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Aiwei Yao-Borengasser
University of Arkansas for Medical Sciences

Neda Rasouli
University of Colorado

Vijayalakshmi Varma
University of Arkansas for Medical Sciences

Lesliie M. Miles
University of Arkansas for Medical Sciences

Bounleut Phanavanh
University of Arkansas for Medical Sciences

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Authors
Aiwei Yao-Borengasser, Neda Rasouli, Vijayalakshmi Varma, Lesliie M. Miles, Bounleut Phanavanh, Tasha Starks, Jack Phan, Horace J. Spencer III, Robert E. McGehee Jr., Karen Reue, and Philip A. Kern

Original Article

Lipin Expression Is Attenuated in Adipose Tissue of Insulin-Resistant Human Subjects and Increases With Peroxisome Proliferator–Activated Receptor γ Activation

Aiwei Yao-Borengasser,[1] Neda Rasouli,[1] Vijayalakshmi Varma,[1] Leslie M. Miles,[1] Bounleut Phanavanh,[1] Tasha N. Starks,[1] Jack Phan,[2] Horace J. Spencer III,[3] Robert E. McGehee, Jr.,[4] Karen Reue,[2] and Philip A. Kern[1]

Lipin-α and -β are the alternatively spliced gene products of the *Lpin1* gene, whose product lipin is required for adipocyte differentiation. Lipin deficiency causes lipodystrophy, fatty liver, and insulin resistance in mice, whereas adipose tissue lipin overexpression results in increased adiposity but improved insulin sensitivity. To assess lipin expression and its relation to insulin resistance in humans, we examined lipin-α and -β mRNA levels in subjects with normal or impaired glucose tolerance. We found higher expression levels of both lipin isoforms in lean, insulin-sensitive subjects. When compared with normal glucose-tolerant subjects, individuals with impaired glucose tolerance were more insulin resistant, demonstrated higher levels of intramyocellular lipids (IMCLs), and expressed ~50% lower levels of lipin-α and -β. In addition, there was a strong inverse correlation between adipose tissue lipin expression and muscle IMCLs but no evidence for an increase in muscle lipid oxidation. After treatment of the impaired glucose-tolerant subjects with insulin sensitizers for 10 weeks, pioglitazone (but not metformin) resulted in a 60% increase in the insulin sensitivity index (S_i) and a 32% decrease in IMCLs (both $P < 0.01$), along with an increase in lipin-β (but not lipin-α) expression by 200% ($P < 0.005$). Lipin expression in skeletal muscle, however, was not related to obesity or insulin resistance. Hence, high adipose tissue lipin expression is found in insulin-sensitive subjects, and lipin-β expression increases following treatment with pioglitazone. These results suggest that increased adipogenesis and/or lipogenesis in subcutaneous fat, mediated by the *LPIN1* gene, may prevent lipotoxicity in muscle, leading to improved insulin sensitivity. *Diabetes* 55:2811–2818, 2006

Along with the increasing prevalence of obesity is a parallel increase in diabetes (1–3). With progressive obesity, there is a significant worsening of insulin resistance and the development of features of the metabolic syndrome (4), which include abnormalities in insulin secretion, hepatic steatosis, dyslipidemia, and atherosclerosis (5,6). A common element in obesity and its complications is the accumulation of lipids in adipose tissue and in other organs, such as liver, islets, and muscle (7–9). However, the pathophysiology of these relationships is not well understood.

Important insight into the role of adipose tissue in metabolic syndrome comes from studies of lipodystrophic animals and humans. Whereas excess adipose tissue in the form of obesity leads to insulin resistance, the absence of adipose tissue leads to a severe syndrome characterized by ectopic deposition of lipids in other organs (10). This condition can be treated and partially reversed by the transplantation of adipose tissue in mice (11) or by the treatment of mice or humans with recombinant leptin (12,13). These studies suggest that adipose tissue secretory proteins may be important for normal glucose and lipid homeostasis and also suggest that the absence of adipose tissue deprives the animal of an important lipid storage depot, resulting in inappropriate lipid storage and lipotoxicity in other organs. Indeed, several studies (9,14,15) in humans have demonstrated a significant relationship between muscle lipid accumulation and peripheral insulin resistance.

Further insight into the role of adipose tissue in insulin resistance comes from studies using thiazolidinediones (TZDs). These drugs are high-affinity ligands for peroxisome proliferator–activated receptor (PPAR)γ, a transcription factor activated early in the process of adipocyte differentiation (16), and result in improved insulin sensitivity, a process that predominantly involves glucose uptake in skeletal muscle (17,18). In recent studies, the treatment of subjects with the TZD pioglitazone resulted in an increase in subcutaneous adipose tissue, a decrease in intramyocellular lipids (IMCLs), along with an improve-

From the [1]Department of Medicine, Division of Endocrinology, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, Arkansas; the [2]Departments of Medicine and Human Genetics, VA Greater Los Angeles Healthcare System, University of California at Los Angeles, Los Angeles, California; the [3]Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, Arkansas; and the [4]Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Address correspondence and reprint requests to Philip A. Kern, MD, Associate Chief of Staff, Research, Central Arkansas Veterans Healthcare System, 598/151 LR, 4300 West 7th St., Little Rock, AR 72205. E-mail: kernphilipa@uams.edu.

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CPT, carnitine palmitoyltransferase; DMEM, Dulbecco's modified Eagle's medium; FSIGT, frequently sampled intravenous glucose tolerance test; IMCL, intramyocellular lipid; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione.

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ment in insulin sensitivity (19). Such studies with TZDs suggest that the redistribution of lipids into adipose tissue results in the loss of ectopic lipids from other organs and thus improves insulin action due to a relief of lipotoxicity.

The fatty liver dystrophy (*fld*) mouse has features of human lipodystrophy, and this phenotype results from a mutation in the *Lpin1* gene, whose gene product is lipin (20). Lipin expression is required for normal adipocyte differentiation, such that lipin-deficient mice fail to develop lipid-filled mature adipocytes (21). In contrast, transgenic mice with enhanced lipin expression in adipose tissue become obese, exhibiting increased lipogenic gene expression and hypertrophic adipocytes (22). Despite increased adipose tissue accumulation, the lipin transgenic mice remain insulin sensitive, likely due to a redistribution of lipids from ectopic sites, such as liver and skeletal muscle, into adipose tissue. On the other hand, lipin overexpression in muscle yielded a mouse that was obese, insulin resistant, and with decreased muscle lipid oxidative capacity (22).

In recent studies, the *Lpin1* gene has been shown to generate two alternatively spliced mRNA forms, referred to as lipin-α and -β, which differ by 33 amino acids encoded by an alternatively spliced exon. The two lipin isoforms appear to have distinct roles in the adipocyte in terms of expression dynamics, subcellular localization, and induction of adipogenic and lipogenic gene expression (23). Thus, lipin-α is primarily present in the nucleus and stimulates expression of genes involved in adipocyte differentiation such as PPARγ and CCAAT/enhancer-binding protein α. On the other hand, lipin-β is predominantly localized to the cytoplasm and is associated with the expression of genes involved in lipogenesis and triglyceride storage in adipocytes, including fatty acid synthase and diacylglycerol acyltransferase (23).

The molecular function of lipin is not known, but studies of yeast lipin homologs have revealed interactions with proteins involved in nuclear transport and chromosome segregation (24) and a potential role for lipin in phospholipid biosynthesis at the nuclear/endoplasmic reticulum membrane (25). In mammals, there is evidence that lipin may be a downstream target of insulin action in the mTOR (mammalian target of rapamycin) pathway. In rat adipocytes, lipin becomes phosphorylated in response to incubation with insulin or amino acids, and this effect is abolished by treatment with a specific inhibitor of mTOR (26). Thus, lipin may act as an effector in this important pathway that is known to integrate signals from nutrients and energy status to regulate cell growth and metabolism (27).

Except for a brief report (28) of lipin expression in 19 subjects, no studies have yet measured lipin expression in insulin-resistant humans. To determine the possible relationship between lipin expression and insulin resistance, we studied *LPIN1* gene expression in adipose tissue and muscle of subjects who were well characterized in terms of obesity and insulin resistance. We also examined the influence of pioglitazone, a TZD drug agonist for PPARγ, on lipin expression in subjects with impaired glucose tolerance.

RESEARCH DESIGN AND METHODS

Healthy subjects without diabetes were recruited by local advertisement. All subjects provided written informed consent under protocols that were approved by the local institutional review board, and studies were conducted at the University of Arkansas for Medical Sciences/Central Arkansas Veterans Health Care System General Clinical Research Center. None of the subjects had a history of coronary artery disease or were being treated with fibrates, ACE inhibitors, or angiotensin II receptor blockers. Subjects were included if fasting glucose was <110 mg/dl and 2-h postchallenge glucose was <200 mg/dl, as determined by an initial 75-g oral glucose tolerance test. Based on the oral glucose tolerance test, subjects were defined as either normal (fasting blood glucose <110 mg/dl, 2-h glucose <140 mg/dl) or impaired (2-h glucose 140–199 mg/dl) glucose tolerant. A total of 51 subjects were recruited (44 women and 7 men; 48 Caucasians and 3 African Americans; aged 20–66 years), of which 27 were normal glucose tolerant and 24 were impaired glucose tolerant. All subjects were weight stable and underwent subcutaneous adipose tissue and muscle biopsies and insulin sensitivity testing using a frequently sampled intravenous glucose tolerance test (FSIGT). Impaired glucose-tolerant subjects were then randomized to receive either metformin or pioglitazone for a 2-week dose escalation followed by 8 weeks at a maximum dose (1,000 mg metformin twice a day or 45 mg pioglitazone daily). After 10 weeks of treatment, the oral and intravenous glucose tolerance tests and biopsies were repeated.

Insulin sensitivity measurement. Insulin sensitivity was measured by an insulin-modified FSIGT using 11.4 g/m^2 glucose and 0.04 units/kg insulin (29). Plasma insulin was determined using a chemoluminescent assay (Molecular Light Technology Research, Cardiff, Wales, U.K.), and plasma glucose was measured by a glucose oxidase assay in duplicate. Insulin sensitivity was calculated according to the insulin and glucose data using the MinMod program (30). In five normal glucose-tolerant subjects, the FSIGT was performed with an injection of tolbutamide (125 mg/m^2) instead of insulin. However, tolbutamide was subsequently discontinued by the manufacturer. Although the tolbutamide-modified FSIGT and the insulin-modified FSIGT correlate well with each other and with the euglycemic clamp (31), the insluin sensitivity index (S_i) values obtained by the tolbutamide method are different from the values obtained by insulin injection. Hence, the S_i data of those five normal glucose-tolerant subjects were excluded.

Adipose tissue and cells. Adipose cell and stromal fractions were isolated from adipose tissue as previously described (32). Briefly, subcutaneous adipose tissue was digested with collagenase, and the adipocytes were separated from the stromal vascular fractions by centrifugation. Cultured human adipocytes were prepared by differentiation of preadipocytes obtained from discarded adipose tissue from normal women undergoing liposuction (32,33). Differentiation was assessed by Oil Red O staining and the detection of adipocyte-specific mRNA and/or protein expression.

Total RNA isolation and real-time RT-PCR. Total RNA from adipose tissue was isolated using an RNAeasy Lipid Tissue Mini kit from Qiagen (Valencia, CA), following the manufacturer's instruction. Total RNA from muscle biopsies was isolated using an Ultraspec RNA Isolation System kit from Biotex (Houston, TX), according to the manufacturer's instruction. The quantity and quality of the isolated RNA was determined by ultraviolet spectrophotometry and formaldehyde–agarose gel electrophoresis, respectively. One microgram of total RNA was reverse transcribed using random hexamer primers with TaqMan Reverse transcription reagents (Applied Biosystems, Foster City, CA). Reverse-transcribed RNA was amplified with 1× SYBR Green PCR Master Mix (Applied Biosystems) plus 0.3 μmol/l of gene-specific upstream and downstream primers during 55 cycles on a Rotor-Gene 3000 Real-Time Thermal Cycler (Sydney, Australia). Each cycle consisted of denaturation at 94°C for 20 s, annealing at 58°C for 20 s, and extension at 72°C for 20 s. Amplified 18S ribosomal RNA expression was used as a standard to normalize the differences in individual samples. All data were expressed in relation to 18S RNA, where the standard curves were generated using pooled RNA from the samples assayed. The strategy for identification and measurement of lipin-α, lipin-β, and total lipin was similar to the strategy for measurement of mouse lipin isoforms, which was previously described (23). The primer sequences were as follows: 18S forward TTCGAACGTCTGCCCTATCAA, 18S reverse ATGGTAGGCACGGCGACTA, lipin forward TGCTGGAGAGCAGCA GAACTC, lipin-α reverse GAACCGGAAGGACTGGGAGTG, lipin-β reverse AAGACTGTGGAGGGCAAGAAC, and total lipin reverse TAGGGTATGAG GCTGACTGAG. Each primer set was validated for specificity by visualization of products by agarose gel electrophoresis before use in these studies.

Muscle biopsy, immunohistochemistry, and measurement of IMCLs. Muscle biopsies from the vastus lateralis were performed under local anesthesia and immediately processed for fiber typing and lipid content (IMCLs) as previously described (19). Muscle lipid content was determined by Oil Red O staining (19,34), and images of the muscle sections were analyzed using the NIH Image program v. 1.60. The total area of lipid droplets in a muscle fiber was divided by the total area of the same muscle fiber, yielding a percentage of lipid content in the muscle fiber. To assess the oxidative capacity of the muscle, we measured the activity of succinate dehydrogenase and carnitine palmitoyltransferase (CPT)-1 mRNA, as previously described (19,35).

TABLE 1
Lipin mRNA in human cells and tissue

Cell/tissue	Lipin*	Lipin-α*	Lipin-β*	Lipoprotein lipase*
Whole adipose tissue	0.59 ± 0.11	0.45 ± 0.08	0.71 ± 0.23	4.06 ± 0.86
Adipocytes from adipose tissue	1.59 ± 0.31	1.33 ± 0.23	1.81 ± 0.39	5.03 ± 0.85
Stromal fraction from adipose tissue	0.23 ± 0.03	0.15 ± 0.04	0.28 ± 0.06	0.68 ± 0.17
Adipocytes from cultured preadipocytes	1.81 ± 0.25	1.45 ± 0.15	2.56 ± 0.29	0.76 ± 0.31
Cultured preadipocytes	0.36 ± 0.04	0.21 ± 0.00	0.50 ± 0.01	ND
Muscle tissue	1.32 ± 0.23	1.72 ± 0.37	0.63 ± 0.04	0.11 ± 0.03

Data are means ± SE. *The pooled RNA from all the samples was used for a standard curve. Hence, the data are expressed relative to each other. ND, not detectable.

Cell culture. To study lipin expression in response to TZDs in vitro, the mouse 3T3-L1 cell line was used. Human preadipocytes were not used because these cells require the addition of TZDs for differentiation to mature adipocytes. 3T3-L1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS until confluent and then refed with fresh medium for 2 more days. Adipocyte differentiation was induced by treating the cells with DMEM containing 5 μg/ml insulin, 0.25 mmol/l 3-isobutyl-1-methylxanthine, 1 μg/ml dexamethasone, and 10% FCS, with or without 1 μmol/l rosiglitazone. For a subset of cells, RNA expression was assessed 10 h later. For the remaining cells, the differentiation medium was removed after 48 h and the cells were maintained in DMEM supplemented with 10% FCS with insulin until day 6. On day 6, the cells were subsequently refed with DMEM supplemented with 10% FCS, with or without 1 μmol/l rosiglitazone for 24 h. Afterward, the cells were lysed and homogenized for RNA isolation, and real-time PCR quantitation of mouse lipin mRNA was performed using previously described primers (23). All experiments were performed in triplicate.

Statistical analysis. Student's two-sample t tests were used to compare groups with respect to continuous variables, while paired t tests were used to compare baseline and posttreatment measures within a group. Pearson's correlation coefficients were used to describe the linear association between variables. For each of these analyses, distributional assumptions were evaluated and appropriate transformations were applied as necessary. Results based on analyses requiring transformations will be noted. All data from human samples are expressed as means ± SE.

This study involved predominantly Caucasian women. Although this study lacked power to detect differences in lipin expression related to sex and race, none of the relationships presented were significantly changed by the elimination of men or African Americans.

The data described below include <51 subjects for each analysis. In some instances, this is due to the limitation of the analysis to subgroups of subjects (e.g., impaired glucose tolerance). In other instances, we wished to compare mRNA data among subjects where the reverse transcription reaction was performed at the same time to minimize experimental error.

RESULTS

Detection of lipin in human tissue and cell fractions. To determine the specificity of lipin expression in different cell types, mRNA levels of total lipin, lipin-α, and lipin-β were measured using real-time RT-PCR in human whole adipose tissue, adipose tissue fractionated into adipocytes and stromal cells, cultured preadipocytes and differentiated adipocytes, and muscle tissue (Table 1). The expression of lipoprotein lipase was also measured as an indicator of adipocyte gene expression. All data were expressed as arbitrary units in relation to 18S RNA, and all of the samples were from normal glucose-tolerant subjects. As shown in Table 1, all forms of lipin from adipose tissue were expressed at levels at least sixfold higher in the adipocyte fraction compared with the stromal fraction. Similarly, in cultured cells, all forms of lipin were expressed more than fivefold higher in differentiated adipocytes compared with preadipocytes. In agreement with mouse studies, lipin was also highly expressed in human muscle.

Adipose tissue lipin expression is associated with obesity and insulin sensitivity. In previous studies in mice, lipin overexpression in either adipose tissue or skeletal muscle promoted obesity and altered insulin sensitivity (22). To determine whether lipin expression levels are associated with obesity and insulin sensitivity in humans, total lipin, lipin-α, and lipin-β mRNA levels were measured in human adipose tissue using real-time RT-PCR and normalized to 18S rRNA. Thirty-nine human subjects (36 women and 3 men, aged 42 ± 1.7 years), including subjects with normal and impaired glucose tolerance and having a wide range of BMI (19–55 kg/m^2), percent body fat (15.5–54.1%), and S_i (0.1–14.6 × 10^{-5} × min^{-1} per pmol/l), were included in the analyses.

All forms of lipin mRNA (lipin-α, lipin-β, and total lipin) tended to be decreased with obesity and increased in subjects who were more insulin sensitive. There was a significant negative association between lipin and BMI ($r = -0.33$, $P < 0.05$ for total lipin; $r = -0.39$, $P < 0.05$ for lipin-α; $r = -0.53$, $P < 0.001$ for lipin-β), and the relationships between lipin and percent body fat were similar to that of BMI. For both measures of obesity (BMI and percent body fat), lipin-β had the strongest inverse relationship with obesity, and this relationship is shown in Fig. 1*A*. Obese subjects tend to be insulin resistant, and in the subjects chosen for these studies, there was a significant relationship between BMI and S_i ($r = -0.65$, $P < 0.001$), as expected. As shown in Fig. 1*B*, lipin-β expression in adipose tissue demonstrated a positive association with insulin sensitivity, and the relationship between S_i and lipin-α ($r = 0.44$, $P < 0.02$) and lipin-β were similar. Lipin expression was also inversely related to waist circumference ($r = -0.34$, $P < 0.05$ for lipin-α and $r = -0.459$, $P = 0.008$ for lipin-β), and there was no association between lipin and age. Thus, lipin expression in adipose tissue was strongly linked to both obesity and S_i.

Reduced lipin expression in adipose tissue from impaired versus normal glucose-tolerant subjects. The studies described above were performed in a group of subjects that included both normal and impaired glucose-tolerant individuals. Impaired glucose-tolerant subjects tend to be more insulin resistant, and we therefore wished to compare lipin expression in normal versus impaired glucose-tolerant subjects without the confounding effects of obesity. Therefore, we selected from our group of subjects Caucasian women between the ages of 34 and 59 years, with a BMI between 28 and 43 kg/m^2, and divided them into normal and impaired glucose-tolerant groups. There was no significant difference in menopausal status between the women in these two groups. As shown in

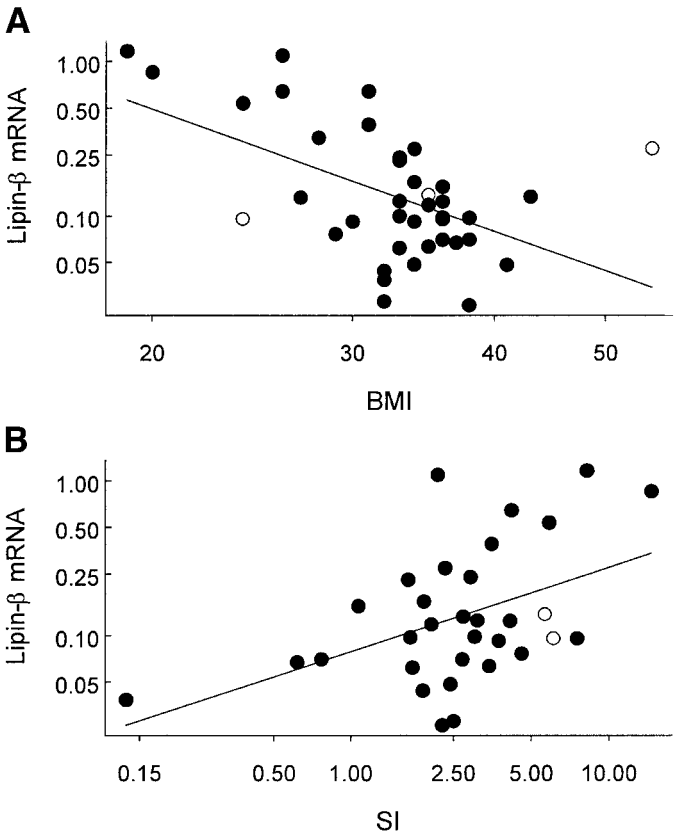

FIG. 1. Relationships between adipose lipin-β expression and BMI (n = 39) (*A*) and S_i (SI; n = 32) (*B*). Lipin-β mRNA level was determined by real-time RT-PCR analysis and was expressed in relation to endogenous 18S RNA. S_i value was determined by the method of FSIGT. The natural log of lipin-β was plotted against the natural log of BMI (*A*) and S_i (*B*). All relationships remain statistically significant after removal of apparent "outliers." Male subjects are indicated by open symbols.

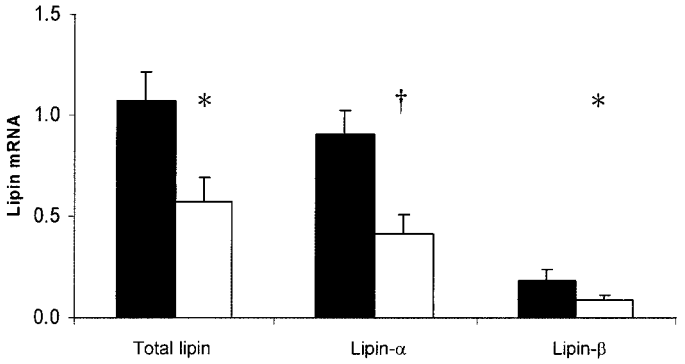

FIG. 2. Adipose lipin expression profile in normal (NGT; n = 15) and impaired (IGT; n = 13) glucose-tolerant subjects. Total lipin, lipin-α, and lipin-β mRNA levels were measured in adipose tissue as described in RESEARCH DESIGN AND METHODS. The values represent relative values, normalized by 18S RNA. *P < 0.05 vs. impaired glucose tolerance; †P < 0.005 vs. impaired glucose tolerance. ■, normal glucose tolerance; □, impaired glucose tolerance.

Table 2, there were no differences in BMI between groups. As expected, normal and impaired glucose-tolerant subjects differed in their 2-h glucose levels and S_i. As shown in Fig. 2, the expression levels of total lipin, lipin-α, and lipin-β were approximately twofold higher in normal glucose-tolerant subjects compared with impaired glucose-tolerant subjects of the same age, sex, race, and BMI.

Pioglitazone increases lipin-β expression in adipose tissue. Pioglitazone is an agonist for PPARγ, which is highly expressed in adipose tissue, and treatment with pioglitazone improves insulin sensitivity. To determine whether pioglitazone affects lipin expression, 21 impaired glucose-tolerant subjects (mean age 50.5 years; mean BMI 33.4 kg/m^2) were randomized to receive treatment for 10 weeks with either pioglitazone or metformin. There were no significant differences in baseline characteristics between the pioglitazone and metformin groups, and there were no significant changes in serum lipids following either treatment. Pioglitazone treatment resulted in an increase in S_i from 2.2 to 3.1 $\times$ 10^{-5} $\times$ min^{-1} per pmol/l ($P < 0.005$), whereas metformin had no effect on insulin sensitivity.

TABLE 2
Characteristics of BMI-matched normal and impaired glucose-tolerant subjects

	Impaired glucose tolerance	Normal glucose tolerance
n	13	13
Age (year)	48 ± 2	43 ± 2
BMI (kg/m^2)	35.2 ± 0.6	33.9 ± 1.1
S_i (10^{-5} $\times$ min^{-1}/pmol/l)	1.8 ± 0.2	3.7 ± 0.6*†
Fasting glucose (mmol/l)	5.0 ± 0.14	4.6 ± 0.10
2-h glucose (mmol/l)	10 ± 0.4	6.1 ± 0.3†
Triglycerides (mmol/l)	1.9 ± 0.2	1.5 ± 0.2
LDL (mmol/l)	2.4 ± 0.2	3.0 ± 0.2†
HDL (mmol/l)	1.3 ± 0.1	1.5 ± 0.1†

Data are means ± SE. *n = 9 (four S_i values were excluded, see RESEARCH DESIGN AND METHODS). †$P < 0.05$ vs. impaired glucose tolerance.

Lipin mRNA levels were measured in adipose tissue obtained before and after treatment. As shown in Fig. 3, metformin treatment yielded no change in lipin expression. In the pioglitazone-treated subjects, however, total lipin expression was increased 92% (P = 0.05) (Fig. 3). When levels of lipin-α and lipin-β mRNA were examined separately, pioglitazone treatment resulted in no change in lipin-α expression, but lipin-β mRNA level increased 200% (P = 0.02). Therefore, pioglitazone treatment improved insulin sensitivity in adipose tissue and caused increased expression specifically of the lipin-β isoform.

The observation that human adipose tissue lipin mRNA levels increase in response to pioglitazone treatment raised the question of whether this was a direct response to the TZD or to changes that occur as a consequence of treatment, such as increased insulin sensitivity. To distinguish between these, we assessed the effect of TZD on lipin mRNA expression in cultured 3T3-L1 adipocytes. The experiment was performed in adipocytes at two stages of development and with two TZD drugs, pioglitazone and rosiglitazone. As shown in Fig. 4, treatment of 3T3-L1 preadipocytes with rosiglitazone for 10 h during the differentiation period resulted in a significant 40% increase in lipin-α and more than twofold increase in lipin-β mRNA levels. In mature adipocytes cultured for 6 days and treated with rosiglitazone for 24 h, there was only a slight increase in lipin-α, but as with the preadipocytes, a twofold increase in lipin-β expression. Similar results in human mature adipocytes were observed with pioglitazone treatment (data not shown). Thus, results in mature 3T3-L1 adipocytes mirror those observed in vivo and suggest that this represents a direct effect of the TZD on lipin mRNA transcription and/or mRNA splicing.

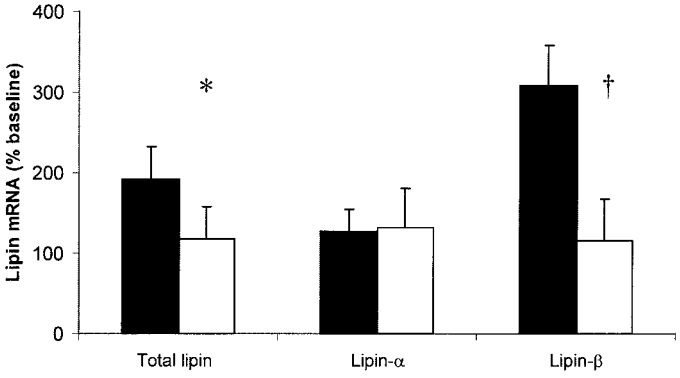

FIG. 3. Effects of pioglitazone (n = 10) and metformin (n = 11) treatment on lipin expression in adipose tissue. Total lipin, lipin-α, and lipin-β mRNA levels were determined as described in RESEARCH DESIGN AND METHODS. The baseline lipin level (before treatment) was expressed as 100%, and the data shown are the percent of baseline data following drug treatment. *P < 0.05 vs. baseline. †P < 0.005 vs. baseline. □, metformin; ■, pioglitazone.

Adipose tissue lipin expression levels are inversely correlated with IMCLs. In previous studies, insulin resistance has been associated with elevated IMCLs, and in mice, lipin overexpression in adipose tissue resulted in improved insulin sensitivity (22). Therefore, we wondered whether elevated lipin expression in adipose tissue would be associated with decreased IMCLs in humans. To assess this, we measured IMCLs in 31 subjects, including normal and impaired glucose-tolerant subjects, and examined the relationship between adipose tissue lipin expression and IMCLs in both type 1 and type 2 fibers. As shown in Table 3, adipose tissue lipin expression was significantly inversely related to IMCLs, and this inverse correlation between lipin-α expression in adipose tissue and IMCLs is illustrated in Fig. 5. However, both obesity and insulin resistance are associated with higher IMCLs. To determine whether adipose lipin expression was associated with IMCLs independent of obesity and insulin resistance, the relationship between lipin and IMCLs was adjusted for BMI and S_i, and the partial correlation coefficients are shown in Table 3. The relationship between lipin and IMCLs in type 1 fibers was no longer significant after adjustment for BMI and S_i. However, IMCLs in type 2 fibers remained significantly associated with total lipin

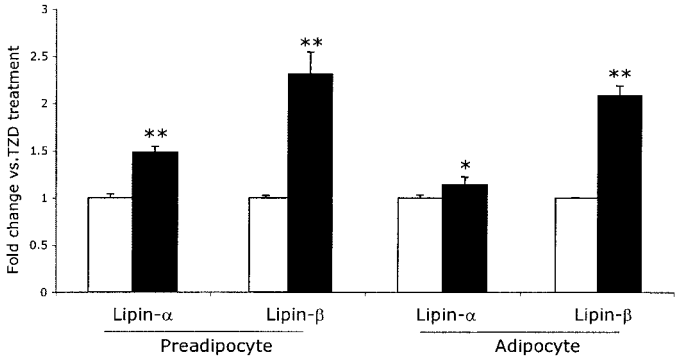

FIG. 4. Effect of the TZD and rosiglitazone on lipin mRNA isoform expression in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were treated with TZD for 10 h during incubation with differentiation cocktail containing insulin, dexamethasone, and isobutylmethylxanthine. Mature adipocytes were cultured for 6 days after initiation of differentiation and treated with rosiglitazone for 24 h. Each bar represents real-time PCR quantitation in triplicate of three independent wells. Error bars show SD. *P < 0.05; **P < 0.005. □, −TZD; ■, +TZD.

TABLE 3
Correlation coefficients of adipose tissue lipin with IMCLs before and after adjusting for BMI and S_i

	Lipin-α*	Lipin-β*	Lipin total*
Before adjustment for BMI and S_i			
IMCLs type 1	−0.44†	−0.45†	−0.44†
IMCLs type 2	−0.59‡	−0.51§	−0.58‡
After adjustment for BMI and S_i			
IMCLs type 1	−0.12	−0.04	−0.22
IMCLs type 2	−0.43‖	−0.06	−0.49†

*Lipin and IMCL measures are logarithmically transformed. †P < 0.02; ‡P < 0.001; §P < 0.005; ‖P < 0.05.

(r = −0.49, P < 0.02) and lipin-α (r = −0.43, P < 0.05) after adjustment for obesity and insulin resistance.

Changes in IMCLs could be due to increased lipid oxidation in muscle or could be due to a partitioning of lipids into adipose tissue and away from skeletal muscle. To determine whether adipose tissue lipin was affecting muscle lipid oxidation, we examined the relationship between adipose tissue lipin expression and muscle lipid oxidation. We found no significant relationship between lipin expression (total, lipin-α, or lipin-β) and succinate dehydrogenase activity or CPT-1 mRNA expression (data not shown). The lack of association between adipose tissue lipin levels and muscle lipid oxidation suggests that the association of lipin with IMCLs may be due to a diversion of lipids into adipose tissue and away from skeletal muscle.

Muscle lipin expression. Previous studies in mice demonstrated abundant lipin expression in muscle, and overexpression of lipin in muscle resulted in obesity and changes in muscle energy utilization (22). Therefore, we measured lipin expression in muscle from the normal and impaired glucose-tolerant subjects described above. In contrast to adipose tissue lipin expression, there was no significant relationship between muscle lipin (either total lipin, lipin-α, or lipin-β) and obesity (BMI or percent body fat) or insulin sensitivity (S_i). In addition, there was no significant difference in muscle lipin expression between impaired or normal glucose-tolerant subjects, nor was there any significant change in lipin expression following treatment of impaired glucose-tolerant subjects with either pioglitazone or metformin (data not shown). Because overexpression of lipin in the muscle of mice resulted in increased fatty acid storage and decreased lipid oxidation (22), we examined our data carefully for any relationship between muscle lipin expression and muscle lipid storage or oxidation. We found no significant relationship between muscle lipin and IMCLs, CPT-1 expression, or fiber type (data not shown).

DISCUSSION

Lipin is a novel gene that was discovered as the etiologic mutation causing the phenotype in the *fld* mouse (20). The *fld* mouse is lipodystrophic, with nearly absent adipose tissue, a large fatty liver during the neonatal period, insulin resistance, and hyperlipidemia. In subsequent studies, lipin-β was overexpressed in adipose tissue and muscle (22). With adipose lipin overexpression, mice gained modest amounts of weight but, importantly, became more insulin sensitive and demonstrated less lipids in muscle and liver. Muscle lipin-β overexpression, however, tended

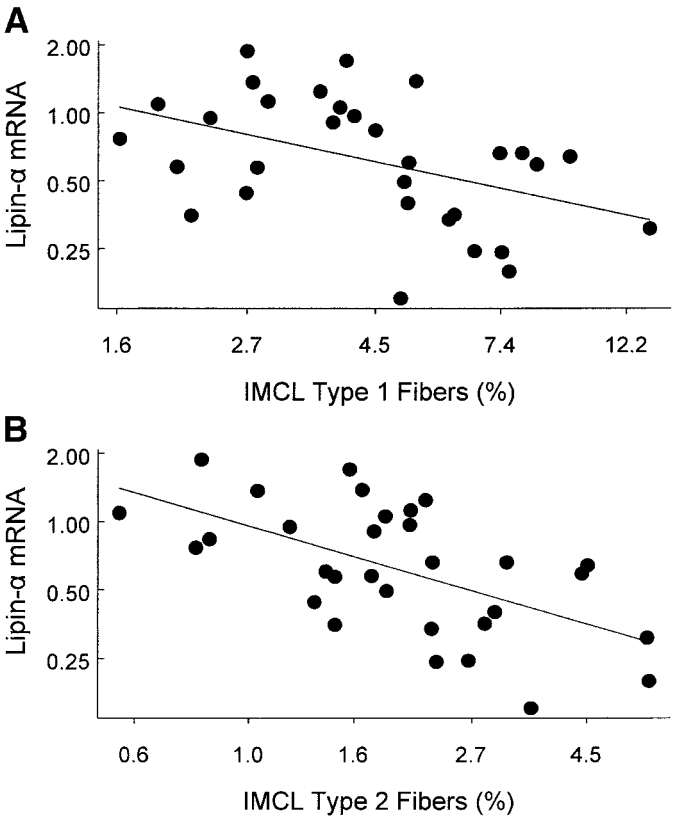

FIG. 5. Relationship between adipose tissue lipin expression and IMCLs (n = 22). *A*: Type 1 muscle fiber IMCLs. *B*: Type 2 muscle fiber IMCLs. Data are plotted on a log/log scale.

to have the opposite effect. Such mice became very obese, and their skeletal muscle demonstrated a diminished ability to oxidize lipids, leading to lipid accumulation and insulin resistance (22).

The mouse studies described above were provocative and suggested that lipin expression in both adipose tissue and muscle could be related to insulin resistance and other features of the metabolic syndrome (22). In addition, a recent study quantitated adipose total lipin mRNA using arrays in 19 subjects and demonstrated an inverse relationship with fasting insulin, glucose, and triglycerides (28). To determine whether these findings could be translated to a broader spectrum of humans with insulin resistance, we investigated whether there was any regulation of lipin expression in humans and whether the physiologic changes induced in mice were relevant to humans with obesity and insulin resistance. In nondiabetic human subjects covering a range of BMI and insulin sensitivity, adipose tissue lipin expression was inversely correlated with obesity and positively correlated with S_i; hence, lipin was highest in lean, insulin-sensitive subjects. However, the regulation of lipin expression was not a simple association with obesity. The subjects in this study were all nondiabetic, but some had impaired glucose tolerance. Such subjects typically have more features of metabolic syndrome, are more insulin resistant, and have a higher likelihood of eventually developing type 2 diabetes than normal glucose-tolerant subjects (36,37). When we compared lipin expression in normal versus impaired glucose-tolerant subjects who were matched for BMI, age, sex, and race, adipose tissue lipin levels were significantly higher in the normal glucose-tolerant subjects. Both lipin-α and lipin-β were increased in normal glucose-tolerant subjects, reflecting an adipose tissue profile of normal glucose-tolerant subjects that may be primed for both increased adipogenesis (lipin-α) and lipogenesis (lipin-β) (23). This study was unable to analyze differences in ethnic or sex subgroups. Overall, however, these data are consistent with studies in transgenic mice, where enhanced lipin expression in adipose tissue resulted in improved insulin sensitivity (22).

In studies with mice, enhanced lipin expression in adipose tissue leads to increased lipid accumulation in adipose tissue and increased insulin sensitivity (22), and it has been proposed that efficient partitioning of lipids into adipose tissue may "protect" muscle from lipid accumulation and adverse effects on insulin sensitivity. We therefore examined the relationship between adipose lipin expression and IMCLs and found that adipose lipin expression was inversely associated with IMCLs, and the relationship between lipin-α expression and IMCLs in type 2 fibers was independent of obesity and insulin sensitivity. This variation in IMCLs was not associated with changes suggestive of muscle lipid oxidative capacity, consistent with data from adipose-specific lipin transgenic mice (22). Another example in humans of improved insulin sensitivity through partitioning of lipids into adipose tissue, with no change in muscle lipid oxidation, occurs with TZD treatment (19). Thus, the data from mice and humans both point to a mechanism whereby increased lipin expression in adipose tissue influences insulin sensitivity through preferential lipid storage in adipose tissue and diversion away from muscle, rather than an effect on lipid oxidation in muscle.

The TZD drugs are agonists for PPARγ and result in improved insulin sensitivity. In a previous study, we demonstrated that pioglitazone increased subcutaneous (but not visceral) adipose tissue volume and decreased IMCLs (19), suggesting that this PPARγ agonist promoted adipose tissue lipid accumulation as a mechanism for decreasing IMCLs and improving insulin sensitivity. In this study, impaired glucose-tolerant subjects treated with pioglitazone demonstrated increased expression of lipin-β, but not lipin-α, along with improved insulin sensitivity. Subjects treated with metformin, an insulin sensitizer that predominantly affects hepatic glucose production, had no effect on adipose tissue lipin expression. These data are consistent with the concept that the mechanism of action of TZDs is an induction of adipose tissue lipid accumulation, resulting in a diversion of lipids into adipose tissue and away from muscle. The increase in lipin-β expression may be an important component of the adipose tissue response to TZDs. Because lipin-β expression stimulates lipogenic gene expression (23), increased adipose tissue lipid accumulation in response to pioglitazone may be associated with both PPARγ activation and enhanced lipin-β expression.

The relationship between lipin levels and obesity appears to be complex. Although overexpression of lipin in adipose tissue of transgenic mice promotes increased lipogenic gene expression and obesity, this situation may not be analogous to that in a genetically heterogeneous human population where numerous environmental and genetic factors interact to influence body mass. In the current study, we observed an inverse relationship between lipin mRNA levels in the complete subject group, which included individuals ranging from lean to obese and normal to impaired glucose tolerance. Thus, lower levels of lipin in subjects with higher BMI may reflect several

factors, including insulin resistance or abnormal adipocyte function that may occur in obesity. Furthermore, it is important to consider that in addition to the levels of lipin mRNA produced, lipin activity in the cell may be dependent on posttranslational modification, as demonstrated by insulin-stimulated lipin phosphorylation in rat adipose tissue (26). It will be of great interest to investigate protein levels and phosphorylation status in human adipose tissue once adequate antibodies are available.

Although the regulation of adipose tissue lipin generally mirrors the physiologic changes in transgenic mice with lipin overexpression (22), the regulation of muscle lipin expression is more complex. Whereas overexpression of lipin in mouse muscle led to impressive obesity and insulin resistance, there was no relationship between muscle lipin and obesity or insulin resistance in humans, and improved insulin sensitivity following pioglitazone treatment had no effect on muscle lipin. Another feature of transgenic lipin overexpression in mouse muscle, however, was a decrease in muscle lipid oxidation, manifested by an increase in lipid storage enzymes, and a decrease in lipid oxidation enzymes. In human muscle, there was no association between muscle lipin expression and IMCLs and also no association with CPT-1. However, it is possible that a larger study with a more homogeneous population may detect evidence of lipin regulation in muscle.

Taken together, our data demonstrate that adipose tissue *LPIN1* gene expression is associated with insulin sensitivity and other features of the metabolic syndrome. These results are consistent with the role of lipin in promoting adipocyte differentiation and lipid accumulation, which could result in a diversion of lipids into adipocytes and away from ectopic sites such as skeletal muscle. The specific induction of lipin-β, but not lipin-α, in response to pioglitazone provides evidence that the two isoforms are differentially regulated. In contrast to adipose tissue lipin expression, muscle lipin expression did not vary with insulin sensitivity or respond to pioglitazone.

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REFERENCES

1. Flegal KM, Carroll MD, Ogden CL, Johnson CL: Prevalence and trends in obesity among US adults, 1999–2000. *JAMA* 288:1723–1727, 2002
2. Mokdad A, Serdula MK, Dietz WH, Bowman B, Marks J, Koplan J: The spread of the obesity epidemic in the United States, 1991–1998. *JAMA* 282:1519–1522, 1999
3. Harris MI: Diabetes in America: epidemiology and scope of the problem. *Diabetes Care* 21 (Suppl. 3):C11–C14, 1998
4. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 285:2486–2497, 2001
5. Carr DB, Utzschneider KM, Hull RL, Kodama K, Retzlaff BM, Brunzell JD, Shofer JB, Fish BE, Knopp RH, Kahn SE: Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes* 53:2087–2094, 2004
6. Karelis AD, St. Pierre DH, Conus F, Rabasa-Lhoret R, Poehlman ET, Poehlman ET: Metabolic and body composition factors in subgroups of obesity: what do we know? *J Clin Endocrinol Metab* 89:2569–2575, 2004
7. Friedman J: Fat in all the wrong places (Letter). *Nature* 415:268–269, 2002
8. Unger RH: Lipotoxic diseases. *Annu Rev Med* 53:319–336, 2002
9. Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H–13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48:1600–1606, 1999
10. Agarwal AK, Simha V, Oral EA, Moran SA, Gorden P, O'Rahilly S, Zaidi Z, Gurakan F, Arslanian SA, Klar A, Ricker A, White NH, Bindl L, Herbst K, Kennel K, Patel SB, Al Gazali L, Garg A: Phenotypic and genetic heterogeneity in congenital generalized lipodystrophy. *J Clin Endocrinol Metab* 88:4840–4847, 2003
11. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, Vinson C, Eckhaus M, Reitman ML: Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* 105:271–278, 2000
12. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A: Leptin-replacement therapy for lipodystrophy. *N Engl J Med* 346:570–578, 2002
13. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
14. Phillips DI, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, Taylor R: Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metab Clin Experimental* 45:947–950, 1996
15. Krssak M, Falk PK, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI: Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42:113–116, 1999
16. Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, Flier JS: Peroxisome proliferator-activated receptor gene expression in human tissues: effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99:2416–2422, 1997
17. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP: The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30:1000–1007, 1981
18. Saltiel AR, Olefsky JM: Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 45:1661–1669, 1996
19. Rasouli N, Raue U, Miles LM, Lu T, Di Gregorio GB, Elbein SC, Kern PA: Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue. *Am J Physiol Endocrinol Metab* 288:E930–E934, 2005
20. Peterfy M, Phan J, Xu P, Reue K: Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat Genet* 27:121–124, 2001
21. Phan J, Peterfy M, Reue K: Lipin expression preceding peroxisome proliferator-activated receptor-gamma is critical for adipogenesis in vivo and in vitro. *J Biol Chem* 279:29558–29564, 2004
22. Phan J, Reue K: Lipin, a lipodystrophy and obesity gene. *Cell Metab* 1:1–11, 2005
23. Peterfy M, Phan J, Reue K: Alternatively spliced lipin isoforms exhibit distinct expression pattern, subcellular localization and role in adipogenesis. *J Biol Chem* 280:32883–32889, 2005
24. Tange Y, Hirata A, Niwa O: An evolutionarily conserved fission yeast protein, Ned1, implicated in normal nuclear morphology and chromosome stability, interacts with Dis3, Pim1/RCC1 and an essential nucleoporin. *J Cell Sci* 115:4375–4385, 2002
25. Santos-Rosa H, Leung J, Grimsey N, Peak-Chew S, Siniossoglou S: The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. *EMBO J* 24:1931–1941, 2005
26. Huffman TA, Mothe-Satney I, Lawrence JC Jr: Insulin-stimulated phosphorylation of lipin mediated by the mammalian target of rapamycin. *Proc Natl Acad Sci U S A* 99:1047–1052, 2002
27. Sarbassov DD, Ali SM, Sabatini DM: Growing roles for the mTOR pathway. *Curr Opin Cell Biol* 17:596–603, 2005
28. Suviolahti E, Reue K, Cantor RM, Phan J, Gentile M, Naukkarinen J, Soro-Paavonen A, Oksanen L, Kaprio J, Rissanen A, Salomaa V, Kontula K, Taskinen MR, Pajukanta P, Peltonen L: Cross-species analyses implicate lipin 1 involvement in human glucose metabolism. *Hum Mol Genet* 15:377–386, 2006
29. Bergman RN, Prager R, Volund A, Olefsky JM: Equivalence of the insulin

sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest* 79:790–800, 1987
30. Bergman RN, Ider YZ, Bowden CR, Cobelli C: Quantitative estimation of insulin sensitivity. *Am J Physiol* 236:E667–E677, 1979
31. Saad MF, Steil GM, Kades WW, Ayad MF, Elsewafy WA, Boyadjian R, Jinagouda SD, Bergman RN: Differences between the tolbutamide-boosted and the insulin-modified minimal model protocols. *Diabetes* 46:1167–1171, 1997
32. Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA: Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* 54:2305–2313, 2005
33. Halvorsen YD, Bond A, Sen A, Franklin DM, Lea-Currie YR, Sujkowski D, Ellis PN, Wilkison WO, Gimble JM: Thiazolidinediones and glucocorticoids synergistically induce differentiation of human adipose tissue stromal cells: biochemical, cellular, and molecular analysis. *Metabolism* 50:407–413, 2001
34. 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 Obes* 25:1316–1321, 2001
35. Kern PA, Simsolo RB, Fournier M: Effect of weight loss on muscle fiber type, fiber size, capillarity, and succinate dehydrogenase activity in humans. *J Clin Endocrinol Metab* 84:4185–4190, 1999
36. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, the Diabetes Prevention Program Research Group: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403, 2002
37. Festa A, D'Agostino RJR, Hanley AJG, Karter AJ, Saad MF, Haffner SM: Differences in insulin resistance in nondiabetic subjects with isolated impaired glucose tolerance or isolated impaired fasting glucose. *Diabetes* 53:1549–1555, 2004