Supplementary Materials and Methods

Subjects

Sixteen obese subjects (14 women and 2 men; age, 42.0 ± 10.8 y), scheduled to undergo bariatric surgery at Barnes-Jewish Hospital in St Louis, MO, participated in this study (Supplementary Table 1). All subjects completed a comprehensive medical evaluation, which included a detailed history and physical examination, routine blood tests, a 2-hour oral glucose tolerance test, and assessment of body composition by using dual-energy X-ray absorptiometry (iDXA; GE Lunar, Madison, WI). Intrahepatic triglyceride content was measured in 10 of the 16 subjects by using magnetic resonance spectroscopy (3T Siemens Magnetom Trio scanner; Siemens, Erlanger, Germany).[1] Potential participants who smoked cigarettes, consumed ≥20 g/d of alcohol, had diabetes, or severe organ dysfunction were excluded. All subjects had been weight-stable (≤2% change in body weight) and sedentary (exercise <1 h/wk) for at least 2 months before enrollment. Subjects were not advised to follow any special diet before surgery, and were instructed to maintain their usual weight throughout the study procedures until surgery (mean body weight, 141 ± 33, 141 ± 34, and 142 ± 34 kg at 9, 8, and 2 weeks before surgery). Subjects provided their written informed consent before participating in the study, which was approved by the Washington University Human Research Protection Office.

Experimental Protocol

Subjects were admitted to the Clinical Research Unit in the afternoon and were given a standard meal, containing approximately 12 kcal/kg fat-free mass (50% of total energy from carbohydrate, 30% from fat, and 20% from protein), which was consumed between 6:00 and 7:00 PM. The following morning, one catheter was inserted into a forearm vein to infuse stable isotopically labeled tracers (Cambridge Isotope Laboratories, Andover, MA), dextrose and insulin, and a second catheter was inserted into a radial artery to obtain blood samples. At approximately 6:00 AM, a primed-continuous infusion of [6,6-$^2H_2$]glucose (0.22 μmol/kg/min; priming dose, 22 μmol/kg) was started. At 9:30 AM, insulin was infused at a rate of 50 mU/m^2 body surface area (BSA)/min for 4 hours, initiated with a 2-step priming dose of 200 mU/m^2 BSA/min for 5 minutes and then 100 mU/m^2 BSA/min for 5 minutes. The infusion rate of [6,6-$^2H_2$]glucose was reduced by 50% during the insulin infusion to account for the expected insulin-mediated decrease in glucose production. Euglycemia was maintained at a blood glucose concentration of approximately 100 mg/dL by infusing 20% dextrose solution enriched to 2.5% with [6,6-$^2H_2$]glucose. Blood samples were obtained immediately before starting the tracer infusion and every 10 minutes during the final 30 minutes of the basal period and the insulin infusion period. After completing the clamp procedure, subjects underwent laparoscopic bariatric surgery (Roux-en-Y gastric bypass, adjustable gastric banding, or sleeve gastrectomy). During surgery, liver tissue was obtained by Tru-Cut needle biopsy before gastric stapling, intestinal anastomosis, or band placement. One sample of liver tissue was frozen immediately in liquid nitrogen and stored at −80°C until analysis of DAG, ceramide, and acylcarnitine contents. Another piece of tissue was placed in formalin for subsequent histologic analysis.

Sample Analyses

The tracer-to-tracee ratio of plasma glucose was determined by using gas chromatography–mass spectrometry.[2,3] Intrahepatic DAG, ceramide, and acylcarnitine contents were determined by using mass spectrometry.[4–6] Plasma concentrations of branched-chain amino acids (leucine, isoleucine, and valine) were measured by using a commercially available kit for free amino acid analysis by gas chromatography–mass spectrometry (EZ:faast; Phenomenex, Torrance, CA).[7] The concentrations of tumor necrosis factor α, interleukin 6 (both from R&D Systems, Minneapolis, MN), and total adiponectin (Millipore, Billerica, MA) in plasma were measured by using commercially available enzyme-linked immunosorbent assays. Liver biopsy specimens were evaluated for steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis according to a modification of a standardized histologic scoring system for NAFLD[8] by using H&E and Masson trichrome staining. The NAFLD activity score was calculated as the unweighted sum of the scores for steatosis, lobular inflammation, and ballooning.[8]

Calculations

Isotopic steady-state conditions were achieved during the final 30 minutes of the basal state and the insulin infusion period. Endogenous glucose Ra in plasma was calculated by dividing the glucose tracer infusion rate by the average plasma glucose tracer-to-tracee ratio during the last 30 minutes of the basal state and the insulin infusion period.[2,3] Hepatic insulin sensitivity was estimated as the relative suppression of glucose Ra during insulin infusion.[3,9]

Statistical Analysis

Data were examined for normality according to the Shapiro–Wilks criteria. Non-normally distributed variables were ranked for analyses. Data are presented as means ± SD for normally distributed variables and medians with quartiles for non-normally distributed variables. The Pearson correlation coefficient (on ranks, when necessary) was used to examine the relationships between specific variables. A 2-tailed P value ≤.05 was considered statistically significant.

Supplementary References

1. Frimel TN, et al. J Cardiometab Syndr 2007;2:136–138.
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4. Leiker TJ, et al. Int J Mass Spectrom 2011;305:103–109.
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7. Kugler F, et al. J Agric Food Chem 2006;54:4311–4318.
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Supplementary Table 1. Characteristics of the Study Subjects

Characteristic	Mean ± SD	Range
Body mass index, *kg/m²*	48.1 ± 9.3	37.1–68.6
Body fat mass, *% body weight*	52.0 ± 4.2	44.5–59.0
Fat-free mass, *kg*	64.3 ± 13.0	47.3–87.4
Glucose, *mg/dL*	95.3 ± 8.3	80.5–108.0
Insulin, *μU/mL*	20.0 ± 7.7	7.5–35.3
HOMA-IR score	4.8 ± 2.1	1.6–8.7
Free fatty acids, *μmol/L*	638 ± 154	437–892
Triglyceride level, *mg/dL*	143 ± 56	71–258
HDL cholesterol, *mg/dL*	40 ± 9	26–64
Total cholesterol, *mg/dL*	169 ± 32	114–210
LDL cholesterol, *mg/dL*	100 ± 21	59–130
Branched-chain amino acids, *μmol/L*	509 ± 116	271–724
Tumor necrosis factor α, *pg/mL*	1.63 ± 0.44	1.02–2.79
Interleukin 6, *pg/mL*	4.46 ± 2.48	1.58–9.88
Adiponectin, *μg/mL*	6.6 ± 3.3	1.6–13.7

HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.