Regulation of the Fibrosis and Angiogenesis Promoter SPARC/Osteonectin in Human Adipose Tissue by Weight Change, Leptin, Insulin, and Glucose

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OBJECTIVE—Matricellular Secreted Protein, Acidic and Rich in Cysteine (SPARC), originally discovered in bone as osteonectin, is a mediator of collagen deposition and promotes fibrosis. Adipose tissue collagen has recently been found to be linked with metabolic dysregulation. Therefore, we tested the hypothesis that SPARC in human adipose tissue is influenced by glucose metabolism and adipokines.

RESEARCH DESIGN AND METHODS—Serum and adipose tissue biopsies were obtained from morbidly obese nondiabetic subjects undergoing bariatric surgery and lean control subjects for analysis of metabolic markers, SPARC, and various cytokines (RT-PCR). Additionally, 24 obese subjects underwent a very-low-calorie diet of 1,883 kJ (450 kcal)/day for 16 weeks and serial subcutaneous-abdominal-adipose tissue (SCAT) biopsies (weight loss: 28 ± 3.7 kg). Another six lean subjects underwent fast-food–based hyperalimentation for 4 weeks (weight gain: 7.2 ± 1.6 kg). Finally, visceral adipose tissue explants were cultured with recombinant leptin, insulin, and glucose, and SPARC mRNA and protein expression determined by Western blot analyses.

RESULTS—SPARC expression in human adipose tissue correlated with fat mass and was higher in SCAT. Weight loss induced by very-low-calorie diet lowered SPARC expression by 33% and increased by 30% in adipose tissue of subjects gaining weight after a fast-food diet. SPARC expression was correlated with leptin independent of fat mass and correlated with homeostasis model assessment–insulin resistance. In vitro experiments showed that leptin and insulin potently increased SPARC production dose dependently in visceral adipose tissue explants, while glucose decreased SPARC protein.

CONCLUSIONS—Our data suggest that SPARC expression is predominant in subcutaneous fat and its expression and secretion in adipose tissue are influenced by fat mass, leptin, insulin, and glucose. The profibrotic effects of SPARC may contribute to metabolic dysregulation in obesity. ***Diabetes*** **58:1780–1788, 2009**

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Secreted Protein, Acidic and Rich in Cysteine (SPARC), a 34-kDa matricellular glycoprotein, is also known as osteonectin and BM-40. It was initially found to be secreted from bone (1), but SPARC is expressed in most tissues and was the first extracellular matrix protein described in adipose tissue (2,3).

SPARC is a multifunctional protein: it is involved in osteogenesis, angiogenesis, wound healing, tumorigenesis, and the pathogenesis of fibrosis involving the kidney (4,5) and liver (6). SPARC also contributes to collagen fibril formation in the dermis, and SPARC knockout mice have reduced collagen content in the dermis (7). More recent evidence suggests that fibrosis in adipose tissue impairs metabolic function and reduces the capacity of fat expansion (3). SPARC is secreted from human adipose tissue and is predominantly derived from adipocytes, where it has a role in adipocyte differentiation, adipogenesis, and adipose tissue hyperplasia (2,8); however, it is unknown whether SPARC contributes to the pathogenesis of insulin resistance or the metabolic syndrome. The aim of this study was therefore to study *1*) the depot-specific expression of SPARC, *2*) the association of SPARC with markers of insulin resistance, *3*) the association of SPARC adipose tissue expression with the adipokines leptin and adiponectin, and *4*) the effect of weight loss and weight gain on adipose tissue SPARC expression.

We determined the adipose tissue depot expression in visceral fat (VAT) and subcutaneous abdominal adipose tissue (SCAT) in lean and morbidly obese subjects in association with adipokine levels and markers of insulin resistance and explored the gene expression of SPARC in a longitudinal intervention study involving obese subjects treated with a very-low-calorie diet (VLCD) to induce weight loss. Cell culture studies confirmed the regulation of SPARC by insulin, glucose, and leptin.

RESEARCH DESIGN AND METHODS

Subjects for adipose tissue depot study. Volunteers were recruited from adults undergoing bariatric surgery (obese group) and routine abdominal surgery (lean group) at the University Hospital Aintree, Liverpool, after ethical permission from the local research ethics committee. Subjects with diabetes, inflammatory disorders, or infectious or malignant diseases and subjects on drug treatments that would likely affect body weight or any study variables, including endocrine diseases and treatments with systemic glucocorticoids, were excluded. Lean and obese participants were of similar age and had the following patient characteristics: obese group—13 men and 26 women, age 44.5 ± 1.4 years (mean ± SD), BMI 46.8 ± 1.9 kg/m^2, n = 39; lean group—10 men and 8 women, age 42.3 ± 4.1 years, BMI 23.6 ± 0.8 kg/m^2, n = 18. Volunteers attended the clinical investigation unit after an overnight fast from

TABLE 1
Subject characteristics from Aintree study population

	Lean	Obese	Significance P value
Sex (male/female)	10/8	13/26	NS
Age (years)	42.3 ± 17.3	44.6 ± 9	NS
BMI (kg/m^2)	23.6 ± 2.1	46.9 ± 11.7	<0.001
Waist (cm)	88.6 ± 12	130 ± 28	<0.001
Systolic blood pressure (mmHg)	129 ± 13	143 ± 19	<0.01
Diastolic blood pressure (mmHg)	76 ± 9	88 ± 14	<0.01
HOMA-IR	1.8 ± 0.3	4.3 ± 0.6	<0.001
C-reactive protein (ng/ml)	1.55 ± 1	11.9 ± 5.7	<0.001

Characteristics of lean and obese subjects recruited for biopsies of SCAT and VAT. Subjects differ in metabolic variables including blood pressure and HOMA-IR. $n = 56$.

midnight for a physical examination and venesection before admission for surgery. Blood pressure, weight, and height were recorded by the same observer, and BMI was calculated. Waist circumferences were recorded by measurement at the midpoint between iliac crest and lowest point of the costal margin. Body composition was estimated by electrical bio-impedance measurement using a four-pole system (Bodystat, Isle of Man). Patient characteristics are shown in Table 1.

VLCD study. Subjects for the VLCD study that aims to identify gene expression changes in adipose tissue of obese subjects undergoing weight loss from caloric restriction were recruited after approval by the ethics review board at University of Gothenburg, Göteborg Sweden. As previously described (9), a total of 40 obese (BMI >30) men and women age 25–61 years participated in the study. Smokers and those with pharmacological treatment of diabetes or lipid-lowering medication were excluded. Of the 40 subjects recruited, 21 met the criteria for metabolic syndrome based on the modified World Health Organization criteria (10): type 2 diabetes or impaired glucose tolerance as measured by oral glucose tolerance test, lipid disturbance [triglycerides >1.7 mmol/l or HDL <0.9 mmol/l in men and HDL <1.0 mmol/l in women], and hypertension (blood pressure >140/90 mmHg). All subjects were treated with three VLCD meals daily from Cambridge Manufacturing (Northants, U.K.); the daily energy intake was 1,883 kJ (450 kcal) for 16 weeks followed by a 2-week period when regular food was gradually reintroduced. Study assessments were performed at the start of VLCD treatment (week 0), twice during the VLCD phase (weeks 8 and 16), and 2 weeks after the end of VLCD treatment (week 18). Anthropometrical measurements, blood pressure recording, blood sampling, oral glucose tolerance test, and a SCAT biopsy were performed at each of the four time points. Computed tomography performed at weeks 0 and 16 was used for adipose tissue area calculations. SCAT biopsy samples were obtained with a syringe with manually applied vacuum under local anesthesia and immediately frozen in liquid nitrogen and stored at −80°C until analysis. Microarray analysis of SCAT biopsies was performed on 24 patients of which 12 subjects (three women and nine men) were obese but healthy and 12 subjects (three women and nine men) were dysmetabolic according to the above criteria. Patient characteristics are shown in Table 2. Biochemical and anthropometrical measurements and examinations were performed as described (9). Computed tomography was used to determine body composition as previously described (11).

Hyperalimenation study. The fast-food study included a total of 18 lean subjects who accepted a fast-food diet–induced increase in body weight of 5–15% during a period of 4 weeks as described previously (12). The goal for each individual was to double his or her calorie intake by a diet rich in protein and saturated animal fat combined with a sedentary lifestyle. The participants were free from current diseases as judged by medical checkup and history. Subcutaneous abdominal fat biopsies from six of the subjects were collected at baseline and after 4 weeks (four men, two women; age 24.1 ± 3 years; BMI 21.4 ± 2.5 kg/m^2) for microarray analysis. Adipocytes were isolated from the adipose tissue samples by collagenase digestion (collagenase type 1, Worthington, NJ) as described previously (13). Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA) and then further purified with RNeasy mini columns (Qiagen, Hilden, Germany). High-quality RNA was confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Labeled cRNA from reverse transcription of total RNA was fragmented and hybridized to HG-U133 Plus 2.0 arrays according to manufacturers' instructions (Affymetrix, Santa Clara, CA). Fat mass was assessed by dual-energy X-ray absorptiometry at baseline and after 4 weeks. Patient characteristics are shown in Table 3.

Characteristics of fat sample donors for in vitro studies. VAT biopsies were obtained (0800–1000 h) from six women undergoing elective abdominal surgery [age (mean ± SD): 27.5 ± 7 years, BMI: 23.8 ± 2.8 kg/m^2] for adipose tissue explant studies. Exclusion criteria included known cardiovascular disease, thyroid disease, neoplasms, current smoking, diabetes, hypertension (blood pressure >140/90 mmHg), and renal impairment (serum creatinine >120 μmol/l). None of the subjects were on any medications for at least 6 months before the study. The local research ethics committee approved the study, and all patients involved gave their informed consent, in accordance with the guidelines in the Declaration of Helsinki 2000.

Serum analysis. The subjects recruited at Aintree Hospital for the adipose tissue depot study had blood taken for analysis of adipokines, glucose (glucose oxidase method, Yellow Springs), insulin (insulin ELISA, BioSource Europe S.A.; sensitivity 0.15 μIU/ml, intra-assay coefficients of variation 2.5–5.3%; human specific and with 0% crossreactivity with proinsulin), and high-sensitivity C-reactive protein (hsCRP, ELISA ImmunDiagnostik AG, Bensheim, Germany; sensitivity, 0.124 ng/ml, intra-assay coefficients of variation 5.0–6.0%). The computer-solved homeostasis model assessment method (14) was used to determine insulin sensitivity and β-cell function. Serum levels

TABLE 2
VLCD study patient characteristics

	Baseline	8 weeks	16 weeks	18 weeks
Weight (kg)	119 ± 20	101 ± 17	91 ± 16	91 ± 16***
BMI (kg/m^2)	37.6 ± 4.9	31.8 ± 4.1	28.6 ± 4.1	28.9 ± 3.9***
Waist (cm)	123 ± 12	110 ± 12	101 ± 13	101 ± 13***
Waist-to-hip ratio	1.0 ± 0.08	0.99 ± 0.08	0.95 ± 0.08	0.95 ± 0.08***
Fasting glucose (mmol/l)	6.0 ± 1.6	4.5 ± 0.7	4.5 ± 0.7	5.0 ± 1.0***
OGTT 2-h glucose (mmol/l)	8.2 ± 3.8	7.0 ± 1.9	7.0 ± 2.6	5.9 ± 2.3**
Fasting insulin (mU/l)	16 ± 7.4	7.0 ± 4.1	4.3 ± 2.2	6.3 ± 3.7***
HOMA-IR	4.4 ± 2.7	1.4 ± 0.9	0.9 ± 0.5	1.5 ± 1.3***
Total abdominal fat area (cm^2)	778 ± 191	—	416 ± 171**	—
SCAT area (cm^2)	526 ± 166	—	308 ± 135**	—
VAT area (cm^2)	241 ± 76	—	101 ± 48**	—
Systolic blood pressure (mmHg)	138 ± 17	121 ± 12	117 ± 14	124 ± 16***
Total cholesterol	5.7 ± 1.1	3.9 ± 0.9	4.4 ± 0.8	4.9 ± 0.8***
Serum leptin	38.4 ± 19	9.8 ± 7.5	6.3 ± 5.9	8.8 ± 6.7***
Serum adiponectin	9.0 ± 5.3	11.5 ± 7.3	13.6 ± 6.4	15.2 ± 7.9***
C-reactive protein (mg/l)	5.3 ± 5.8	4.6 ± 5.4	2.4 ± 1.5	2.4 ± 2.2*
SPARC (signal units)	1,857 ± 66	1,186 ± 82***	1,087 ± 80***	1,217 ± 82***

Data are means ± SD. A computed tomography was performed at weeks 0 and 16 for adipose tissue area calculations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared with baseline week 0 with week 16. $n = 24$. OGTT, oral glucose tolerance test.

TABLE 3
Hyperalimentation study

	Baseline	Week 4	Significance P value
Sex (male/female)	4/2	—	—
Age (years)	24 ± 3	—	—
Weight (kg)	64.2 ± 9.3	71.5 ± 13.0	<0.01
BMI (kg/m^2)	21.4 ± 2.5	23.7 ± 3.3	<0.01
Fat mass (kg)	11.6 ± 6.6	15.8 ± 5.3	<0.01
Systolic blood pressure (mmHg)	115 ± 8	125 ± 14	NS
Fasting glucose (mmol/l)	5.15 ± 0.72	5.80 ± 0.77	NS
Fasting Insulin (mU/l)	4.2 ± 2.7	8.8 ± 2.9	<0.05
HOMA-IR	0.7 ± 0.5	1.63 ± 0.50	<0.05
C-reactive protein (ng/ml)	0.28	0.61	NS
Serum leptin (ng/ml)	6.4 ± 10.9	14.8 ± 17.7	<0.05
SPARC (signal units)	3.5 ± 0.8	6.2 ± 0.4	<0.05

Patient characteristics of the participants in the fast-food study, their metabolic profile, and changes in serum leptin and subcutaneous abdominal adipose tissue expression of SPARC in signal units; statistical comparison is performed with use of the paired Student's t test. $n = 6$.

were measured for adiponectin with the Quantikine ELISA (R&D Systems, Abingdon, Oxon, U.K.) that has a sensitivity of 0.25 ng/ml and intra-assay coefficients of variation of 2.5–4.7%. Serum leptin was measured by ELISA from DRG Diagnostics (Marburg, Germany); sensitivity 1.0 ng/ml, intra-assay coefficients of variation 3.5–5.0%. All ELISA-based measurements were performed according to manufacturers' instructions.

Adipose tissue processing: fat depot study. Adipose tissue was obtained during surgery from subcutaneous and visceral compartments from each patient (paired samples). Tissue was collected immediately and frozen at −70° for further analysis. Total RNA was isolated from frozen adipose tissue samples with the RNeasy lipid tissue kit (Qiagen, Crawley, U.K.). The isolated total RNA was quantified by measurement of absorbency at 260 and 280 nm, and its integrity was verified using agarose gels (1%) stained with ethidium bromide. Reverse transcription to first-strand cDNA was carried out using the ImProm-II Reverse Transcription System (Promega, Madison, WI), according to the manufacturer's instructions. Real-time quantification of adipokines and SPARC mRNA was performed using QuantiTect Multiplex PCR kits (Qiagen), with commercially available prevalidated and assay-on demand primers and Quantiprobes, on a ROTORGENE 2000 analyzer (Corbett Research, Cambridge, U.K.) and compared with the housekeeping gene β-actin. SPARC primers were forward 5′ATTAGGCTGTTGGTTCAAA 3′ and reverse 5′ AGCC CTGGTTCTCCAAAA 3′. The use of a duplex configuration allowed simultaneous amplification of the adipokine gene of interest with the β-actin housekeeping gene. In brief, 1 μl cDNA (50 μg) was added to a mixture of 2× QuantiTec Multiplex PCR Master Mix, 20× Primer Mix for target gene, 20× Quantiprobe for target gene, 10× Assay mix for β-actin gene, and RNAase-free water to a final volume of 25 μl, in different 100-μl PCR tubes and in duplicates. Thermocycling conditions were: 15 min initial activation step followed by 50 cycles of 45 s of denaturation at 94°C, 45 s of annealing at 56°C, and 45 s of extension at 76°C. Data were obtained as threshold cycle (computed tomography) values, which is defined as the fractional cycle number at which the fluorescence reached 10 times the standard deviation of the baseline. The increase in fluorescence was measured in real time and analyzed using the Rotorgene Application Software v6.0 (Build 38). The relative gene expression of adipokines was calculated according to the method of Pfaffl as a relation to β-actin that takes into account the differing amplification efficiencies of each gene during the PCR (15).

RNA preparation and DNA microarray analysis (VLCD study). Total RNA from human tissues was prepared with Qiagen Lipid tissue kit (Qiagen). Total RNA from adipocytes was prepared with the phenol-chloroform extraction method of Chomczynski and Sacchi (16). After further purification with RNeasy (Qiagen), the RNA concentration was measured spectrophotometrically. The A260/A280 ratio was 1.8–2.0 and the quality of the RNA was verified by agarose gel electrophoresis before reverse transcription into cDNA. Preparation of cRNA and hybridization in the different studies was performed according to standard Affymetrix protocols as previously described (9).

Gene expression was measured using the Human Genome U133A DNA microarray (Affymetrix), composed of 22,283 probe sets representing almost 14,000 expressed genes. Arrays were scanned with a confocal laser scanner (GeneArray scanner G2500A Hewlett Packard, Palo Alto, CA). The Affymetrix probesets 200665_at and for control 212667_at were used for SPARC analyses, and the data from probeset 200665_at were reported.

Tissue distribution analysis. SPARC gene expression in different human tissues was assessed using DNA microarrays. Duplicate GeneChip HG U133A expression profiles from 17 different tissues were downloaded from the SymAtlas dataset (http://symatlas.gnf. org/SymAtlas/), as previously described (17). In addition, our own expression profiles, originating from small and large adipocytes, were included and normalized, as previously described (17).

Primary explant culture. Surgical adipose tissue samples were placed into sterile containers containing Medium 19 (Sigma Aldrich, Gillingham, U.K.) for primary adipose tissue culture following a protocol that was a modification of the method described by Fried and Moustaid-Moussa (18). Briefly, 1–3 g of adipose tissue was minced into 5–10 mg (~1 mm^3) fragments, washed with a 230 μm mesh (Filter no. 60; Sigma Aldrich, Gillingham, U.K.) and rinsed with sterile PBS warmed to 37°C. Samples were then transferred to six-well plates (~50 mg/well) containing 3 ml of Media 199 (Invitrogen, Paisley, U.K.) supplemented with 50 μg/ml gentamicin and 1% FCS (containing insulin at a concentration of 10^{-14} M) and cultured for 24 h with or without the addition of insulin or D-glucose in a 37°C incubator under an atmosphere of 5% CO_2/95% air.

Western blotting. Protein lysates were prepared by homogenizing adipose tissue in radioimmunoprecipitation lysis buffer (Upstate, Lake Placid, NY) according to manufacturer's instructions. Protein samples (40 μg/lane) containing SDS sample buffer (5 mol/l urea, 0.17 mol/l SDS, 0.4 mol/l dithiothreitol, and 50 mmol/l Tris-HCl, pH 8.0) were subjected to SDS-PAGE (10% resolving gel) and transferred to polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were incubated with primary rabbit–anti-human antibody for SPARC (Abcam, Cambridge, U.K.) [1:500 dilution] or primary rabbit–anti-human antibody for β-actin (Cell Signaling Technology, Beverly, MA) [1:1,000 dilution] overnight at 4°C. The membranes were washed thoroughly for 60 min with TBS-0.1% Tween before incubation with the secondary anti-rabbit horseradish peroxidase–conjugated Ig (Dako, Ely, U.K.) [1:2,000] for 1 h at room temperature. Antibody complexes were visualized using chemiluminescence (ECL+; GE Healthcare, Little Chalfont, U.K.). Band densities were measured using a scanning densitometer coupled to scanning software Scion Image (Scion Corporation, Frederick, MD), and the membranes were reprobed with the β-actin antibody (Cell Signaling Technology; 1:10,000 dilution) to determine equal protein loading.

Statistical methods. Data were analyzed using the package SPSS version 15 (SPSS, Chicago, IL). Positively skewed data were log transformed where possible, and then analyzed parametrically, or else nonparametric methods were used. Student's paired t test and Pearson or Spearman correlation coefficients were used as appropriate, and multiple regression analysis was used for correction for dependent variables. For the cell culture comparison, we used group comparison by Friedman's ANOVA and post hoc Dunn's test. Statistical significance was regarded as $P < 0.05$ (two tailed).

RESULTS

SPARC expression in adipose tissue and its depots. Our data showed that SPARC was expressed in various human tissues but especially in adipocytes where the expression was higher in larger cells (Fig. 1*A*). The expression in adipocytes was greater than the expression in stroma cells of adipose tissue (data not shown). When comparing adipose tissue depot expression, SPARC expression was higher in SCAT than VAT (0.81 ± 0.06 vs. 0.50 ± 0.03 signal units, $P < 0.001$, $n = 47$, Fig. 1*B*) similar to the expression of the adipokine leptin in SCAT (1.53 ± 0.1 vs. 0.6 ± 0.03 signal units, $P < 0.001$, $n = 47$, Fig. 1*A*).

SPARC and indicators of obesity and obesity-related inflammation. We did not find a sex difference in expression of BMI-matched men and women. Both SCAT- and VAT-derived SPARC showed a positive correlation with fat mass (Fig. 2) and with waist circumference (Table 4) in the depot study. SPARC was strongly correlated with high-sensitivity C-reactive protein but not with circulating tumor necrosis factor-α (TNF-α) or interleukin-6 (IL-6). SPARC was correlated with local adipose tissue expression

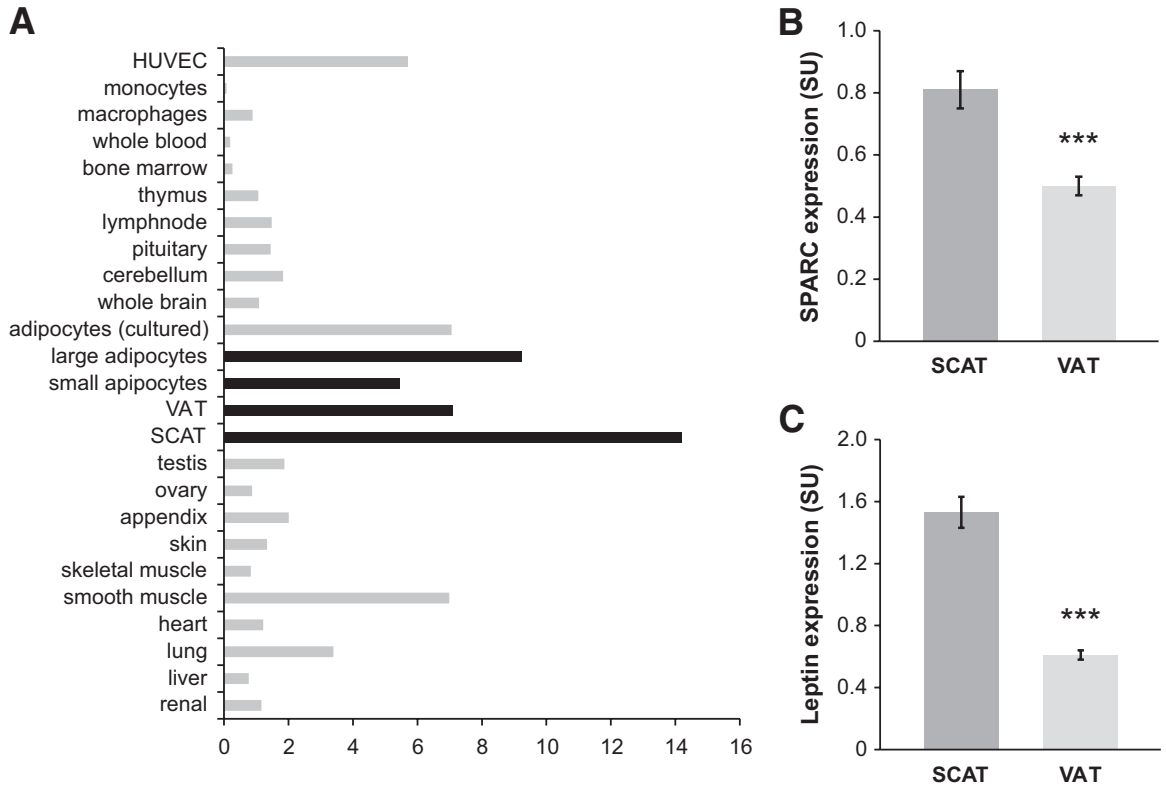

FIG. 1. ***A*: Expression intensity of SPARC in human tissues adapted from the Symatlas in comparison to human adipose tissue. *B*: SPARC depot expression in adipose tissue. *C*: Leptin expression in adipose tissue. SU, signal units; $n = 47$. ***$P < 0.01$.**

of the macrophage-migration inhibitory factor (MMIF; $r = 0.3$, $P < 0.05$), but only SCAT expression of SPARC correlated with local IL-6 expression ($r = -0.3$, $P < 0.05$). TNF-α, macrophage inflammatory protein-1 (MIP-1), monocyte chemoattractant protein-1 (MCP-1), or regulated upon activation normal T-cell expressed and secreted expression showed no association with SPARC expression (Table 4).

SPARC and markers of insulin resistance. SPARC was associated with fasting insulin levels (SCAT: $r = 0.31$, $P = 0.04$; VAT: $r = 0.34$, $P = 0.02$) and homeostasis model assessment–insulin resistance (HOMA-IR; SCAT: $r = 0.32$, $P = 0.03$; VAT: $r = 0.36$, $P = 0.016$). In addition, VAT-SPARC expression but not SCAT-SPARC was correlated with fasting glucose ($r = 0.29$, $P = 0.046$, Table 4).

SPARC and the adipokines leptin and adiponectin. VAT-SPARC correlated with VAT leptin ($r = 0.55$, $P < 0.001$) and SCAT-SPARC with SCAT leptin ($r = 0.68$, $P < 0.001$); this remained significant after correction for fat mass, waist-to-hip ratio, and HOMA-IR. VAT-SPARC was negatively correlated with serum adiponectin ($r = -0.37$, $P < 0.01$) and SCAT-SPARC but not VAT-SPAC negatively correlated with SCAT adiponectin ($r = -0.3$, $P < 0.05$; Fig. 2*C*, Table 4).

SPARC and weight loss. Obese subjects in the VLCD study lost 18 kg in the first 8 weeks and an additional 10 kg by week 16. There was no further weight loss from week 16 to 18. The average SPARC expression in the adipose tissue of these subjects decreased by 33% in the first 8 weeks ($P < 0.0001$, paired *t* test). However, despite further weight loss, there was no change in SPARC expression between 8 and 16 weeks ($P = 0.74$, paired *t* test). Between 16 and 18 weeks, during which period the subjects went from VLCD to regular food while remaining weight stable, the SPARC expression increased significantly (20% rise, $P < 0.0001$, paired *t* test) and remained significantly correlated with insulin, glucose, and leptin levels but not adiponectin levels (data not shown). There was no difference in SCAT-SPARC expression in the group with the metabolic syndrome, but there was a tendency to a further decline of SPARC at week 16 (Fig. 3).

SPARC and weight gain. The fast food study subjects gained on average of 7.2 ± 1.6 kg with resulting BMI of 23.7 ± 3.3 kg/m^2 and fat mass increment of 4.2 ± 5.8 kg. This resulted in a significant increase in fasting insulin by 52%, and HOMA-IR and serum leptin were more than doubled. SPARC-expression in SCAT increased with hyperalimentation in 4 weeks from 3.5 ± 0.8 to 6.2 ± 0.4 signal units ($P < 0.05$; Table 3), and the increment in SPARC correlated with the amount of weight gain ($r = 0.77$, $P < 0.01$).

SPARC regulation by insulin and glucose. Cell culture studies were performed to determine whether the observed correlations of SPARC with metabolic parameters are because of direct regulation by glucose, leptin, or insulin. Increasing doses of glucose (5, 10, 20, and 40 mmol/l) decreased SPARC protein expression (5 mmol/l: 100 ± 8 optical density units; 10 mmol/l: 93 ± 6 optical density units; 20 mmol/l: 24 ± 6 optical density units; 40 mmol/l: 19 ± 4 optical density units [SPARC/β-actin], respectively, $n = 6$; $P < 0.01$, Fig. 4*A*). In contrast, culture of VAT with insulin (0, 0.01, 1, and 100 nmol/l) increased SPARC expression (C: 100 ± 20 optical density units; 0.01 nmol/l: 105 ± 35 optical density units; 1 nmol/l: 156 ± 27 optical density units; 100 nmol/l: 292 ± 30 optical density units, $n = 6$; $P < 0.001$; Fig. 4*B*).

SPARC regulation by leptin. Similar to insulin, leptin treatment of visceral fat explants showed a dose-

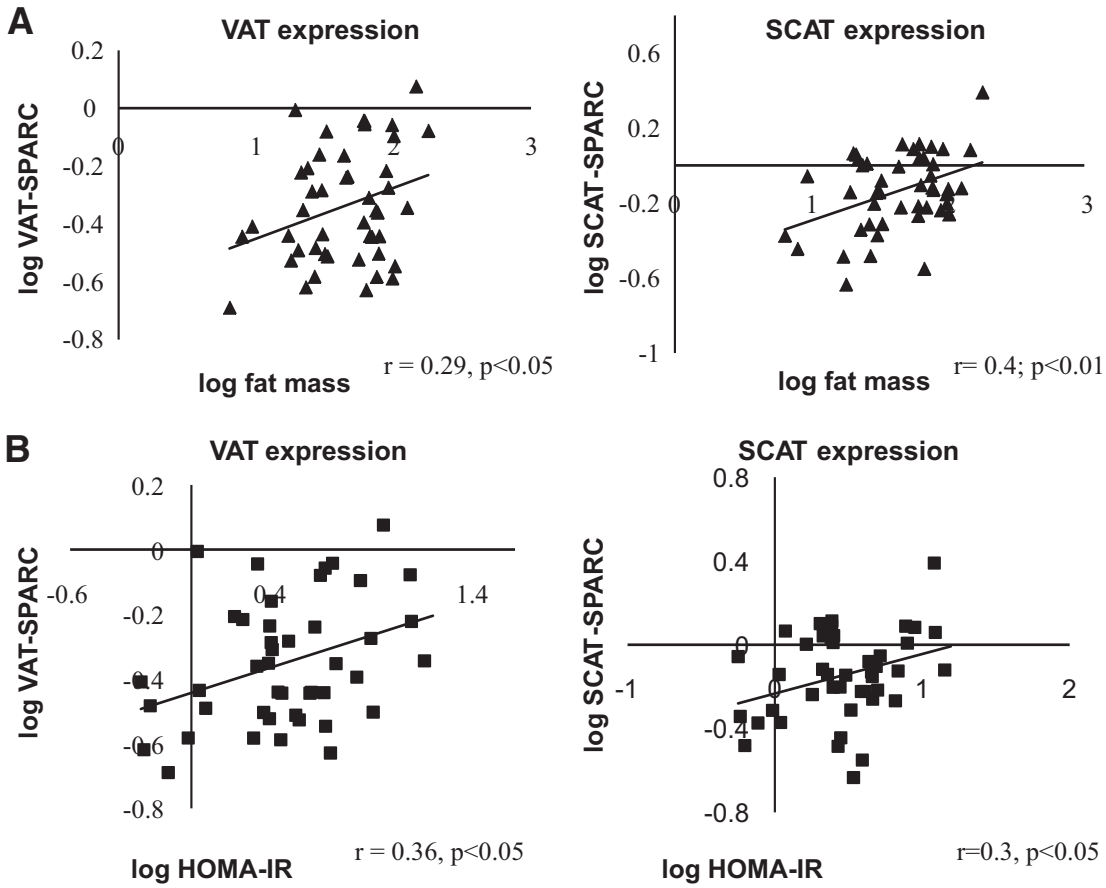

FIG. 2. SPARC and relation to fat mass, HOMA-IR, and adipokine expression. *A*: Depot expression of SPARC and its correlation with fat mass. *B*: VAT and SCAT-SPARC expression and HOMA-IR. *C*: VAT expression of adiponectin and SPARC. *D*: The correlation of SPARC expression with leptin. Values are expressed in signal units.

dependent increment of SPARC expression (C: 100 ± 20 optical density units; 0.1 nmol/l: 271 ± 30 optical density units; 10 nmol/l: 465 ± 52 optical density units; $P < 0.001$; Fig. 4*C*).

DISCUSSION

Adipose tissue plays a key role in energy homeostasis both as an energy store and as an endocrine organ. Dysfunction of adipose tissue fat storage leads to fat deposition in other organs as ectopic fat and results in disadvantageous metabolic consequences as most apparent in subjects with familial lipodystrophy (19). The cause of impaired adipose tissue storage capacity is unclear and SPARC that influences matricellular composition could contribute to its development by inhibition of adipocyte differentiation. Disturbance of the 3D extracellular matrix (ECM), for example, increased rigidity by increased collagen 1 content and has previously been shown to compromise in vitro adipocyte differentiation (20), and collagen dysregulation has recently been attributed to metabolic dysregulation (3).

The following attributes make SPARC a likely candidate to limit fat deposition in adipose tissue itself. Although it is an evolutionarily conserved collagen-binding glycoprotein, it does not contribute to the structure of the ECM, but it was recently shown to be a ligand of the integrin receptor α5-β1–integrin (21) and inhibit adipogenesis through stimulation of β-catenin signaling (8), which is part of the Wnt pathway that enhances osteoblastogenesis alongside the inhibition of adipogenesis (22). SPARC null mice have a phenotype marked by an increased subcutaneous fat deposition, reduction in collagen 1 in SPARC-null fat, adipocytes of higher diameters, and fat pads with an increase in adipocyte number (23).

The results of our study showed an increase of SPARC expression with increased fat mass that is consistent with previous reports showing a higher expression of SPARC in rodent obesity (24) and elevated SPARC levels in obese subjects (25). In addition, this is the first study to highlight the depot-specific expression of SPARC in humans. SPARC/osteonectin is expressed in various human tissues with particularly high expression in subcutaneous abdominal fat where is appears to be secreted primarily by adipocytes in comparison to the adipose tissue stromal fraction that is consistent with previous findings (2). Subjects with familial lipodystrophy lack SCAT, and the metabolic consequences could be attributed to the limitation of subcutaneous tissue expansion to which SPARC as an inhibitor of adipogenesis (8) with higher expression in SCAT may contribute.

This is the first study to assess metabolic parameters in combination with adipose tissue SPARC expression. We found a positive correlation with fasting insulin, fasting glucose, and HOMA-IR and waist circumference as well as hsCRP for subcutaneous and visceral depots. However, we did not find a correlation of SPARC with fasting lipids or blood pressure, which may in part be masked by treatment

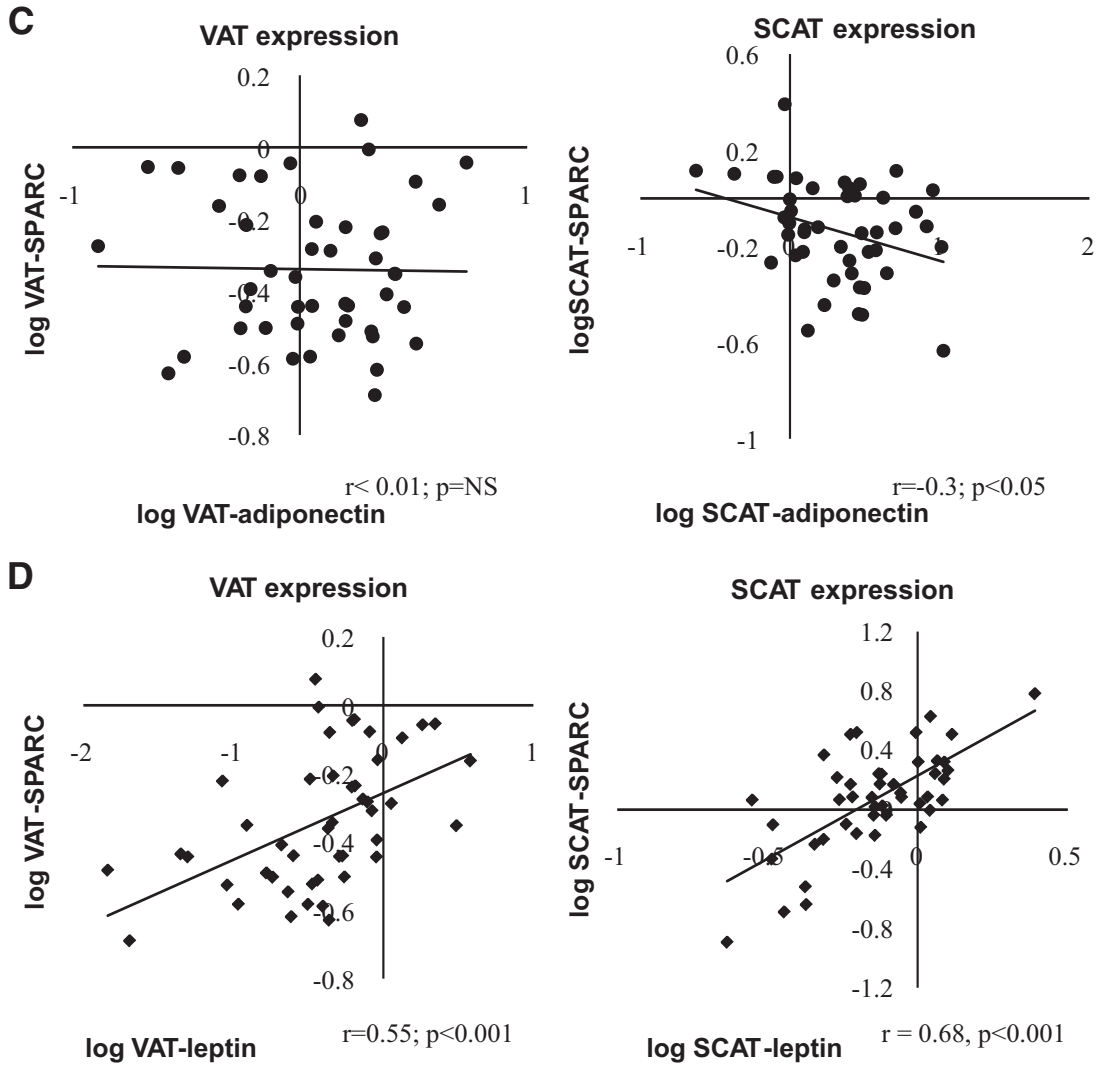

FIG. 2. Continued.

of subjects with hypercholesterolemia and hypertension (not shown).

Higami et al. (26) examined genes downregulated by energy restriction in epididymal fat in mice, and SPARC was one of the prominently affected genes lowered by long-term energy restriction. Consistent with this we have found a strong downregulation of SPARC with weight loss after 8 weeks of a VLCD and an increment of SPARC with weight gain. Although HOMA-IR improved with weight loss in our VLCD study population, and SCAT-SPARC expression correlated with fasting insulin and HOMA-IR, the SPARC expression was no different in the BMI-matched groups with and without the metabolic syndrome, which is explained and consistent with the lack of correlation of SPARC with blood pressure and lipids. Leptin is a strong predictor of SPARC in the depot study as well as the VLCD study in which it is independent of the BMI at baseline. SPARC remains correlated with leptin expression but loses its association with weight loss after 8 weeks, after which subjects continued to lose weight. The strong correlation with insulin, glucose, and leptin in the depot and the VLCD study prompted us to study and confirm their regulation by further in vitro studies, while adiponectin showed an inverse correlation with SPARC expression in SCAT but not VAT and thus appeared less likely to be because of a direct effect.

Culture of visceral explants showed that glucose lowered and insulin and leptin increased SPARC expression that apart from the glucose correlation is consistent with the findings in the clinical studies. However, circulating glucose in vivo showed a positive correlation with SPARC-AT expression that contradicts the in vitro study in which supraphysiological glucose levels lower SPARC. The lack of influence of circulating glucose levels may be explained by the insulin resistance of adipocytes for glucose transport in obese individuals. In diabetic subjects, we would expect the glucose levels required to lower SPARC to be supraphysiological as found in marked hyperglycemia that is usually avoided by antidiabetic treatment. Although we show that SPARC is regulated by leptin, SPARC in turn suppresses leptin gene expression in mouse preadipocytes (21), which may explain the high leptin levels observed in SPARC knockout mice (23).

It was suggested that SPARC may be involved in inflammatory processes (27,28), but data from our study show only a correlation of adipose tissue SPARC expression with the local expression of the MMIF-1—a proinflammatory adipocytokine (29). An association of SPARC with MMIF was to our knowledge only reported in connection

TABLE 4
SPARC and metabolic parameters and cytokines

	SCAT	VAT
Waist circumference (cm)	**$R = 0.37$, $P < 0.01$**	**$R = 0.23$, $P < 0.05$**
Fat mass (kg)	**$R = 0.4$, $P < 0.01$**	**$R = 0.3$, $P < 0.05$**
Total cholesterol (mmol/l)	$R = 0.008$, $P =$ NS	**$R = 0.23$, $P < 0.05$**
Fasting insulin (μIU/ml)	**$R = 0.31$, $P < 0.05$**	**$R = 0.34$, $P < 0.05$**
Fasting glucose (mmol/l)	$R = 0.23$, $P =$ NS	**$R = 0.3$, $P < 0.05$**
HOMA-IR	**$R = 0.32$, $P < 0.05$**	**$R = 0.36$, $P < 0.05$**
Circulating leptin (ng/ml)	**$R = 0.38$, $P < 0.05$**	$R = 0.17$, $P =$ NS
Circulating adiponectin (ng/ml)	$R = -0.22$, $P =$ NS	**$R = -0.37$, $P < 0.05$**
Circulating IL-6 (ng/ml)	$R = 0.26$, $P =$ NS	$R = 0.01$, $P =$ NS
Circulating TNF-α (ng/ml)	$R = 0.16$, $P =$ NS	$R = 0.03$, $P =$ NS
hsCRP (mmol/l)	**$R = 0.44$, $P < 0.01$**	**$R = 0.31$, $P < 0.05$**
Adipose tissue leptin	**$R = 0.68$, $P < 0.001$**	**$R = 0.55$, $P < 0.001$**
Adipose tissue adiponectin	**$R = -0.3$, $P < 0.05$**	$R < 0.01$, $P =$ NS
Adipose tissue IL-6	**$R = -0.3$, $P < 0.05$**	$R = -0.014$, $P =$ NS
Adipose tissue MMIF	**$R = -0.3$, $P < 0.05$**	**$R = 0.3$, $P < 0.05$**
Adipose tissue TNF-α	$R = 0.07$, $P =$ NS	$R = 0.03$, $P =$ NS
Adipose tissue MIP-1	$R = 0.03$, $P =$ NS	$R = -0.47$, $P =$ NS
Adipose tissue MCP-1	$R = 0.055$, $P =$ NS	$R = -0.14$, $P =$ NS
Adipose tissue RANTES	$R = 0.077$, $P =$ NS	$R = 0.017$, $P =$ NS

SPARC depot-specific expression in relation to metabolic parameters, circulating cytokine levels, and adipose tissue expression of adipokine/cytokine corresponding to the depot studied. RANTES, regulated upon activation normal T-cell expressed and secreted. $n = 56$, significant correlations are shown in bold.

with malignant melanoma cells that showed overexpression of both (30). Thus, the interaction of SPARC and MMIF remains to be confirmed but may explain a potential association of SPARC with diabetes with MMIF being associated with the pathogenesis of type 1 and type 2 diabetes and latter among others through a decrease in insulin signal transduction (31).

Much pathology related to diabetes and obesity has been linked with SPARC. An increased SPARC expression was found in rodent models of diabetic nephropathy (32), and SPARC null mice were shown to be protected from renal fibrosis (27). Increased circulating levels of SPARC have been observed in subjects with cardiovascular disease (25). SPARC is also expressed in human retinal endothelial cells (33), and with its close interaction with VEGF and PAI-1 (34,35) it may further the progression of diabetic retinopathy. Furthermore, SPARC is linked with tumorigenesis and appears to favor certain tumors (36), although its exact role in tumor development is controversial (37) and it remains to be shown whether SPARC could contribute to the association of obesity and some cancers (38).

Apart from focusing on the potential advantages of lowering SPARC levels, the inducers of SPARC excess such as hyperleptinemia in subjects with leptin resistance and hyperinsulinemia in type 2 diabetes require similar

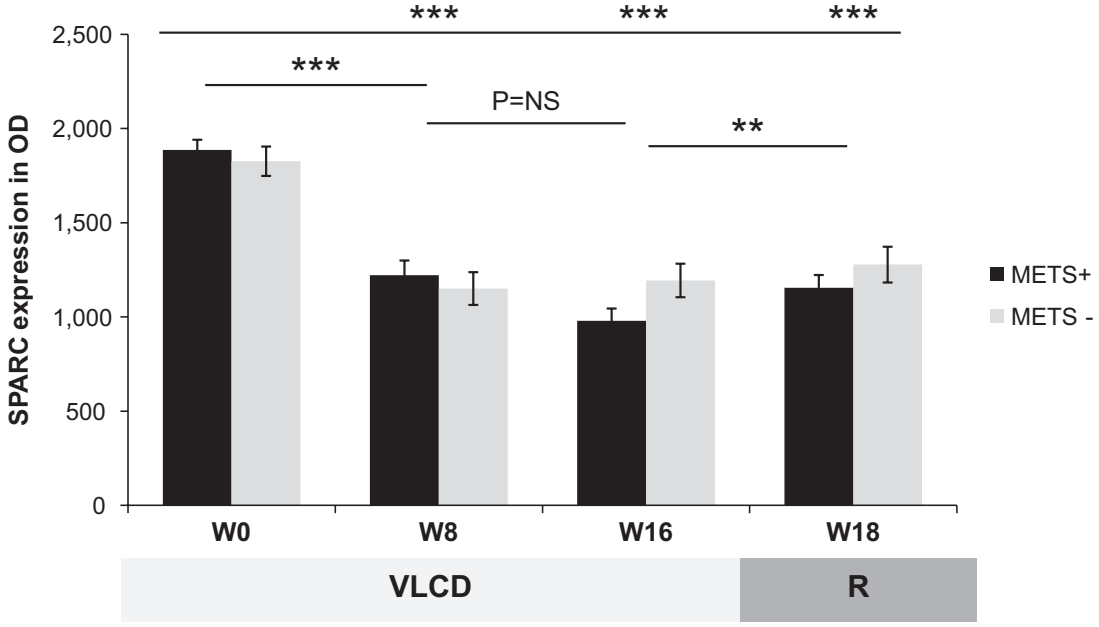

FIG. 3. SPARC during VLCD 16-week 450 kcal/day in subjects with (METS+) and without (METS−) the metabolic syndrome. Data from SPARC adipose tissue expression of subjects of both groups were compared with their baseline levels (*top*) and the within-patient variation with the previous time point. R, refeeding; W, week. **$P < 0.01$, ***$P < 0.001$, $n = 24$.

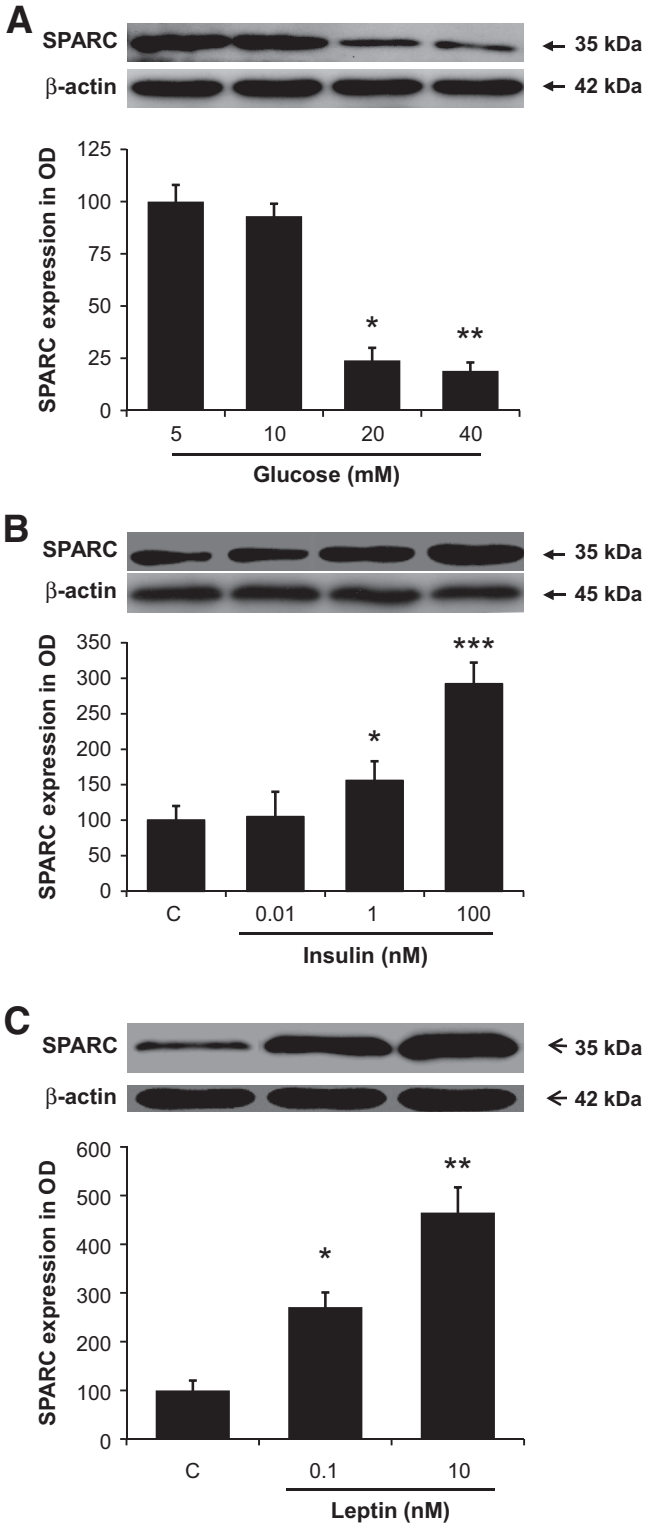

FIG. 4. *A*: Dose-dependent effects of D-glucose on SPARC protein production in VAT explants assessed by Western blotting. Densitometric analysis of SPARC immune complexes (35 kDa) were normalized to β-actin (40 kDa). Data are expressed as percentage difference of median of basal. *B*: Dose-dependent effects of insulin on SPARC protein production. *C*: Dose-dependent effects of leptin on SPARC production. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $n = 6$.

attention. Leptin is a proinflammatory molecule, and it has been suggested that leptin is permissive in the pathogenesis of liver fibrosis as shown by protection of leptin-deficient mice from fibrosis during steatohepatitis or in response to chronic toxic liver injury (39). As such, hyperleptinemia in adipose tissue may through upregulation of SPARC also induce adipose tissue fibrosis that requires further study.

In summary, our study showed that SPARC-AT expression is related to human metabolism and SPARC expression that is predominant in SCAT is upregulated by insulin and leptin. Together with the previous reported consequences of SPARC in other tissues, a role for SPARC in the development of obesity- and diabetes-related complications is likely. Further research is required to show whether increased adipose tissue SPARC limits the expansion of normal adipose tissue in response to energy excess and promotes ectopic fat deposition and associated metabolic dysfunction.

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REFERENCES

1. Termine JD, Kleinman HK, Whitson SW, Conn KM, McGarvey ML, Martin GR. Osteonectin, a bone-specific protein linking mineral to collagen. Cell 1981;26:99–105
2. Chavey C, Boucher J, Monthouel-Kartmann MN, Sage EH, Castan-Laurell I, Valet P, Tartare-Deckert S, Van Obberghen E. Regulation of secreted protein acidic and rich in cysteine during adipose conversion and adipose tissue hyperplasia. Obesity (Silver Spring) 2006;14:1890–1897
3. Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia M, Abate N, Zhang BB, Bonaldo P, Chua S, Scherer PE. Metabolic dysregulation and adipose tissue fibrosis: the role of collagen VI. Mol Cell Biol 2008;29:1571–1591
4. Reed MJ, Sage EH. SPARC and the extracellular matrix: implications for cancer and wound repair. Curr Top Microbiol Immunol 1996;213:81–94
5. Bradshaw AD, Reed MJ, Sage EH. SPARC-null mice exhibit accelerated cutaneous wound closure. J Histochem Cytochem 2002;50:1–10
6. Camino AM, Atorrasagasti C, Maccio D, Prada F, Salvatierra E, Rizzo M, Alaniz L, Aquino JB, Podhajcer OL, Silva M, Mazzolini G. Adenovirus-mediated inhibition of SPARC attenuates liver fibrosis in rats. J Gene Med 2008;10:993–1004
7. Bradshaw AD, Puolakkainen P, Dasgupta J, Davidson JM, Wight TN, Sage HE. SPARC-null mice display abnormalities in the dermis characterized by decreased collagen fibril diameter and reduced tensile strength. J Invest Dermatol 2003;120:949–955
8. Nie J, Sage EH. SPARC inhibits adipogenesis by its enhancement of β-catenin signaling. J Biol Chem 2009;284:1279–1290
9. Gummesson A, Jernås M, Svensson PA, Larsson I, Glad CA, Schéle E, Gripeteg L, Sjöholm K, Lystig TC, Sjöström L, Carlsson B, Fagerberg B, Carlsson LM. Relations of adipose tissue CIDEA gene expression to basal metabolic rate, energy restriction, and obesity: population-based and dietary intervention studies. J Clin Endocrinol Metab 2007;92:4759–4765
10. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–553
11. Gabrielsson BG, Johansson JM, Jennische E, Jernås M, Itoh Y, Peltonen M, Olbers T, Lönn L, Lönroth H, Sjöström L, Carlsson B, Carlsson LM, Lönn M. Depot-specific expression of fibroblast growth factors in human adipose tissue. Obes Res 2002;10:608–616
12. Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindström T, Nystrom FH, Fast Food Study Group. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut 2008;57:649–654
13. Strålfors P, Honnor RC. Insulin-induced dephosphorylation of hormone-

sensitive lipase: correlation with lipolysis and cAMP-dependent protein kinase activity. Eur J Biochem 1989;182:379–385
14. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:673–674
15. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acid Res 2002;30:e36
16. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–159
17. Jernås M, Palming J, Sjöholm K, Jennische E, Svensson PA, Gabrielsson BG, Levin M, Sjögren A, Rudemo M, Lystig TC, Carlsson B, Carlsson LM, Lönn M. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. FASEB J 2006;20:1540–1542
18. Fried SK, Moustaid-Moussa N. Culture of adipose tissue and isolated adipocytes. Methods Mol Biol 2001;155:197–212
19. Wong SP, Huda M, English P, Bargiotta A, Wilding JP, Johnson A, Corrall R, Pinkney JH. Adipokines and the insulin resistance syndrome in familial partial lipodystrophy caused by a mutation in lamin A/C. Diabetologia 2005;48:2641–2649
20. Chun TH, Hotary KB, Sabeh F, Saltiel AR, Allen ED, Weiss SJ. A pericellular collagenase directs the 3-dimensional development of white adipose tissue. Cell 2006;125:577–591
21. Nie J, Chang B, Traktuev DO, Sun J, March K, Chan L, Sage EH, Pasqualini R, Arap W, Kolonin MG. IFATS collection: combinatorial peptides identify alpha5beta1 integrin as a receptor for the matricellular protein SPARC on adipose stromal cells. Stem Cells 2008;26:2735–2745
22. Kennell JA, MacDougald OA. Wnt signaling inhibits adipogenesis through beta-catenin-dependent and -independent mechanisms. J Biol Chem 2005; 280:24004–24010
23. Bradshaw AD, Graves DC, Motamed K, Sage EH. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc Natl Acad Sci U S A 2003;100:6045–6050
24. Tartare-Deckert S, Chavey C, Monthouel MN, Gautier N, Van Obberghen E. The matricellular protein SPARC/osteonectin as a newly identified factor up-regulated in obesity. J Biol Chem 2007;276:22231–22237
25. Takahashi M, Nagaretani H, Funahashi T, Nishizawa H, Maeda N, Kishida K, Kuriyama H, Shimomura I, Maeda K, Hotta K, Ouchi N, Kihara S, Nakamura T, Yamashita S, Matsuzawa Y. The expression of SPARC in adipose tissue and its increased plasma concentration in patients with coronary artery disease. Obes Res 2001;9:388–393
26. Higami Y, Barger JL, Page GP, Allison DB, Smith SR, Prolla TA, Weindruch R. Energy restriction lowers the expression of genes linked to inflammation, the cytoskeleton, the extracellular matrix, and angiogenesis in mouse adipose tissue. J Nutr 2006;136:343–352
27. Socha MJ, Manhiani M, Said N, Imig JD, Motamed K. Secreted protein acidic and rich in cysteine deficiency ameliorates renal inflammation and fibrosis in angiotensin hypertension. Am J Pathol 2007;171:1104–1112
28. Sangaletti S, Stoppacciaro A, Guiducci C, Torrisi MR, Colombo MP. Leukocyte, rather than tumor-produced SPARC, determines stroma and collagen type IV deposition in mammary carcinoma. J Exp Med 2003;198: 1475–1485
29. Skurk T, Herder C, Kräft I, Müller-Scholze S, Hauner H, Kolb H. Production and release of macrophage migration inhibitory factor from human adipocytes. Endocrinology 2005;146:1006–1011
30. Rumpler G, Becker B, Hafner C, McClelland M, Stolz W, Landthaler M, Schmitt R, Bosserhoff A, Vogt T. Identification of differentially expressed genes in models of melanoma progression by cDNA array analysis: SPARC, MIF and a novel cathepsin protease characterize aggressive phenotypes. Exp Dermatol 2003;12:761–771
31. Atsumi T, Cho YR, Leng L, McDonald C, Yu T, Danton C, Hong EG, Mitchell RA, Metz C, Niwa H, Takeuchi J, Onodera S, Umino T, Yoshioka N, Koike T, Kim JK, Bucala R. The proinflammatory cytokine macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation. J Immunol 2007;179:5399–5406
32. Taneda S, Pippin JW, Sage EH, Hudkins KL, Takeuchi Y, Couser WG, Alpers CE. Amelioration of diabetic nephropathy in SPARC-null mice. J Am Soc Nephrol 2003;14:968–980
33. Munjal ID, McLean NV, Grant MB, Blake DA. Differences in the synthesis of secreted proteins in human retinal endothelial cells of diabetic and nondiabetic origin. Curr Eye Res 1994;13:303–310
34. Kupprion C, Motamed K, Sage EH. SPARC (BM-40, osteonectin) inhibits the mitogenic effect of vascular endothelial growth factor on microvascular endothelial cells. J Biol Chem 1998;273:29635–29640
35. Yunker CK, Golembieski W, Lemke N, Schultz CR, Cazacu S, Brodie C, Rempel SA. SPARC-induced increase in glioma matrix and decrease in vascularity are associated with reduced VEGF expression and secretion. Int J Cancer 2008;122:2735–2743
36. Wiese AH, Auer J, Lassmann S, Nährig J, Rosenberg R, Höfler H, Rüger R, Werner M. Identification of gene signatures for invasive colorectal tumor cells. Cancer Detect Prev 2007;31:282–295
37. Podhajcer OL, Benedetti LG, Girotti MR, Prada F, Salvatierra E, Llera AS. The role of the matricellular protein SPARC in the dynamic interaction between the tumor and the host. Cancer Metastasis Rev 2008;27:691–705
38. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008;371:569–578
39. Leclercq IA, Farrell GC, Schriemer R, Robertson GR. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. J Hepatol 2002;37: 206–213