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

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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 ~84% (23.3 ± 3.8 vs. 3.8 ± 0.5 mU/ml), and insulin sensitivity, as determined by minimal model, increased by ~360% (1.2 ± 0.3 vs. 4.1 ± 0.5 min^{-1} · [$\mu U/kg^{-1}$]) indicating enhanced insulin action. Muscle palmityl CoA (16:0; 0.54 ± 0.08 vs. 0.35 ± 0.04 nmol/g wet wt) concentration decreased by ~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 ^{1}H 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 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

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

TABLE 1
Selected characteristics of the morbidly obese subjects before intervention

Variable	Mean ± SE
Age (years)	39.8 ± 3.1
Stature (m)	1.71 ± 0.02
BMI (kg/m^2)	48.6 ± 1.2
Fasting insulin (μU/ml)	23.3 ± 3.8
Fasting glucose (mg/dl)	104.3 ± 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/m^2 body surface area. Insulin, at a dose of 1.5 units/m^2 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 ([fasting glucose (mg/dl) × 0.05551] × fasting insulin [μU/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 C^{17} 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 $[M\text{-}^2H]^{2-}/[M\text{-}H\text{-}80]^-$ 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 ± 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/m^2) 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 ~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 μU/ml; $P < 0.001$) by ~84% for before versus after weight 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 ~360% with weight loss, indicating enhanced insulin sensitivity [1.2 ± 0.3 vs. 4.1 ± 0.5 $min^{-1} \cdot (\mu U/kg^{-1})$; 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.

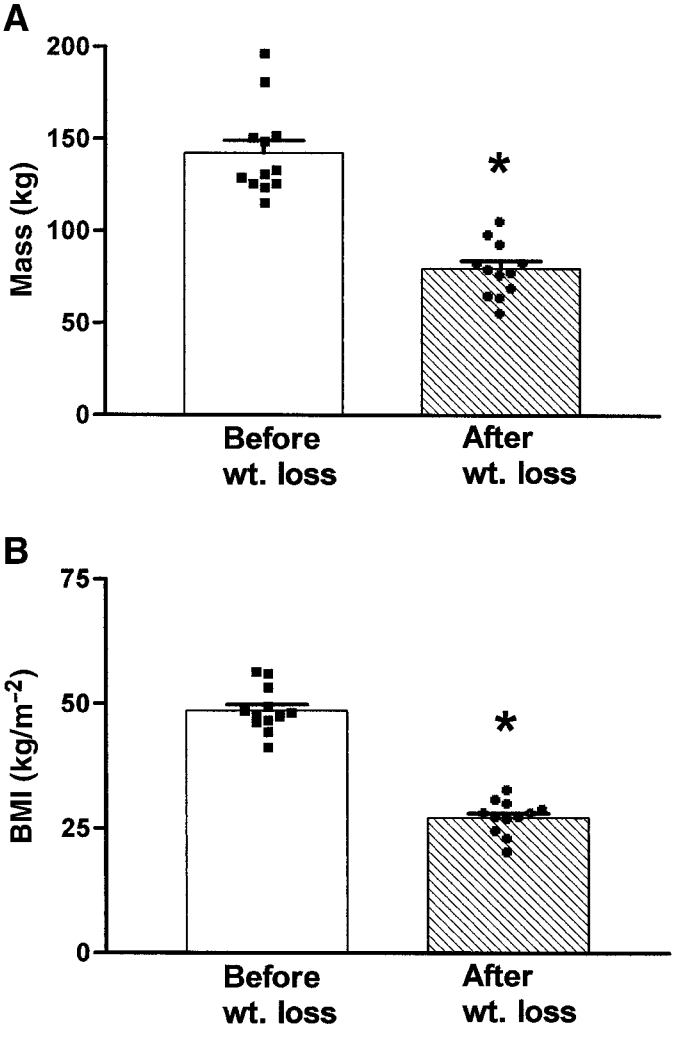

FIG. 1. Individual and mean body mass (*A*) and BMI (*B*) before and after weight (wt.) loss intervention (n = 12; means ± SE). *Significantly different ($P < 0.001$) from before weight loss.

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 palmityl CoA (16:0) concentration with weight loss (0.54 ± 0.08 vs. 0.35 ± 0.04 nmol/g wet wt) of

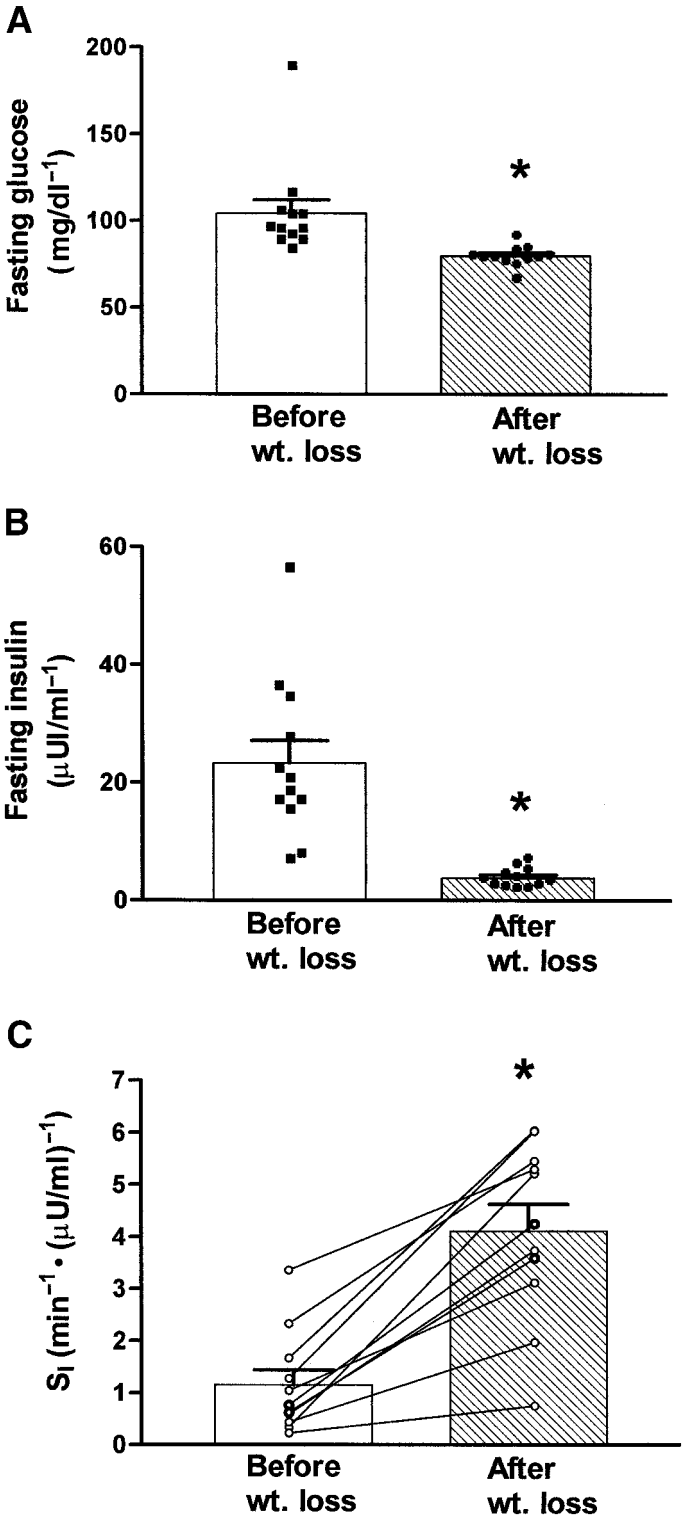

FIG. 2. Changes in individual and mean fasting glucose (*A*), fasting insulin (*B*), and insulin S_I derived from an intravenous glucose tolerance test (*C*) with weight (wt.) loss. Fasting data are from 12 subjects, and S_I data are from 11 subjects (means ± SE). *Significantly different ($P < 0.01$)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

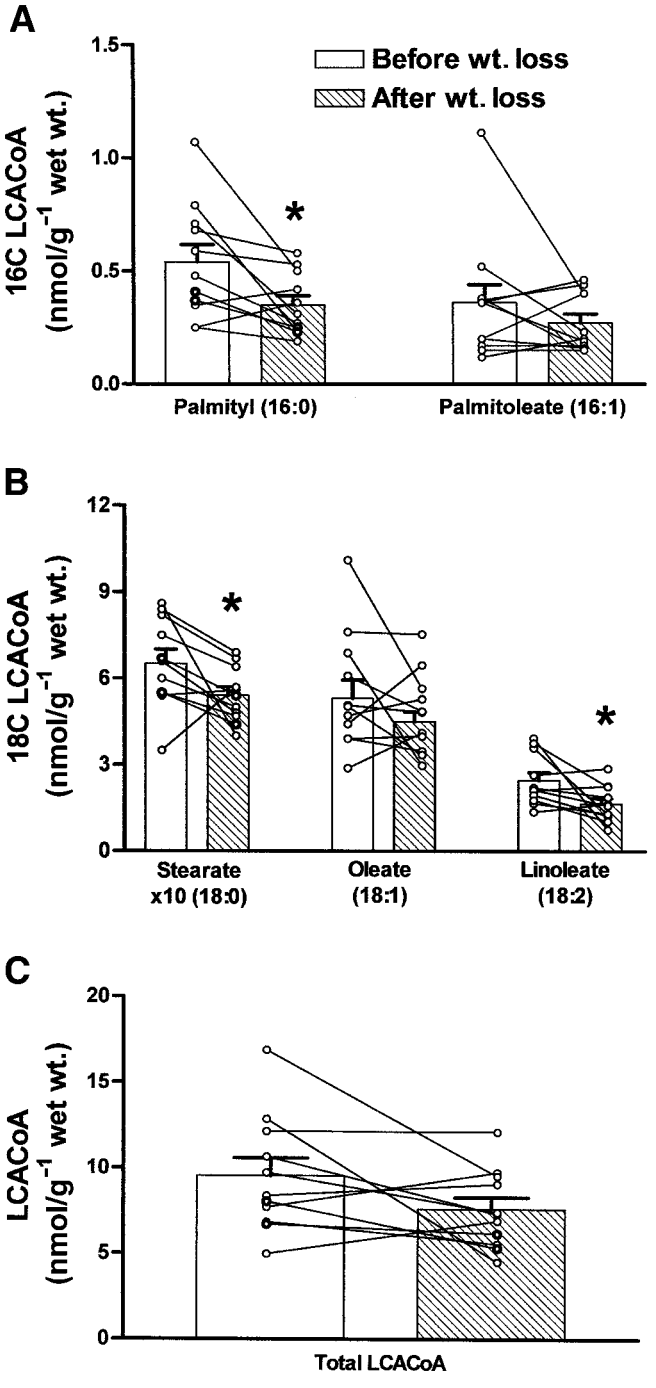

FIG. 3. Changes in individual and mean muscle LCACoA content with weight (wt.) loss. *A*: Data for 16-chain LCACoAs. *B*: Data for 18-chain LCACoAs. *C*: data for total muscle LCACoA content ($n = 11$; means ± SE). *Significantly different ($P < 0.01$) from before weight loss for that LCACoA species.

~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 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).

There was a trend ($r = -0.58$, $P = 0.08$) for a greater increase in insulin sensitivity (S_I) to be associated with a larger decline in palmityl CoA (16:0) with weight loss. No other correlations between changes in LCACoAs with changes in insulin action and body mass were evident.

DISCUSSION

The main finding of the present study was that skeletal muscle LCACoA content decreased in conjunction with enhanced insulin action in obese patients after weight loss. This observation is relevant to discerning mechanisms by which weight loss enhances insulin action, as the accumulation of LCACoA esters induces cellular alterations associated with insulin resistance. LCACoA esters can lead to activation of protein kinase Cθ, which can subsequently activate a serine kinase cascade that phosphorylates the insulin receptor (19) and/or insulin receptor substrate-1 at serine sites (20,21). Serine phosphorylation of the insulin receptor and insulin receptor substrate-1 reduces the ability of the cell to respond to ligand binding and produces insulin resistance (19–24). An intervention that decreases intramuscular LCACoA concentrations thus would be hypothesized to improve insulin action via enhancing insulin signal transduction. LCACoA esters also serve as regulatory molecules that control the activity of key enzymes in glucose metabolism, such as glycogen synthase (8–10,25), hexokinase (26), and nuclear factors that regulate gene transcription (8–10,14). These findings indicate the potential importance of LCACoA and suggest that a reduction in muscle LCACoA concentrations would contribute, at least in part, to enhancing insulin action; the current data support this hypothesis. The novel aspect of the present study was that a reduction in LCACoA was specifically observed in human skeletal muscle with a clinical intervention (weight loss) used to enhance insulin action (Figs. 2 and 3).

As presented in Fig. 3, palmityl CoA, stearate CoA, and linoleate CoA decreased significantly with weight loss, whereas there was no statistically significant reductions in muscle palmitoleate CoA or oleate CoA. It is of particular interest that both saturated fatty acyl CoAs (palmityl CoA and stearate CoA) decreased significantly with weight loss. Stein et al. (13) reported that saturated fatty acids (palmitic, stearic) promoted the hypersecretion of insulin more dramatically than unsaturated fatty acids of similar chain lengths (linoleic, oleic). These findings prompted the speculation that saturated fatty acids and their derivatives contribute to the development of insulin resistance (13). In support, the consumption of saturated fats is significantly associated with hyperinsulinemia and subsequent disease states such as diabetes, hypertension, and obesity (27). Such data suggest that saturated fatty acids and their metabolic products are relatively potent in terms of functioning as intracellular signaling molecules that induce insulin resistance. Although we cannot discern cause and effect, in the present study we did observe a tendency for a larger reduction in palmityl CoA in human skeletal muscle to be associated with a greater improvement in insulin action with weight loss (RESULTS). Palmityl CoA can be synthesized into ceramide (1) and/or possibly function through other mechanisms that induce insulin resistance (22). These findings emphasize that saturation status and chain length are important factors to consider when examining the role that LCACoAs play in controlling insulin action in skeletal muscle.

Previous experiments have studied the effects of weight loss on muscle lipid content. Using quantitative histochemical staining, Goodpaster et al. (28) reported that intramuscular (vastus lateralis) triglyceride concentration decreased with weight loss (~15 kg) in obese subjects with and without diabetes. Using computed tomography to estimate muscle lipid content, this same research group (29) reported a 36% reduction in muscle lipid content in obese individuals after weight loss. These relatively sparse data indicate that muscle triglyceride content is reduced in obese individuals with weight loss. However, although muscle triglyceride content is negatively associated with insulin action (4–6), it is likely that other intramyocellular components of lipid metabolism have a more mechanistic and direct link with the pathophysiology of insulin resistance. Recent evidence indicates that LCACoA esters play a vital role in controlling carbohydrate metabolism and influence insulin action in skeletal muscle (8,11,14,22,24,25). In support of a relationship between LCACoA and insulin action, correlations between insulin action and muscle LCACoA content have been demonstrated in both rodents (30,31) and humans (11). However, the present data are the first, to our knowledge, to demonstrate that LCACoA content is reduced in human skeletal muscle with an intervention that concomitantly enhances insulin action.

Although cytosolic triglyceride concentration may serve as an useful index of muscle fat content, an accurate determination of intramuscular triglyceride concentration is technically difficult because of possible contamination from adipose tissue interspersed between individual muscle cells (4,11,32). The concentrations of LCACoAs of various chain lengths and saturations are virtually undetectable in adipose tissue; the measurement of LCACoA thus has been proposed as a reliable indicator of intramuscular lipid content (11). In support of LCACoA reflecting muscle lipid content, high-fat feedings increased LCACoA concentration in rodent skeletal muscle (11,33). In addition, tissue-specific overexpression of lipoprotein lipase in liver and muscle promoted increased fatty acyl CoA deposition in these tissues, which was associated with tissue-specific insulin resistance (3). The decrease in LCACoA reported in the current study with weight loss thus also indicates that total intramyocellular lipid content was significantly reduced with this intervention in obese individuals.

In summary, increases in intramyocellular LCACoAs have been implicated in the pathogenesis of insulin resistance in skeletal muscle. In the present study, muscle palmityl CoA (−35%), stearate CoA (−17%), and linoleate CoA (−30%) decreased significantly with weight loss in obese subjects. Conversely, muscle palmitoleate CoA and oleate CoA were not significantly altered. There was a trend for a larger decrease in palmityl acyl CoA to be associated with enhanced insulin action with weight loss. These findings 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.

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