression was enhanced by the ketogenic diet in the PNPLA3-148MM group (37 ± 5% before and 51 ± 4% after; P < 0.05) and in the PNPLA3-148II group (35 ± 7% before and 47 ± 4% after; P >
No statistical difference, however, was found between the groups with respect to the degree of change in suppression of lipolysis.

**Rates of substrate oxidation and energy expenditure**

At baseline in all subjects, insulin increased the NPRQ from 0.73 ± 0.02 to 0.77 ± 0.02 (P = 0.03), and carbohydrate oxidation increased from 0.25 ± 0.13 to 0.53 ± 0.16 mg · kg⁻¹ · min⁻¹ (P = 0.004). Acute hyperinsulinemia decreased lipid oxidation from 1.02 ± 0.06 to 0.91 ± 0.08 mg · kg⁻¹ · min⁻¹ (P = 0.007) in all subjects. The responses to insulin before the diet did not differ between the study groups.

In all subjects, the rate of protein oxidation was similar before (0.59 ± 0.07 mg · kg⁻¹ · min⁻¹) and after (0.64 ± 0.07 mg · kg⁻¹ · min⁻¹) the diet. Protein oxidation rates were comparable between the groups both before and after the diet (data not shown). The NPRQ was significantly lower after the diet (0.67 ± 0.01) than before the diet (0.73 ± 0.02) in all subjects (P = 0.001). The PNPLA3 genotype did not influence the change in NPRQ.

Correlates of changes in liver fat during the diet

We calculated the correlation coefficients between changes in liver fat and insulin sensitivity of glucose metabolism with those of serum FFA concentrations and glycerol fluxes. The antilipolytic effects of insulin, measured as percentage (absolute data not shown) suppression of serum FFA, correlated with the M-value both before (ρ = −0.67, P = 0.002) and after (ρ = −0.75, P = 0.003) the diet. The percentage change in liver fat correlated significantly with the change in absolute glycerol Ra during hyperinsulinemia (ρ = 0.53, P = 0.028).

**PNPLA3 mRNA and protein expression**

PNPLA3 mRNA was detectable in all adipose tissue samples. In all subjects, expression of the PNPLA3 gene decreased by 27% after the diet (P = 0.044, Figure 4). PNPLA3 expression did not differ significantly between the PNPLA3-148MM and PNPLA3-148II groups either before or after the diet (data not shown).

**DISCUSSION**

**Summary**

The rs738409 minor G allele of PNPLA3 has been suggested to impair triglyceride hydrolysis in in vitro studies (14). Recently, 5 independent studies have shown that homozygous carriers of this allele have an increased risk of nonalcoholic steatohepatitis (9–13), for which weight loss is currently considered the perhaps best treatment (26). We therefore reasoned that it would be important

![FIGURE 1. Mean (±SEM) change in body weight as measured by scale (A) and change in liver fat content (B), absolute change in liver fat content (C), and percentage change in liver fat content (D) as measured by proton magnetic resonance spectroscopy (1H-MRS) after a 6-d hypocaloric, low-carbohydrate diet in the PNPLA3-148MM (n = 8) and PNPLA3-148II (n = 10) genotype groups. Unpaired t test and 2-factor ANOVA for repeated measures were used for data analysis with Bonferroni post hoc test for the effect of time, as appropriate. *P < 0.05, **P < 0.01, ***P < 0.001.](image)

**FIGURE 2.** Mean (±SEM) whole-body insulin sensitivity of glucose metabolism (M-value) before and after the dietary intervention in the PNPLA3-148MM (n = 8) and PNPLA3-148II (n = 10) genotype groups. Two-factor ANOVA for repeated measures was used for data analysis with Bonferroni post hoc test for the effect of time. Effect of time: P = 0.01. Time × genotype interaction: P = 0.15.
to establish whether weight loss is able to decrease liver fat in homozygous carriers of the rs738409 G allele (PNPLA3-148MM group). In response to a 6-d hypocaloric low carbohydrate diet, both groups lost similar amounts of body weight and ketone body concentrations increased in a comparable manner. Liver fat content decreased significantly more both in absolute and relative units in the PNPLA3-148MM than the PNPLA3-148II group. These data show that weight loss is an effective means for reducing liver fat content in subjects with PNPLA3-148MM.

Selection and matching of subjects

Previous studies have found liver fat content to be significantly (1.3–2.4-fold) higher but fasting insulin concentrations similar in carriers of PNPLA3-148MM as compared with PNPLA3-148II (3, 5–7). In the present study, we compared groups with similar baseline liver fat content to allow reliable comparison of its changes during a hypocaloric low carbohydrate diet. This matching resulted, expectedly, in a baseline difference in insulin sensitivity as measured using fasting insulin concentrations. Liver fat decreased significantly more in absolute and relative units in subjects with PNPLA3-148MM than the PNPLA3-148II group. These data show that weight loss is an effective means for reducing liver fat content in subjects with PNPLA3-148MM.

Change in lipolysis, ketones, and NPRQ in the entire group

In the present study, we measured whole-body rates of lipolysis, as at least 2 large studies have shown that serum FFA are closely correlated with liver fat content under hyperinsulinemic, but not fasting conditions (27, 28). Because lipolysis is suppressed by very low insulin concentrations even in insulin-resistant subjects (21, 29, 30), we chose a low insulin infusion dose

![FIGURE 3. Mean (±SEM) plasma β-hydroxybutyrate concentrations (A and B), serum free fatty acid concentrations (S-FFA; C and D), and rate of appearance (Rg) of glycerol (E and F) during the euglycemic hyperinsulinemic clamp before (A, C, and E) and after (B, D, and F) the dietary intervention in the PNPLA3-148MM (●; n = 8) and PNPLA3-148II (○; n = 10 for β-hydroxybutyrate and FFA, and n = 9 for glycerol Rg) genotype groups. Two-factor ANOVA for repeated measures was used for data analysis. None of the data points differ significantly between the PNPLA3-148MM and the PNPLA3-148II groups.](image-url)

![FIGURE 4. Mean (±SEM) percentage change from baseline (set to equal 100%) in PNPLA3 mRNA concentrations. n = 18. *Significant difference between before and after the diet, P < 0.05 (unpaired t test).](image-url)
to prevent complete suppression of lipolysis. We found the low carbohydrate ketogenic diet to decrease serum insulin and increase FFA concentrations significantly and similarly in both groups. The increase in FFA provides fuel for maintenance of gluconeogenesis and ketogenesis during carbohydrate restriction (31). Consistent with enhanced lipid or ketone body oxidation, the NPRQ decreased significantly. In the entire group, the sensitivity of lipolysis to insulin, measured as the % suppression of either FFA or glycerol Ra by insulin increased significantly (Figure 3). These data are novel as we are not aware of studies measuring in vivo rates of lipolysis before and after weight loss in humans. Enhanced insulin suppression of FFA after weight loss has been observed in our own previous study and that of Kelley et al (32, 33).

These are the first data directly quantifying the rate of in vivo lipolysis in homozygous carriers of rs738409 PNPLA3 variants (PNPLA-148MM and PNPLA-148II). A recent cross-sectional study by Santoro et al (34) reported glycerol turnover rates between the carriers of PNPLA-148II and “G allele carriers” (ie, PNPLA-148IM and/or PNPLA-148MM) in 41 obese subjects matched for BMI, but not liver fat. As in the present study, no difference was observed in insulin sensitivity of lipolysis between the study groups.

Although we found no significant difference in rates of lipolysis between the groups, there was a significant correlation between the change in lipolysis measured with [2H5]glycerol during hyperinsulinemia and the change in liver fat content. Because peripheral lipolysis is the major source of intrahepatocellular triglycerides both under fasting and postprandial conditions (35), this result suggests that the greater decrease in liver fat in the PNPLA-148MM group was due to a change in insulin action on FFA delivery to the liver. As insulin action on lipolysis and the M-value were highly significantly correlated before and after the diet, the greater insulin sensitivity of the PNPLA3-148MM than the PNPLA3-148II group could have contributed to the greater decrease in liver fat.

The whole-body glucose infusion rate corrected for changes in the glucose pool size (the M-value) tended to increase more in the PNPLA-148MM than the PNPLA3-148II group. The greater increase, in view of the low insulin concentration used, is likely to reflect greater improvement in hepatic insulin sensitivity due to greater loss of liver fat in the PNPLA-148MM than the PNPLA3-148II group. This interpretation is supported by data of Kirk et al (15), who using a similar hypocaloric ketogenic diet, found an improvement in hepatic but not peripheral insulin sensitivity.

De novo lipogenesis

After peripheral lipolysis, de novo lipogenesis is quantitatively the second most important source of intrahepatocellular triglycerides in NAFLD (35, 36). Insulin stimulates de novo lipogenesis via liver X receptor (LXR) and the sterol regulatory element binding protein 1c (SREBP1c) (37, 38). In rodent models, this pathway has been suggested to be unaffected by insulin resistance leading to its stimulation in hyperinsulinemic animals (39). The 45% lower insulin concentration in the PNPLA3-148MM than the PNPLA3-148II group (Table 1) could, therefore, have been associated with a lower rate of de novo lipogenesis, but this possibility was not directly explored.

Adipose tissue and liver PNPLA3

Consistent with other SREBP1c–activated genes, PNPLA3 is strongly upregulated by insulin and refeeding (40–44). One would, therefore, expect weight loss to decrease PNPLA3 expression. This was found in adipose tissue but the sample size is too low to allow conclusions regarding effects of the PNPLA3 genotype on changes in PNPLA3 gene expression.

We conclude that weight loss is an effective way to decrease liver fat irrespective of the PNPLA3 genotype. Indeed, short-term weight loss decreases liver fat content even more in homozygous carriers of PNPLA3-148MM than in those carrying PNPLA3-148II. Because the PNPLA3-148MM genotype predisposes its carriers to severe liver disease (9–13), these patients might be those particularly benefiting from weight loss–induced decrease in liver fat.

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