

Effect of Weight Loss on Insulin Sensitivity and Intramuscular Long-Chain Fatty Acyl-CoAs in Morbidly Obese Subjects

Joseph A. Houmard¹, Charles J. Tanner¹, Chunli Yu,² Paul G. Cunningham¹, Walter J. Pories¹, Kenneth G. MacDonald¹, and Gerald I. Shulman²

Increases in intramyocellular long-chain fatty acyl-CoAs (LCACoA) have been implicated in the pathogenesis of insulin resistance in skeletal muscle. To test this hypothesis, we measured muscle (vastus lateralis) LCACoA content and insulin action in morbidly obese patients ($n = 11$) before and after weight loss (gastric bypass surgery). The intervention produced significant weight loss (142.3 ± 6.8 vs. 79.6 ± 4.1 kg for before versus after surgery, respectively). Fasting insulin decreased by $\sim 84\%$ (23.3 ± 3.8 vs. 3.8 ± 0.5 mU/ml), and insulin sensitivity, as determined by minimal model, increased by $\sim 360\%$ (1.2 ± 0.3 vs. 4.1 ± 0.5 $\text{min}^{-1} \cdot [\mu\text{U}/\text{kg}^{-1}]$) indicating enhanced insulin action. Muscle palmitoyl CoA (16:0; 0.54 ± 0.08 vs. 0.35 ± 0.04 nmol/g wet wt) concentration decreased by $\sim 35\%$ ($P < 0.05$) with weight loss, whereas stearate CoA (18:0; -17% ; 0.65 ± 0.05 vs. 0.54 ± 0.03 nmol/g wet wt) and linoleate CoA (18:2; -30% ; 2.47 ± 0.27 vs. 1.66 ± 0.19 nmol/g wet wt) were also reduced ($P < 0.05$). There were no statistically significant declines in muscle palmitoleate CoA (16:1), oleate CoA (18:1), or total LCACoA content. These data suggest that a reduction in intramuscular LCACoA content may be responsible, at least in part, for the enhanced insulin action observed with weight loss in obese individuals. *Diabetes* 51: 2959–2963, 2002

Recent studies in rodents and humans have demonstrated a strong relationship between insulin resistance in skeletal muscle and increased intramuscular triglyceride (1–4) and intramyocellular triglyceride content, assessed by ^1H nuclear magnetic resonance (5–7). Because triglyceride is a relatively inert intracellular metabolite, it is likely that it serves more as a marker for other fatty acid–derived metabolites that actually mediate the insulin resistance, such as cytosolic long-chain acyl-CoA (LCACoA). Cytosolic LCACoA esters

From the ¹Departments of Exercise and Sport Science, Surgery, and the Human Performance Laboratory and Diabetes/Obesity Center, East Carolina University, Greenville, North Carolina; and ²Howard Hughes Medical Institute and the Departments of Internal Medicine and Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut.

Address correspondence and reprint requests to Joseph A. Houmard, Human Performance Laboratory, Room 371, Ward Sports Medicine Building, East Carolina University, Greenville, NC 27858. E-mail: houmardj@mail.ecu.edu.

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HOMA, homeostasis model assessment; LCACoA, long-chain fatty acyl-CoA; S_p , sensitivity index.

are intermediates in lipid synthesis/oxidation and are primarily derived from circulating fatty acids or intramuscular lipid sources such as triglyceride and phospholipid. There is accumulating evidence that LCACoAs are involved in controlling a variety of important cellular functions, including cellular metabolism, signal transduction, and gene expression (8–10). In relation to insulin action, rodents that were fed a high-fat diet had increased intramuscular LCACoA content that was associated with insulin resistance (11). Furthermore, mice with tissue-specific overexpression of lipoprotein lipase in muscle and liver have increased concentrations of LCACoA in these tissues, which is associated with tissue-specific insulin resistance (3). Such observations have led to the hypothesis that LCACoA concentration is an important factor in the pathogenesis of insulin resistance. In support of a role for LCACoA in insulin resistance, a negative correlation between insulin action and total LCACoA content has been reported in human skeletal muscle (11). However, to our knowledge, there is no additional evidence to support the hypothesis that LCACoA concentration is involved in the regulation of insulin action in human skeletal muscle. The purpose of the current study was to use a prospective design to test more rigorously the hypothesis that LCACoA is associated with insulin action in human skeletal muscle. This was accomplished by discerning whether LCACoA content decreased in concert with an intervention, weight loss, known to enhance insulin action (12). In addition, total LCACoA content may not reflect individual alterations in LCACoAs of different chain lengths and/or degrees of saturation; there is also evidence that individual LCACoA species serve different regulatory roles (9,10,13,14). We therefore also measured the concentrations of prevalent acyl-CoA species before and after weight loss.

RESEARCH DESIGN AND METHODS

Design and subjects. Morbidly obese (BMI >40 kg/m^2 and/or at least 100 lb over ideal body weight) subjects (10 women, 2 men; 1 black, 11 white) were examined before and 1 year after elective gastric bypass surgery to induce weight loss (12). Descriptive data for the subjects are presented in Table 1. We have previously characterized responses to gastric surgery in morbidly obese subjects; the 12-month postsurgery time was selected as body mass stabilizes and remains significantly depressed compared with the presurgery condition; there is also a dramatic improvement in insulin action (12). One subject was diabetic (type 2); all others had normal fasting glucose concentrations.

Subjects reported to the laboratory at ~ 0800 h after a 12-h fast. For the 3 days before the study, subjects were instructed to consume at least 250 g of carbohydrate per day. Body mass and stature were measured upon entering the laboratory. A muscle sample was obtained from the vastus lateralis in the fasted state using the needle biopsy technique. A minimal model (15) was then

TABLE 1

Selected characteristics of the morbidly obese subjects before intervention

Variable	Mean \pm SE
Age (years)	39.8 \pm 3.1
Stature (m)	1.71 \pm 0.02
BMI (kg/m ²)	48.6 \pm 1.2
Fasting insulin (μ U/ml)	23.3 \pm 3.8
Fasting glucose (mg/dl)	104.3 \pm 7.7

Subjects consisted of 1 black individual and 11 white individuals; 2 of the subjects were men, and 10 were women.

performed for calculating an insulin sensitivity index (S_I). Identical procedures were performed when subjects were morbidly obese (before surgery) and after weight loss (1 year after surgery). All procedures were approved by the East Carolina University Institutional Review Board, and informed consent was obtained before any experimental procedures were performed.

Insulin action. Insulin action was determined with a 3-h intravenous glucose tolerance test (minimal model) (15). Glucose and insulin dosages were calculated on the basis of body surface area, because of the high body mass of morbidly obese subjects (R.N. Bergman, personal communication). After fasting samples were obtained, glucose (50%) was injected into a catheter placed in an antecubital vein at a dose of 12 g/m² body surface area. Insulin, at a dose of 1.5 units/m² body surface area, was injected at minute 19. Blood samples were obtained at minutes 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, and 160 and centrifuged, and plasma was frozen at -80°C for the subsequent determination of insulin and glucose. Insulin was determined with immunoassay (Access Immunoassay System; Beckman Coulter, Fullerton, CA), and glucose was determined with an oxidation reaction (YSI Model 2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH). An insulin S_I was calculated on the basis of the minimal model as described by Bergman et al. (15). S_I is an index of the ability of insulin to promote the disposal of glucose, with a higher S_I indicating enhanced insulin sensitivity. Insulin action was also determined from fasting glucose and insulin concentrations by calculating a homeostasis model assessment (HOMA) value ($[\text{fasting glucose (mg/dl)} \times 0.05551] \times \text{fasting insulin } [\mu\text{U/ml}]/22.1$) (16).

Liquid chromatography tandem mass spectrometry. A needle biopsy of the vastus lateralis was obtained under local anesthesia in the fasted condition, immediately before the minimal model. Visible fat and/or connective tissue was removed from the sample (50–100 mg) and frozen in liquid nitrogen for subsequent analyses. LCACoAs were extracted from the biopsy sample by solid-phase extraction and C¹⁷ CoA was added as an internal standard as previously described (17,18). A tandem mass spectrometer API 3000 (Perkin-Elmer Sciex) interfaced with TurboIonSpray ionization source was used for mass spectrometry/mass spectrometry analysis (18). Fatty acyl-CoAs were ionized in a negative electrospray mode, and the transition pairs $[\text{M}^{-2}\text{H}]^{-2}/[\text{M}-\text{H}-80]^{-}$ were monitored in multiple reaction monitoring mode. Doubly charged ions and corresponding product ions (precursor minus phosphate group) were chosen as a transition pair for multiple reactions monitoring for quantitation (18). Total LCACoA content was calculated as the sum of the LCACoA species measured.

Statistics. Data are presented as mean \pm SE. Repeated measures ANOVA was used to compare data before and after surgery. Pearson product correlation coefficients were used to determine whether relationships existed between selected variables. Statistical significance was denoted at $P < 0.05$.

RESULTS

Weight loss. The gastric bypass surgery induced substantial weight loss in the morbidly obese subjects. As presented in Fig. 1, body mass decreased significantly ($P < 0.0001$) by ~ 60 kg (142.3 ± 6.8 vs. 79.6 ± 4.1 kg). BMI also decreased (48.6 ± 1.2 vs. 27.1 ± 0.9 kg/m²) significantly ($P < 0.0001$).

Insulin action. Insulin action, as determined by a variety of indexes, improved with weight loss. As presented in Fig. 2, fasting glucose concentration decreased ($P < 0.01$) by $\sim 24\%$ with weight loss (104.3 ± 7.7 vs. 79.6 ± 1.6 mg/dl). Fasting insulin was also reduced (23.3 ± 3.8 vs. 3.8 ± 0.5 $\mu\text{U/ml}$; $P < 0.001$) by $\sim 84\%$ for before versus after weight

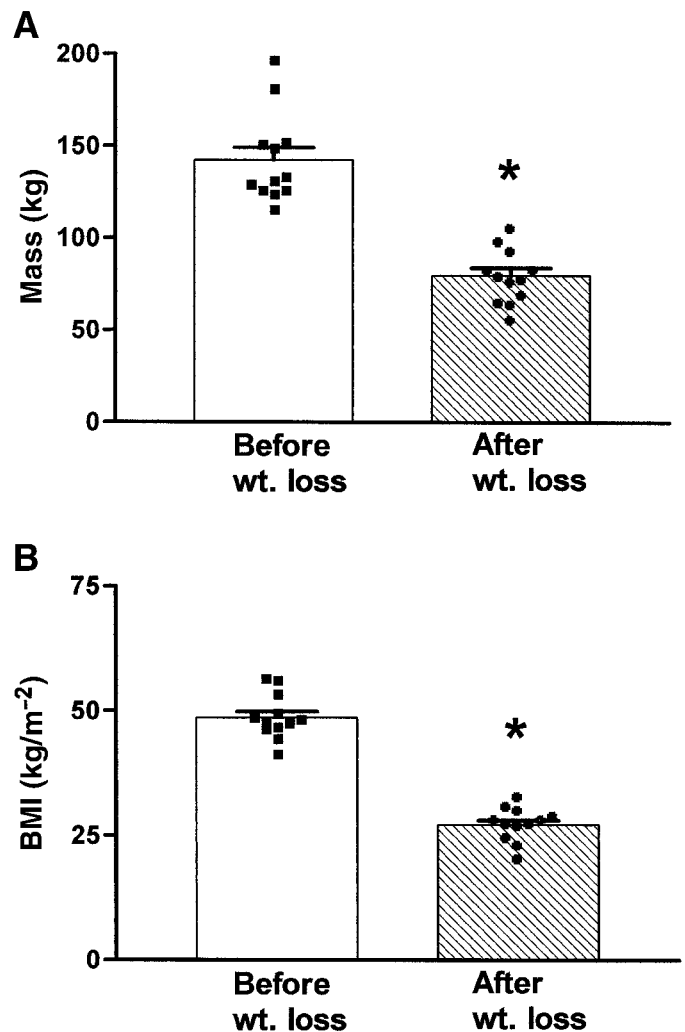


FIG. 1. Individual and mean body mass (A) and BMI (B) before and after weight (wt.) loss intervention ($n = 12$; means \pm SE). *Significantly different ($P < 0.001$) from before weight loss.

loss, respectively. Because both glucose and insulin decreased, there was a reduction ($P < 0.001$) in HOMA (5.9 ± 0.9 vs. 0.8 ± 0.1) with weight loss. S_I as determined from the minimal model (15) increased ($P < 0.0001$) by $\sim 360\%$ with weight loss, indicating enhanced insulin sensitivity [1.2 ± 0.3 vs. 4.1 ± 0.5 $\text{min}^{-1} \cdot (\mu\text{U/kg}^{-1})$; Fig. 2]. An S_I was not calculated for one female subject because of an inability to obtain venous access during the entire minimal model procedure; S_I data are thus presented for 11 subjects. Improvements in these indexes of insulin action remained statistically significant when the diabetic subject was excluded; data from this subject was thus included in all statistical analyses.

Muscle LCACoA content. Data for LCACoA concentrations are presented in Fig. 3. Sufficient material was not obtained in 1 individual; data are presented for 11 subjects (9 women, 2 men). There was no evidence for sex differences in relation to insulin action, body composition, and changes in the total and individual LCACoA species with weight loss intervention. All data are thus presented irrespective of sex. There was a significant ($P < 0.05$) decline in muscle palmitoyl CoA (16:0) concentration with weight loss (0.54 ± 0.08 vs. 0.35 ± 0.04 nmol/g wet wt) of

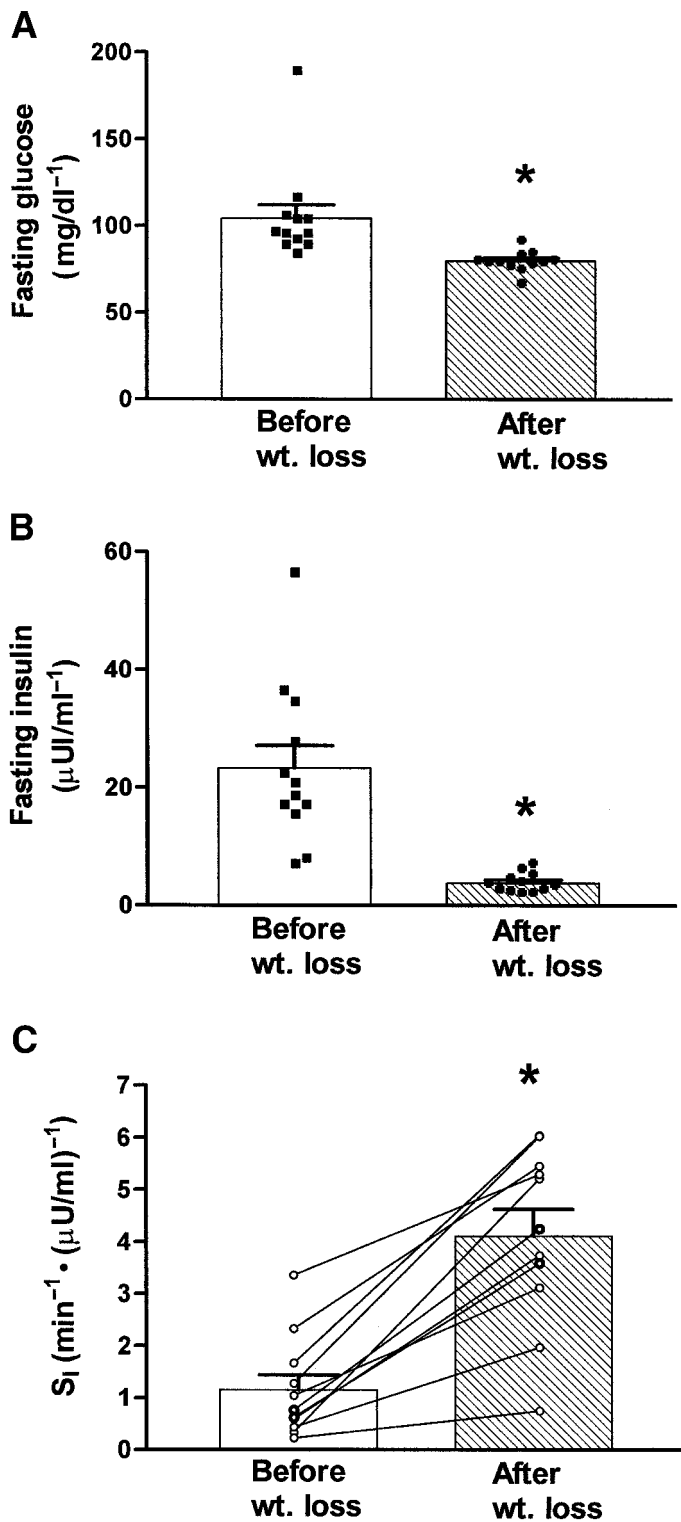


FIG. 2. Changes in individual and mean fasting glucose (A), fasting insulin (B), and insulin S_1 derived from an intravenous glucose tolerance test (C) with weight (wt.) loss. Fasting data are from 12 subjects, and S_1 data are from 11 subjects (means \pm SE). *Significantly different ($P < 0.01$) from before weight loss.

~35%. Muscle palmitoleate CoA (16:1) did not change ($P = 0.23$) with weight loss (0.36 ± 0.08 vs. 0.27 ± 0.04 nmol/g wet wt). In terms of 18 carbon fatty acids, muscle stearate CoA (18:0) decreased ($P < 0.05$) with weight loss (0.65 ± 0.05 vs. 0.54 ± 0.03 nmol/g wet wt) by ~17%. Muscle oleate

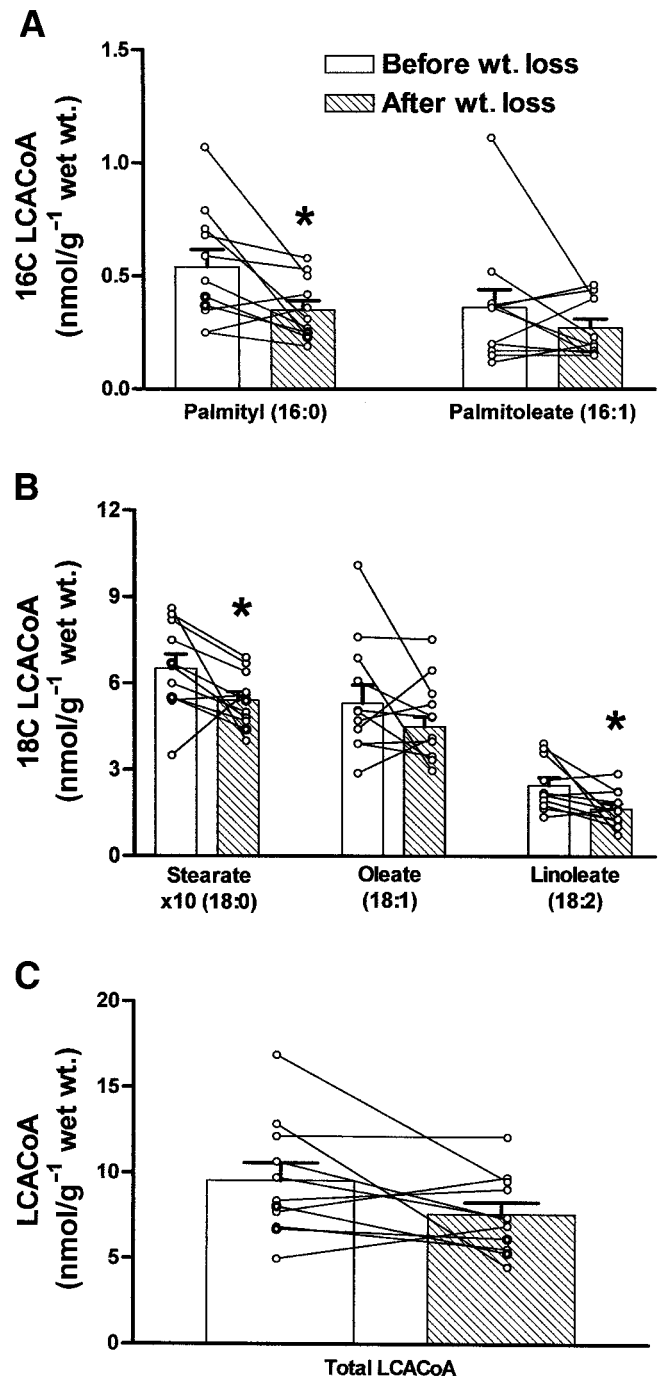


FIG. 3. Changes in individual and mean muscle LCACoA content with weight (wt.) loss. A: Data for 16-chain LCACoAs. B: Data for 18-chain LCACoAs. C: data for total muscle LCACoA content ($n = 11$; means \pm SE). *Significantly different ($P < 0.01$) from before weight loss for that LCACoA species.

CoA (18:1) was not altered ($P = 0.25$) with weight loss (5.31 ± 0.64 vs. 4.50 ± 0.35 nmol/g wet wt). Linoleate CoA (18:2) was also reduced ($P < 0.05$) with weight loss (2.47 ± 0.27 vs. 1.66 ± 0.19 nmol/g wet wt) by ~30%. The relative percentages of total LCACoA content (mean of before and after weight loss) were palmityl CoA 5.2%, palmitoleate CoA 3.6%, stearate CoA 7.2%, linoleate CoA 23.4%, and oleate CoA 60.8%. There was a trend ($P = 0.09$) for total LCACoA concentration to decrease by ~20% with weight loss (9.54 ± 1.03 vs. 7.60 ± 0.69 nmol/g wet wt).

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