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Context and Objective: Stearoyl-coenzyme A desaturase (SCD1) is the rate-limiting enzyme that converts palmitoyl- and stearoyl-coenzyme A to palmitoleoyl- and oleoyl-cownzyme A, respectively. SCD-deficient mice are protected from obesity, and the ob/ob mouse has high levels of SCD. This study was designed to better characterize SCD1 gene and protein expression in humans with varying insulin sensitivity.

Design, Participants, and Setting: In a university hospital clinical research center setting, SCD1 gene expression was measured in sc adipose and vastus lateralis muscle of 86 nondiabetic subjects; 10 wk of pioglitazone (45 mg daily) and metformin (1000 mg twice daily) treatment were assessed in 36 impaired glucose-tolerant subjects. Adipocytes were treated with pioglitazone, and SCD1 expression was attenuated with small interfering RNA (siRNA) to examine other adipocyte genes.

Results: There was no significant relationship between adipose or muscle SCD1 mRNA and either body mass index or insulin sensitivity. After pioglitazone (but not metformin) treatment, there was a 2-fold increase in SCD1 mRNA and protein in adipose tissue. Pioglitazone also increased SCD1 *in vitro*. There were significant positive correlations between SCD1 and peroxisomal proliferator-activated receptor γ (PPARγ) as well as other PPARγ-responsive genes, including lipin-β, AGPAT2, RBP4, adiponectin receptors, CD68, and MCP1. When SCD1 expression was inhibited with a siRNA, lipin-β, AGPAT2, and the adiponectin R2 receptor expression were decreased, and adipocyte MCP-1 was increased.

Conclusions: SCD1 is closely linked to PPARγ expression in humans, and is increased by PPARγ agonists. The change in expression of some downstream PPARγ targets after SCD1 knockdown suggests that PPARγ up-regulation of SCD1 leads to increased lipogenesis and potentiation of adiponectin signaling. **(*J Clin Endocrinol Metab* 93: 4431–4439, 2008)**

In both humans and rodents, high intake of dietary saturated fatty acids promotes dyslipidemia, obesity, and other features of the metabolic syndrome, including insulin resistance (1, 2). Upon entry into the cell, saturated fatty acids are converted into mono- and eventually polyunsaturated fatty acids by the action of various desaturases, which include stearoyl-Co A desaturase (SCD), of which humans have two isoforms, SCD1 and SCD5 (3, 4). SCD1 has high homology with rodents SCDs, whereas SCD5 is unique to primates. SCD1 is a δ-9 desaturase and is the rate-limiting enzyme responsible for converting

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Abbreviations: BMI, Body mass index; IGT, impaired glucose tolerant; LPL, lipoprotein lipase; PPARγ, peroxisomal proliferator-activated receptorγ; SCD, stearoyl-Co A desaturase; SGBS, Simpson-Golabi-Behmel syndrome; S_I, insulin sensitivity; siRNA, small interfering RNA; TZD, thiazolidinedione.

palmitic (16:0) and stearic acid (18:0) to palmitoleic (16:1) and oleic (18:1) acids, respectively, and the ratio of palmitoleic to palmitic acid (16:1 to 16:0) has been called the desaturation index (4, 5). Because saturated fatty acids have the potential for causing lipotoxicity in many tissues (6, 7), one would expect that a desaturase such as SCD1 would protect cells against lipotoxicity due to overaccumulation of saturated fat. However, the monounsaturated products of SCD1 are preferred substrates for the synthesis of triglycerides, cholesterol esters, and phospholipids and are major components of all cellular lipids (4).

New insights regarding the role of SCD in lipogenesis, obesity, and insulin resistance were gained from the genetic manipulation of SCD in animal models. SCD knockout mice were protected against obesity and insulin resistance and also demonstrate increased lipid oxidation (8). In addition, the lowering of hepatic SCD1 levels with an antisense oligonucleotide resulted in improved hepatic insulin sensitivity (9), and a diet high in saturated fatty acids did not induce insulin resistance in SCD1 knockout mice (10). There was increased SCD1 gene expression in skeletal muscle of Zucker diabetic rats suggests that there is a link between the SCD enzyme and insulin resistance (11). Furthermore, leptin-deficient ob/ob mice were shown to have high level of SCD that normalized after treatment with leptin, supporting the role of SCD in energy homeostasis (12, 13).

Despite the considerable animal data suggesting a strong link between obesity, insulin resistance, and SCD1, our knowledge regarding the link between SCD and obesity in humans is limited. In obese insulin-resistant subjects, an elevated 18:1 to 18:0 desaturation index (but not 16:1 to 16:0), suggestive of elevated SCD1 activity, was found (14), and another study showed that rectus abdominus muscle SCD1 gene expression was increased in extremely obese subjects (15). SCD1 polymorphisms were associated with insulin sensitivity and body fat distribution in Swedish men (16). However, rosiglitazone treatment increased SCD1 mRNA in diabetic subjects (17, 18), an effect that is opposite to what would be expected from a positive association between SCD1 and insulin resistance. No study has yet examined adipose SCD1 expression in nondiabetic humans covering a spectrum of lean to obese.

Because of the predominance of data on SCD1, we measured SCD1 gene expression in adipose tissue of nondiabetic subjects, covering a wide range of body mass index (BMI) and insulin sensitivity, and we also assessed the effects of two insulin-sensitizing drugs, pioglitazone and metformin, on SCD1 expression in adipose and muscles tissues. In addition, we examined the effects of SCD1 knockdown by small interfering RNA (siRNA) in adipocytes. We found that SCD1 expression was highly correlated with the expression of a number of other genes that are responsive to peroxisomal proliferator-activated receptor γ (PPARγ) agonists and involved in different components of adipogenesis.

Subjects and Methods

Human subjects and muscle biopsies

Generally healthy human subjects were recruited by local advertisement. All subjects signed informed consent forms under protocols that were approved by the local institutional review board. The studies were performed at the University of Arkansas for Medical Sciences/Central Arkansas Veterans Health Care System General Clinical Research Center. Some of the subjects in this study were also included in previous reports (19, 20). Subjects with a history of coronary artery disease and those who were treated with fibrates, statins, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, or antiinflammatory medications were excluded. Subjects were included if fasting glucose was less than 126 mg/dl and the 2-h postchallenge glucose was less than 200 mg/dl, as determined by an initial 75-g oral glucose tolerance test. Based on this test, subjects were defined as either normal glucose tolerant ($n = 55$) (2 h glucose < 140 mg/dl) or impaired glucose tolerant (IGT; $n = 31$) (2 h glucose 140–199 mg/dl). A total of 86 subjects were recruited (70 women and 16 men; 72 Caucasians, 13 African Americans, and one Hispanic); the age range was 21–66 yr, and there was a wide range of BMI (19–55 kg/m^2) and insulin sensitivity (S_I; 0.51–10.94 $\times$ 10^{-4}/min$^{-1}\cdot\mu$U per milliliter). All subjects were weight stable and underwent sc adipose tissue biopsies from the lower abdominal wall and muscle biopsies from the vastus lateralis and insulin sensitivity testing using the insulin-modified frequently sampled iv glucose tolerance test, as described previously (21). IGT subjects were then randomized to receive either metformin or pioglitazone for a 2-wk dose escalation followed by 8 wk at a maximum dose (1000 mg metformin twice a day or 45 mg pioglitazone daily). After 10 wk of treatment, the oral and iv glucose tolerance tests and biopsies were repeated. Percent body fat was measured by dual-emission x-ray absorptiometry.

Human adipose tissue fractions and cells

To examine gene expression in different adipose tissue fractions, adipocyte and stromal fractions were isolated from adipose tissues by collagenase digestion, as described previously (22). Two different human adipocyte cell systems were used. Simpson-Golabi-Behmel syndrome (SGBS) cells were originally derived from the stromal fraction of sc adipose tissue of an infant with SGBS and were cultured and differentiated as described earlier (23, 24), and cells were 90% differentiated after differentiation. The other cultured cell system was human adipose-derived human stem cells isolated from discarded adipose tissue from normal women undergoing liposuction, as described previously (22, 25, 26). After induction of differentiation, at least 80% of cells were differentiated, and the cells were changed to medium without any thiazolidinedione (TZD) for a further 4 d. The differentiated cells were then treated with pioglitazone (1.5 μM) for 24 and 48 h. Differentiation in both cell lines was confirmed by Oil Red O staining and the detection of adipocyte-specific mRNA and/or protein expression.

Insulin sensitivity measurement

Insulin sensitivity was determined by an insulin-modified frequently sampled iv glucose tolerance test using 11.4 g/m^2 of glucose and 0.04 U/kg of insulin (21). Plasma insulin was measured using a chemoluminescent assay (Molecular Light Technology Research, Ltd., Cardiff, Wales, UK), 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 Millennium program (27).

RNA isolation and real-time RT-PCR

Total RNA from human adipose tissue and cultured cells was isolated using an RNAeasy lipid tissue minikit from QIAGEN (Valencia, CA), and total RNA from muscle biopsies was isolated using an Ultraspec RNA isolation system kit (Biotex, Houston, TX). The quantity and quality of the isolated RNA was determined by Agilent 2100 bioanalyzer (Palo Alto, CA). Real-time RT-PCR and primer sequences of 18S, CD68 and MCP-1, RBP4, lipin-α, lipin-β, and TSP1 were as published previously (19, 22, 28, 29). The other primer sequences were as follows: SCD1, CCGGGAGAATATCCTGGTTT (forward), GCGGTACTCAACTGGCAGAGT (reverse); adiponectin R1, TTCTTCCTCATGGCTGTGATGGT (forward), AAGAAGCGCTCAGGAATTCG (reverse); adiponectin R2, ATAGGGCAGATAGGCTGGTTGA (forward), GGATCCGGGCAGCATACA (reverse); AGPAT2, CCCGTGGTG-

TACTCTTCCTT (forward), GCACCTGCACTGTGACTGTT (reverse); and PPARγ2, CCAGAAAGCGATTCCTTCAC (forward), GAGAGATCCACGGAGCTGAT (reverse). All data were expressed in relation to 18S RNA, in which the standard curves was generated using pooled RNA from the samples assayed. The use of other housekeeping genes, such as β-actin and β-2-microglobulin, gave results that were essentially identical with the use of 18S.

SCD1 siRNA treatment of adipocytes

Differentiated SGBS cells were transfected with 30 nM of siSCD1 (Ambion, Austin, TX) or negative control siRNA using siPORTT-MAmine transfection reagent (Ambion) for 48 h following the manufacturer's instruction. The percentage of knockdown target gene expressions was determined using quantitative real-time RT-PCR.

SCD1 protein measurement

SCD1 protein level in adipose tissue was measured by Western blot with a monoclonal antibody to human SCD1 (Abcam Co., Cambridge, MA). Frozen adipose tissue (150 mg) was lysed in 500 μl of M-PER mammalian protein extraction reagent (Pierce, Rockford, IL) containing protease inhibitors, and protein (10 μg) was loaded on a 10% polyacrylamide gel, transferred onto a nitrocellulose membrane, and blotted with anti-SCD1 (1:1000), followed by horseradish peroxidase antimouse IgG.

Statistical analysis

Student's two-sample *t* tests were used to compare groups with respect to continuous variables. Paired *t* tests were used to compare baseline and treatment measurements within a group. Pearson's correlation coefficients were used to describe the linear association between variables. All data from samples were expressed as mean ± SEM.

Results

SCD1 mRNA expression profile in human adipose tissue and cell fractions

To determine the expression level in different cell fractions, SCD1 mRNA was measured by real-time PCR in human whole adipose tissue, adipocytes, and the stromal vascular fraction. As shown in Table 1, SCD1 mRNA level in the adipocyte fractions were at least 18-fold higher than that of the stromal fractions. To further characterize SCD1 expression with regards to adipocyte differentiation, SCD1 expression was measured in human adipose-derived human stem cells, both before and after induction of differentiation into adipocytes, as described in *Subjects and Methods*. As shown in Table 1, the expression of SCD1 was very low in preadipocytes and was mainly expressed in differentiated adipocytes, similar to the level of expression in whole adipose tissue. SCD1 was expressed at a very low level only in muscle (data not shown). Lipoprotein lipase (LPL) gene expression was also measured as an indicator of adipocyte gene expression. As expected, LPL expression was low in the stromal fraction of adipose tissue and in preadipocytes.

TABLE 1. SCD1 mRNA in human cells and tissue

Cell/tissue	SCD1[a]	LPL[a]
Whole adipose tissue	2.79	4.92
	1.42	3.20
Adipocytes from adipose tissue	3.30	4.19
	3.50	5.87
Stromal fraction from adipose tissue	0.18	0.51
	0.18	0.51
Adipocytes from cultured preadipocytes	2.16	1.07
	2.52	0.44
Cultured stem cells (preadipocytes)	0.10	ND
	0.06	

Data are from two independent experiments. ND, Not detectable.

[a] The pooled RNA from all the samples was used for a standard curve. Hence, the data are expressed relative to each other.

SCD1 gene expression: role of obesity and insulin resistance

Because of the link between increased adipose tissue lipid desaturation and obesity, we examined the relationship between SCD1 expression in adipose tissue and markers of obesity and insulin resistance in 86 nondiabetic humans, as described in *Subjects and Methods*. As shown in Table 2, SCD1 mRNA levels did not demonstrate any correlation with BMI, percent body fat, S_I, or plasma triglycerides. Although there was no significant relationship between SCD1 and S_I in this overall group of subjects, we examined SCD1 and insulin resistance in a more homogeneous population consisting of Caucasian women between the ages of 21 and 59 yr, with a BMI between 23 and 36 kg/m^2. Among the 86 subjects described above, 42 subjects met these criteria and were divided into two groups according to the median S_I. The S_I group (BMI 30 ± 0.84, S_I 6.12×10^{-5} min/μIU ± 0.56) and the insulin-resistant group (BMI 32 ± 0.64, S_I 2.61×10^{-5} min/μIU ± 0.15) differed only by their S_I. There was no significant difference in SCD1 mRNA level between these two subgroups (data not shown), again suggesting there was no relationship between SCD1 expression and S_I. We also measured SCD1 mRNA level in skeletal muscle of 74 subjects; muscle SCD1 expression was very low, and there was no significant correlation with either BMI or S_I (data not shown).

TABLE 2. Correlation coefficient of SCD1 mRNA with different plasma and adipose tissue factors

Variable (n)	r	*P*
BMI (86)	0.13	NS
S_I (81)	−0.01	NS
Percent body fat (86)	0.23	NS
Plasma triglycerides (77)	−0.11	NS
Plasma leptin (49)	0.18	NS
Plasma TNFα (46)	−0.07	NS
Plasma IL-6 (53)	−0.15	NS
Adipose tissue		
MCP1 mRNA (86)	0.48	<0.0001
CD68 mRNA (85)	0.30	<0.01
PPARγ2 mRNA (85)	0.51	<0.0001
Lipin-α mRNA (85)	0.18	NS
Lipin-β mRNA (84)	0.58	<0.0001
AGPAT2 mRNA (85)	0.64	<0.0001
RBP4 mRNA (85)	0.50	<0.0001
Adiponectin R1 (84)	0.48	<0.0001
Adiponectin R2 (83)	0.58	<0.0001
TSP1 mRNA (83)	0.17	NS

NS, Not significant; TSP, thrombospondin.

SCD1 expression was associated with the expression of other genes in adipose tissue

Whereas there were no discernible direct associations between SCD1 gene expression and obesity or insulin resistance, it was important to examine other factors associated with insulin resistance. Because inflammation plays an important role in insulin resistance, we examined the expression of MCP1 and CD68 in adipose tissue to determine whether there was a correlation between human SCD1 and inflammatory markers. CD68 is a macrophage marker, and MCP-1 is expressed predominantly in macrophages but also in adipocytes (22). As shown in Fig. 1A, a strong positive correlation was observed between SCD1 expression and MCP1 mRNA level in adipose tissue ($r = 0.48$, $P < 0.0001$). As shown in Table 2, there was a significant relationship between SCD1 expression and CD68 mRNA level ($r = 0.30$, $P < 0.01$), although this relationship was not as strong as for MCP-1. Despite these correlations between SCD1 and adipose tissue inflammatory markers, there was no correlation between SCD1 expression and plasma TNFα or IL-6 (Table 2).

In previous studies and as shown below, SCD1 gene expression was increased by PPARγ agonists (17, 18). Therefore, we examined the relationship between SCD1 expression and PPARγ. As observed in Table 2, SCD1 mRNA in adipose tissue correlated well with PPARγ2 mRNA ($r = 0.51$, $P < 0.0001$). The expression of other genes involved in adipocyte lipid accumulation, and PPARγ responsiveness, was also examined. As seen in Table 2 and Fig. 1, SCD1 demonstrated a strongly positive correlation with lipin-β (but not lipin-α), AGPAT2, RBP4, and both the R1 and R2 adiponectin receptors. However, TSP1, an adipocyte gene involved in inflammation and that is decreased by TZD treatment (19), was not correlated with SCD1 expression.

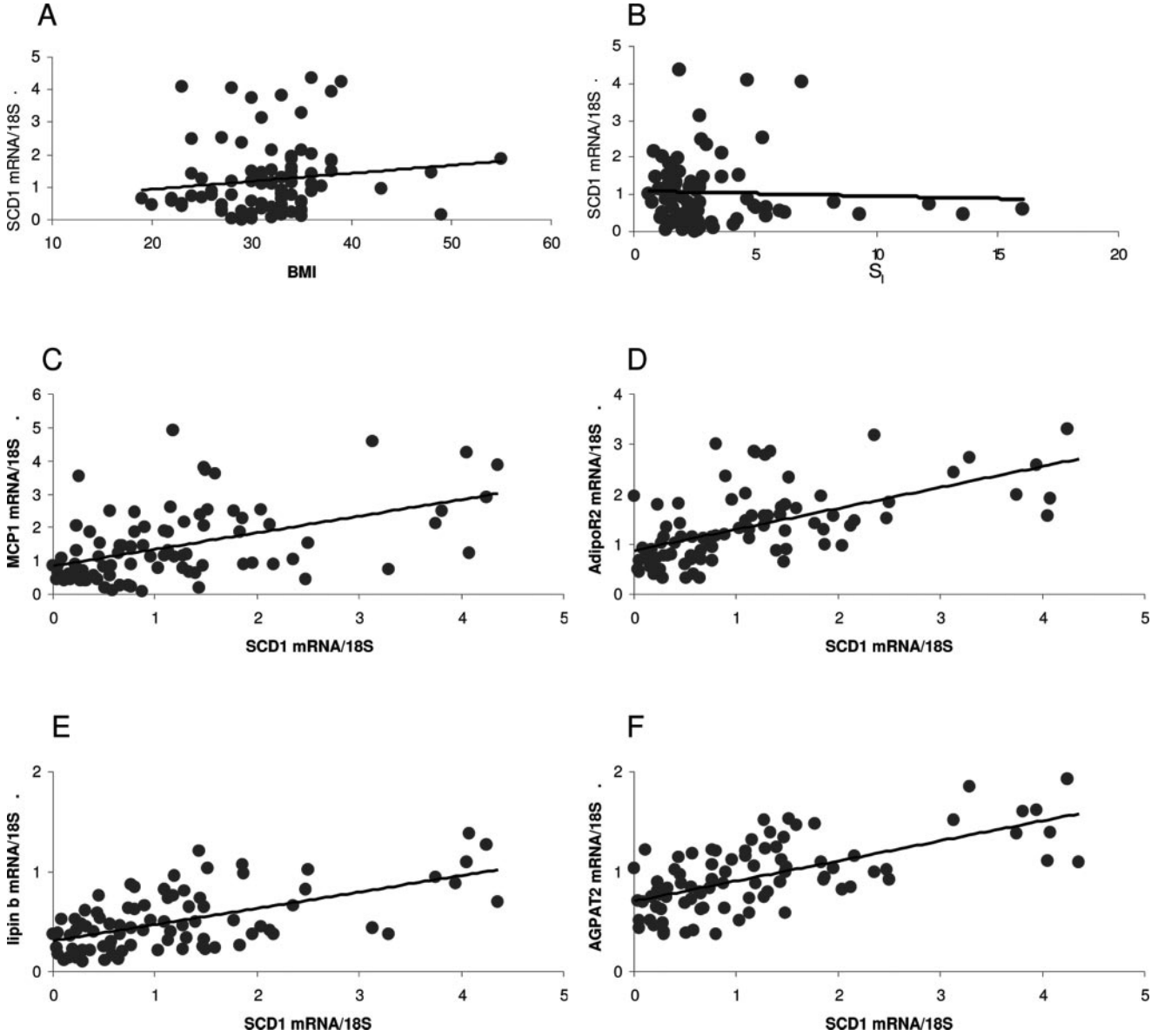

FIG. 1. Adipose tissue expression of SCD1. Relationship to A, BMI. B, S_I. C, MCP-1 mRNA. D, Adiponectin R2 mRNA. E, Lipin-β mRNA. F, AGPAT2 mRNA. See Table 2 for correlation coefficients.

Pioglitazone increased SCD1 mRNA and protein level in adipose tissue along with the expression of other adipogeneic genes

To determine the effects of PPARγ stimulation on SCD1, IGT individuals were treated with two different classes of insulin sensitizers. As described in *Subjects and Methods*, subjects were randomized to treatment with either pioglitazone or metformin for 10 wk. The baseline characteristics of these individuals have been described previously (30). The mean age, BMI, and S_I was 49 yr, 34 kg/m^2, and 1.44 $10^{-5} \times \text{min}^{-1}/\mu\text{IU}$, respectively. S_I increased from 1.44 ± 0.13 to 2.21 ± 0.21 ($P < 0.05$) in the pioglitazone treatment group, but there was no significant change in S_I in the metformin treatment group. Treatment with pioglitazone resulted in a 2-fold increase in SCD1 mRNA levels in adipose tissue ($P = 0.01$, Fig. 2A) and a 10-fold increase in muscle SCD1 ($P = 0.04$) (Fig. 2B). No significant change in SCD1 expression was observed with metformin in either tissue. To determine whether the increase in SCD1 after pioglitazone treatment in human subjects was a direct effect of the drug or secondary to the improved insulin sensitivity, SGBS adipocytes were treated with pioglitazone *in vitro* for 48 h, and SCD1 mRNA was measured. As shown in Fig. 2C, the addition of pioglitazone to adipocytes resulted in a significant increase in SCD1 mRNA, suggesting a direct effect on the adipocyte.

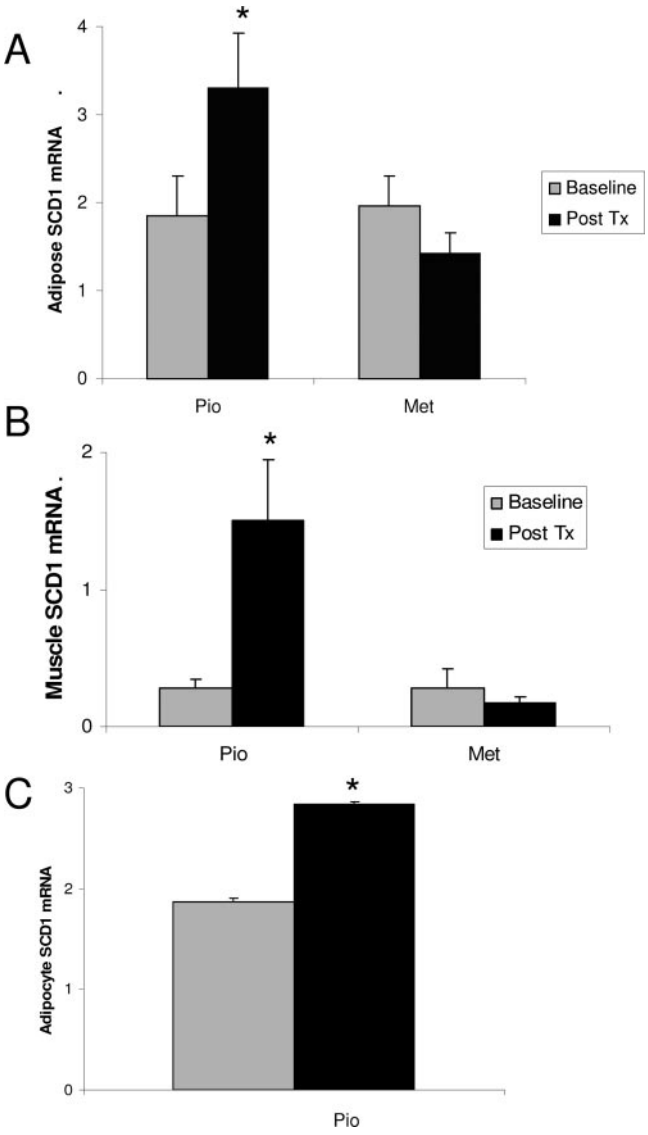

FIG. 2. Effects of pioglitazone (Pio) and metformin (Mel) treatment on SCD1 mRNA expression. A, Adipose tissue SCD1 mRNA expression was measured in the adipose tissue of IGT subjects before and after 10 wk of treatment with either pioglitazone or metformin. B, SCD1 mRNA expression in muscle from IGT subjects treated for 10 wk with either pioglitazone or metformin. C, SGBS adipocytes were treated with pioglitazone 1 μM for 48 h and SCD1 expression measured. *, $P < 0.05$.

To determine whether the pioglitazone-induced increase in SCD1 mRNA was also associated with an increase in SCD1 protein, Western blotting was performed on adipose tissue extracts. As shown in Fig. 3, the treatment of pioglitazone resulted in an increase of SCD1 protein level in adipose tissue. Interestingly, we observed no association between SCD1 and insulin sensitivity, yet an improvement of insulin sensitivity by pioglitazone treatment resulted in a significant increase in SCD1 mRNA and protein expression levels. There was no change in SCD1 protein expression after treatment with metformin (data not shown).

Previous studies have described changes in the expression of MCP-1, CD68, lipin-β, and RBP4 after pioglitazone treatment (22, 28, 29). As noted above and in Table 2, the expression of a number of mRNAs were highly associated with the expression of SCD1, and we wanted to determine whether these genes were also responsive to PPARγ stimulation in human adipose tissue. To assess the effects of pioglitazone and metformin treatment on the expression of adiponectin receptors and AGPAT2, the mRNA levels of these genes were examined in the adipose samples from the same subjects described above for SCD1. Results from these experiments revealed that pioglitazone (but not metformin) treatment resulted in a significant increase in expression of the adiponectin R2 (but not R1) receptor mRNA in adipose tissue (Fig. 4A). In a similar pattern, there was also a pioglitazone-specific increase in AGPAT2 mRNA expression (Fig. 4B).

Effect of SCD1 inhibition on gene expression

The correlations between SCD1 expression and other adipose tissue genes could be a direct effect of SCD1 or could be secondary to other changes in adipose tissue. To better understand the effects of SCD1 expression in adipocytes, differentiated SGBS adipocytes were treated with an SCD1-specific siRNA to inhibit SCD1 expression. SGBS adipocytes were induced to differentiate and then treated with a siRNA to SCD1 (described in *Subjects and Methods*). After treatment for 48 h, cell extracts were prepared, RNA extracted, and the expression of a number of adipocyte mRNAs examined. The siRNA treatment successfully inhibited expression of SCD1 mRNA by 65% (Fig. 5) and had no effect on GAPDH mRNA levels (data not shown). Further examination of other adipocyte mRNAs after SCD1 inhibition revealed no change in expression of leptin, RBP4, PPARγ, or TSP1. In contrast, there was a significant decrease in the expression of AdipoR2, AGPAT2, and lipin-β mRNAs as well as a significant increase in expression of MCP-1. Therefore, SCD inhibition by siRNA selectively affected a subset of PPARγ-responsive mRNAs in adipose tissue.

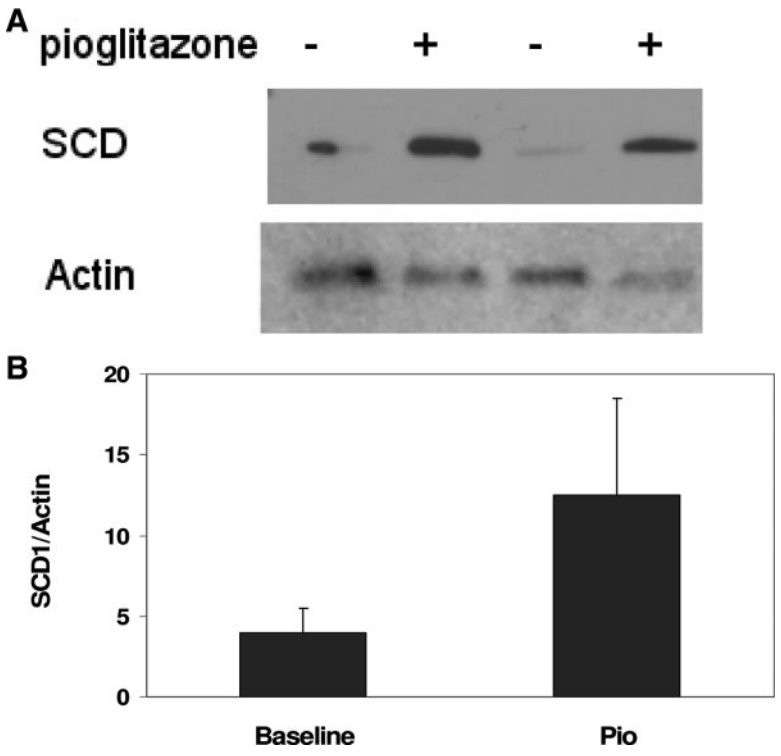

FIG. 3. Effects of pioglitazone on SCD1 protein levels in adipose tissue. Adipose tissue samples from IGT subjects were extracted and analyzed by SDS-PAGE and Western blotting, as described in *Subjects and Methods*. A, Representative Western blots of adipose samples from subjects before and after treatment with pioglitazone. Gels were blotted for SCD1 and actin as a loading control. B, Densitometric analysis of Western blots from adipose tissue of seven subjects from baseline and after pioglitazone (Pio) treatment ($P < 0.05$).

Discussion

SCD1 is highly expressed by adipose tissue and is rate limiting in the desaturation of cellular lipids into monounsaturated fatty acids. Several studies in rodents have identified SCD1 as an important component of obesity and insulin resistance. Mice with a naturally occurring mutation in SCD resulting in no expression have impaired synthesis of hepatic cholesterol esters and triglycerides and are lean and hypermetabolic (13, 31, 32), and SCD1 knockout mice demonstrated a similar phenotype (8). Similarly, rodents treated with SCD antisense oligonucleotide did not gain weight on high-fat diet and showed improvement in diet-induced hepatic insulin resistance and hepatic steatosis (9, 33). The mechanism by which SCD1 exerts these effects on insulin resistance and lipid metabolism in mice is not clear. SCD1 deficiency is associated with increased AMP kinase activity (34) along with increased expression of uncoupling proteins in brown adipose tissue (35), all of which may account for the increased thermogenesis and hypermetabolism. There is also evidence that leptin exerts some metabolic effects through the reduction of SCD1 activity (8, 13, 34). Other studies have suggested that the cellular deprivation of oleate, which is a product of the enzymatic activity of SCD1, leads to a down-regulation of sterol regulatory element-binding protein-1 and PPARγ coactivator 1β, which would lead to a decrease in lipogenesis (10). On the other hand, the absence of SCD1 would be expected to lead to an accumulation of palmitate and stearate, which are associated with lipotoxicity and the development of insulin resistance.

Despite the link between SCD1, obesity, and insulin resistance in various animal models, the role of SCD1 in humans remains uncertain. An elevated adipose tissue desaturation index, suggestive of elevated SCD1 activity, was found in obese insulin-resistant subjects (14), and another study demonstrated that muscle SCD protein expression levels and enzymatic activity in muscle were increased in a small number of very obese subjects (15). Several studies found elevated plasma triglyceride desaturation ratios in obese subjects; however, plasma triglyceride desaturation is likely more reflective of hepatic desaturation (36–38). Although no study has measured adipose SCD1 expression in a broad spectrum of subjects, previous studies reported an increase in insulin sensitivity and an increase in SCD1 mRNA after rosiglitazone treatment of diabetic subjects (15, 17). This increase in SCD1 after TZD-mediated improvement in insulin sensitivity would appear to contradict the apparent inverse relationship between SCD1 and insulin sensitivity in rodent studies or, alternatively and more likely, suggest that the role of SCD1 is more complicated and involves adipocyte lipid accumulation and PPARγ responsiveness.

In the present study, we examined SCD1 expression in nondiabetic human subjects over a broad range of BMI. In adipose tissue, SCD1 was predominantly expressed by the adipocytes, whereas the stromal fraction, containing macrophages and preadipocytes, had very low levels of SCD mRNA, a result corroborated using *in vitro* human adipocyte cultures. The desaturation index of adipose tissue was not measured. Insulin sensitivity was measured in these subjects; however, no significant correlation was observed between adipose tissue SCD1 expression and insulin sensitivity (using S_I) or obesity (using BMI and percent body fat). The expression of a number of other genes was examined in the same adipose tissue samples. Although there was no relationship between SCD1 and obesity or insulin resistance, there were significant relationships between SCD1 and other mRNAs encoding proteins involved in either lipogenesis or inflammation. Along with PPARγ2, SCD1 mRNA expression exhibited strong positive correlations with lipin-β, AGPAT2, RBP4, and adiponectin receptors R1 and R2. Lipin and AGPAT are both genes whose products are involved with lipid synthesis in adipocytes and have been associated with lipodystrophy in mice or humans (39, 40). High levels of plasma RBP4 have been linked to insulin resistance in some (41), but not all (28), studies, and the R1 and R2 receptors for adiponectin have been reported to mediate the lipid oxidative effects of adiponectin in target tissues (42).

Because of the significant relationship between SCD1 and PPARγ and with genes known to be affected by PPARγ agonists, we examined the adipose tissue and muscle of human subjects

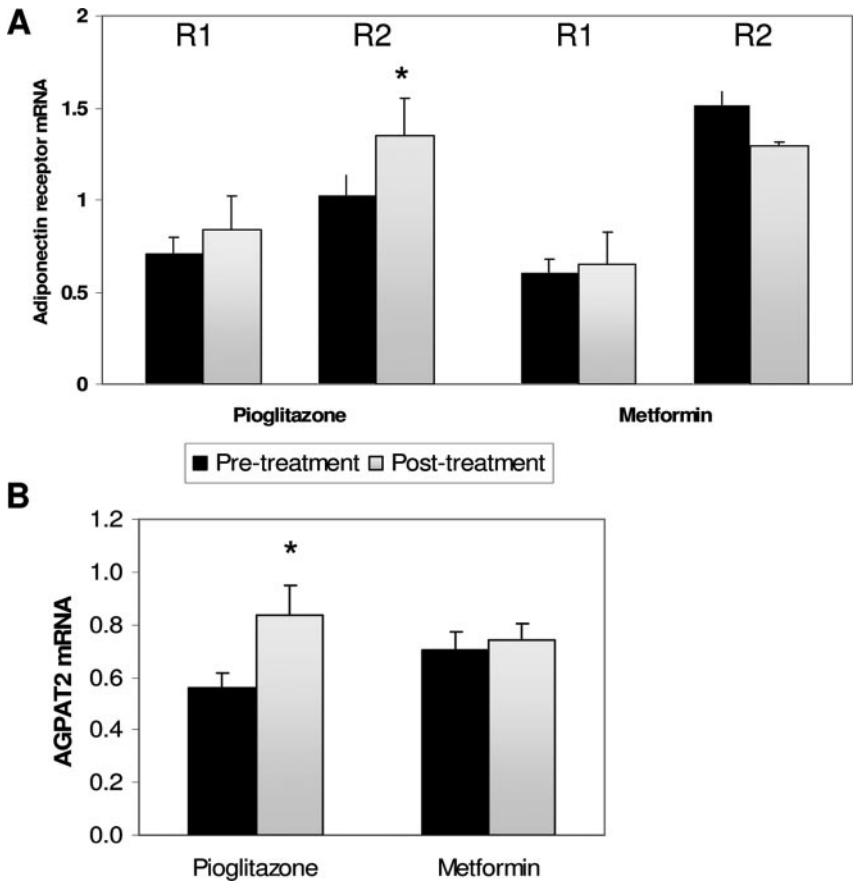

FIG. 4. Effects of pioglitazone and metformin on adipose tissue mRNA expression. Adipose tissue biopsies were performed in subjects before and after treatment with pioglitazone (n = 18) or metformin (n = 21). A, adiponectin R1 and R2 receptor mRNA expression. B, AGPAT2 mRNA expression in adipose tissue before and after treatment with pioglitazone or metformin. *, $P < 0.05$ *vs.* before treatment.

treated with the PPARγ agonist pioglitazone. The effects of pioglitazone were compared with metformin, which improves insulin sensitivity through hepatic effects, with relatively little effect on adipose tissue. SCD1 mRNA and protein expression in adipose tissue increased after pioglitazone but not metformin treatment. The increase in SCD1 may have been due to a direct effect of pioglitazone or may have been secondary to the improvement in insulin sensitivity. To determine whether there was a direct cellular effect, cultured adipocytes were treated with pioglitazone, yielding a similar increase in SCD1. Although expressed at much lower levels, there was a similar increase in muscle SCD1 in response to pioglitazone treatment as well. The expression of a number of other genes was examined after pioglitazone treatment. A number of lipogenic genes were increased by PPARγ agonists (43), and we have previously reported an increased expression of lipin-β and RBP4 after pioglitazone treatment (28, 29). In the current study, we examined the expression of AGPAT2 and adiponectin receptors R1 and R2 after pioglitazone treatment and observed an increase in adiponectin R2 (not R1) and AGPAT. One previous study found no change in adiponectin receptors after pioglitazone in diabetic subjects (44), whereas no previous study has examined AGPAT2 expression in humans in response to TZDs.

The parallel changes between SCD1 and PPARγ, along with a number of PPARγ-responsive genes, suggested a relationship between SCD1- and PPARγ-mediated gene expression. Alternatively, SCD1 and PPARγ may be linked due to an overall increase in adipocyte differentiation in certain subjects. Such a relationship may explain, in part, the phenotype of the SCD1 knockout mice (8). If the knockout of SCD1 were to result in a decrease in a number of other PPAR-responsive, lipogenic, or adipogenic genes, then this may lead to leanness. Both lipin and AGPAT, when deficient, are associated with lipodystrophy, and a decrease in expression of these genes as a result of decreased SCD1 expression would be expected to lead to weight loss. However, a severe inhibition of adipogenesis would lead to lipodystrophy, which was not a feature of the SCD1 knockout mouse.

To identify PPARγ-responsive genes dependent on SCD1 expression, adipocytes were treated with a siRNA designed to inhibit SCD1 expression. The siRNA significantly inhibited SCD1 mRNA expression by 65%, and similarly, mRNA levels for lipin-β, AGPAT2, and adiponectin R2 receptor were also decreased. However, several mRNAs of genes known to be PPARγ responsive, such as RBP4, TSP1, and PPARγ itself, were unaffected by SCD1 inhibition. Together these data would suggest that SCD1 and PPARγ are closely linked through a selective mechanism that regulates a subset of adipocyte signaling molecules. PPARγ agonists directly increase SCD1, and perhaps SCD1 is a downstream effector of PPARγ action or alters PPARγ coactivator function to alter downstream gene expression. Indeed, previous studies in muscle cells demonstrate that palmitate induces insulin resistance through a decrease in the PPARγ coactivator PPARγ coactivator 1, resulting in an increase in inflammatory pathways (7). On the other hand, it is possible that the desaturated lipids formed by SCD1 activity are themselves endogenous ligands for PPARγ and thus aid in augmenting and modulating the PPARγ response.

Previous studies have demonstrated interactions between PPARγ and SCD. A PPARγ response element is located on the SCD1 promoter (45, 46), supporting the effects of PPARγ agonists on SCD1 gene expression. Other studies, however, have suggested that SCD may play an important role in PPARγ responsiveness. In 3T3-L1 adipocytes, a recent study knocked down both SCD1 and SCD2 with siRNA and found that SCD2 silencing inhibited adipogenesis and decreased PPARγ protein levels (47). Although this effect was seen only with SCD2, and not SCD1, humans do not express SCD2, and SCD1 has very high homology to mouse SCD2 (3). Therefore, it is possible that the SCD1 silencing in human adipocytes also inhibited PPARγ responsiveness, resulting in the decreased expression of lipin-β and AGPAT2.

Obesity and insulin resistance are associated with increased adipose tissue inflammation, manifested by increased numbers

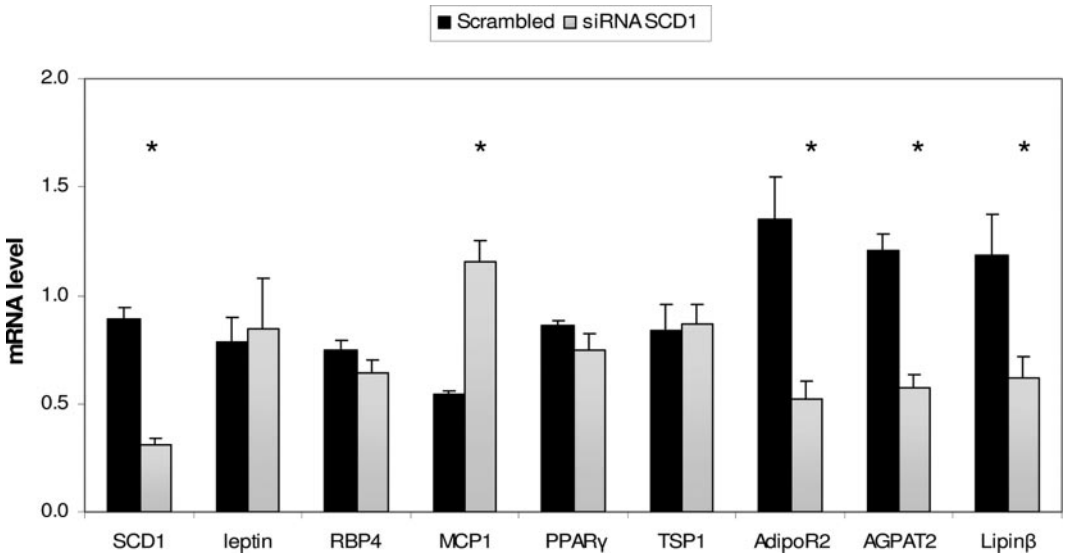

FIG. 5. Effects of SCD1 siRNA treatment on adipocyte mRNA gene expression. Differentiated SGBS adipocytes were treated with SCD1 siRNA, as described in *Subjects and Methods*, and RNA was extracted for gene expression analysis 48 h later (n = 3 for each mRNA expression level). *, $P < 0.05$.

of macrophages and macrophage-associated genes, such as CD68 and MCP-1 (48, 49). Previous studies have demonstrated a decrease in CD68 and MCP-1 expression from adipose tissue after pioglitazone treatment along with a decrease in macrophage number (22, 23). In the subjects described above, significant positive associations were noted between SCD1 expression and the adipose tissue expression of MCP-1 and CD68, although there was no association between SCD1 and plasma markers of inflammation, such as IL-6 or TNFα. In adipose tissue, MCP-1 is expressed by both macrophages and adipocytes, and pioglitazone treatment of human subjects decreased MCP-1 in whole adipose tissue (22). This decrease in MCP-1 could be due to the decrease in adipose tissue macrophages (23) but may also be due to a specific reduction in adipocyte MCP-1. SCD1 mRNA was associated with high levels of MCP-1 mRNA in whole adipose tissue samples of human subjects; however, the knockdown of SCD1 by the siRNA in cultured adipocytes resulted in an increase in MCP-1 expression. Although these data would appear to be inconsistent, the differences in MCP-1 response from adipose tissue *vs.* adipocytes may be due to the cellular source of MCP-1 production. MCP-1 was positively associated with SCD1 in whole adipose tissue, in which MCP-1 is predominantly derived from macrophages, but MCP-1 was inversely associated with SCD1 in cultured adipocytes in the knockdown experiments. It is possible that MCP-1 is regulated differently in adipocytes, compared with whole adipose tissue, and these studies suggest that adipocyte MCP-1 is also PPARγ responsive and linked to PPARγ-SCD1 signaling.

In summary, unlike animal models, we did not find a link between SCD1 and obesity in human adipose tissue. Nevertheless, the association in expression of SCD1 and a number of other mRNAs involved in adipogenesis and inflammation, especially those responsive to PPARγ agonists, raises the possibility that SCD1 may be involved in complex signal transduction pathways that interact with PPARγ.

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