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Dysregulation of sterol regulatory element binding protein–1c in livers of morbidly obese women is associated with altered suppressor of cytokine signaling–3 and signal transducer and activator of transcription–1 signaling

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Abstract

We compared hepatic expression of genes that regulate lipid biosynthesis and metabolic signaling in liver biopsy specimens from women who were undergoing gastric bypass surgery (GBP) for morbid obesity with that in women undergoing ventral hernia repair who had experienced massive weight loss (MWL) after prior GBP. Comprehensive metabolic profiles of morbidly obese (MO) (22 subjects) and MWL (9 subjects) were also compared. Analyses of gene expression in liver biopsies from MO and MWL were accomplished by Affymetrix microarray, real-time polymerase chain reaction, and Western blotting techniques. After GBP, MWL subjects had lost on average 102 lb as compared with MO subjects. This was accompanied by effective reversal of the dyslipidemia and insulin resistance that were present in MO. As compared with MWL, livers of MO subjects exhibited increased expression of sterol regulatory element binding protein (SREBP)–1c and its downstream lipogenic targets, fatty acid synthase and acetyl-coenzyme A-carboxylase–1. Livers of MO subjects also exhibited enhanced expression of suppressor of cytokine signaling–3 protein and attenuated Janus kinase signal transducer and activator of transcription (JAK/STAT) signaling. Consistent with these findings, we found that the human SREBP-1c promoter was positively regulated by insulin and negatively regulated by STAT3. These data support the hypothesis that suppressor of cytokine signaling–3–mediated attenuation of the STAT signaling pathway and resulting enhanced expression of SREBP-1c, a key regulator of de novo lipid biosynthesis, are mechanistically related to the development of hepatic insulin resistance and dyslipidemia in MO women.
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1. Introduction

Both insulin insensitivity and the accompanying hyperinsulinemia and dsylipidemia are key features of obesity [1]. In obesity, dyslipidemia associated with insulin resistance results, in large part, from increased hepatic synthesis and secretion of very low-density lipoprotein (VLDL) [2]. Although increased availability of fatty acids from adipose tissue as a result of impaired ability of insulin to inhibit lipolysis is, in part, responsible for enhanced hepatic triglyceride

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(TG) synthesis in obesity, enhanced hepatic de novo lipogenesis also appears to play an important role. In addition, in obesity, enhanced production of TG by the liver often exceeds its ability to secrete TG, thereby leading to the development of nonalcoholic fatty liver disease, steatohepatitis, fibrosis, and cirrhosis [3,4].

Although de novo hepatic lipogenesis is a relatively minor pathway in the production of VLDL-TG in lean humans [5], metabolic turnover studies performed in obese subjects [6] and in those consuming high-carbohydrate diets indicate that de novo lipogenesis becomes a major source of VLDL-TG [7-9]. In obese hyperinsulinemic rodents, increased hepatic lipid synthesis results from up-regulation of the lipogenic regulator sterol regulatory element binding protein (SREBP)–1c and its downstream lipogenic enzyme gene targets [10,11]. The SREBP-1c expression in the liver is directly enhanced by insulin at both the transcriptional and posttranslational levels [12-15]. The rodent SREBP-1c promoter contains a multicomponent insulin response element that mediates rapid induction of SREBP-1c transcription [12]. Conversely, insulin also enhances the transport of nascent SREBP-1c and its chaperone, sterol cleavage activating protein (SCAP), to the Golgi where it undergoes proteolytic cleavage to release the transcriptionally active n-terminal SREBP-1c fragment [15,16]. Enhanced hepatic expression of SREBP-1c in response to hyperinsulinemia in obesity seems somewhat paradoxical, however, given the attenuation of other effects of insulin including suppression of gluconeogenesis and lipolysis as a result of obesity-related insulin resistance [17].

The mechanisms of insulin resistance in obesity involve many factors, including elevated levels of proinflammatory adipokines such as tumor necrosis factor–α and interleukin-6 [18]. Recent studies have begun to shed light on the mechanisms by which adipokines oppose insulin action; specifically, the suppressor of cytokine signaling (SOCS) proteins have been implicated in the induction of insulin resistance in obese rodents [19]. Ueki and coworkers [20] demonstrated that increased expression of SOCS-1 and SOCS-3 in liver and muscle of obese mice was associated with decreased tyrosine phosphorylation of insulin receptor substrate (IRS) proteins. In these animal studies, attenuation of Janus kinase signal transducer and activator of transcription (JAK/STAT)–3 signaling by SOCS-1 and SOCS-3 was associated with enhanced expression of SREBP-1c [21].

It is unclear whether enhanced hepatic de novo lipogenesis in obese humans also arises from aberrant regulation of SREBP-1c and the JAK/STAT signaling pathway. Examination of the effect of obesity on hepatic lipid gene expression in humans has been limited by the inherent difficulty in obtaining nondiseased liver tissue from obese and nonobese controls. In this regard, morbidly obese (MO) patients who are undergoing gastric bypass surgery (GBP) for weight loss provide a unique opportunity to study hepatic gene expression in the human. Furthermore, a subset of these individuals subsequently undergoes ventral hernia repair after massive weight loss (MWL) as a result of prior GBP. This provides an opportunity to sample liver tissue in MO subjects who have experienced reversion to the preobese state after MWL. These individuals can serve as a postobese control for MO insofar as MWL after GBP is accompanied by effective reversal of both obesity-associated insulin resistance and dyslipidemia [22]. To gain insight into the altered metabolic signaling and lipid homeostasis that accompany morbid obesity, we compared gene expression profiles in liver biopsy samples from MO subjects undergoing GBP as compared with women undergoing ventral hernia repair as a result of MWL after GBP. Using gene ontology and hierarchical clustering analysis of microarray-based gene expression, we recently reported altered expression of many genes involved in wound healing, bile acid transport, and xenobiotic metabolism in MO women [23]. Because only a limited number of genes related to lipid and energy homeostasis met the stringent criteria for differential gene expression set in this previous analysis [23], in the present analysis, we specifically mined the microarray data to address the question of altered expression of many key genes related to lipid synthesis and metabolic signaling. The microarray analyses were further supplemented by real-time polymerase chain reaction (PCR) measurements of selected messenger RNAs (mRNAs) and Western blotting measurements of the putative protein products encoded by these mRNAs. We report here that expression of SREBP-1c and its downstream regulatory targets, fatty acid synthase (FAS), diacylglycerol acyltransferase (DGAT), stearoyl-coenzyme A desaturase–1 (SCD-1), acetyl-coenzyme A carboxylase–1 (ACC-1), and malic enzyme (ME), is increased in livers of MO women. Furthermore, morbid obesity is also associated with enhanced expression of the signaling inhibitors SOCS-1 and SOCS-3 and concomitant attenuation of JAK/STAT signaling.

2. Experimental procedures

2.1. Treatment of study subjects, tissue sample collection, and clinical laboratory assessments

Institutional Review Boards of the University of Tennessee Health Science Center (UTHSC) and Memphis Baptist Memorial Hospital approved all study procedures. All participants gave written informed consent. Subjects were excluded from participating in the study if they had a history of TG-related pancreatitis; or elevated creatinine, liver enzymes, or bilirubin; or hypoalbuminemia [23]. Subjects were also excluded if there was a history of malignancy, diabetes mellitus, recent major illness, or current treatment with corticosteroids, androgens, or lipid-lowering agents. The study participants were admitted to the UTHSC General Clinical Research Center (GCRC) 1 day before surgery for preoperative care and clinical profiling. Participants were

provided a diet diary and were instructed by the GCRC dietician to record their food intake for the 3 days before admission. The GCRC nutritionist reviewed the 3-day diet diary, and dietary composition was analyzed by computer program (Nutritionist Pro, Axxya Systems, Stafford, TX).

Blood samples were obtained in the fasting state for baseline determinations of plasma lipoproteins, glucose, insulin, and free fatty acid (FFA). Serum glucose, insulin, and FFA were assessed in MO and WL patients over a 3-hour period after initiation of oral glucose tolerance test (OGTT). Insulin sensitivity was estimated using an index of whole-body insulin sensitivity based on OGTT-derived glucose and insulin levels as previously described [24]. The formula used was as follows: 10 000/square root of (fasting glucose × fasting insulin) × (mean OGTT glucose × mean OGTT insulin). Plasma lipoproteins were isolated from fasting plasma samples; apolipoprotein (apo) and lipid content were analyzed using high-performance liquid chromatography and enzymatic assays after separation of lipoprotein fractions by ultracentrifugation as described previously [25]. After completion of the OGTT, subjects were allowed intake of clear liquids ad libitum as part of their preoperative care. The patients were then transferred to the surgical units of the participating hospitals and remained fasting overnight until completion of surgery.

Gastric bypass surgery in the MO women was accomplished by an extended Roux-en-Y gastric bypass coupled with a horizontal gastric pouch as described previously [23]. A separate group of patients who were, on average, 1 year post-GBP and who had lost greater than 80 lb of body weight who were undergoing abdominoplasty to repair ventral hernias and diastasis recti served as postobese controls (MWL). A wedge biopsy specimen of liver was removed under direct operative visualization from patients undergoing GBP and from MWL subjects undergoing ventral hernia repair. A portion of the liver biopsy sample was immediately placed in RNAzol (Tel-Test, Friendswood, TX) solution, and the remainder of the tissue was snap-frozen in liquid nitrogen and stored at −70°C for subsequent protein analysis.

Samples were analyzed by the GCRC core laboratory and by commercial laboratory. Serum chemistry was determined by standard autoanalyzer techniques in a commercial laboratory. Insulin and C-peptide were measured using commercially available radioimmunoassay.

2.2. Determination of hepatic gene expression by microarray analysis

Total RNA was isolated by chloroform-phenol extraction and isopropanol precipitation after the manufacturers' recommended protocol. The RNA samples were redissolved in diethyl pyrocarbonate–treated water and were processed for microarray analysis using Affymetrix oligonucleotide arrays (HG-133A) as previously described [23]. The expression data were analyzed according to published methods [26]. Genes were arranged in decreasing order of their mean detection P value, and all of the genes possessing a value of 95% or greater ($P \leq .05$) were copied into a new file. The resulting 3576 genes were matched with their identification numbers from Unigene, Locus Link, and GenBank databases that were used to confirm the identity of the gene. This probe set database was then queried to identify specific genes related to fatty acid metabolism and lipid-related signaling, and mean signal ratios for expression of these genes in livers of MO vs MWL were generated.

2.3. Determination of hepatic gene expression by real-time PCR

The expression of a subset of differentially expressed genes, as revealed by microarray analysis, along with a number genes involved in lipid metabolism that were not detected by microarray-based analyses, was determined by real-time PCR. Real-time PCR was performed with a LightCycler 480 System (Roche Diagnostics, Indianapolis, IN) using SYBR Green 1 dye (LightCycler 480 SYBR Green 1 Master Mix; Roche Diagnostics) intercalation to detect DNA amplification. Expression of both the target gene and control gene (cyclophilin D) within each sample was quantified based on their respective threshold cycle (Ct) values. Target gene Ct values were normalized to cyclophilin D control gene Ct values. The ratios of target gene expression of MO vs MWL were derived. The Universal Probe Library from Roche Applied Science (Indianapolis, IN) was used to design the primers used for real-time PCR (Table 1).

Table 1
Sequences of primers used for real-time PCR

Gene	Oligonucleotide sequence	
FASN	Forward	5′-ACAGGGACAACCTGGAGTTCT-3′
	Reverse	5′-CTG TGG TCC CAC TTG ATG AGT-3′
SREBP-1	Forward	5′-GCTCCTCCATCAATGACAAAA-3′
	Reverse	5′-TGCGCAAGACAGCAGATTTA-3′
SREBP-1c	Forward	5′-GGAGGGGTAGGGCCAACGGCCT-3′
	Reverse	5′-CATGTCTTCGAAAGTGCAATCC-3′
SREBP-2	Forward	5′-ATCTGGATCTCGCCAGAGG-3′
	Reverse	5′-CCAGGCAGGTTTGTAGGTTG-3′
HMG-R	Forward	5′-CTGAAGCTGGCAAATCAAAAG-3′
	Reverse	5′-CTTTGCATGCTCCTTGAACA-3′
DGAT-1	Forward	5′-ACTACCGTGGCATCCTGAAC-3′
	Reverse	5′-ATAACCGGGCATTGCTCA-3′
PGC-1a	Forward	5′-GCAGGAGCAGAGCAAAGAGG-3′
	Reverse	5′-AAAGTTGTTGGTTTGGCTTGTAAG T-3′
SCD-1	Forward	5′-GTACCGCTGGCACATCAACTT-3′
	Reverse	5′-TTGGAGACTTTCTTCCGGTCAT-3′
ACC-1	Forward	5′-GGACAACACCTGTGTGGTAGAA-3′
	Reverse	5′-CGTGGGGATGTTCCCTCT-3′
DGAT-1	Forward	5′-ACTACCGTGGCATCCTGAAC-3′
	Reverse	5′-ATAACCGGGCATTGCTCA-3′

Primers were designed using the Universal Probe Library from Roche Applied Science. Primers were obtained from Integrated DNA Technologies (Coralville, IA). Primers for SOCS3 and MAP2K6 were purchased from Qiagen (Valencia, CA). QuantiTect Primer Assay (proprietary).

2.4. Western blot analysis of liver proteins

Liver biopsy samples (2 g) were homogenized in a protein lysis buffer (150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mmol/L Tris-HCl pH 8.0, 2 mmol/L DTT) containing protease and phosphatase inhibitors and then centrifuged at 10,000 × g for 10 min at 4°C. The supernatants were collected and their protein concentrations were determined. Equal aliquots of hepatic proteins were size-fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, subjected to Western blot analysis, and quantified according to published protocols [15]. The anti–SREBP-1 (SC-13551), anti–SREBP-2 (SC-13552), anti-FAS (SC-20140), anti–ACC-1 (SC-26817), anti–SOCS-3 (SC-9023), anti–SOCS-1 (SC-9021), anti–STAT-3 (SC-482), anti–apo A (SC-13549), and anti–apo E (SC-13521) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti–phospho-STAT-3 (9131S) was from Cell Signaling Technology (Beverly, MA). Anti–β-actin antibody (SC-1616) was used to determine protein loading.

2.5. Determination of response of the human SREBP-1c promoter-luciferase construct response to insulin and STAT3

To evaluate the potential significance of altered hepatic SREBP-1c and SOCS/STAT3 expression in MO, the response of the human SREBP-1c promoter to both insulin and STAT3 was evaluated in vitro. The BAC-CTD2502C3 clone (Open Biosystems, Huntsville, AL) containing the human SREBP-1c genomic DNA was used to amplify promoter DNA encompassing −1498 to +1 (SREBP-1c transcription start point denoted as +1) by PCR primers terminated with *Kpn*I and *Bgl*II sites. The amplified DNA was ligated into the polylinker upstream of the luciferase coding sequence of pGL3 vector (Promega, Madison, WI) to generate the hSREBP-1c-luc plasmid. Rat primary hepatocytes were transfected with 1 μg of hSREBP-1c-luc plus 1 μg of pRL-TK (Promega), and luciferase expression was quantified as reported previously [12,27] after treatment with either insulin (100 nmol/L) or the LXR agonist T0901317 (10 μmol/L). To determine the effect of altered STAT3 expression on hSREBP-1c promoter activity, primary rat hepatocytes were transfected with the hSREBP-1c-luc construct along with plasmids expressing wild-type or dominant negative STAT3 proteins, or empty vector. Twenty-four hours after transfection, cells were harvested; and the luciferase activity was measured. Cells were cotransfected with a control *Renilla* expression plasmid, pRL-TK.

2.6. Statistical analyses

Significance of differences between plasma analytes, mRNA, and protein expression and anthropometric variables between MWL and MO was determined by Student *t* test (continuous variables) or C^2 (Fisher exact test for noncontinuous variables) using JMP Statistical Software (Version 5; SAS, Cary NC).

3. Results

3.1. Clinical and metabolic characteristics of MO women compared with those with MWL after GBP

Metabolic profiling was performed before GBP and ventral hernia repair in 22 MO and 9 MWL subjects, respectively. The MWL group was on average 102 lb lighter (199 vs 301 lb for MO), reflecting weight loss as a result of prior GBP (Table 2). Similarly, body mass index (BMI) was significantly lower in MWL (32.0 vs 54.5 kg/m^2 for MO) (Table 2). Total plasma cholesterol and TG levels were higher and high-density lipoprotein (HDL) cholesterol was lower than expected in MO subjects, given their age and sex (Table 2). Correspondingly, in women with MWL after prior GBP, plasma levels of total and low-density lipoprotein (LDL) cholesterol and TG were significantly lower and HDL cholesterol was significantly higher compared with their pre-GBP (MO) counterparts (Table 2). Similarly, plasma apo B was markedly lower in MWL subjects; as was the VLDL apoprotein apo C-III (Table 2). On the other hand, plasma

Table 2
Clinical and metabolic characteristics of MO women undergoing GBP and of those experiencing MWL after GBP

Variable	MO (n = 22)	MWL (n = 9)	P[a]
Age (y)	36.2 ± 1.4	39.2 ± 2.5	.27
African American (%)	22.7%	44.0%	.38
Weight (lb)	301 ± 17	199 ± 13	.002
BMI (kg/m^2)	54.5 ± 3.9	32.0 ± 1.8	.001
Cholesterol (total)	171 ± 7	114 ± 9	.0002
LDL cholesterol	101 ± 6	65 ± 12	.008
HDL cholesterol	40 ± 2	50 ± 2	.002
TG (total)	157 ± 26	63 ± 9	.04
Apo A-I (mg/dL)	146 ± 9	151 ± 7	.74
Apo A-II (mg/dL)	44 ± 3	41 ± 6	.66
Apo B (mg/dL)	73 ± 3	44 ± 6	.0002
Apo C-III (mg/dL)	12 ± 1	7 ± 1	.016
Glucose (mg/dL)	105 ± 5	84 ± 4	.03
Insulin (μU/mL)	20 ± 2	5 ± 0.9	.0002
FFA (μEq/mL)	889 ± 54	674 ± 98	.047
FFA (post-OGTT)	611 ± 44	285 ± 75	.002
Insulin sensitivity index[b]	0.027 ± 0.008	0.220 ± 0.094	<.001
AST (U/L)	23.0 ± 3.5	26.2 ± 3.0	.57
ALT (U/L)	35.2 ± 5.0	32.4 ± 4.6	.73

Anthropometric measures, plasma lipoproteins, hepatic transaminase, and an index of insulin sensitivity were assessed in MO before GBP and in another group of women undergoing ventral hernia repair after MWL following GBP. Data are mean ± SEM for demographic, anthropometric, and metabolic parameters. AST indicates aspartate aminotransferase; ALT, alanine aminotransferase.

[a] Significance of differences between obese and lean participants determined by Student *t* test for continuous variables and χ^2 (Fisher exact test) for noncontinuous variables (JMP).

[b] Whole-body insulin sensitivity (glucose utilization) was estimated based on OGTT insulin and glucose determinations as described by Matsuda and DeFronzo [24].

levels of the HDL apoproteins, apo A-I and apo A-II, were not significantly different between MWL and MO (Table 2). Thus, reduced HDL cholesterol appeared to result from reduced cholesterol content of HDL particles rather than reduced particle number. In summary, the comparison between MWL and MO indicated a significant elevation in plasma levels of apo B–containing lipoproteins, in particular TG-rich particles (VLDL, intermediate-density lipoprotein), and LDL and decreased cholesterol content of HDL in MO.

Dietary analysis confirmed higher daily intake of total calories in MO women compared with those who had previously undergone GBP (2268 vs 1604 cal/d, respectively; P = .012). Although total intake of protein, fat, and carbohydrate was lower in MWL subjects, the most striking change in their diet was a marked reduction in carbohydrate intake (157 g/d in MWL vs 272 g/d in MO, P = .003). In particular, daily intake of simple sugars was dramatically reduced in MWL from 19.7% of total calories in MO to 10.9% (P = .02).

3.2. GBP effectively reverses insulin resistance and hyperinsulinemia in MO women

We assessed the glycemic status and insulin levels in MO and MWL patients both in the fasting state and via an OGTT that was administered after overnight fasting. As shown in Table 2, both fasting plasma glucose and insulin levels were higher in MO subjects; and insulin sensitivity was significantly reduced (Table 2). After oral glucose challenge, MO subjects exhibited significant postprandial hyperglycemia and hyperinsulinemia with elevated 2-hour levels of glucose vs MWL (165 vs 75 mg/dL, respectively) and insulin (125 vs 30 mIU/mL, respectively) (Fig. 1). Both fasting and postprandial levels of C-peptide were also higher in MO, indicating increased insulin secretion (Fig. 1). There was an almost 10-fold difference in insulin sensitivity (an index of whole-body glucose disposal) between MO and MWL groups as estimated by the method of Matsuda and DeFronzo [24] (0.028 ± 0.008 vs 0.220 ± 0.094, respectively) (Table 2). Fasting plasma levels of FFA were higher and the reduction in plasma FFA after OGTT was attenuated in MO subjects, indicating impaired suppression of lipolysis by insulin (Table 2).

3.3. Hepatic expression of genes related to fatty acid metabolism and metabolic signaling: results of microarray analysis

We have recently defined the hepatic transcriptome of MO and MWL women using Affymetrix gene arrays and identified a number of genes that potentially linked the susceptibility of MO women to a wide range of diseases [23]. Because of the stringent selection criteria needed for this initial transcriptomewide analysis, this prior analysis did not provide information on key genes related to lipid synthesis and metabolic signaling. For the present study, we reinterrogated the hepatic transcriptome database to focus on a number of genes with well-known involvement in the regulation of metabolic signaling and fatty acid and lipid biosynthesis. This analysis indicated that a panel of genes regulating fatty acid oxidation was expressed at a higher

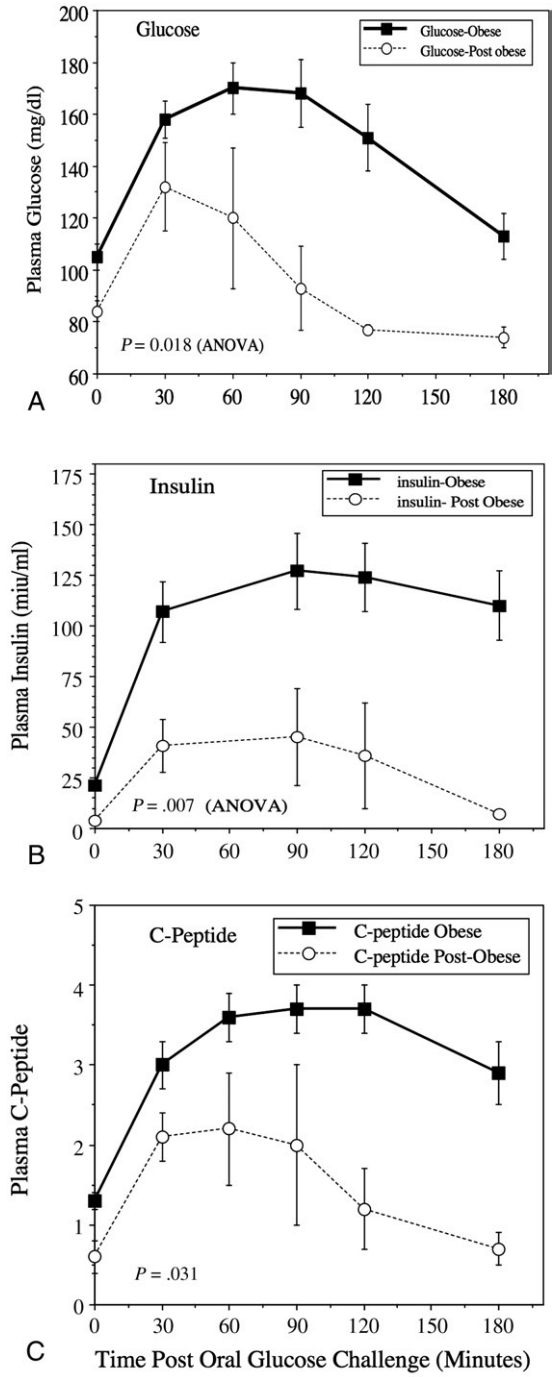

Fig. 1. Weight loss after GBP effectively reverses abnormal glucose and insulin tolerance after oral glucose challenge in MO women. Data are mean ± SEM of (A) plasma glucose, (B) insulin, and (C) C-peptide after OGTT in MO (obese) women undergoing GBP and in those undergoing ventral hernia repair after experiencing significant weight loss (postobese) after prior GBP. Significance of differences between responses to oral glucose challenge was assessed by 2-way analysis of variance (group vs time).

Table 3
Hepatic expression of genes related to fatty acid metabolism and metabolic signaling in liver biopsy samples of MO and MWL women as assessed by Affymetrix microarray analysis

Affymetrix ID	Symbol	Gene Name	Fold change (MO/MWL)	*P*
218285_s_at	*BDH2*	3-Hydroxybutyrate dehydrogenase type 2	1.35	.014
211569_s_at	*HADH*	Hydroxyacyl-coenzyme A dehydrogenase	1.67	.009
203658_at	*SLC25A20*	Carnitine/acylcarnitine translocase	1.26	.037
201135_at	*ECHS1*	Enoyl coenzyme A hydratase, short chain, 1	1.51	.001
202025_x_at	*ACAA1*	Peroxisomal 3-oxoacyl-coenzyme A thiolase	1.38	.031
204059_s_at	*ME1*	Malic enzyme 1	1.47	.038
220029_at	*ELOVL2*	Elongation of very long chain fatty acids	0.34	.021
204686_at	*IRS1*	Insulin receptor substrate 1	1.31	.023
209541_at	*IGF1*	Insulin-like growth factor 1 (somatomedin C)	0.64	.028
209542_x_at	*IGF1*	Insulin-like growth factor 1 (somatomedin C)	0.41	.001
211354_s_at	*LEPR*	Leptin receptor	0.49	.002
211355_x_at	*LEPR*	Leptin receptor	0.50	.005
203372_s_at	*SOCS2*	Suppressor of cytokine signaling–2	0.32	.003
203373_at	*SOCS2*	Suppressor of cytokine signaling–2	0.26	.002
201331_s_at	*STAT6*	Signal transducer and activator of transcription 6	0.71	.008
205698_s_at	*MAP2K6*	Mitogen-activated protein kinase-kinase–6	1.9	.011
201627_s_at	*INSIG1*	Insulin-induced gene–1	1.1	.722
209566_at	*INSIG2*	Insulin-induced gene–2	0.7	.165
212329_at	*SCAP*	SREBP cleavage activating protein	0.9	.277

Gene expression was assessed in liver biopsy samples from MO women undergoing GBP (n = 13) and women undergoing ventral hernia repair following MWL after previous GBP (n = 5) using Affymetrix oligonucleotide gene arrays (HG133A). Genes related to fatty acid metabolism and metabolic signaling were identified from the gene expression data set and assessed for significance of differences in signal intensity between MO and MWL by unpaired Student *t* test (JMP). Genes detected by multiple probe sets are presented individually.

level in livers of MO subjects (Table 3). These included enzymes mediating mitochondrial β-oxidation including hydroxyacyl-coenzyme A dehydrogenase and enoyl coenzyme A hydratase. Similarly, peroxisomal 3-oxoacyl-coenzyme A thiolase and the ketogenic enzyme 3-hydroxybutyrate dehydrogenase, type 2, were also up-regulated in MO (Table 3). Expression of the lipogenic enzyme ME1 was increased in MO (Table 3). Conversely, expression of the fatty acid–modifying enzyme elongation of very long-chain fatty acids (ELOVL2) was reduced in MO as compared with MWL (Table 3). Although expression of IRS1 was modestly increased in MO livers, expression of IRS2 and expression of mRNA encoding the insulin receptor were unaltered (data not shown). On the other hand, expressions of key genes underlying signal transduction pathways evoked by insulin-like growth factor (IGF)–1 (or somatomedin C), leptin (leptin receptor), and proinflammatory cytokines (SOCS2 and STAT6) were differentially regulated in livers of MO and MWL subjects (Table 3).

3.4. Hepatic expression of genes related to fatty acid metabolism and metabolic signaling: results of real-time PCR analysis

We noted that a number of genes known to be critically involved in lipid synthesis and metabolic signaling were absent from our Affymetrix microarray-derived transcriptome. Therefore, to extend the microarray experiments, we further analyzed expression of a subset of genes by real-time PCR. This analysis demonstrated that, in addition to ME1, an entire panel of insulin- and SREBP-1c–responsive genes that mediate both de novo lipogenesis and TG synthesis was expressed at higher levels in livers of MO subjects (Table 4). These include ACC-1, *FASN*, DGAT, and SCD-1. Based on the observed up-regulation of these SREBP-1c–dependent genes and previous findings of increased SREBP-1c mRNA expression in livers of obese rodents [10,11], we expected to see increased SREBP-1c mRNA expression in livers of MO subjects. Unexpectedly, expression of neither total SREBP-1 (1a plus 1c) mRNA nor that encoding the SREBP-1c isoform was increased in livers of MO subjects (Table 4). Expression of mRNA encoding the cholesterol-regulating SREBP isoform SREBP-2 was higher in MO; however, expression of its target gene, hydroxymethylglutaryl-coenzyme A reductase (HMGR),

Table 4
Hepatic expression of genes related to fatty acid metabolism and metabolic signaling in liver biopsy samples of MO and MWL women as assessed by quantitative real-time PCR

Gene name	Symbol	Fold change (MO/MWL)
Diacylglycerol acyltransferase	*DGAT*	2.51
Stearoyl-coenzyme A desaturase–1	*SCD-1*	2.66
Acetyl-coenzyme A carboxylase–1	*ACC-1*	1.34
Fatty acid synthase	*FASN*	2.67
Hydroxymethylglutaryl coenzyme A reductase	*HMGR*	0.87
Sterol regulatory element binding protein–1	*SREBP-1*	0.91
Sterol regulatory element binding protein–1c	*SREBP-1c*	0.82
Sterol regulatory element binding protein–2	*SREBP-2*	1.89
Peroxisomal proliferator activated receptor–α	*PPARα*	0.89
Peroxisomal proliferator activated receptor–γ	*PPARγ*	0.66
PPARγ coactivator-1α	*PGC-1α*	0.67
Mitogen-activated protein kinase-kinase–6	*MAP2K6*	6.11
Suppressor of cytokine signaling–3	*SOCS-3*	0.96

Data are relative expression of selected genes related to fatty acid metabolism and metabolic signaling determined by real-time PCR in liver biopsy samples derived from women undergoing GBP for morbid obesity (n = 7-12) and in those undergoing ventral hernia repair after MWL following GBP (n = 3-6). Real-time PCR was carried out using primer sets outlined in Table 1. Primer sets used to analyze MAP2K6 and SOCS-3 were obtained from Qiagen. For SREBP-1c, primers were used that identified both 1a and 1c isoforms (SREBP-1) and that were specific for the 1c isoform (SREBP-1c).

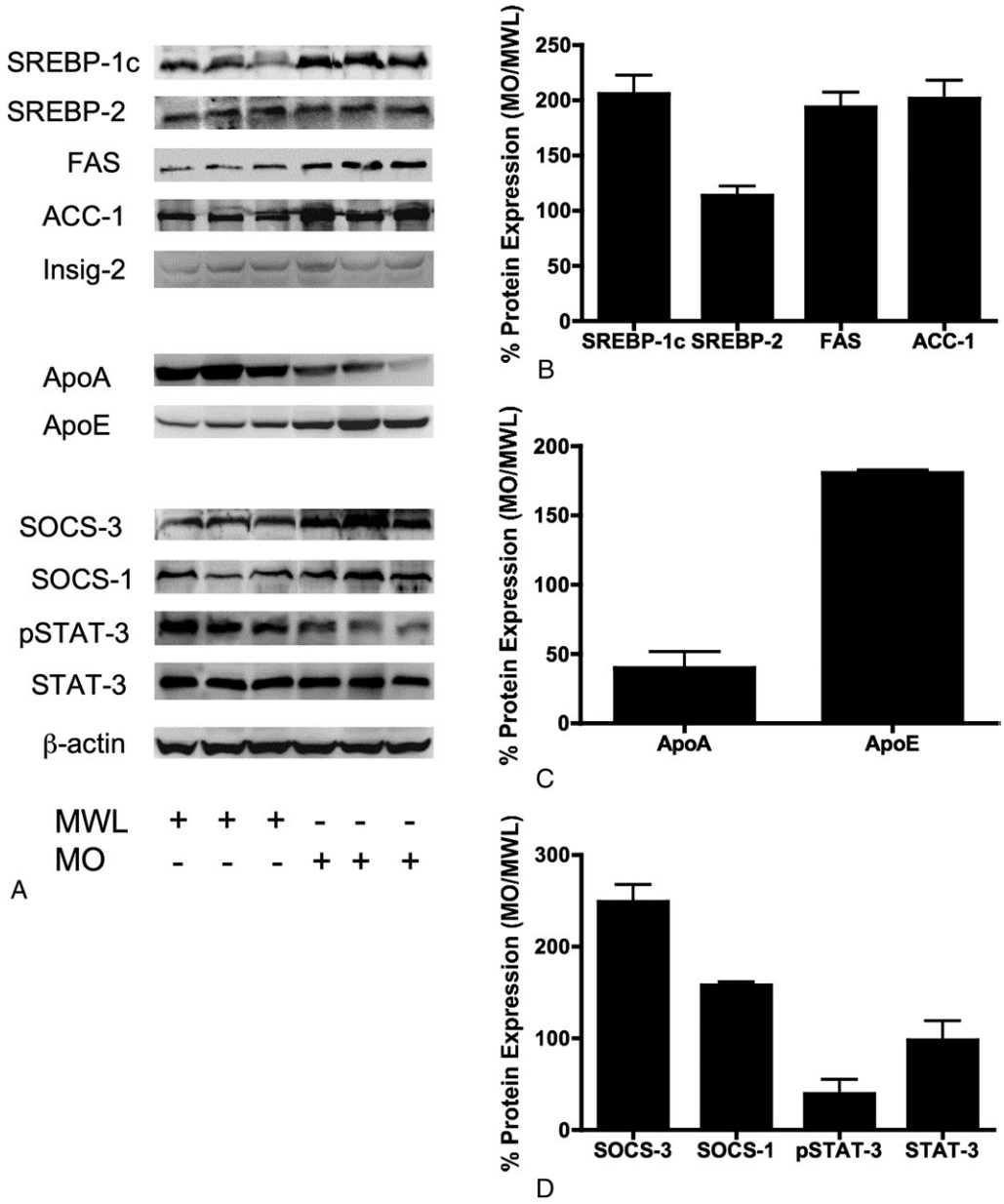

Fig. 2. Expression of hepatic proteins related to lipid metabolism and metabolic signaling in livers of MO and MWL patients. A, Data are Western blots of total cell lysate from liver of MO (n = 11) and MWL (n = 4) subjects. Representative Western blots from 6 subjects are shown. Blots show expression of SREBP-1c/2, FAS, ACC-1, INSIG-2, apo A-1, apo E, SOCS-1, SOCS-3, STAT-3, and pSTAT3. Results of densitometry scanning for lipogenic genes (B), apo genes (C), and STAT signaling genes (D). *$P < .05$ MO vs MWL.

was not elevated (Table 4). Although expression of a panel of peroxisomal proliferator activator (PPAR)α–dependent genes related to fatty acid oxidation was somewhat increased (Table 3), that of PPARα itself was not (Table 4). Conversely, expression of PPARγ and that of the coactivator PGC-1α were reduced in livers of MO subjects (Table 4).

In the microarray analysis (Table 3), expression of mRNA encoding the signaling proteins SOCS2 and STAT6 was significantly lower and expression of mitogen-activated protein kinase-kinase–6 (MAP2K6) was higher in MO livers; however, SOCS-3 was not detected on the microarray. Based on the observations of Ueki et al [28] and Bode et al [29], both SOCS-3 and MAP2K6 appear to play a central role in development of hepatic insulin resistance in obesity. Therefore, we examined mRNA expression of SOCS-3 and MAP2K6 using real-time PCR (Table 4). Concordant with the microarray findings, expression of MAP2K6 was 6-fold higher in livers of MO subjects (Table 4). Conversely, mRNA expression of the SOCS-3 isoform, a known negative regulator of insulin signaling [20], was unaltered in MO as compared with MWL subjects (Table 4).

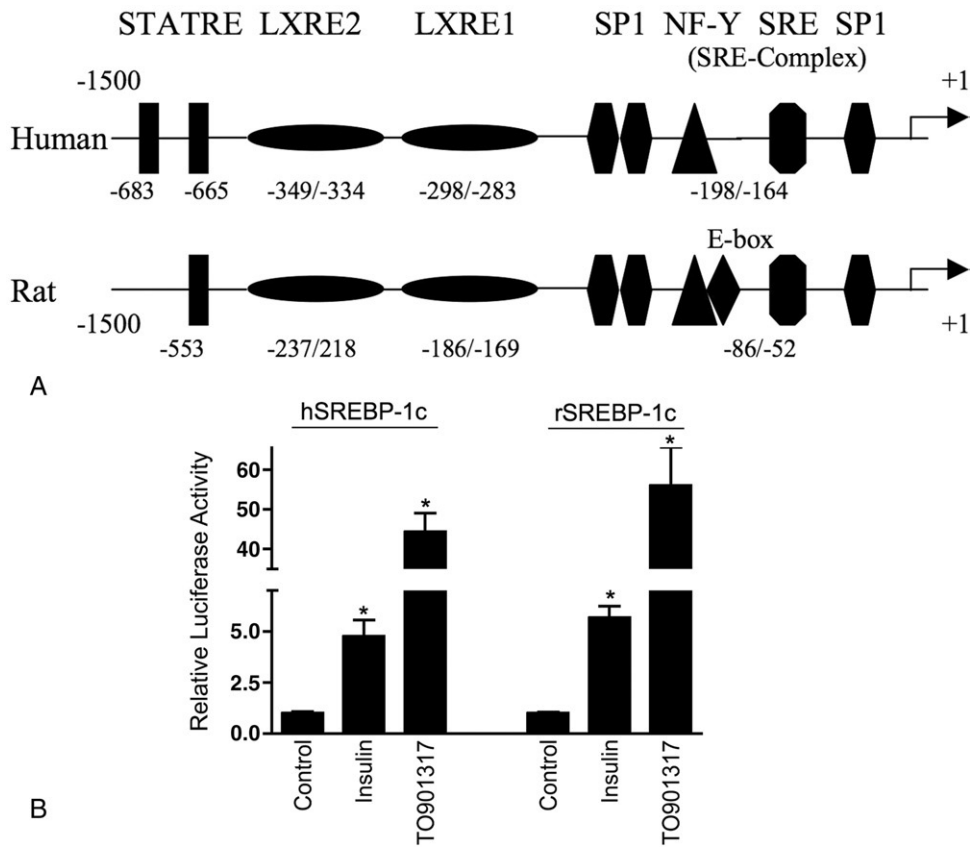

Fig. 3. The human SREBP-1c promoter is responsive to insulin treatment in vitro. A, A schematic representation of the major *cis*-acting elements of the human and rat SREBP-1c promoter. The transcription initiation site of the human promoter, determined by TRANSFAC analysis, was designated as *+1*. Insulin-mediated activation of the human SREBP-1c promoter may involve a number of binding sites known to regulate the rat and mouse promoter. These include the following: STATRE, signal transducer and activator of transcription response element; LXRE, liver X receptor response element; SRE, sterol regulatory element; SP1, specificity protein-1; NF-Y, nuclear factor-Y. The locations of these *cis*-acting elements are not depicted to scale on the promoter models. B, Primary rat hepatocytes were transfected with either human (hSREBP-1c) or rat (rSREBP-1c) SREBP-1c promoter-luciferase reporter constructs and incubated with and without insulin (100 nmol/L) or LXR agonist TO901317 (10 μmol/L) for 24 hours. Luciferase activity was measured as described in "Experimental procedures." Both human and rat SREBP-1c promoter constructs responded comparably to both insulin and TO901317. Data are mean ± SEM of luciferase activity relative to untreated (control) hepatocytes (n = 12 determinations from 4 separate hepatocyte preparations). $P < .05$ vs control.

3.5. Effect of morbid obesity on expression of genes related to fatty acid metabolism and metabolic signaling: results of Western blot protein expression analyses

Because many genes are regulated by posttranscriptional mechanisms, we extended the microarray and real-time PCR analysis by assessing expression of proteins transcribed from these key genes by Western blotting of liver proteins from MO and MWL subjects. Insulin is known to accelerate proteolytic processing of nascent SREBP-1c, thereby increasing nuclear content of its transcriptionally active n-terminal fragment [14,15]. Although we were not able to detect differential expression of SREBP-1c mRNA in livers of MO and MWL, expression of the n-terminal protein fragment of SREBP-1c (nSREBP-1c) was 2-fold higher in livers of MO. Similarly, protein expression of the down-stream lipogenic enzyme targets of SREBP-1c, FAS and ACC-1, was also increased in MO (Fig. 2A, B). In contrast, protein expression of the cholesterol-regulating isoform, SREBP-2, was unaltered in MO subjects (Fig. 2A, B).

Before its transport to the Golgi and release of its n-terminal fragment by proteolytic processing, ER-bound nascent full-length SREBP-1c is associated with the chaperone protein SCAP (SREBP cleavage activating protein) and the ER retention protein insulin-induced gene (Insig)–2a [16]. Insulin is thought promote the ER to Golgi transport and subsequent proteolytic processing of SREBP-1c by reducing levels of Insig-2a [30,31]. The observation of increased nSREBP-1c in livers of MO prompted examination of the microarray database for expression of these SREBP-1c–associated proteins. Although expression of the insulin-responsive gene isoform INSIG-2 trended lower in MO, a condition that would be expected to favor enhanced proteolytic processing, this did not achieve statistical significance (Table 3). In addition, expression of Insig-2a protein was unaltered in livers of MO subjects (Fig. 2A). Furthermore, expression of mRNA encoding SCAP and Insig-1 was comparable in livers of MO and MWL subjects (Table 3).

Importantly, we found that hepatic expression of SOCS-1 and SOCS-3 proteins was increased in MO (1.8-fold and 2.5-

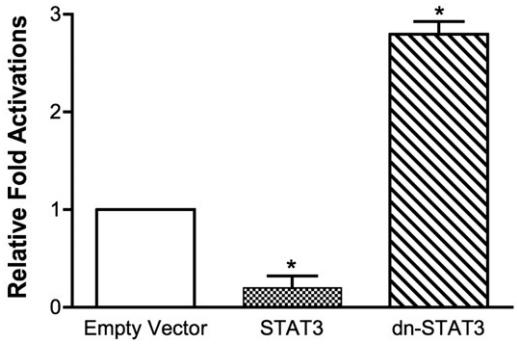

Fig. 4. Signal transducer and activator of transcription 3 represses the human SREBP-1c promoter. Primary rat hepatocytes were transfected with hSREBP1c-luc DNA along with plasmids expressing wild-type or dominant negative STAT3 proteins, or empty vector. Twenty-four hours after, transfection cells were harvested; and the luciferase activity was measured. Cells were cotransfected with a control *Renilla* expression plasmid, pRL-TK; and transfection results were normalized to *Renilla* luciferase activity. The results represent the mean ± SEM for 5 independent experiments, with fold induction over the level observed with the reporter construct alone.

fold higher, respectively) (Fig. 2A, D). Although STAT-3 protein levels were unaltered in MO, STAT-3 signaling was, in fact, attenuated as evidenced by reduced levels of active (phosphorylated) form pSTAT-3 (Fig. 2A, D).

Of additional interest, although microarray analysis showed that expression of mRNA encoding major apoproteins including apo A-I, apo A-II, apo B, apo C-II, apo E, and apo C-III was unchanged (data not shown), protein expression of 2 apoproteins, apo A-I and apo E, was reduced and increased, respectively, in livers of MO (Fig. 2A, B). Despite repeated attempts, we were not able to measure expression of apo B or apo Cs by Western analysis.

3.6. The human SREBP-1c promoter is responsive to insulin and LXR agonists

As stated earlier, the finding of comparable levels of mRNA encoding SREBP-1c in livers of MO vs MWL subjects was both unexpected and discordant with both prior animal studies and the Western blot findings of enhanced expression of nSREBP-1c protein and both mRNA and protein of its downstream lipogenic targets in MO. Although this may reflect rapid decay of SREBP-1c mRNA during the obligatory overnight fast required as preparation for surgery in our subjects, we also considered the possibility that these findings may reflect attenuated response of the SREBP-1c promoter to insulin in the human as compared with the rodent. The human SREBP-1c promoter has been cloned and found to be responsive to insulin treatment when transfected into HEK-293 cells [13]; however, its response to insulin has not been examined relative to the rodent ortholog in primary hepatocyte cultures. We therefore compared the response of human and rat SREBP-1c-luciferase reporter constructs to insulin and the LXR ligand T0901317 in transient transfection of primary (rat) hepatocytes. Although there are minor differences in spatial organization of the rat and human SREBP-1c promoter, the major response elements are preserved (Fig. 3A). Accordingly, the human SREBP-1c promoter exhibited comparable response to both insulin (5- to 8-fold induction) and T0901317 (45- to 55-fold induction) as the rat promoter (Fig. 3B). Thus, the human SREBP-1c promoter is highly responsive to insulin.

3.7. The human SREBP-1c promoter is negatively regulated by STAT3

Based on our in vivo observations of differential hepatic regulation of SOCS1 and 3 as well as pSTAT3 in MO humans, together with the findings of the animal studies of Ueki et al [28] as well as those of Endo et al [32], we reasoned that the corresponding changes in the expression of SREBP-1c protein and attenuated JAK/STAT signaling in MO and WL patients may be mechanistically related. To directly test this hypothesis, we transfected primary hepatocytes with full-length hSREBP-1c-luc either alone or cotransfected with vectors designed to express wild-type STAT3 or dominant negative STAT3. As shown in Fig. 4, coexpression of wild-type STAT3 led to a 5-fold decrease in luciferase expression driven by the SREBP-1c promoter, indicating repression of SREBP-1c transcription by STAT3. Conversely, mimicking the effect of SOCS-3 to suppress pSTAT3 via exogenous expression of a dominant negative form of STAT3 significantly increased hSREBP-1c promoter activity (Fig. 4). These data support the hypothesis that SOCS-mediated down-regulation of JAK/STAT signaling may be mechanistically related to up-regulation of SREBP-1c expression in obesity.

4. Discussion

Morbid obesity is associated with insulin resistance and hypertriglyceridemia that results, at least in part, from increased synthesis and secretion of VLDL by the liver [33,34]. We report here that expression of the lipogenic regulator SREBP-1c and its downstream targets that mediate both de novo lipogenesis and TG synthesis is significantly increased in livers of MO women. These findings support the hypothesis that morbid obesity leads to a widespread up-regulation of enzymes mediating synthesis of fatty acids and TG, as previously observed in obese animal models [11,35].

Until recently, the prevailing opinion was that, under most circumstances, plasma FFAs were the predominant source of FAs for VLDL-TG and that the rate of delivery of FAs to the liver from exogenous sources (dietary and lipolysis) was the major determinant of VLDL-TG secretion in the human [36]. However, recent in vivo metabolic turnover studies have shown that enhanced de novo lipogenesis assumes a greater role in overproduction of VLDL-TG in the obese human [6-9,33,37]. Increased de novo lipogenesis and enhanced VLDL-TG secretion were also seen in hyperinsulinemia caused by hypercaloric carbohydrate feeding in humans [38]. We show here that

expression of both mRNA and protein of enzymes mediating de novo lipogenesis is, in fact, increased in livers of obese women. Thus, enhanced VLDL-TG production in obesity and carbohydrate feeding in humans also result from enhanced de novo lipogenesis. Reminiscent of findings in the obese rat [35], we observed modest increases in hepatic expression of mRNAs encoding enzymes mediating fatty acid oxidation in MO as compared with MWL. This likely represents an adaptive response to increased dietary intake of fat and carbohydrate. Because SREBP-1c strongly up regulates the enzymes that incorporate fatty acid into TG [39], one would predict, however, that the predominant effect would be preferential utilization of fatty acids for esterification into TG. Increased expression of these PPARα-dependent genes was not related to increased PPARα expression but rather appears to result from increased ligand-dependent activation of PPARα. Conversely, expression of PPARγ and PGC-1α was reduced in livers of MO, similar to the previous observations of reduced levels of PGC-1α in skeletal muscle of insulin-resistant diabetic humans [40]. The significance of reduced expression of PPARγ in livers of MO is not entirely clear.

Interestingly, we did not detect increased expression of mRNA encoding SREBP-1c in MO. This is in striking contrast to prior findings of enhanced SREBP-1c expression in liver of obese rodents [10,11,35]. This finding may be related to a significant difference in nutritional state of the subjects in the present study as compared with that in prior animal studies. In the present study, liver biopsy samples were obtained after an overnight fast required as part of preparation for surgery in humans, whereas in prior animal studies liver samples were collected in the fed state. Under fasting conditions, mRNA transcripts with short half-life such as SREBP-1c (6-8 hours) will have rapidly declined. Insofar as the human and rodent SREBP-1c promoters are structurally similar and exhibit comparable response to insulin in vitro, it is likely that our findings reflect the nutritional state of our subjects. On the other hand, it is possible that up-regulation of SREBP-1c in the human may be more tightly linked to enhanced posttranslational proteolytic processing, which is also insulin dependent [15]. In addition, lipogenic enzymes (ie, FAS) may be induced in the presence of hyperglycemia independent of the effects of insulin and SREBP-1c via the carbohydrate response element [41].

A second major finding of the present study was the presence of increased expression of the inhibitory signaling protein SOCS-3 and the reduced levels of its target, phosphorylated STAT3, in MO. These findings are highly significant in light of studies of Ueki et al [21] who reported that hepatic expression of SOCS-1 and SOCS-3 was elevated in livers of obese, insulin-resistant mice and that exogenous expression of either SOCS-1 or SOCS-3 proteins induced both insulin resistance and SREBP-1c expression. Our findings of concomitant up-regulation of SOCS-3 with decreased STAT-3 phosphorylation in livers of MO humans combined with the demonstration that the human SREBP-1c promoter is negatively regulated by STAT3 strengthen the putative mechanistic link between SOCS-3–induced impaired JAK/Stat signaling and enhanced SREBP-1c expression in obesity. In this regard, our finding of increased hepatic expression of the dual-specificity protein kinase *MAP2K6* in MO subjects is particularly intriguing because *MAP2K6* phosphorylates p38 MAP kinase in response to inflammatory cytokines or environmental stress and may induce insulin resistance by a similar mechanism in obesity [29].

Although some alterations in expression of genes related to lipid metabolism in MO may be attributed to hyperinsulinemia, it is likely that many observed differences may also arise from alterations in the nutritional and hormonal milieu that accompanies morbid obesity. For example, the dietary intake of the 2 groups was very different. In addition to reduced overall caloric intake, MWL subjects exhibited a markedly reduced intake of sucrose and fructose as has been reported previously [42]. Higher intake of simple sugars in general and fructose in particular in MO is significant in view of the known effects of fructose on hepatic lipogenesis and insulin resistance [43] and SREBP-1c gene expression [44].

Altered expression of enzymes related to modification of fatty acids, SCD-1 and the fatty acid elongase ELOVL2, in livers of MO is also of considerable interest. Our finding of higher expression of SCD-1 is reminiscent of earlier observations of increased SCD expression in livers of obese hyperinsulinemic *ob*/*ob* mice [10] and may be important in view of the postulated role of this enzyme in insulin insensitivity and hepatic lipid synthesis in obesity [45]. Hepatic expression of the gene ELOVL2 that encodes an enzyme that mediates elongation of 20- and 22-carbon long-chain polyunsaturated fatty acids [46] was reduced in MO subjects. This, together with the reported linkage of the related gene, ELOV16, with insulin resistance in rodents [47], suggests a potential role for altered ELOVL2 in the metabolic complications of obesity in humans.

The general implications of the data presented here must be tempered by a number of caveats arising from the inherent limitations of our study. First, it must be noted that GBP surgery is reserved for individuals with an extreme form of obesity. Second, although the MWL subjects had experienced significant weight loss and resolution of metabolic abnormalities after GBP, their BMI was not normal. Third, although many changes in gene expression can be attributed to amelioration of insulin resistance and hyperinsulinemia after GBP, many changes may be due to additional effects of weight loss and altered dietary intake.

This cautionary note notwithstanding, we conclude that enhanced de novo synthesis of lipids is critically involved in the dyslipidemia of obesity in humans. We further postulate that enhanced expression of SREBP-1c and its downstream gene targets is mechanistically linked to SOCS-mediated attenuation of STAT signaling in morbid obesity.

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