Human Obesity and Type 2 Diabetes Are Associated With Alterations in SREBP1 Isoform Expression That Are Reproduced Ex Vivo by Tumor Necrosis Factor-α

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Sterol regulatory element binding protein (SREBP)-1 is a transcription factor with important roles in the control of fatty acid metabolism and adipogenesis. Little information is available regarding the expression of this molecule in human health or disease. Exposure of isolated human adipocytes to insulin enhanced SREBP1 gene expression and promoted its proteolytic cleavage to the active form. Furthermore, 3 h of in vivo hyperinsulinemia also significantly increased SREBP1 gene expression in human skeletal muscle. Transcript levels of SREBP1c, the most abundant isoform in adipose tissue, were significantly decreased in the subcutaneous adipose tissue of obese normoglycemic and type 2 diabetic subjects compared with that of nonobese normoglycemic control subjects. In skeletal muscle, SREBP1 expression was significantly reduced in type 2 diabetic subjects but not in obese subjects. Within the diabetic group, the extent of SREBP1 suppression was inversely related to metabolic control and was normalized by 3 h of in vivo hyperinsulinemia. Exposure of isolated human adipocytes to tumor necrosis factor-α (TNF-α) produced a marked and specific decrease in the mRNA encoding the SREBP1c isoform and completely blocked the insulin-induced cleavage of SREBP1 protein. Thus, both the expression and proteolytic maturation of human SREBP1 are positively modulated by insulin. The specific reduction in the SREBP1c isoform seen in the adipose tissue of obese and type 2 diabetic subjects can be recapitulated ex vivo by TNF-α, suggesting a possible mechanism for this association. ***Diabetes* 51:1035–1041, 2002**

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ADD1, adipocyte determination differentiation factor 1; AH, Addenbrooke's Hospital; bHLH, basic helix loop helix leucine zipper; DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemiluminescence; ECU, East Carolina University; FBS, fetal bovine serum; IU, Indiana University; PPARγ, peroxisome proliferator–activated receptor-γ; SCAP, sterol regulatory element binding protein-cleavage–activating protein; SREBP; sterol regulatory element binding protein; UCSD, University of California San Diego.

Sterol regulatory elements binding proteins (SREBPs) (1–3) are a key transcription factor family that play an important role in adipogenesis, insulin sensitivity, and fatty acid homeostasis (4). SREBPs are basic helix loop helix leucine zipper (bHLH) transcription factors that control cholesterol and fatty acid metabolism in mammalian cells (5–7). The SREBP family is comprised of two members, SREBP1 and SREBP2. SREBP1 exists as two isoforms (SREBP1a and SREBP1c) derived from alternative splicing of the first exon of the same gene. SREBP1a and -1c are controlled independently by regulatory regions that appear to respond differentially to organ- and metabolic-specific factors. Rat adipocyte determination differentiation factor 1 gene (ADD1) was identified independently by Tontonoz et al. (8) and is homologous to the human SREBP1c isoform. SREBP1c/ADD1 is predominantly expressed in liver, adrenal gland, adipose tissue, and skeletal muscle, whereas SREBP1a is predominantly expressed in spleen (9). SREBP2 is derived from a separate gene, and no alternative forms have been described. The major role of SREBP2 is in the control of cholesterol biosynthesis (6). To activate transcription, the NH_2-terminal domains of SREBPs are cleaved and released from the endoplasmic reticulum before entering the nucleus. These processing events require specific proteases (10) and a regulatory protein designated SREBP-cleavage–activating protein (SCAP) (11).

In vitro, ADD1/SREBP1c enhances the transcriptional activity of peroxisome proliferator–activated receptor-γ (PPARγ), increasing the proportion of cells undergoing adipose differentiation. It has been suggested that ADD1/SREBP1c increases PPARγ activity either through the induction of enzymes responsible for the generation of its endogenous ligands (12) and/or through increasing the transcription of PPARγ1 itself (13). Furthermore, SREBP1 appears to mediate part of the transcriptional effects of insulin (12,14,15). In vitro studies have shown that ADD1/SREBP1c could function as an insulin response factor through binding E boxes (16). These data suggest that ADD1/SREBP1c expression could be modulated by insulin, eliciting its proadipogenic and insulin-sensitizing activ-

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TABLE 1
Study groups

Group	n	Sex (M/F)	Age (years)	BMI (kg/m^2)	Tissue
Study group A					
Lean	13	F	34 ± 3.7	22.2 ± 0.5	ScWAT
	4	M	27 ± 2.9	23.8 ± 1.1	ScWAT
Obese	18	F	37 ± 2.6	48.1 ± 3.1	ScWAT
	4	M	33 ± 4.8	59.0 ± 8.9	ScWAT
Diabetic subject	7	F	54 ± 3.4	51.5 ± 4.2	ScWAT
Study group B (mature adipocytes)					
Lean					
	4	F	44 ± 2.4	21.0 ± 1.9	Isolated adipocytes
	7	M	68 ± 4.4	20.6 ± 1.2	Isolated adipocytes
Obese					
	6	F	49 ± 4.8	32.0 ± 2.2	Isolated adipocytes
	1	M	55	27.4	Isolated adipocytes
Morbidly obese					
	6	F	38 ± 2.9	59.4 ± 6.1	Isolated adipocytes
	2	M	37 ± 1.5	50.5 ± 1.6	Isolated adipocytes

Group	n	Sex (M/F)	Age (years)	Race	BMI (kg/m^2)	Tissue
Study group C						
Lean	12	9F/3M	48 ± 5.1	11C/1B	23.8 ± 0.8	Rectus abdominus
Obese	15	13F/2M	47 ± 3.1	10C/5B	48.6 ± 2.3*	Rectus abdominus
Weight reduced	6	4F/2M	43 ± 2.9	5C/1B	37.2 ± 2.6*‡	Rectus abdominus

Group	n	Age (years)	BMI (kg/m^2)	Tissue	IRI	HbA_{1c}
Study group D						
Lean	5	40 ± 0.6	24.9 ± 2.4	Vastus lateralis	5.2 ± 2.2	5.0 ± 0.49
Obese	6	45 ± 2.8	34.7 ± 2.3	Vastus lateralis	12.1 ± 3.0	5.4 ± 0.3
Diabetic	6	49 ± 3.3	32.6 ± 1.8	Vastus lateralis	21.5 ± 4.5	9.9 ± 1.0

Data are means ± SD unless otherwise indicated. ScWAT, Subcutaneous white adipose tissue. Race: C, Caucasian; B, Black Americans. *$P < 0.05$, obese vs. lean; ‡$P < 0.05$, obese vs. weight reduced.

ity through PPARγ activation. In vivo overexpression of ADD1/SREBP1c in adipose tissue using transgenic technology paradoxically produces a general lipodystrophy syndrome (17) characterized by disordered adipose tissue, decreased expression of adipose cell differentiation markers, and severe insulin resistance.

Despite the potential relevance of SREBP1 in the pathogenesis of insulin resistance and diabetes, there is a paucity of data regarding expression of SREBP1 in human adipose tissue and skeletal muscle in normal and pathological states. We have studied the effects of insulin on SREBP1 gene expression and protein cleavage in human isolated adipocytes and skeletal muscle. We have also examined whether common states of insulin resistance are associated with altered expression of SREBP1/ADD1. Finally, we have investigated the potential mechanism involved in the dysregulation of SREBP1 observed in morbid obesity and diabetes.

RESEARCH DESIGN AND METHODS

Subjects. The characteristics of the populations studied are summarized in Table 1. Group A comprised 17 lean, 22 obese, and 7 type 2 diabetic patients from Indiana University (IU) who donated subcutaneous adipose tissue biopsies. Group B comprised 11 lean, 7 obese, and 8 morbidly obese (BMI >40 kg/m^2) patients undergoing elective open-abdominal surgery at Addenbrooke's Hospital (AH) who donated subcutaneous adipose tissue biopsies. Group C comprised 12 lean, 15 obese, and 6 weight-reduced formerly obese patients undergoing elective abdominal surgery at East Carolina University (ECU) who donated rectus abdominus muscle samples. Three subjects from the latter group were studied in both the obese and weight-reduced state. Weight reduction in the weight-reduced group was induced by gastric bypass surgery. Five of these patients had stable weight for at least 4 months before the second biopsy. Group D comprised subjects studied at the VA San Diego Healthcare System and University of California San Diego (UCSD). Muscle biopsy specimens were obtained from vastus lateralis of lean, obese, and type 2 diabetic subjects before and after a 3-h hyperinsulinemic (300 mU · m^{-2} · min^{-1}) euglycemic (5.0–5.5 mmol/l) clamp as described in detail previously (18). Protocols involving muscle biopsies were approved by ECU policy and the UCSD Review on Human Research. The Institutional Review Board at IU and the Cambridge Local Research Ethics Committee (at AH) approved protocols involving adipose biopsies. Informed consent was obtained from all patients. For all subjects, obesity was defined as BMI >27.3 kg/m^2 for men and BMI >27 kg/m^2 for women (19).

Reagents. All chemicals and reagents were purchased from Sigma (Dorset, U.K.) unless otherwise stated. Tumor necrosis factor-α (TNF-α) was purchased from R&D Systems Europe (Abingdon, U.K.), and the anti-SREBP1 antibody was obtained from Santa Cruz Biotechnology (Calne, U.K.). Secondary antibodies were from DAKO (Goldstrup, Denmark), and enhanced chemiluminescence (ECL) reagents and Hyperfilm were from Amersham Pharmacia Biotech (Little Chalfont, U.K.). RNeasy extraction kit was obtained from Qiagen (West Sussex, U.K.). Polyethyleneglycol methacrylate-T easy cloning vector and RT reagents were from Promega (Madison, WI), and Cyclophilin RPA internal control was from Ambion (Austin, TX).

RNA preparation. Muscle tissue biopsies at UCSD and abdominal subcutaneous adipose tissue biopsies at IU, ECU, and AH were obtained as previously described (20). Total RNA was obtained by guanidinium thiocyanate-phenol chloroform extraction (21), except samples for AH, which were extracted using the RNeasy mini extraction kit according to the manufacturer's recommendations. RNA samples were quantified by spectrophotometry, and integrity was assessed by agarose gel electrophoresis and ethidium bromide staining. The RNA samples were then diluted as appropriate in RNase-free water and stored at −80°C.

Adipocyte and preadipocyte isolation. Adipocyte isolation for group B was as follows. Adipose tissue biopsies were placed in normal saline and immediately processed (transport time to the laboratory was <5 min). The adipose tissue was finely diced and digested in a collagenase solution (Hank's

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balanced salt solution containing 3 mg/ml type II collagenase and 1.5% BSA) for 1 h in a shaking water bath at 37°C. After digestion, the mature adipocytes were separated from the stromo-vascular cells by centrifugation (10 min, 1,500*g*) of the digestion mixture. The mature adipocytes were removed from the top layer, and then RNA was immediately extracted or adipocytes were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 plus 10% fetal bovine serum (FBS) at 37°C/5% CO_2 in the absence or presence of 10 or 100 ng/ml TNF-α, 100 nmol/l insulin, or 10 ng/ml TNF-α plus 100 nmol/l insulin. After 24 h, total RNA or protein was extracted. Protein extraction was carried out using RIPA buffer (1% NP40, 1% sodium deoxycolate, 0.1% SDS, 0.9% NaCl, 250 mmol/l Tris HCl [pH 6.8], 1 mmol/l EDTA, and 1 × protease cocktail inhibitor; Boehringer Mannheim, Mannheim, Germany).

Preadipocyte isolation and culture. The stromo-vascular pellet containing the preadipocytes was treated with erythrocyte lysis solution (154 mmol/l NH_4Cl, 10 mmol/l $KHCO_3$, and 0.1 mmol/l EDTA) for 5 min at room temperature and centrifuged (5 min, 1,500*g)*. The preadipocyte pellet was cultured in DMEM/Ham's F12 supplemented with 10% FBS, 2 mmol/l glutamine, 100 units penicillin, and 0.1 mg/ml streptomycin at 37°C in a humidified 95% air and 5% CO_2 incubator. Cultures were passaged four times and grown to confluence (day 0). At confluence, the medium was changed to a serum-free hormonally modified differentiating medium (DMEM/Ham's F12 supplemented with 2 mmol/l glutamine, 100 units penicillin, 0.1 mg/ml streptomycin, 33 μmol/l Biotin, 17 μmol/l pantothenic acid, 10 μg/ml human apo-transferrin, 0.2 nmol/l tri-iodothyronine, 100 nmol/l cortisol, and 500 nmol/l insulin) with the addition of the following compounds: 10^{-7} mol/l BRL 49653 and 10^{-7} mol/l LG 100268. For the first 3 days only, 250 μmol/l iso-butylmethylxanthine was added to the differentiating medium. Medium was replaced every 2–3 days. After 15 days postdifferentiation, TNF-α was added to the medium (10 ng/ml). Control samples were maintained in medium without TNF-α after 24 h RNA was extracted.

Analysis of mRNA expression. Expression of mRNA was analyzed using two different methods, depending on RNA availability.

Solution-hybridization nuclease protection assay. The studies described were performed using a partial human ADD1/SREBP1 cDNA probe common to both isoforms. This probe was generated as previously described (22,23) by RT-PCR using total RNA from human adipose tissue using primers (5′TCT ACC ATA AGC TGC ACC AGC TG3′ and 5′CAG TCC CCA TCC ACG AAG AAA CG3′) designed to amplify 319 bp of the hSREBP1 sequence. A 103-bp cDNA corresponding to human cyclophilin (Ambion, Austin, TX) was used as internal control. SREBP1 and cyclophilin were quantitated using previously described solution hybridization RNase protection assay methods and phosphorimager analysis (22,23).

Real-time quantitative PCR. Real-time quantitative PCR was used to analyze the RNA samples from group B. This PCR-based method was developed to overcome the problem of limiting amounts of human RNA. Total RNA (100 ng) was reverse-transcribed for 1 h at 37°C in a 20-μl reaction containing 1× RT buffer (50 mmol/l Tris-HCl, 75 mmol/l KCl, 3 mmol/l $MgCl_2$, and 10 mmol/l dithiothreitol), 100 ng random hexamers, 1 mmol/l dNTPs, and 100 units Moloney murine leukemia virus RT (Promega). Reactions in which RNA was omitted served as negative controls. A reaction containing 500 ng of adipocyte total RNA was also included as a standard. Following first-strand cDNA synthesis, this standard was serially diluted 1:2 in DNase-free water to generate a standard curve for the PCR analysis.

Oligonucleotide primers and taqman probe for SREBP1a and -1c were designed using Primer Express, Version 1.0 (Applied Biosystems, Warrington, U.K.) and sequences from the Genebank database (accession nos. U00968 and S66167). For quantitation of SREBP1a and -1c isoforms, the same reverse primer and fluorogenic probe but different forward primers were used. The sequences were as follows: SREBP1c forward–5′ CCATGGATTGCACTTTCGAA 3′, SREBP1a forward–5′ TGCTGACCGACATCGAAGAC 3′, reverse–5′CCAGCAT-AGGG TGGGTCAAA 3′, and probe–5′ TATCAACA ACCAAGACAG TGACTTC-CCTGGC3′. The taqman probe was labeled at the 5′ end with the reporter dye FAM (6-carboxy-fluorescein) and at the 3′ end with the quencher TAMRA (6-carboxy-tetramethyl-rhodamine). Oligonucleotide primers and taqman probes for the glyceraldehyde-3-phosphate dehydrogenase and β-Actin internal controls were purchased from Perkin-Elmer. PCR was carried out in duplicate for each sample on an ABI 7,700-sequence detection system (PE Applied Biosystems). Each 25-μl reaction contained 2 μl first-strand cDNA, 1× PCR master mix, forward (900 or 300 nmol/l for SREBP1a/1c, respectively) and reverse (900 or 300 nmol/l for SREBP1a/1c, respectively) primers, and Taqman probe (125 or 50 nmol/l for SREBP1a/1c, respectively). All reactions were carried out using the following cycling parameters: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. After PCR, standard curves were constructed from the standard reactions for each target gene and internal control by plotting Ct values, i.e., the cycle number at which the fluorescence signal exceeds background, versus log cDNA input (ng). The Ct readings for each of the unknown samples were then used to calculate the amount of either target or internal control relative to the standard. Because the amplification efficiency of SREBP1a and -1c were equal, the 1c-to-1a ratio in each sample could be calculated using the formula $2^{-\Delta\Delta Ct}$ where $\Delta\Delta Ct$ = 1c Ct − 1a Ct. For each sample, results were normalized by dividing by the amount of target by the amount of internal control. Reproducibility of the PCR was examined by measuring the Ct readings of five samples in two different assays. Intra- and interassay coefficients of variation ranged from 0.19 to 4.2% and from 0.54 to 3.0%, respectively.

SDS-PAGE and immunoblotting. In brief, protein concentrations were measured using the Coomassie Plus Protein Assay reagent (Pierce, Rockford, IL). Equal amounts of total protein were resolved through an 8% SDS-polyacrylamide gel and transferred to Immobilon-P polyvinylidene fluoride membranes (Millipore , Bedford, MA). After a 2.5-h blocking time in blotto A (Tris-buffered saline, 0.05% Tween, and 5% milk), membranes were probed overnight with anti-SREBP1 antibodies detecting both forms of SREBP1, uncleaved and mature (cleaved) form. Identity of these bands was confirmed previously by analyzing nuclear and cytoplasmic fractions separately. The membrane was probed with an appropriate horseradish peroxidase–coupled secondary antibody. Antibody binding was visualized by ECL (Amersham Pharmacia Biotech, Little Chalfont, U.K.).

Statistical analysis. Total SREBP1 and cyclophilin were expressed as phosphoimager arbitrary units. Levels of ADD1/SREBP1c were expressed as arbitrary units of mRNA/cyclophilin. All results are presented as the means ± SE (unless specifically noted). Statistical significance was assessed by ANOVA and specific differences among groups using Bonferroni/Dunn post hoc test. Protein data were standardized to the amount seen in the control experiment. All analyses were performed with Statview statistical package. Comparisons between subjects were analyzed using nonpaired Wilcoxon's nonparametric test or Student's t test. The level of significance was set at $P < 0.05$.

RESULTS

Effects of insulin on SREBP1 expression ex vivo and in vivo. In isolated human adipocytes, SREBP1 mRNA levels increased by approximately threefold in response to 24 h of exposure to 100 nmol/l insulin (Fig. 1*A*). Under the same experimental conditions, there was a modest increase in the expression of full-length SREBP1 protein (130 ± 12%), whereas the abundance of the mature, cleaved form of SREBP1 was greatly increased (338 ± 51%, $P = 0.0015$; $n = 6$) (Fig. 1*B*). The in vivo effect of 3 h of hyperinsulinemia on SREBP1 mRNA expression in skeletal muscle (vastus lateralis) from healthy subjects was also examined. Hyperinsulinemia significantly increased levels of SREBP1 mRNA in skeletal muscle (1,031 ± 82 vs. 1,961 ± 197 AU, $P = 0.016$; $n = 4$ for previous postclamp) (Fig. 1*C*).

SREBP1/ADD1 mRNA expression in adipose tissue and isolated adipocytes of lean, obese, and diabetic patients. SREBP1 mRNA levels were measured by RNase protection assay in subcutaneous adipose tissue from lean, obese, and obese diabetic individuals (study group A). Adipose tissue expression of SREBP1 mRNA was decreased in the obese versus lean subjects (0.11 ± 0.02 vs. 0.2 ± 0.02 AU, $P < 0.0001$) (Fig. 2*A*) and further reduced in obese diabetic patients (0.07 ± 0.01 AU, $P < 0.0001$ vs. control subjects) (Fig. 2*B*).

Having established that obesity was associated with reduced expression of total SREBP1 in whole adipose tissue, we examined whether this effect of obesity was seen in isolated mature adipocytes. Real-time, quantitative PCR assays were developed to allow RNA measurement in small amounts of human tissue and to permit specific quantitation of mRNAs encoding the human SREBP1a and -1c splice variants.

Consistent with previous studies, SREBP1c (ADD1) was the predominant isoform expressed in human subcutaneous adiopocytes (ratio of SREBP1c to -1a 20.0 ± 7.3) and

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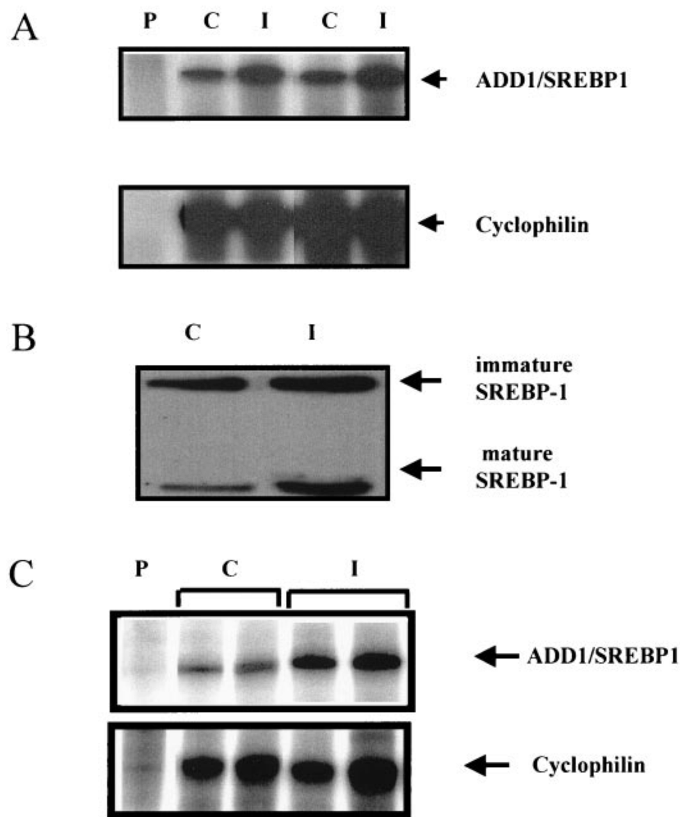

FIG. 1. Effect of insulin on human SREBP1 expression and cleavage. *A*: Effect of 24-h insulin treatment (100 nmol/l) on SREBP1 mRNA expression in isolated human subcutaneous adipocytes. Representative example of two subjects from study group B. *B*: Effect of insulin on SREBP1 protein in human isolated adipocytes. Western blot analysis. Adipocyte cell lysates from mature subcutaneous adipocytes cultured for 24 h in the presence or absence of 100 nmol/l insulin for 24 h. Lysates were obtained from patients in study group B. *C*: RNase protection analysis comparing SREBP1 mRNA in vastus lateralis of lean subjects before and after a 3-h hyperinsulinemic clamp. Samples were obtained from patients in study group D. C, control; I, insulin; P, probe.

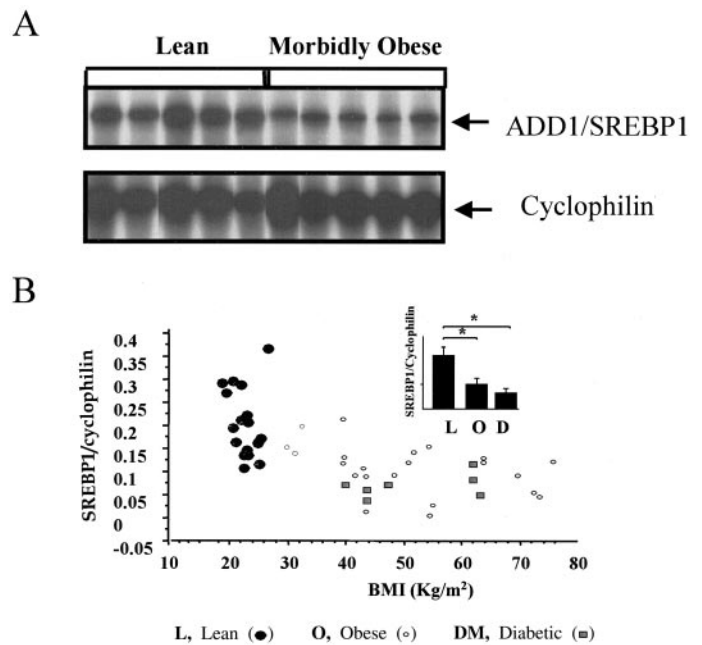

FIG. 2. SREBP1 mRNA expression in adipose tissue of lean, obese, and diabetic patients. *A*: Representative RNase protection analysis comparing SREBP1 mRNA (319 bp) expression in subcutaneous adipose tissue of lean and morbidly obese (BMI >40 kg/m^2) (group A) subjects. Cyclophilin (103 bp) mRNA was used as an internal control. *B*: Relation between adipose tissue SREBP1 mRNA levels and BMI in lean (n = 17), obese (n = 22), and type 2 diabetic (n = 7) subjects. A highly significant (*P < 0.0001) reduction in SREBP1 levels was seen in both the obese and diabetic groups compared with the lean control subjects (inset).

skeletal muscle tissue (ratio of SREBP1c to -1a 10.3 ± 2.7). RNA from patient-group B (see above) was used to examine the specific expression of the two SREBP1 isoforms in isolated human adipocytes (Fig. 3). An inverse correlation between SREBP1c and BMI in both depots ($r = -0.63$, $P = 0.0008$) was observed. SREBP1a was expressed at much lower level than SREBP1c in isolated adipocytes, and its expression was not significantly correlated with BMI.

SREBP1 mRNA expression in skeletal muscle in lean, obese, and type 2 diabetic subjects. In contrast to adipose tissue, SREBP1 mRNA levels were similar in biopsy samples from rectus abdominus of lean (0.23 ± 0.02 AU, n = 12), obese (0.24 ± 0.02 AU, n = 15), and stable weight-reduced patients (0.23 ± 0.02 AU, n = 6) (study group C). Similarly, in an independent set of patients (study group D), SREBP1 mRNA expression levels were similar in vastus lateralis of lean (0.24 ± 0.02 AU, n = 5) and obese individuals (0.22 ± 0.03 AU, n = 6) (Fig. 4)

In contrast to the findings in obese subjects, SREBP1 mRNA levels in skeletal muscle (vastus lateralis) of diabetic patients (n = 6) were significantly reduced compared with those of control subjects (0.17 ± 0.04 vs. 0.24 ± 0.02, $P < 0.05$) (Fig. 4*A* and *B*). Notably, the three patients with poor metabolic control, as indicated by markedly elevated HbA_{1c} levels (>10%), had the greatest reduction is SREBP1, and a negative correlation between SREBP1 and HbA_{1c} levels (−0.339, $P = 0.036$) was established (Fig. 4*C*). Interestingly, exposure of diabetic subjects to 3 h of supraphysiological hyperinsulinemia restored the expression of SREBP1 mRNA to levels similar to control subjects (Fig. 4*A* and *B*). Although a similar trend was observed in obese patients, this difference did not reach statistical significance.

Effects of TNF-α on SREBP1 expression and cleavage in human adipocytes. As SREBP1 expression was decreased in adipose tissue of morbidly obese and diabetic patients, we examined whether TNF-α, a cytokine whose production is increased in the adipose tissue of obese patients and which has been implicated in the induction of obesity-associated insulin resistance, could affect SREBP1 expression. Isolated mature subcutaneous adipocytes were cultured in the absence or presence of 10 or 100 ng/ml TNF-α for 24 h, and SREBP1 isoform expression was measured by real-time RT-PCR. SREBP1c mRNA expression was reduced by 63.7 ± 18.8 and 47.2 ± 11.1% by treatment with 10 or 100 ng/ml TNF-α, respectively ($P < 0.01$) (Fig. 5*A*). In contrast, TNF-α had no effect on SREBP1a mRNA expression (Fig. 5*B*). The effect of TNF-α on SREBP1a and -1c was also determined in human preadipocytes that had been differentiated in vitro. As in mature adipocytes, TNF-α decreased SREBP1c mRNA expression by 83.1 ± 6.4% ($P = 0.03$) but had no effect on SREBP1a mRNA expression.

In contrast to its effect on SREBP1c mRNA, 24 h

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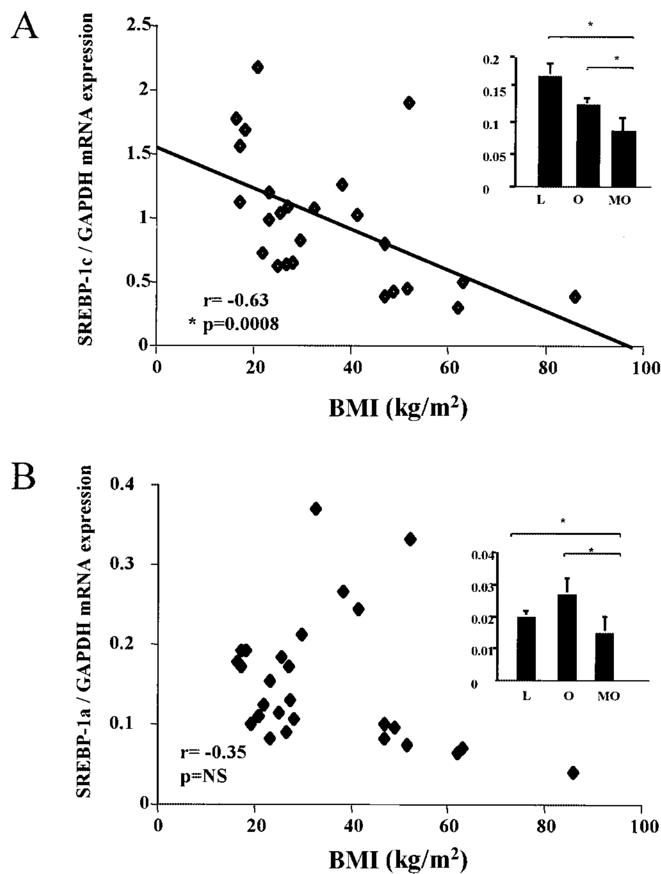

FIG. 3. Effect of obesity on SREBP1a/c mRNA expression in isolated adipocytes. SREBP1c (*A*) and SREBP1a (*B*) mRNA levels were measured using real-time quantitative RT-PCR in freshly isolated subcutaneous adipocytes from lean (L) (n = 11), obese (O) (n = 7), and morbidly obese (MO) (n = 8) subjects (*P < 0.0001). Results expressed as correlation between SREBP1a and -1c isoforms and BMI.

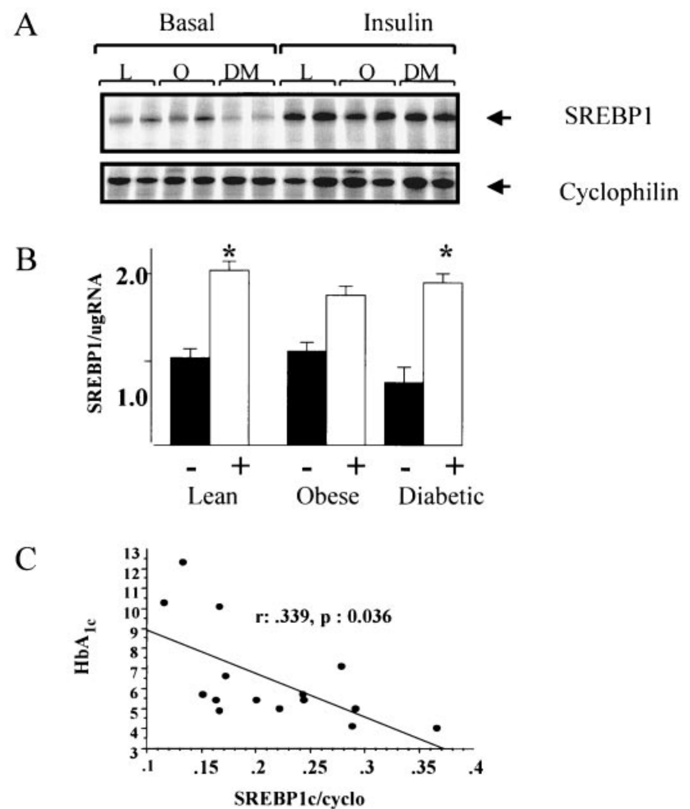

FIG. 4. Effects of insulin and/or diabetes on human skeletal muscle SREBP1 mRNA. SREBP1 mRNA levels were measured by RNase protection analysis in skeletal muscle (vastus lateralis) biopsies from lean, obese, and diabetic patients before and after a 3-h hyperinsulinemic clamp (study group D). *A*: Representative RNase protection assay demonstrating two diabetic subjects with low basal levels of SREBP1 and restoration of levels to normal by 3 h of hyperinsulinemia. L, lean; O, obese; DM, diabetic patients. *B*: SREBP1 mRNA levels (means ± SE) in skeletal muscle (vastus lateralis) biopsies taken before and after hyperinsulinemic clamp in lean (n = 4), obese (n = 5), and diabetic patients (n = 4). Insulin significantly increased SREBP1 expression in lean (P = 0.016) and diabetic subjects (P = 0.005). *C*: Correlation between SREBP1c/cyclophilin and HbA_{1c}.

exposure of isolated adipocytes to TNF-α treatment had no significant effects on the expression levels of either the full-length or cleaved form of SREBP1 protein (Fig. 5*C*). However, TNF-α completely blocked the ability of insulin to promote cleavage of SREBP1 protein to the mature form (Fig. 5*D*).

DISCUSSION

SREBP1 is a transcription factor, highly expressed in metabolically active tissues, with important functions in the regulation of processes such as lipid metabolism, adipogenesis, and insulin action. Despite the growing appreciation of its importance in metabolic regulation, little information is available regarding the regulation of SREBP1 expression in human tissues, either in normal or insulin-resistant states. In the studies of human adipose tissue and skeletal muscle reported here, we have *1*) established that insulin positively modulates human SREBP1 expression and proteolytic cleavage; *2*) demonstrated that altered SREBP1 isoform expression is found in human insulin-resistant states, such as obesity and type 2 diabetes; and *3*) shown that TNF-α blocks the effects of insulin on SREBP1 in human adipocytes and recapitulates, at least in part, the altered SREBP1 isoform expression seen in human metabolic disease states.

The ability of insulin to increase SREBP1 mRNA expression in the liver has been repeatedly demonstrated in rodents (16,24). In isolated rat hepatocytes, insulin and glucose together have been shown to induce the expression of SREBP1c (15). Our studies provide new evidence that insulin also induces SREBP1 gene expression in human isolated adipocytes ex vivo and in skeletal muscle in vivo. Furthermore, we have demonstrated that incubation of isolated adipocytes with insulin increases the amount of cleaved, transcriptionally active SREBP1 protein.

Having established the effect of insulin on human SREBP1 gene expression, we explored whether common insulin-resistant states such as obesity and type 2 diabetes were associated with impaired expression of SREBP1. Our data show that both obese normoglycemic and obese type 2 diabetic patients have decreased expression of SREBP1 mRNA in subcutaneous adipose tissue. Using a PCR-based method, we showed that SREBP1c is the most abundant isoform in subcutaneous human adipocytes and that this isoform is markedly reduced in isolated adipocytes from obese patients. Indeed, we observed that SREBP1c is negatively correlated with BMI. In contrast, SREBP1a mRNA was also detectable in human adipocytes, but it was expressed at much lower levels, and its expression

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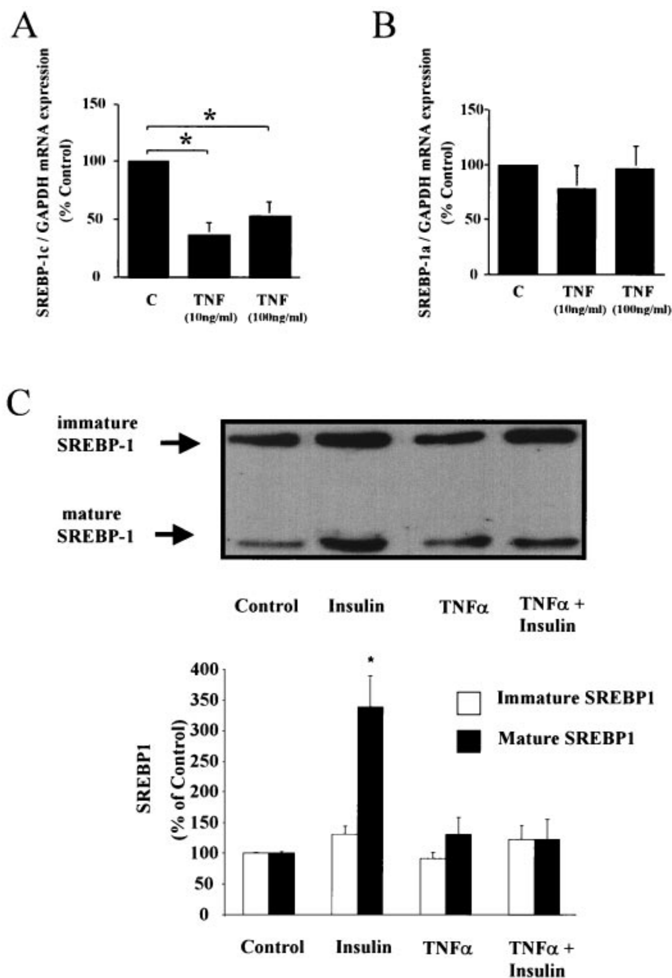

FIG. 5. Effect of TNF-α on SREBP1 mRNA and protein levels. Isolated human subcutaneous adipocytes from six independent subjects were incubated for 24 h with either TNF-α or vehicle, after which cells were lysed and SREBP1c (*A*) and SREBP1a (*B*) mRNA levels were measured by real-time quantitative RT-PCR. Results are expressed as percentage of the levels seen in vehicle-treated control cells (P* = 0.004). *C*: Subcutaneous human adipocytes were isolated, cultured for 24 h, and then treated for 24 h with 100 nmol/l insulin (*n* = 6), 10 ng/ml TNF-α (*n* = 6), or 100 nmol/l insulin plus 10 ng/ml TNF-α or vehicle (*n* = 4, **P* = 0.0015). Cell lysates were immunoblotted with anti-SREBP1 antibody and visualized by ECL. Results are expressed as percentages of the values obtained in the vehicle-treated cells.**

level was not correlated with BMI. Altogether, these data indicate that in states of insulin resistance or deficiency, there is a specific decrease in the expression of SREBP1c, suggesting a specific role of this isoform in the mediation of the effect of insulin on lipid metabolism. The mechanism whereby insulin promotes SREBP1c expression is unclear, although recent evidence suggests that, at least in hepatocytes, increased glucose flux in response to insulin may also have a role in SREBP1 regulation (26).

Consistent with our findings in adipose tissue, SREBP1 mRNA levels in skeletal muscle of type 2 diabetic patients were also reduced, particularly in those with markedly impaired metabolic control. Interestingly, 3 h of hyperinsulinemia was able to restore SREBP1 expression to levels comparable with those of nondiabetic individuals. In contrast to the subjects with type 2 diabetes, SREBP1 levels in skeletal muscle of obese subjects was not reduced. As insulin resistance is generally more severe in type 2 diabetes than obesity, it is possible that there may be quantitative differences in the sensitivity of particular tissues to the effects of insulin resistance on SREBP1 expression. Alternatively, the regulation of SREBP1 by insulin may be fundamentally different in different tissues.

In this regard, recent studies have shown that SREBP1c expression in the liver of the highly insulin-resistant *ob/ob* mouse is not altered, despite the presence of profound resistance of other processes to insulin in the same tissue (27). Furthermore, we have recently observed opposite regulation of SREBP1c mRNA in adipose and hepatic tissues of *ob/ob* mice in response to insulin sensitizing drugs (H. Roche and A.J.V.-P., unpublished observations). By preventing lipid accumulation in adipose tissue, the selective downregulation of adipocyte SREBP1 in obesity might promote the partitioning of free fatty acids toward other tissues, such as skeletal muscle, pancreas, or liver, where through 'lipotoxicity' they may impair insulin action and/or secretion (28,29). While this manuscript was in preparation, Ducluzeau et al. (25) reported that SREBP1c gene expression was decreased in adipose tissue of obese and type 2 diabetic patients. Similarly, this group showed that SREBP1c was not altered in skeletal muscle of obese individuals (5) and only slightly decreased in diabetic patients. Also, Ducluzeau et al. (25) reported insulin-induced SREBP1c expression in adipose tissue and skeletal muscle of lean and obese individuals but not in diabetic patients. Our study supports these results, although we also observed restoration of SREBP1 expression in skeletal muscle of diabetic patients in response to insulin. The reason for this difference is unclear but may be related to the different degree of previous metabolic control of these patients.

TNF-α production by adipocytes increases in obese states and, through a paracrine and autocrine effects, has been implicated in the causation of obesity-associated insulin resistance (30,31). We therefore examined whether TNF-α might be capable of mediating the alterations in SREBP1 isoform expression seen in adipose tissue of obese human subjects. Incubation of human isolated adipocytes and preadipocytes differentiated in vitro with TNF-α markedly decreased the expression of SREBP1c but not SREBP1a, a pattern that recapitulates the alterations in SREBP1 isoform expression seen in vivo in obese and type 2 diabetic individuals. TNF-α also completely blocked the effects of insulin to increase SREBP1 protein in human adipocytes and inhibited insulin's effect on cleavage of the protein to its active form. TNF-α is thought to induce insulin resistance through the production of serine phosphorylated signaling molecules that act as inhibitors of the insulin receptor tyrosine kinase (32,33). Thus, it is possible that all of the effects of TNF-α on SREBP1 expression and cleavage are mediated through its inhibitory effects on insulin signaling. In other systems, however, TNF-α has been reported to have effects on SREBPs independent of insulin signaling. For example, Lawler et al. (34) reported that SREBP cleavage occurs as part of the TNF-α–induced apoptotic program in hepatocytes. Thus, the "discrepancies" between RNA and protein data in vitro are intriguing and suggest that the regulation of SREBP1 by TNF-α may involve several pathways. However, TNF-α–specific effects on SREBP1c mRNA expression and inhibition of the insulin-induced SREBP1

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cleavage indicates that TNF-α interferes with insulin regulation of SREBP1.

In summary, we have shown for the first time that insulin induces SREBP1 gene expression in isolated human adipocytes and skeletal muscle and also promotes SREBP1 cleavage in human isolated adipocytes. We have also provided evidence that common insulin-resistant states, such as obesity and type 2 diabetes, are characterized by decreased expression of SREBP1c mRNA and indicated a potential mechanism whereby TNF-α could contribute to the dysregulation of SREBP1. As SREBP1c/ADD1 appears to play such a key role in the coordination of fuel metabolism, we suggest that the decreased expression of this molecule in obese and diabetic human subjects could play an important role in the induction and/or maintenance of the insulin resistance seen in these disorders.

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