SUPPLEMENTAL METHODS.

Experimental Procedures.

Subjects were admitted to the GCRC at about 1800 h and consumed a meal containing ~8 kcal/kg body weight, which contained 55% of total energy as carbohydrates, 30% as fat, and 15% as protein. The following morning, after subjects fasted overnight (12 h), blood samples were obtained to determine plasma glucose and insulin concentrations. Blood obtained to determine plasma glucose concentrations was immediately centrifuged and analyzed by using an automated glucose analyzer (Yellow Springs Instruments Inc., Yellow Springs, OH). Blood was collected in chilled tubes containing EDTA and aprotinin (Trasylol) to determine insulin concentrations. Insulin resistance (IR) was calculated by using the homeostasis model assessment [HOMA-IR = fasting glucose (mg/dL) x fasting insulin (μU/mL) / 17.7 / 22.5] (1).

Subcutaneous abdominal adipose tissue biopsies were obtained after blood samples were collected. The skin was cleaned and draped with a sterile towel. After anesthetizing the skin and subcutaneous fat with 1% lidocaine, adipose tissue was aspirated from the periumbilical area by using a 14-gauge needle connected to a 5-cc syringe. Adipose tissue was immediately rinsed in sterile saline, frozen in liquid nitrogen, and stored at -80°C until RNA extraction.

Gastric bypass surgery was performed the day after blood and tissue samples were obtained. Liver tissue was obtained by needle biopsy during the surgical procedure, before gastric stapling and intestinal resection were performed. Hepatic tissue was rinsed in sterile saline, immediately frozen in liquid nitrogen, then stored at -80°C until RNA extraction.

One year after GBS, 11 subjects were re-admitted to the GCRC, where blood samples and adipose tissue biopsies were obtained by using the same procedures performed before surgery. Percutaneous liver biopsies were obtained by using a 15-gauge, suction needle technique with physical examination guidance.

HepG2 studies. For PGC-1α and lipin 1β overexpression in HepG2 cells, previously-described adenoviral constructs were employed (2; 3). Briefly, a *myc*-tagged (C-terminus) mouse PGC-1α cDNA or an HA-tagged (N-terminus) lipin 1β cDNA was cloned into the Ad-track CMV vector. The Ad-track CMV vector drives expression of a green fluorescent protein (GFP) cDNA and the cDNA of interest through distinct CMV promoters (4). The Ad-track construct was then recombined into the Ad-EASY system, transfected into 293 cells, and viral particles harvested as specified (4).

Sub-confluent cultures of HepG2 cells were maintained in DMEM with 10% fetal calf serum (FCS). HepG2 cells were seeded at 75% confluence in 6-well culture dishes. Upon reaching 90% confluence (approximately 18 h), cells were infected with 1000 particles per cell of adenovirus driving expression of PGC-1α and GFP, lipin 1β and GFP, or GFP only (control). Twenty-four hours later, the medium was replaced with new DMEM/10% FCS. RNA was collected 48 h later. For studies involving insulin treatment, hepatocytes were cultured in DMEM/10% FCS containing vehicle (100 nM bovine serum albumin) or bovine insulin (100 nM) from time of infection until total cellular RNA was collected.

Sample Analyses

RNA isolation. Total RNA was isolated from adipose tissue using the TRI-REAGENT protocol (Sigma Chemical Co.) with an additional centrifugation step to

remove excess triglyceride (according to the manufacturer's instructions). For isolating RNA from liver tissues or cultured HepG2 hepatocytes, RNAzol B (Tel-test) was used according the manufacturers instructions.

Real-time RT-PCR and lipin 1 splice variant PCR. Real-time RT-PCR was performed using Applied Biosystems (Foster City, CA) reverse transcription (RT) kit, PowerSYBR PCR mix, and the ABI PRISM 7500 sequence detection system. Arbitrary units of target gene mRNA were corrected to 36B4 RNA content to control for loading. The primers used in these analyses can be found in Supplemental Table 1. To distinguish the lipin 1α and 1β splice variants (Figure 1A), the method of Peterfy et al (5) was adapted for use with human RNA using primers found in Supplemental Table 1. For studies designed to detect the presence of the splice variants only, RT was pooled (4 obese individuals per pooled sample).

1. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419, 1985
2. Finck BN, Gropler MC, Chen Z, Leone TC, Croce MA, Harris TE, Lawrence JC, Jr., Kelly DP: Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. *Cell Metab* 4:199-210, 2006
3. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP: Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* 106:847-856, 2000
4. He TC, Zhou S, da Costa LT, Yu J, Kinzler KW, Vogelstein B: A simplified system for generating recombinant adenoviruses. *Proc Natl Acad Sci U S A* 95:2509-2514, 1998
5. Peterfy M, Phan J, Reue K: Alternatively spliced lipin isoforms exhibit distinct expression pattern, subcellular localization, and role in adipogenesis. *J Biol Chem* 280:32883-32889, 2005

Supplemental Table 1. Body weight and metabolic characteristics of the study subjects (n=27).

	Mean	Range
Weight (kg)	179.6 ± 6.9	117.9-262.0
BMI (kg/m²)	60.8 ± 2.0	41.5-80.6
Insulin μU/mL	30.1 ± 2.9	2.9-61.9
HOMA-IR	8.1 ± 0.9	0.59-20.8

Mean values are ± SEM.
HOMA-IR = Homeostasis model assessment of insulin resistance.

Supplemental Table 2. Body weight and metabolic characteristics of the GBS study subjects (n=11).

	Before GBS	1 year after GBS
Weight (kg)	167.2 ± 15.8	108.3 ± 10.4†
BMI (kg/m²)	59.1 ± 4.8	38.5 ± 3.85†
Insulin μU/mL	34.7 ± 9.1	9.0 ± 1.7†
HOMA-IR	10.1 ± 3.08	2.0 ± 0.4*

Values are means ± SEM.
HOMA-IR = Homeostasis model assessment of insulin resistance.
Value significantly different from Before gastric bypass surgery value, *$p<0.02$, †$p<0.005$

Supplemental Table 3. RT-PCR primer sequence

Primer	
lipin 1 fwd:	AGTCAGCCTCATACCCTAATTCGG
lipin 1α rev:	GAACCGGAAGGACTGGGAGTG
lipin 1β rev:	GGCAAGAACTAGACAGACCTCCCT
lipin 1 splice rev:	GCTCAATGGGCTGGACTCTTTCATC
PGC-1α fwd:	TGAGGACTGCTAGCAAGTTTG
PGC-1α rev:	AGTGACCAATCAGAAATAATATCCAATC
SDHA fwd:	TGGGAACAAGAGGGCATCTG
SDHA rev:	CCACCACTGCATCAAATTCATG
MCAD fwd:	CTGTGCCAGCCCAGAACACT
MCAD rev:	TGACCAGCCCAAAGGAGAAG
36B4 fwd:	GCAGACAACGTGGGCTCCAAGCAGAT
36B4 rev:	GGTCCTCCTTGGTGAACACGAAGCCC

Supplemental Figure 1