Increased fat accumulation in liver may link insulin resistance with subcutaneous abdominal adipocyte enlargement, visceral adiposity, and hypoadiponectinemia in obese individuals[1–3]

Juraj Koska, Norbert Stefan, Paska A Permana, Christian Weyer, Mina Sonoda, Clifton Bogardus, Steven R Smith, Denis R Joanisse, Tohru Funahashi, Jonathan Krakoff, and Joy C Bunt

ABSTRACT

Background: Enlargement of adipocytes from subcutaneous abdominal adipose tissue (SAT), increased intrahepatic lipid content (IHL), intramyocellular lipid content (IMCL), and low circulating adiponectin concentrations are associated with insulin resistance.

Objective: Because adiponectin increases fat oxidation in skeletal muscle and liver, and the expression of the adiponectin gene in SAT is inversely associated with adipocyte size, we hypothesized that hypoadiponectinemia links hypertrophic obesity with insulin resistance via increased IMCL and IHL.

Design: Fifty-three obese Pima Indians with a mean (±SD) age of 27 ± 8 y, body fat of 35 ± 5%, and normal glucose regulation (normal fasting and 2-h glucose concentration per WHO 1999 criteria) underwent euglycemic-hyperinsulinemic clamp, biopsies of SAT and vastus lateralis muscle, and magnetic resonance imaging of the abdomen.

Results: Adipocyte diameter (AD) correlated positively with body fat ($P < 0.0001$) and IHL (estimated from magnetic resonance imaging intensity of liver; $P = 0.047$). No association was found between AD and plasma adiponectin or IMCL. Plasma adiponectin negatively correlated with type II IMCL (IIA, $P = 0.004$; IIX, $P = 0.009$) or IHL ($P = 0.02$). In a multivariate analysis, plasma adiponectin, AD, and visceral adipose tissue (VAT) independently predicted IHL. Low insulin-mediated glucose disposal was associated with low plasma adiponectin ($P = 0.02$) and high IHL ($P = 0.0003$), SAT ($P = 0.02$), and VAT ($P = 0.04$). High IHL was the only predictor of reduced insulin-mediated suppression of hepatic glucose production ($P = 0.02$) and the only independent predictor of insulin-mediated glucose disposal in a multivariate analysis.

Conclusions: Increased lipid content in the liver may independently link hypoadiponectinemia, hypertrophic obesity, and increased visceral adiposity with peripheral and hepatic insulin resistance. *Am J Clin Nutr* 2008;87:295–302.

KEY WORDS Obesity, adipocyte size, adiponectin, intrahepatic fat, intramyocellular fat, insulin action

INTRODUCTION

Obesity is characterized by a growing adipose tissue mass associated with increases in adipocyte size and number (1). It has been known for a few decades that obese people with enlarged subcutaneous adipocytes (ie, with hypertrophic obesity) are, on average, more hyperinsulinemic than those with a similar degree of adiposity but relatively smaller adipocytes (2). More recent analyses have shown that the enlargement of subcutaneous abdominal adipocytes is associated with reduced insulin-mediated glucose disposal independent of overall adiposity (3, 4).

It has been hypothesized that enlargement of subcutaneous adipocytes is a sign of reduced adipogenic capacity of subcutaneous adipose tissue (SAT), which in turn leads to increased accumulation of fat in visceral adipose tissue (VAT), skeletal muscle, and liver and a subsequent worsening of insulin action and glucose tolerance (5). In support of this hypothesis, implantation of adipose tissue in lipodystrophic mice (mice with no

[1] From the Obesity and Diabetes Clinical Research Section, NIDDK/NIH/DHHS, Phoenix, AZ (JK, NS, CW, CB, JK, and JCB); Carl T Hayden Veterans Affairs Medical Center, Phoenix, AZ (PAP); the Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Osaka, Japan (MS and TF); Pennington Biomedical Research Center, Baton Rouge, LA (SRS); and the Department of Kinesiology, Laval University, Quebec, Canada (DRJ).

[2] Supported by an intramural research program of the NIDDK/NIH/DHHS (to JK, NS, PAP, CW, CB, JK, and JCB) and a new investigator award from the Fonds de la Recherche en Santé du Québec (to DRJ).

[3] Reprints not available. Address correspondence to J Krakoff, ODCRS/NIDDK/NIH/DHHS; 4212 N 16th Street, Phoenix, AZ 85016. E-mail: jkrakoff@mail.nih.gov.

Received June 8, 2007.

adipogenic potential) is followed by reversal of hepatic steatosis, insulin resistance, and hyperglycemia (6, 7). Moreover, administration of thiazolidinediones (TZDs), a class of antihyperglycemic medications that increase proliferation of adipose tissue (8), leads to a reduction of fat content in the liver and improvement of insulin action despite increased body weight (9).

The mechanism underlying abnormal partitioning of lipids in hypertrophic obesity, and metabolic derangements reminiscent of those seen in lipodystrophic syndromes, may involve other factors in addition to simple overflow of fat from large dysfunctional adipocytes. One such factor may be the adipocyte-derived, insulin-sensitizing hormone adiponectin. It has been suggested that large adipocytes are characterized by reduced production and secretion of adiponectin compared with small newly differentiated adipocytes (10). Hypoadiponectinemia is a common characteristic of both lipodystrophy and obesity (11, 12). Administration of adiponectin to mice with lipodystrophic diabetes reduces the fat content in liver and skeletal muscle, improves insulin action, and reverses hyperglycemia (13). In nondiabetic humans, low plasma adiponectin concentrations are associated with increased intramyocellular and intrahepatic fat content (14, 15). Finally, increases in circulating adiponectin concentrations in patients with type 2 diabetes mellitus treated with TZDs are associated with improvements in insulin sensitivity and reduction of intrahepatic fat (9).

In the present study we tested *1*) whether larger subcutaneous abdominal adipocyte size is associated with increased visceral, hepatic, and intramyocellular fat accumulation in obese individuals with normal glucose tolerance; *2*) whether those relations might be mediated by low plasma adiponectin concentrations; and *3*) whether increased accumulation of fat in VAT, liver, and skeletal muscle might explain the association of increased adipocyte size and low adiponectin concentrations with impairment of insulin action.

SUBJECTS AND METHODS

Subjects

Subjects were at least 3 quarters Pima Indian, between 18 and 45 y of age, nonsmokers at the time of the study, and healthy on the basis of medical history, physical examination, and routine laboratory tests. The protocol was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases, and all subjects provided written informed consent before participation. The female subjects were studied during the follicular phase of the menstrual cycle.

Methods

All subjects were admitted to a clinical research unit and were placed on a weight-maintaining diet (containing 50% of energy as carbohydrates, 30% as fat, and 20% as protein). Body composition was measured by dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) (16) to confirm obesity status [percentage body fat (BF) ≥25 in men and ≥30 in women]. At least 3 d after admission and after a 12-h overnight fast, the subjects underwent a 2-h 75-g oral-glucose-tolerance test to exclude impaired glucose regulation or diabetes according to the World Health Organization 1999 criteria. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured with an automated immunoassay (Access; Beckman Instruments). Blood samples for the measurement of fasting plasma adiponectin concentrations were drawn at 0700 with prechilled syringes, transferred into prechilled EDTA-coated tubes, and immediately placed on ice. All tubes were centrifuged at 4 °C within several minutes of collection and were stored at −70 °C until assayed. Total plasma adiponectin concentrations were determined with the use of a validated sandwich enzyme-linked immunosorbent assay that uses an adiponectin-specific antibody (intraassay and interassay CVs of 3.3% and 7.4%, respectively).

Insulin action was assessed at physiologic insulin concentrations during a hyperinsulinemic-euglycemic glucose clamp (17). Briefly, after the subjects fasted overnight, a primed (30 μCi) continuous (0.3 μCi/min) 3-[^{3}H]glucose infusion was started to determine endogenous glucose production (EGP). Two hours after the isotope infusion was started, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 mU·m^{-2} body surface area·min^{-1}. Blood samples for measurement of 3-[^{3}H] glucose specific activity were collected from the end of the basal period to and every 10 min during the final 40 min of insulin infusion. Under basal conditions, EGP was calculated as the 3-[^{3}H] glucose infusion rate divided by the steady state plasma 3-[^{3}H] glucose specific activity. During the insulin clamp, EGP was calculated from Steele's equation (18). The degree of hepatic insulin sensitivity was calculated as the ratio of absolute differences (clamp minus fasting) in EGP and plasma insulin concentrations. The rate of total insulin-stimulated glucose disposal was calculated for the last 40 min of the insulin infusion and was corrected for the rate of EGP. Individual variation in plasma glucose and insulin concentrations during the clamp was taken into account in the calculation of the insulin-mediated glucose disposal (17, 19). All measurements derived from the clamp were normalized to estimated metabolic body size (or fat-free mass + 17.7 kg) (20).

Abdominal SAT was removed from the periumbilical region by percutaneous needle biopsy under local anesthesia (1% lidocaine). The specimen was placed on a sterile nylon mesh, rinsed with sterile 0.9% NaCl solution, and cleaned of visible connective tissue and blood vessels in Hank's Buffered Saline Solution (HBBS) supplemented with 5.5 mmol/L glucose. Adipose tissue was then digested in HBBS buffer containing 5.5 mmol/L glucose, 5% fatty acid–free bovine serum albumin (Introgen/Serologicals, Norcross, GA) and 3.3 mg/mL type I collagenase (Worthington Biochemical Corp, Lakewood, NJ) for 30 min in a 37 °C water bath. The digestion mixture was passed through a sterile 230-μm stainless steel tissue sieve (Thermo EC, Holbrook, NY). Adipocytes were allowed to float by gravity, and an aliquot of the supernatant containing adipocytes was collected for adipocyte size measurement (21). Briefly, packed adipocytes were mixed at a ratio of 1:2 with Medium 199 (Life Technologies, Grand Island, NY) containing 1% heat-inactivated fetal bovine serum (Life Technologies), 1% bovine serum albumin (Introgen/Serologicals), and 50 nmol/L adenosine (Sigma-Aldrich, St Louis, MO) on a chambered slide covered with a cover slip. Representative pictures of the adipocytes were taken

Accepted for publication September 13, 2007.

TABLE 1
Anthropometric and metabolic characteristics of the study population[1]

Variable	All subjects	Men	Women
Age (y)[2]	25 (21, 34)[3]	27 (22, 34)	24 (20, 32)
BMI (kg/m^2)[2]	36 ± 4[4]	35 (4)	36 (5)
Body fat (%)[2]	35 (5)	32 (4)	40 (4)[5]
Mean adipocyte diameter (μm)[6]	68 (7)	66 (7)	73 (6)[7]
Subcutaneous adipose tissue (cm^2)[8]	578 (457, 693)	524 (411, 689)	634 (520, 713)
Visceral adipose tissue (cm^2)[8]	120 (42)	129 (43)	105 (37)[9]
Intrahepatic lipid content (AU)[8]	17 (7)	17 (7)	18 (6)
Intramyocellular lipid content I (AU)[10]	18 (4)	18 (4)	20 (4)
Intramyocellular lipid content IIA (AU)[10]	12 (3)	11 (3)	13 (3)[9]
Intramyocellular lipid content IIX (AU)[10]	10 (3)	9 (3)	11 (2)[9]
Fasting plasma adiponectin (mg/L)[2]	5.6 (4.5, 6.5)	6.1 (5.3, 7.9)	4.8 (4.0, 5.9)[9]
Fasting plasma glucose (mg/L)[2]	90 (8)	90 (8)	90 (8)
2-h plasma glucose (mgl/L)[2]	109 (19)	109 (21)	111 (17)
Fasting plasma insulin (mU/L)[2]	9.0 (6.9, 13.0)	8.5 (6.3, 11.5)	9.9 (7.7, 16.3)
Steady state plasma insulin (mU/L)[2]	57 (49, 63)	51 (48, 63)	56 (50, 63)
M (mg/kg$_{EMBS}$ · min)[2]	2.6 (2.3, 3.2)	2.7 (2.3, 3.4)	2.6 (2.3, 3.1)
EGP$_{basal}$ (mg/kg$_{EMBS}$ · min)[2]	1.7 (0.2)	1.7 (0.2)	1.8 (0.2)
EGP$_{insulin}$ (mg/kg$_{EMBS}$ · min)[2]	0.2 (0, 0.5)	0 (0, 0.5)	0.3 (0.1, 0.6)

[1] M, insulin-mediated glucose disposal; EGP, endogenous glucose production; EMBS, estimated metabolic body size; AU, arbitrary units.
[2] $n = 53$ (21 F).
[3] Median; 25th and 75th percentiles in parentheses (all such values).
[4] $\bar{x}$ ± SD (all such values).
[5,7,9] Significantly different from men (Student's t test for means and Mann-Whitney U test for medians): [5] $P < 0.0001$, [7] $P < 0.001$, [9] $P < 0.05$.
[6] $n = 48$ (17 F).
[8] $n = 51$ (20 F).
[10] $n = 41$ (15 F).

with a Polaroid Microcam (Polaroid, Waltham, MA) on an inverted microscope (Eclipse TE200), and the adipocyte cell perimeters on scanned images were measured by using Scion Image (Scion, Frederick, MD) by 3 independent readers (interreader variability: 4.7%). Only samples with ≥100 sized cells ($n = 48$) were included in further analyses (22).

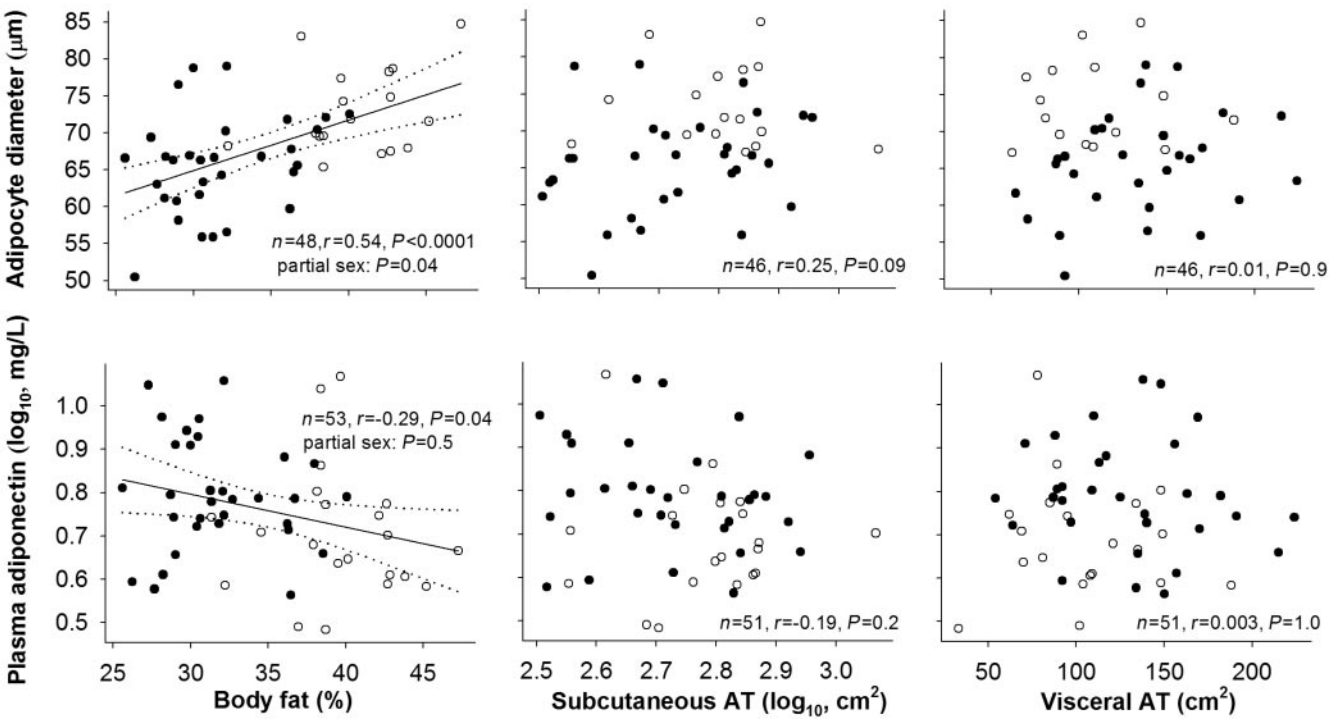

FIGURE 1. Correlation of mean subcutaneous abdominal adipocyte diameter and fasting plasma adiponectin concentration with measures of total and abdominal adiposity in men (●) and women (○). Data are Pearson correlation coefficients. Solid lines represent the regression, and the dotted lines represent 95% CIs for significant correlations only.

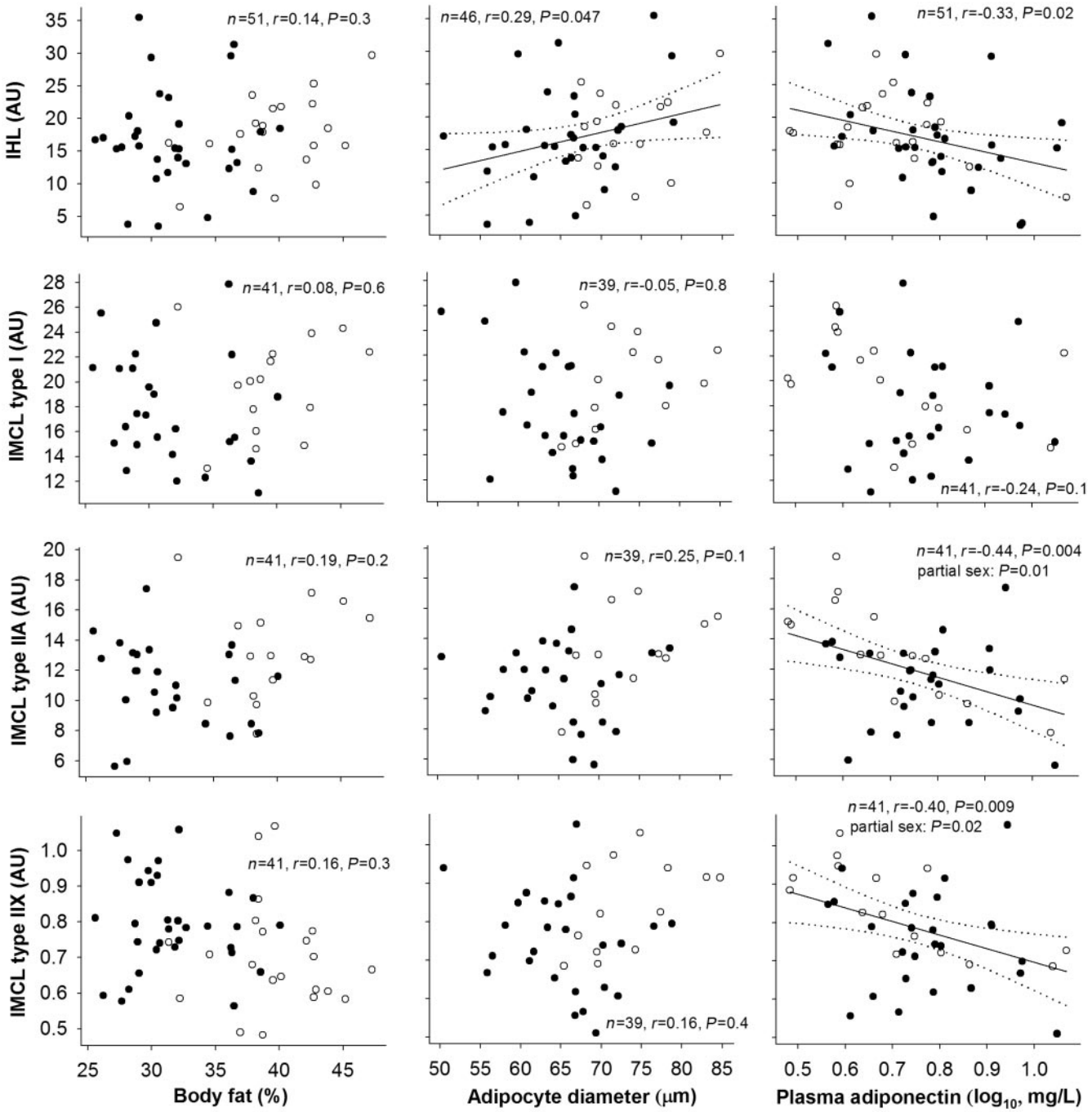

FIGURE 2. Correlation of intramyocellular lipid content (IMCL) and intrahepatic lipid content (IHL) with adiposity, mean subcutaneous abdominal adipocyte diameter, and fasting plasma adiponectin concentration in men (●) and women (○). Data are Pearson correlation coefficients. Solid lines represent the regression, and the dotted lines represent 95% CIs for significant correlations only.

Muscle samples were obtained under local anesthesia (1% lidocaine) from the vastus lateralis muscle via needle biopsy. Muscle was rapidly trimmed of visible connective tissue and blotted free of blood, and small pieces were mounted on cork-board, frozen by immersion in isopentane cooled on liquid nitrogen (−160 °C), and stored at −70 °C until analyzed.

The amount and quality of the sample allowed histochemical analyses in 41 subjects. Muscle cross sections (10 μm) obtained with a microtome at −20 °C were stained for myofibrillar ATPase by established techniques (23) for the determination of fiber types (I, IIA, and IIX). The intramyocellular lipid content (IMCL) of muscle fibers was measured by using the oil-red-O Sudan-type dye staining technique, which mainly reveals triacylglycerols (23). Briefly, a stock solution was prepared by solubilizing 300 mg oil-red-O dye (Sigma, St Louis, MO) in 100 mL concentrated isopropanol (99%). Muscle sections were incubated at room temperature for 10 min in 12 mL freshly prepared stock solution (filtered through a Whatmann paper no. 42) diluted in 8 mL distilled water. Thereafter, stained samples were immersed 4 times in distilled water and rinsed with running water

TABLE 2
Multiple linear regression analysis of intrahepatic fat content

Independent variable	Estimate	SE	*P*
Model 1[1]			
Intercept	32	16	0.06
Age ($\log_{10}$, y)	−13	8	0.1
Sex (M = 1, F = 2)	−4.7	2.4	0.06
Adipocyte diameter (μm)	0.40	0.15	0.01
Plasma adiponectin (mg/L)	−22	7	0.004
Model 2[2]			
Intercept	32	12	0.009
Age ($\log_{10}$, y)	−19	9	0.04
Sex (M = 1, F = 2)	2.0	1.9	0.3
Visceral adipose tissue (cm^2)	0.07	0.03	0.007
Model 3[3]			
Intercept	35	16	0.04
Age ($\log_{10}$, y)	−20	8	0.03
Sex (M = 1, F = 2)	−3.0	2.5	0.2
Visceral adipose tissue (cm^2)	0.05	0.03	0.05
Adipocyte diameter (μm)	0.3	0.1	0.03
Plasma adiponectin (mg/L)	−20	7	0.008

[1] $n = 48$, model $R^2 = 0.29$, $P = 0.007$.
[2] $n = 51$, model $R^2 = 0.16$, $P = 0.04$.
[3] $n = 46$, model $R^2 = 0.35$, $P = 0.003$.

for another 10 min. Glass slides were overlaid with a glycerol drop (to preserve lipids) and sealed with a cover slip and an acetone-based nail polish. All sections were examined under a light microscope (Leitz Dialux 20, Wetzlar, Germany) connected to a CCD camera (Sony C-350) with an analog-to-digital conversion system for image capture. Image analysis was performed by using the public domain NIH Image program (Internet: http://rsb.info.nih.gov/nih-image/). Myosin ATPase and oil-red-O–stained fibers were matched, and the oil-red-O staining intensity of type I, IIA, and IIX muscle fibers was quantified as the average intensity signal over the entire surface of the fiber.

Magnetic resonance imaging (MRI) of the abdomen was performed by using a 1.5-T Sigma scanner (General Electric, Milwaukee, WI), which acquired a series of cross-sectional T1-weighted scans centered on L4–L5 for measurement of fat distribution and around portal hilus for estimation of the relative intensity of liver. MRI images were not acquired in 2 subjects because of scanner unavailability at the time of their admission. VAT and abdominal SAT areas were measured by using ImageJ software (Internet: http://rsb.info.nih.gov/ij/) as the sum of pixels above the intensity corresponding to the nadir between the lean and fat peaks within and outside the abdominal wall (24). Liver fat content was estimated from the relative intensity of liver to that of SAT and spleen (assumed to be fat free). Regions of interest were drawn by using the auto-trace tool, which enables threshold-based segmentation by connecting all pixels within a specified threshold range around a selected seed pixel using Analyze Direct software (Mayo Clinic, Rochester, MN). Intrahepatic lipid content (IHL) was estimated by using the formula

$$IHL = 100 \times (AI_{liver} - AI_{spleen})/(AI_{adipose} - AI_{spleen}) \quad (1)$$

where AI is average intensity.

Statistical analysis

Statistical analyses were performed by using SAS (version 8.02; SAS Institute, Cary, NC). The values of nonnormally distributed variables were logarithmically transformed to approximate normal distribution. Student *t* test was used for comparisons between men and women, and Pearson correlations were used to test for simple or partial correlations between the variables. General linear regression analysis was used to test for independent predictors of the outcomes after adjustment for confounders. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Clinical characteristics of the study population are presented in **Table 1**. On average, women had a higher percentage of body fat (BF), larger adipocytes, less VAT, a higher IMCL in type IIA and type IIX fibers, and a lower fasting plasma adiponectin concentration than did men. Both mean subcutaneous abdominal adipocyte diameter (AD) and fasting plasma adiponectin concentration correlated with BF but not with SAT or VAT (**Figure 1**). After adjustment for sex in the partial correlation, the association between BF and AD was weaker but still significant, whereas the association between BF and plasma adiponectin concentration disappeared (Figure 1). IHL was positively associated with AD, negatively associated with fasting plasma adiponectin concentration, and not related to BF (**Figure 2**). IMCL in type I fibers was related neither to BF nor to AD or plasma

TABLE 3
Pearson correlation of fasting plasma insulin concentration and peripheral (M) and hepatic (ΔEGP/Δinsulin) insulin action with measures of adiposity and fasting plasma adiponectin concentration[1]

	BF	AD	SAT	VAT	IHL	IMCL I	IMCL IIA	IMCL IIX	Plasma adiponectin
n	53	48	51	51	51	41	41	41	53
Plasma insulin	0.47[2]	0.22	0.52[3]	0.36[4]	0.58[3]	−0.11	−0.09	−0.02	−0.32[5]
M	−0.21	−0.09	−0.32[5]	−0.29[5]	−0.48[2]	0.02	−0.08	0.15	0.32[5]
ΔEGP/Δinsulin	−0.18	−0.19	−0.02	−0.25	−0.32[5]	−0.10	−0.17	−0.19	0.16

[1] BF, body fat; AD, adipocyte diameter; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; IHL, intrahepatic lipid content; IMCL, intramyocellular lipid content; *M,* insulin-mediated glucose disposal; EGP, endogenous glucose production.
[2] $P < 0.001$.
[3] $P < 0.0001$.
[4] $P < 0.01$.
[5] $P < 0.05$.

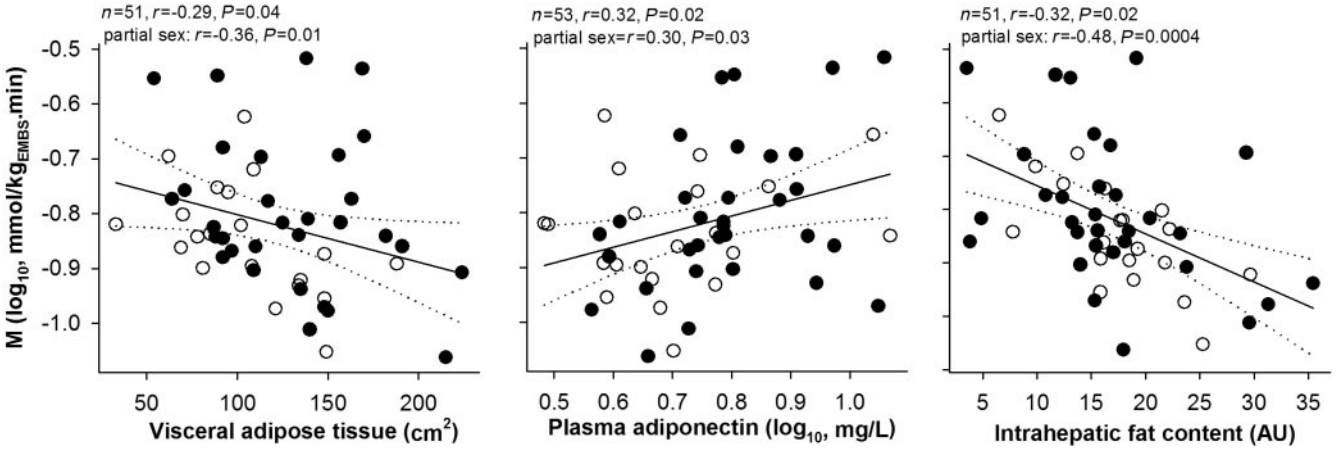

FIGURE 3. Pearson correlation of insulin-mediated glucose disposal (M) with visceral adipose tissue, intrahepatic fat content, and fasting plasma adiponectin concentration in men (●) and women (○). Dotted lines represent 95% CIs.

adiponectin concentration. IMCL in type II fibers was related only to plasma adiponectin concentration (Figure 2). These associations were slightly attenuated after adjustment for sex (Figure 2). After adjustment for age and sex, both AD and fasting plasma adiponectin concentration were independent predictors of IHL before as well as after additional adjustment for VAT, which was a borderline independent predictor of IHL (**Table 2**).

Fasting plasma insulin concentrations correlated positively with BF, SAT, VAT, and IHL and correlated negatively with plasma adiponectin concentration (**Table 3**). Endogenous glucose production (EGP) during insulin infusion was fully suppressed in 22 subjects. Insulin action on glucose uptake and production (ΔEGP/Δinsulin) was not related to AD, BF, or IMCL (Table 3). Insulin-mediated glucose disposal was negatively associated with SAT (Table 3), VAT, and IHL (Table 3 and **Figure 3**) and positively correlated with fasting plasma adiponectin concentration (Table 3 and Figure 3). ΔEGP/Δinsulin was related only to IHL (Table 3). In the multivariate analysis, IHL but not VAT or plasma adiponectin concentration was an independent predictor of insulin-mediated glucose disposal (**Table 4**).

DISCUSSION

In the present study of obese individuals with normal glucose regulation, mean size of adipocytes from abdominal SAT was positively associated with increased lipid content in liver but not in skeletal muscle. Contrary to our hypothesis, mean adipocyte size was not associated with fasting plasma adiponectin concentration. However, both increased mean adipocyte size and low adiponectin concentration were significant predictors of increased fat content in liver, which in multivariate analysis was the only significant predictor of decreased peripheral and hepatic insulin action.

Adipose tissue expands by recruiting new adipocytes and accumulating lipids in existing ones (1). However, even adipocytes ultimately have limited capacity to store lipids (2). Individuals with larger adipocytes in SAT may therefore have a lower capacity for further lipid storage, so the subsequent excesses of fat may be stored in VAT, liver, and skeletal muscle (5). As further increase in adipocyte size becomes limited, increased accumulation of lipid in the visceral organs and in skeletal muscle was suggested to link obesity characterized by large adipocytes with insulin resistance and increased risk of diabetes (5). To test this hypothesis, we studied obese Pima Indians with normal glucose tolerance. We found that mean adipocyte size positively correlated with intrahepatic fat but not with IMLC. A similar finding was recently reported in a cohort of overweight individuals of diverse ethnic origins (4). It is well known that the negative association of insulin action with total body adiposity is attenuated in individuals at the upper spectra of obesity (25–27). In addition, our data indicate that in obese individuals even enlargement of subcutaneous abdominal adipocytes is not a significant predictor of additional worsening of insulin sensitivity.

Although a negative association between insulin-mediated glucose disposal and IMCL was reported by several authors (28, 29), other studies, as well as our present study, have failed to find such an association (4, 30). It has been postulated that muscle triacylglycerols are only a surrogate for other lipid species, having a more direct effect on insulin action, such as diacylglycerol or fatty acid acyl-CoA (30). There is also evidence that muscle

TABLE 4
Multiple linear regression analysis of insulin-mediated glucose disposal (*M*)

Dependent variable: *M* (log_{10}, mg/kg_{EMBS}·min)			
Independent variable	Estimate	SE	*P*
Model 1[1]			
Intercept	0.7	0.2	0.002
Age (log_{10}, y)	0.01	0.2	0.9
Sex (M = 1, F = 2)	−0.06	0.03	0.09
Visceral adipose tissue (cm^2)	−0.0008	0.0004	0.1
Intrahepatic fat content (AU)	−0.008	0.002	0.004
Model 2[2]			
Intercept	0.7	0.3	0.02
Age (log_{10}, y)	−0.1	0.1	0.5
Sex (M = 1, F = 2)	−0.03	0.03	0.4
Plasma adiponectin (log_{10}, mg/L)	0.11	0.13	0.4
Intrahepatic fat content (AU)	−0.008	0.003	0.002

[1] $n = 51$, model $R^2 = 0.31$, $P = 0.002$.
[2] $n = 51$, model $R^2 = 0.28$, $P = 0.002$.

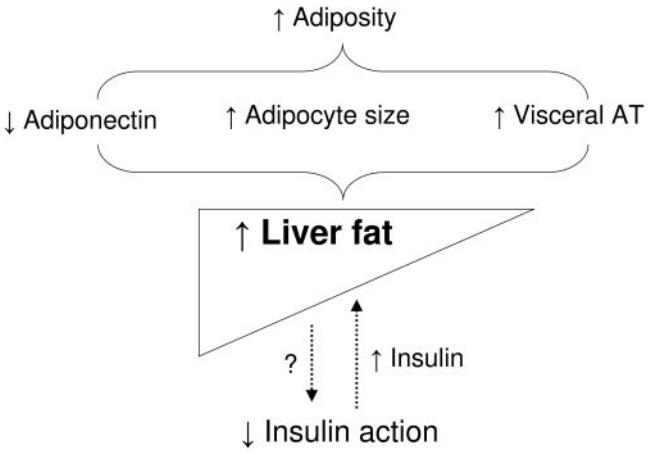

FIGURE 4. Central role of increased liver fat accumulation for impaired insulin action in obesity. Enlargement of adipocytes in the abdominal subcutaneous adipose tissue (AT), decreased production of adiponectin in the adipocytes from subcutaneous AT, and increased intraabdominal adipose tissue mass are independent risk factors for the increased accumulation of fat in the liver, which in turn has a negative effect on both hepatic and peripheral insulin action. Fat accumulation in liver may be further aggravated by the prolipogenic action of excess insulin, which compensates for insulin resistance.

oxidative capacity is the major predictor of peripheral insulin action, not the levels of these lipid metabolites in skeletal muscle (31). It is plausible that insulin resistance and increased IMCL are 2 independent characteristics related to mitochondrial dysfunction.

In the liver, insulin suppresses the endogenous production of glucose. Previous studies in healthy individuals have shown that the suppressibility of EGP is negatively related to intrahepatic fat content (32). In our study, both the hepatic and peripheral action of insulin were inversely associated with IHL. This agrees with data showing that both hepatic and peripheral insulin action are lower in individuals with nonalcoholic fatty liver disease (NAFLD) than in healthy controls (33). Whether increased accumulation of fat in the liver is the cause or the consequence of peripheral insulin resistance is not clear. Rats with a genetically increased accumulation of fat in the liver are characterized by reduced insulin-mediated glucose disposal in the skeletal muscle (34). In addition, fatty liver in animals and humans is associated with the increased production of fetuin-A (35), which impairs insulin signaling in the liver and skeletal muscle (36). The prevailing opinion, however, is that fat increasingly accumulates in the liver secondary to peripheral insulin resistance via insulin-mediated activation of lipogenesis and inhibition of lipid oxidation (37). An increase in IHL may further worsen the inhibitory effect of insulin on hepatic glucose production. In fact, previous studies in Pima Indians showed that serum alanine aminotransferase activity, considered to be a marker of liver fat content, predicted the prospective decline in hepatic but not in peripheral insulin action (38), and that hyperinsulinemia predicted the development of type 2 diabetes mellitus independently of low insulin-mediated glucose disposal (39).

Similar to previous reports (14, 15, 40), a lower fasting plasma adiponectin concentration was associated with reduced peripheral insulin action and with higher IMCL and IHL. These data are supported by experimental evidence that adiponectin increases glucose uptake in skeletal muscle and increases fatty acid oxidation and reduces fatty acid synthesis in both skeletal muscle and liver (41). The activation of the AMP-kinase pathway is thought to provide the mechanistic link for these effects of adiponectin (41). In rat skeletal muscle, AMP-kinase stimulation by adiponectin or synthetic agonist is followed by increased glucose uptake and fatty acid oxidation in fast-twitch but not slow-twitch muscle (42, 43). In lean humans, although a low plasma adiponectin concentration is associated with high IMCL in slow-twitch muscle, it predicts high-fat-diet–induced fat accumulation in fast-twitch muscle (14). In our study, the plasma adiponectin concentration was inversely associated with IMCL in fast-twitch (type II) but not in slow-twitch (type I) muscle fibers. It is conceivable that, with overfeeding or with the development of obesity, type I fibers become easily saturated with lipids because they have a high lipid transport capacity, whereas lipid metabolism in type II fibers depends more on the adiponectin-induced activation of AMP-kinase pathway.

We cannot exclude that the average cell size might have been underestimated in subjects with the largest fat cells due to their increased propensity for disruption or lysis when treated by collagenase (44). It must also be noted that hepatic insulin sensitivity reflects primarily fasting endogenous glucose output in subjects with complete suppression of EGP (43% of the group) warranting some caution when interpreting the results on hepatic insulin sensitivity. Another possible limitation of our study is that a standard MRI has a low sensitivity to detect small amounts of fat and low specificity to distinguish steatosis from other types of liver pathology (45). However, we attempted to minimize those limitations by inclusion of subjects without clinical and laboratory signs of liver dysfunction, which is typical for more advanced liver pathologies and by inclusion of obese subjects who are likely to have a higher liver fat content. In addition, because we have replicated several relations shown by others using gold standard techniques (4, 15, 32, 33), we believe that it provided a valid estimate of relative IHL. Finally, although we used the same biopsy sites as in previous studies reporting the relation of insulin resistance to adipocyte size (3) and skeletal muscle triacylglycerol content (46), our conclusions refer to a small part of a much larger and heterogeneous adipose tissue and skeletal muscle compartment.

In conclusion, our data indicate that the enlargement of subcutaneous abdominal adipocytes is not directly related to insulin action but predicts an increased liver fat content in obese individuals with normal glucose tolerance. This association was independent of fasting plasma adiponectin concentration. Furthermore, liver fat content was the only independent predictor of reduced peripheral and hepatic insulin action in the multivariate analysis, which indicates a pivotal role of increased liver fat accumulation in predicting metabolic risk attributed to subtypes of obesity with enlarged subcutaneous abdominal adipocytes, hypoadiponectinemia, or excess visceral adiposity (**Figure 4**).

We acknowledge Thomas Brookshire, Kathy Trinidad, and the nursing and dietary staff for the care of the volunteers; Josée St-Onge, Emma Rousseau, and Shannon Parrington for technical assistance; and Nicola Pannacciulli, Duc Son Le, and Angelo DelParigi for help with the data evaluation. We are grateful to the members and leaders of the Gila River Indian Community for their continuing cooperation in our studies.

The authors' responsibilities were as follows—JK: data collection and analysis and manuscript writing; NS: study design and data collection; PAP: study design and analyses of adipocytes; CW: study design; MS: analysis of plasma samples (adiponectin); CB: study design; SRS: advice and consultation on magnetic resonance imaging; DRJ: analyses of skeletal muscle samples; TF: design and supervision of the assay (adiponectin); JK: manuscript writing; JCB: study design and manuscript writing. None of the authors had a conflict of interest to disclose.

REFERENCES

1. Salans LB, Cushman SW, Weismann RE. Studies of human adipose tissue. Adipose cell size and number in nonobese and obese patients. J Clin Invest 1973;52:929–41.
2. Stern JS, Batchelor BR, Hollander N, Cohn CK, Hirsch J. Adipose-cell size and immunoreactive insulin levels in obese and normal-weight adults. Lancet 1972;2:948–51.
3. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. Diabetologia 2000; 43:1498–506.
4. Larson-Meyer DE, Heilbronn LK, Redman LM, et al. Effect of calorie restriction with or without exercise on insulin sensitivity, β-cell Function, fat cell size, and ectopic lipid in overweight subjects diabetes care 2006;29:1337–44.
5. Danforth E Jr. Failure of adipocyte differentiation causes type II diabetes mellitus? Nat Genet 2000;26:13.
6. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. J Biol Chem 2000;275: 8456–60.
7. Gavrilova O, Marcus-Samuels B, Graham D, et al. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J Clin Invest 2000;105:271–8.
8. de Souza CJ, Eckhardt M, Gagen K, et al. Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. Diabetes 2001;50:1863–71.
9. Bajaj M, Suraamornkul S, Piper P, et al. Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. J Clin Endocrinol Metab 2004;89:200–6.
10. Yang X, Jansson PA, Nagaev I, et al. Evidence of impaired adipogenesis in insulin resistance. Biochem Biophys Res Commun 2004;317:1045–51.
11. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Comm 1999;257:79–83.
12. Sutinen J, Korsheninnikova E, Funahashi T, Matsuzawa Y, Nyman T, Yki-Jarvinen H. Circulating concentration of adiponectin and its expression in subcutaneous adipose tissue in patients with highly active antiretroviral therapy-associated lipodystrophy. J Clin Endocrinol Metab 2003;88:1907–10.
13. Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941–6.
14. Thamer C, Machann J, Tschritter O, et al. Relationship between serum adiponectin concentration and intramyocellular lipid stores in humans. Horm Metab Res 2002;34:646–9.
15. Bugianesi E, Pagotto U, Manini R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab 2005;90:3498–504.
16. Tataranni PA, Ravussin E. Use of dual-energy X-ray absorptiometry in obese individuals. Am J Clin Nutr. 1995;62:730–4.
17. Lillioja S, Mott DM, Howard BV, et al. Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. N Engl J Med 1988;318:1217–25.
18. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 1959;82:420–30.
19. Best JD, Taborsky GJ, Halter JB, Porte D. Glucose disposal is not proportional to plasma glucose level in man. Diabetes 1981;30:847–50.
20. Lillioja S, Bogardus C. Obesity and insulin resistance: lessons learned from the Pima Indians. Diabetes Metab Rev. 1988;4:517–40.
21. Crandall DL, Armellino DC, Busler DE, McHendry-Rinde B, Kral JG. Angiotensin II receptors in human preadipocytes: role in cell cycle regulation. Endocrinology 1999;140:154–8.
22. Smith U, Sjostrom L, Bjornstorp P. Comparison of two methods for determining human adipose cell size. J Lipid Res 1972;13:822–4.
23. Malenfant P, Joanisse DR, Theriault R, Goodpaster BH, Kelley DE, Simoneau JA. Fat content in individual muscle fibers of lean and obese subjects. Int J ObesRelat Metab Disord 2001;25:1316–21.
24. Gautier JF, Milner MR, Elam E, Chen K, Ravussin E, Pratley RE. Visceral adipose tissue is not increased in Pima Indians compared with equally obese Caucasians and is not related to insulin action or secretion. Diabetologia 1999;42:28–34.
25. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol 1985;248:E286–91.
26. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity in men. J Clin Invest 1995;96:88–98.
27. Ross R, Aru J, Freeman J, Hudson R, Janssen I. Abdominal adiposity and insulin resistance in obese men. Am J Physiol Endocrinol Metab 2002; 282:E657–63.
28. Krssak M, Falk PK, Dresner A, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ^{1}H NMR spectroscopy study. Diabetologia 1999;42:113–6.
29. Virkamaki A, Korsheninnikova E, Seppala-Lindroos A, et al. Intramyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. Diabetes 2001;50:2337–43.
30. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol Metab 2001;86:5755–61.
31. Bruce CR, Anderson MJ, Carey AL, et al. Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. J Clin Endocrinol Metab 2003;88:5444–51.
32. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 2002;87:3023–8.
33. Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844–50.
34. Qi NR, Wang J, Zidek V, et al. A new transgenic rat model of hepatic steatosis and the metabolic syndrome. Hypertension 2005;45:1004–11.
35. Stefan N, Hennige AM, Staiger H, et al. Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. Diabetes Care 2006;29:853–7.
36. Auberger P, Falquerho L, Contreres JO, et al. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. Cell 1989;58:631–40.
37. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. J Clin Invest 2004;114:147–52.
38. Vozarova B, Stefan N, Lindsay RS, et al. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002;51:1889–95.
39. Weyer C, Hanson RL, Tataranni PA, Bogardus C, Pratley RE. A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance: evidence for a pathogenic role of relative hyperinsulinemia. Diabetes 2000;49:2094–101.
40. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930–5.
41. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288–95.
42. Tomas E, Tsao TS, Saha AK, et al. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci U S A 2002;99:16309–13.
43. Buhl ES, Jessen N, Schmitz O, et al. Chronic treatment with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type-specific manner. Diabetes 2001;50:12–7.
44. Hirsch J, Gallian E. Methods for the determination of adipose cell size in man and animals. J Lipid Res 1968;9:110–19.
45. Stark DD, Bass NM, Moss AA, et al. Nuclear magnetic resonance imaging of experimentally induced liver disease. Radiology 1983;148:743–51.
46. Pan DA, Lillioja S, Kriketos AD, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 1997;46:983–8.