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Downregulation of ADIPOQ and PPAR γ 2 Gene-Expression in Subcutaneous Adipose Tissue of Obese Adolescents With Hepatic Steatosis

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Abstract

Hepatic steatosis is associated with hypoadiponectinemia. The mechanism(s) resulting in lower serum adiponectin levels in obese adolescents with fatty liver is unknown. In two groups of equally obese adolescents, but discordant for hepatic fat content, we measured adiponectin, leptin, peroxisome proliferator-activated receptor γ 2 (PPAR γ 2) and tumor necrosis factor- α (TNF α) gene expression in the abdominal subcutaneous adipose tissue (SAT). Twenty six adolescents with similar degrees of obesity underwent a subcutaneous periumbilical adipose tissue biopsy, in addition to metabolic (oral glucose tolerance test, and hyperinsulinemic—euglycemic clamp), and imaging studies (magnetic resonance imaging (MRI), DEXA). Using quantitative real-time-PCR; adiponectin, PPAR γ 2, TNF α , and leptin mRNA were measured. Based on a hepatic fat content (hepatic fat fraction, HFF) >5.5%, measured by fast MRI, the subjects were divided into low and high HFF group. In addition to the hypoadiponectinemia in the high HFF group, we found that the expression of adiponectin as well as PPAR γ 2 in the SAT was significantly decreased in this group. No differences were noted for TNF α and leptin plasma or mRNA levels between the groups. An inverse relationship was observed between adiponectin or PPAR γ 2 expression and hepatic fat content, whereas, adiponectin expression was positively related to PPAR γ 2 expression. Independent of overall obesity, a reduced expression of adiponectin and PPAR γ 2 in the abdominal SAT is associated with high liver fat content, as well as with insulin resistance in obese adolescents.

INTRODUCTION

Concurrent with the worldwide epidemic of childhood obesity, nonalcoholic fatty liver disease has emerged as one of the most common chronic liver diseases in pediatrics (1). Cross-sectional studies from our group in a large multiethnic cohort of obese children and

adolescents, showed a “dose–response” relationship between rising hepatic fat content measured by fast magnetic resonance imaging (MRI) and prediabetes, independent of the degree of obesity (2). Thus, fatty liver may be an early marker of looming diabetes in obese kids. The mechanisms underlying fat accumulation in the human liver are poorly understood.

The adipose tissue secretes several bioactive proteins, or adipokines, that regulate hepatic and peripheral glucose and lipid metabolism. These adipokines include leptin, tumor necrosis factor- α (TNF α), resistin, and adiponectin. There is growing evidence that these adipokines, particularly adiponectin, are critical modulators of insulin resistance (3). Importantly, adiponectin has antilipogenic effects that may protect nonadipose tissues, such as the liver, against lipid accumulation (4). In addition, the conditions most associated with the development of nonalcoholic fatty liver disease, namely obesity (5), insulin resistance (6), type 2 diabetes, (7) and dyslipidemia (8) all have reduced adiponectin levels. Adiponectin also has anti-inflammatory effects that could protect the fatty liver from the development of inflammation and cell injury (necroinflammatory change) (9). Adiponectin and TNF α suppress each other's gene expression, protein synthesis and production in adipocytes (10). Hence, adiponectin and TNF α have opposing effects on insulin sensitivity and inflammation (10), and the balance between these two adipokine systems may be important to the pathogenesis of hepatic steatosis.

Several studies have examined plasma adiponectin and adiponectin mRNA levels in humans and found decreased levels in obese and adult type 2 diabetic subjects (7,11–13). However, there are currently no studies in children and adolescents and studies in adults have not determined the relation between adiponectin expression and hepatic fat content and/ or circulating cytokines, like TNF α and interleukin-6 (IL-6). We hypothesize that independent of obesity, low adiponectin plasma and mRNA level are associated with hepatic steatosis in obese adolescents, mediated by decreased peroxisome proliferator-activated receptor γ 2 (PPAR γ 2) expression in subcutaneous adipose tissue (SAT).

METHODS AND PROCEDURES

Subjects

The Yale Pathophysiology of Type 2 Diabetes in Obese Youth Study is a long-term project examining early alterations in glucose metabolism in relation to fat patterning in obese adolescents. Twenty six obese adolescents participating in this study agreed to have a subcutaneous periumbilical adipose tissue biopsy. Their clinical characteristics are described in **Table 1**. Subjects were not on any medications, had no known major organ system disease as determined by history, physical examination, and standard laboratory tests (hemoglobin, sedimentation rate, and electrolytes plasma glucose). All subjects denied alcohol consumption assessed by a questionnaire and no history of liver diseases. The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The study was approved by the ethics committees of the Yale University Hospital and the National Institutes of Health.

Metabolic phenotyping

Oral glucose tolerance test—All subjects were invited to the Yale Center for Clinical Investigation at 8 am after an overnight fast (14,15). Following placement of an indwelling venous line, baseline samples were obtained for glucose, insulin, C-peptide, lipid profile, liver enzymes, total and high molecular weight adiponectin, leptin, and IL-6 levels. Thereafter, a standard 3-h oral glucose tolerance test was performed (14,15). Impaired fasting glucose, impaired glucose tolerance or combined (impaired fasting glucose +

impaired glucose tolerance) was defined in accordance with the American Diabetes Association guidelines (16). Insulin sensitivity was estimated by calculating the whole body insulin sensitivity index (Matsuda index) (17,18) and the homeostasis model assessment of insulin resistance (19).

Assessment of peripheral insulin sensitivity: the hyperinsulinemic-euglycemic clamp—Peripheral insulin sensitivity was determined in the morning at 8 AM, after an overnight fast of 10–12 h, by the hyperinsulinemic-euglycemic clamp using a prime continuous infusion of 80 mU/m²·min for 120 min, as previously described (20).

Fast MRI: liver fat content—Measurement of liver fat content was performed by MRI using the two-point dixon method as modified by Fishbein *et al.* (21) based on phase-shift imaging where hepatic fat fraction (HFF) is calculated from the signal difference between the vectors resulting from in- and out-of-phase signals. Using the MRIcro software program, five regions of interest were drawn on each image, and the mean pixel signal intensity level was recorded. The HFF was calculated in duplicate from the mean pixel signal intensity data using the formula: $((\text{Sin}-\text{Sout})/(2 \times \text{Sin})) \times 100$. Due to unavoidable image heterogeneity, normal nonfatty livers returned similar signals with in and out-of-phase sequences, resulting in negative HFF values for some subjects. These values were equated with undetectable fat accumulation. The presence or absence of steatosis was determined by a threshold value for HFF of 5.5% (>2.5 s.d. above the mean of our controls). Supporting this cut-off value are the results from a large study that applied a more quantitative measure of hepatic fat content using ¹H-magnetic resonance spectroscopy (MRS) of which the 95th percentile of hepatic fat content was 5.5% (22,23).

Validation of fast MRI—We validated the modified two-point dixon method against ¹H-nuclear magnetic resonance in 34 lean and obese adolescents (26 of these subjects are included in the present study) and found a very strong correlation between the two methods ($r = 0.954$, $P < 0.0001$) (24). To assess its repeatability, measurements were obtained (within the same day) on 12 subjects. The within-subject standard deviation for HFF was 1.9%. This degree of reproducibility is well within the boundaries of that necessary to make this a viable method to assess the relation between HFF and metabolic outcomes. Kim *et al.* (24) demonstrated that a two-point dixon HFF cutoff of 3.6%, provided good sensitivity (80%) and specificity (87%) compared to a ¹H-magnetic resonance spectroscopy reference. Comparisons between the two-point dixon method and histologic determination of fatty liver have been made, albeit only in adults. Fishbein *et al.* (25) found in 38 patients undergoing biopsy for a variety of liver diseases a highly significant correlation between liver histology and MRI determination of HFF, particularly with macrovesicular steatosis ($r = 0.920$, $P < 0.001$).

Abdominal MRI and total body composition (DEXA)—Abdominal MRI studies were performed on a Siemens Sonata 1.5 Tesla system (26,27). Total body composition was measured by dual-energy X-ray absorptiometry with a Hologic scanner (Hologic, Boston, MA).

Adipose tissue biopsy and quantitative real-time-PCR—On a separate day from the clamp study and MRI's, all subjects return to the Yale Center for Clinical Investigation for the biopsy of the abdominal subcutaneous tissue. Adipose tissue was obtained under sterile conditions with administration of 0.25 lidocaine with adrenaline (epinephrine) for local anaesthesia. A 1 cm scalpel incision was made inferior to the umbilicus, from which ~2 g of SAT was removed. Samples of 0.3 g were immediately flash frozen in liquid nitrogen.

Total RNA was extracted using Qiazol (Qiagen, Valencia, CA) and the Adipose Tissue RNAeasy kit (Qiagen) according to the manufacturers' instructions. After DNase treatment, cDNA was synthesized from 2 µg of total RNA using the Maloney murine leukaemia virus reverse transcriptase (New England BioLabs, Ipswich, MA). Relative quantification of mRNAs was performed by real-time-PCR using an ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). In brief, primers for the target genes (adiponectin (ADIPOQ), forward 5'-TGCCCCAGCAAGTGTAACC-3', reverse 5'-TCAGAAACAGGCACACAACCTCA-3'; PPAR γ 2, forward 5'-TCAGGGCTGCCAGTTTCG-3', reverse 5'-GCTTTTGGCATACTCTGTGATCTC-3'; TNF α , forward 5'-GCAGGTCTACTTTGGGATCATTG-3', reverse 5'-GCGTTTGGGAAGGTTGGA-3'; leptin, forward 5'-CGGAGAGTACAGTGAGCCAAGA-3', reverse 5'-CGGAATCTCGCTCTGTCATCA-3') and the reference gene (18S ribosomal RNA, forward 5'-CGAACGTCTGCCCTATCAACTT-3', reverse 5'-ACCCGTGGTCACCATG GTA-3') were purchased from Integrated DNA Technologies (Coralville, IA). Two microliter of cDNA was brought to a final volume of 20 µl in a 96-well plate containing FAST SYBR-Green PCR Master Mix (Applied Biosystems), and primers. PCR was performed with 20 s of initial denaturation at 95 °C, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. A threshold cycle (C_t value) was obtained from each amplification curve and a ΔC_t value was first calculated by subtracting the C_t value for 18S ribosomal RNA from the C_t value for the target gene of the same sample. A $\Delta\Delta C_t$ value was then calculated by subtracting the ΔC_t value of a control subject from the ΔC_t value of the subject. Fold changes compared with the control were then determined by calculating $2^{\Delta\Delta C_t}$, where the control has the value 1.

Statistics

Subjects were classified into two groups according to their hepatic fat content (HFF): "low" (HFF <5.5%), and "high" (HFF >5.5%) liver fat content. We used a cut-off value of 5.5% (>2.5 s.d. above the mean of our control), suggested from previous reports (14,15,26), and supported by a recent study that applied a direct measure of hepatic triglyceride content using magnetic resonance spectroscopy (23). We compared demographic, anthropometric, and metabolic characteristics and outcomes of interest between children with high and low hepatic fat content. Simple unadjusted comparisons were done with the Fisher's Exact test for categorical variables, and with the Mann-Whitney test for continuous variables. Analysis of variance was used to compare anthropometric and metabolic characteristics, as well as the outcomes, adjusting for age, gender and race. Variables with non-normal distribution were log-transformed for analysis of variance. Data are represented as N (%) and mean with 95% confidence intervals. To compare mRNA levels of adiponectin with mRNA levels of PPAR γ 2, TNF α , Leptin and plasma levels of adiponectin, we used the Spearman ρ statistics. The association between HFF% and mRNA levels of adiponectin, PPAR γ 2, TNF α , and Leptin, were also tested with the Spearman ρ statistic. A P value of <0.05 was considered statistically significant. All analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL).

RESULTS

Anthropometric phenotypes according to liver fat content

Fifty percent of the cohort ($n = 13$) had a hepatic fat content below 5.5% (HFF 1.5% (−0.4–3.3%)) and thus were classified as low HFF. On the other hand, 50% had increased liver fat content, and were classified as high HFF (HFF 18.4% (11.4–25.5%)) (**Table 1**). As shown in **Table 1**, age, gender, race, BMI, and % fat were comparable between groups. A significant increased ratio of visceral to visceral + subcutaneous (visceral adipose tissue

(VAT)/VAT + SAT) fat was noted, whereas the amount of visceral fat was not significant different.

Metabolic phenotypes according to liver fat content

Although there was a significant increase in fasting plasma insulin and 2-h C-peptide in the high HFF group, the glucose plasma level didn't reach significance. Peripheral insulin sensitivity, as measured by the clamp, was significantly lower in the group with fatty liver (M/lean body mass; $P = 0.006$) and it was also clearly reduced when estimated by the homeostasis model assessment of insulin resistance ($P = 0.014$), or the whole body insulin sensitivity index ($P = 0.005$) after adjusting for race, gender, and age (**Table 2**). Plasma fatty acids, alanine aminotransferase and aspartate aminotransferase were significantly higher in the high HFF group. Consistent with previous studies, circulating total adiponectin was significantly lower in the group with high liver fat content, even after adjusting for race, gender and age ($P = 0.009$). Levels of leptin and TNF α were not different between the two groups, whereas IL-6 was significantly higher in the high HFF group after adjusting for race, gender and ethnicity ($P = 0.049$).

Adipokines and PPAR γ 2 mRNA in the SAT according to the hepatic fat content

Despite similar degree of overall obesity, the expression of both adiponectin ($P = 0.030$) as well as PPAR γ 2 ($P = 0.013$) in the SAT were significantly lower in the high HFF group compared to the low HFF group and remained significant for PPAR γ 2 after adjusting for age, gender and race ($P = 0.014$), whereas adiponectin slightly missed significance ($P = 0.057$) (**Figure 1**).

In contrast, both leptin and TNF α mRNA level were not different between the two groups.

Relationship between hepatic fat content and gene expression of adiponectin and PPAR γ 2

Besides adiponectin plasma level ($\rho = -0.494$, $P = 0.010$), both adiponectin (*ADIPOQ*; $\rho = -0.400$, $P = 0.043$) and PPAR γ 2 ($\rho = -0.418$, $P = 0.034$) mRNA levels were found to be significantly and inversely correlated with the hepatic fat content (HFF), whereas there was no significant relationship between TNF α or leptin mRNA levels and hepatic fat content (data not shown) (**Figure 2**). There was also a highly significant positive correlation between PPAR γ 2 and adiponectin (*ADIPOQ*) mRNA levels in the SAT ($\rho = 0.954$, $P = 0.000$). Both, *ADIPOQ* and PPAR γ 2 mRNA levels were also positively significant related to mRNA levels of Leptin and TNF α .

The correlation between mRNA level and plasma level were only significant for Leptin ($r = 0.409$, $r = 0.038$), whereas for adiponectin it missed significance ($r = 0.311$, $P = 0.121$).

DISCUSSION

To gain insights into the mechanism(s) that might contribute to low adiponectin levels seen in subjects with fatty liver, we quantified adiponectin, TNF α , leptin, and PPAR γ 2 gene expression by real-time-PCR in biopsies from the abdominal subcutaneous fat tissue of two groups of obese adolescents, well matched for overall adiposity but discordant for the level of hepatic fat content. The novel finding is that, independent of obesity, the amount of liver fat content is associated with lower adiponectin (*ADIPOQ*) and PPAR γ 2 expression in the SAT of obese adolescents.

It should be noted, that while all subjects in the high HFF group had low *ADIPOQ* and PPAR γ 2 mRNA level, we saw subjects with high but also low mRNA levels in the low HFF group. Indeed, the variance in the gene expression for adiponectin and PPAR γ 2 in the low

group may be reflecting another third group that is not yet classified as having an abnormal liver fat content but is at the lower end of insulin-sensitivity and therefore likely at very high risk for accumulating fat in their liver. This however would require a longitudinal follow up of the subjects. Because *ADIPOQ* and PPAR γ 2 expression were also negatively correlated to the visceral to subcutaneous abdominal fat ratio ($P < 0.005$), a reduced adipogenesis in the subcutaneous abdominal layer could explain the decreases in adiponectin, PPAR γ 2 and leptin expression. Paralleling the severity of hepatic steatosis, there was a significant decrease in insulin sensitivity, as previously reported. Due to the cross-sectional nature of our study, we cannot exclude the possibility that insulin resistance might be driving the reduced adiponectin and PPAR γ 2 gene expression in the peripheral adipocyte, leading to lower subcutaneous storage capacity and lipid spill-over into the liver. A limitation of this study is that data shown are only based on the hepatic fat content measured by the MRI, although we do not have evidence of advanced liver disease in our subjects, because liver biopsies are lacking we cannot totally exclude the possibility.

Animal models and uncontrolled human studies have indicated that adiponectin could confer protective effects against alcoholic and nonalcoholic fatty liver diseases (4). In the present study, we confirm the decreased serum levels of adiponectin in adolescents with hepatic steatosis when compared to matched controls, and provide new additional information of impaired adiponectin synthesis in the SAT. These data are consistent with findings of reduced adiponectin gene expression in subcutaneous and VAT of obese adult subjects, patients with type 2 diabetes and patients with nonalcoholic steatohepatitis (12,28,29).

To our surprise, no differences were found in leptin and TNF α plasma or mRNA levels between the two groups. Several *in vitro* studies showed that TNF α inhibits adiponectin mRNA levels in 3T3-L1 adipocytes (30,31) and in human preadipocytes (32). In contrast, we found a significant positive relationship between TNF α mRNA and adiponectin mRNA level ($r = 0.610$, $P = 0.001$). Upregulation of *ADIPOQ* could work as a counter regulatory response to increased TNF α expression in the SAT. Due to a relatively small sample size we can not exclude that an increase in the pro-inflammatory cytokines is associated with hepatic steatosis. An increase in IL-6 plasma levels in the high HFF group would point in this direction. We found a positive correlation of IL-6 plasma level and hepatic fat content ($r = 0.472$, $P = 0.023$), because we had IL-6 level only for 23 of these 26 patients the statistical significance is not clear.

PPAR γ is known to play a key role in the regulation of adiponectin gene expression in adipose tissue (33). Here we showed a highly significant positive correlation between PPAR γ 2 and adiponectin gene expression in the SAT. Thiazolidinediones, potent ligands for PPAR γ , have been found to increase adiponectin levels and prevent alcohol-induced liver injury in rats, and more recently to reduce fatty liver and steato-hepatitis in humans (34–36). Human studies of impaired glucose tolerance or diabetic subjects, using thiazolidinedione therapy, showed 30–70% increase in adipocyte adiponectin expression, but a doubling of the plasma adiponectin level (37–39). Rasouli *et al.* therefore hypothesize that increased plasma adiponectin levels in response to thiazolidinedione treatment result from translational or post-translational changes instead of increased gene expression (37). Results from the present study suggest that lower serum adiponectin levels in obese subjects with fatty liver result, at least in part, from reduced adiponectin synthesis in adipocytes, which is correlated to a reduced PPAR γ 2 expression. Because the correlation between adiponectin plasma level and *ADIPOQ* expression in the SAT didn't reach significance, translational or post-translational changes seem to further contribute to lower adiponectin plasma level. Because the present study measured adiponectin expression only in SAT, the mechanism of gene expression could be different in VAT. Testing of paired samples (subcutaneous and VAT) could help to answer this question.

In conclusion, we showed for the first time that independent of overall adiposity, adiponectin was downregulated in the abdominal subcutaneous fat depot of obese adolescents with hepatic steatosis. Furthermore, we provide evidence that PPAR γ 2 may mediate this effect, because it is not only positively correlated with adiponectin expression, but also negatively correlated with the amount of liver fat.

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Figure 1.

Differences in mRNA concentration in subcutaneous adipose tissue, expressed as relative to 18S RNA, between the low and high liver fat content group (HFF%) (a) ADIPOQ mRNA, (b) PPAR γ 2 mRNA, (c) Leptin mRNA, (d) TNF α mRNA. *P* describes the unadjusted *P* value.

Figure 2.

Correlation between liver fat content in % and (a) ADIPOQ (Spearman $\rho = -0.400$, $P = 0.043$) or (b) PPAR γ 2 (Spearman $\rho = -0.418$, $P = 0.034$) mRNA concentration in subcutaneous adipose tissue expressed as relative to 18S RNA. (c) Correlation between ADIPOQ and PPAR γ 2 mRNA concentration in subcutaneous adipose tissue expressed as relative to 18S RNA (Spearman $\rho = 0.954$, $P = 0.000$).

Table 1

Demographic, anthropometric, and clinical characteristics of subjects with low hepatic fat content compared to subjects with high hepatic fat content (%), adjusted for age, gender, race/ethnicity

	Low hepatic fat content <5.5% (n = 13)	High hepatic fat content >5.5% (n = 13)	P value	P value adjusted
Age (years)	16.2 (14.4, 18.1)	14.8 (13.2, 16.5)	0.352	
Gender-no. (%) ^a			0.430	
Male (n = 14)	6 (46%)	8 (61.5%)		
Female (n = 12)	7 (54%)	5 (38.5%)		
Race/ethnicity-no. (%) ^a			0.625	
White (n = 8)	4 (30.8%)	4 (30.8%)		
African American (n = 9)	5 (38.5%)	4 (30.8%)		
Hispanic (n = 9)	4 (30.8%)	5 (38.5%)		
Weight (kg)	106.8 (93.3, 120.2)	108.9 (93.1, 124.7)	0.739	0.669
BMI (kg/m ²)	36.8 (32.6, 41.0)	39.6 (35.3, 43.8)	0.369	0.280
BMI z-score	2.2 (1.9, 2.5)	2.6 (2.4, 2.8)	0.050	0.196
Waist (cm)	103.2 (89.3, 117.1)	115.0 (105.8, 124.2)	0.184	0.300
DEXA				
%Fat	37.2 (31.7, 42.6)	40.0 (36.7, 43.2)	0.694	0.115
Tissue lipid content				
Liver				
Hepatic fat fraction (HFF%)	1.5 (−0.4, 3.3)	18.4 (11.4, 25.5)	0.000	0.000
Abdominal Region				
VAT (cm ²)	72.5 (49.9, 95.2)	86.5 (67.5, 105.6)	0.228	0.143 ^b
SAT (cm ²)	616.0 (471.3, 760.7)	599.5 (480.3, 718.6)	0.898	0.911 ^b
VAT/VAT + SAT	0.107 (0.084, 0.129)	0.131 (0.099, 0.164)	0.228	0.043^b
DeepSubQ (cm ²)	220.9 (161.1, 280.8)	222.9 (169.0, 276.7)	0.870	0.917 ^b
SupSubQ (cm ²)	142.0 (111.3, 172.6)	145.6 (110.0, 181.1)	0.786	0.758 ^b
Deep/Deep+SupSubQ	0.601 (0.550, 0.652)	0.599 (0.544, 0.653)	0.957	0.583 ^b

Data are presented as means (95% CI). P value in bold indicate significance ($P < 0.05$).

DeepSubQ, deep subcutaneous adipose tissue; SAT, subcutaneous adipose tissue; SupSubQ, superficial subcutaneous adipose tissue; VAT, visceral adipose tissue.

^a Fishers exact test.

^b P value for log transformed data.

Table 2

Metabolic characteristics of subjects with low hepatic fat content compared to subjects with high hepatic fat content (%), adjusted for age, gender, race/ethnicity

	Low hepatic fat content <5.5% (<i>n</i> = 13)	High hepatic fat content >5.5% (<i>n</i> = 13)	<i>P</i> value	<i>P</i> value adjusted
Fasting plasma glucose (mg/dl)	95.6 (92.3, 98.9)	106.6 (95.9, 117.3)	0.081	0.086
2 h-Glucose (mg/dl)	115.6 (103.5, 127.7)	142.9 (114.9, 170.8)	0.095	0.340
Fasting plasma insulin (μU/ml)	27.3 (19.0, 35.7)	51.3 (34.7, 67.9)	0.014	0.023^a
2 h-Insulin (μU/ml)	104.5 (53.5, 155.6)	241.5 (118.8, 364.16)	0.101	0.170 ^a
Fasting C-peptide (pmol/l)	835.8 (380.9, 1,290.7)	1,282.3 (902.1, 1,662.5)	0.059	0.654 ^a
2 h-C-peptide (pmol/l)	2,381.0 (1,784.9, 2,977.1)	4,152.0 (2,665.4, 5,638.6)	0.010	0.013^a
<i>Insulin sensitivity indexes</i>				
HOMA _{IR}	6.5 (4.4, 8.6)	13.5 (9.0, 18.0)	0.010	0.014^a
WBISI (Matsuda index) (10 ⁻⁴ dl/min per μU/ml)	2.6 (1.6, 3.6)	1.2 (0.7, 1.7)	0.002	0.005^a
M/LBM (kg/LBM ^a min)	11.4 (8.2, 14.5)	6.8 (4.9, 8.7)	0.015	0.006^a
<i>Lipids</i>				
HDL-cholesterol (mg/dl)	41.7 (38.1, 45.3)	39.7 (35.2, 44.2)	0.504	0.685 ^a
Triglycerides (mg/dl)	96.7 (75.7, 117.7)	148.3 (94.3, 202.3)	0.091	0.153 ^a
Free fatty acids (FFA) (μmol/l)	178.7 (416.4, 541.0)	638.0 (519.3, 756.7)	0.017	0.023^a
<i>Liver enzymes</i>				
ALT (IU/l)	13.3 (9.4, 17.2)	33.5 (20.8, 46.1)	0.003	0.007^a
AST (IU/l)	16.3 (17.8, 17.9)	25.5 (19.1, 31.8)	0.001	0.005^a
GGT (IU/l)	22.1 (0.4, 43.8)	31.4 (16.7, 46.2)	0.009	0.305 ^a
<i>Adipokines</i>				
Total adiponectin (μg/ml)	9.7 (6.3, 13.0)	5.1 (4.3, 5.9)	0.002	0.009^a
High molecular weight adiponectin (μg/ml)	1.9 (0.6, 3.3)	1.1 (0.8, 1.4)	0.217	0.136 ^a
Leptin (ng/ml)	35.9 (24.4, 47.4)	31.1 (22.8, 39.4)	0.342	0.723 ^a
TNFα (pg/ml)	1.95 (1.40, 2.50)	2.24 (1.61, 2.87)	0.473	0.479 ^a
IL-6 (pg/ml)	1.47 (0.91, 2.04)	2.32 (1.45, 3.20)	0.097	0.049^a

Data are presented as means (95% CI). *P* values in bold indicate significance.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; HOMA_{IR}, homeostasis model assessment of insulin resistance; IL-6, interleukin-6; LBM, lean body mass; TNF-α, tumor necrosis factor-α; WBISI, whole body insulin sensitivity index.

^a*P* value for log transformed data.