Adipose Tissue Monomethyl Branched-Chain Fatty Acids and Insulin Sensitivity: Effects of Obesity and Weight Loss

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Objectives: An increase in circulating branched-chain amino acids (BCAA) is associated with insulin resistance. Adipose tissue is a potentially important site for BCAA metabolism. It was evaluated whether monomethyl branched-chain fatty acids (mmBCFA) in adipose tissue, which are likely derived from BCAA catabolism, are associated with insulin sensitivity.

Methods: Insulin-stimulated glucose disposal was determined by using the hyperinsulinemic-euglycemic clamp procedure with stable isotope glucose tracer infusion in nine lean and nine obese subjects, and in a separate group of nine obese subjects before and 1 year after Roux-en-Y gastric bypass (RYGB) surgery (38% weight loss). Adipose tissue mmBCFA content was measured in tissue biopsies taken in the basal state.

Results: Total adipose tissue mmBCFA content was ~30% lower in obese than lean subjects (P = 0.02) and increased by ~65% after weight loss in the RYGB group (P = 0.01). Adipose tissue mmBCFA content correlated positively with skeletal muscle insulin sensitivity (R2 = 35%, P = 0.01, n = 18).

Conclusions: These results demonstrate a novel association between adipose tissue mmBCFA content and obesity-related insulin resistance. Additional studies are needed to determine whether the association between adipose tissue mmBCFA and muscle insulin sensitivity is causal or a simple association.

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Introduction

Insulin resistance is a common metabolic complication of obesity and an important risk factor for the development of type 2 diabetes, the metabolic syndrome, and coronary heart disease (1,2). It has been proposed that an increase in circulating branched-chain amino acids (BCAA), valine, leucine, and isoleucine, is involved in the pathogenesis of insulin resistance, because increased plasma BCAA concentrations are often observed in obese and insulin resistant states (3,4), and weight loss leads to decreased plasma BCAA concentrations and improved insulin action (5,6). However, the underlying mechanism(s) responsible for the relationship between BCAA metabolism and insulin resistance is not known.

Monomethyl branched-chain fatty acids (mmBCFA) could provide a link between BCAA metabolism and metabolic dysfunction. In most peripheral tissues, BCAA are deaminated by mitochondrial branched-chain aminotransferase (BCAT2 or BCATm) to generate branched-chain α-ketoacids (7), which are then decarboxylated by the branched-chain α-ketoacid dehydrogenase complex (8). The resulting short-chain branched acyl moieties can be exported out of mitochondria (9) and undergo conventional de novo fatty acid biosynthesis, catalyzed by fatty acid synthase (FAS), to produce mmBCFA (10). Alternatively, the fatty acyl chain could be extended within mitochondria by using the mitochondrial fatty acid synthesis (FAS II) system (11) (Supporting Information Figure S1).

The predominant branching in mmBCFA is near the terminal end of the carbon chain with an isopropyl or isobutyl group denoted as iso- or anteiso-BCFA, respectively. mmBCFA are present in a large range of organisms from bacteria to mammals, indicating conserved...
metabolic pathways for their synthesis and function. The enzymes involved in BCAA metabolism are key regulators of both the degradation of BCAA and the synthesis of mmBCFA. Skeletal muscle and adipose tissue are the primary sites for BCAA degradation (12), whereas BCAA catabolism in the liver is minimal because of low levels of BCAATm (7). A study conducted in a rodent model demonstrated that adipose tissue BCAA metabolism can modulate circulating BCAA concentrations (13), presumably because adipose tissue is a major site for plasma BCAA uptake and conversion to lipids (14). Whole tissue assessments of BCAA catabolic activities and kinetics also suggest that adipose tissue could play an important role in regulating whole body BCAA homeostasis in people (15,16).

Adipose tissue gene expression of enzymes involved in BCAA catabolism is lower in obese and insulin resistant mice and people than in their lean counterparts (17,18). Therefore, it is possible that increased catabolism of BCAA and conversion to mmBCFA in adipose tissue could improve insulin sensitivity by clearing BCAA from plasma. The purpose of the present study was to evaluate the possibility that adipose tissue mmBCFA metabolism is associated with whole-body (primarily skeletal muscle) insulin sensitivity in obese subjects. Accordingly, we conducted: (i) a cross-sectional study to assess the relationship between adipose tissue mmBCFA content and insulin sensitivity in lean and obese subjects, and (ii) a longitudinal study to assess the effects of marked weight loss on adipose tissue mmBCFA metabolism and insulin sensitivity.

Methods
Study subjects
A total of 27 subjects (33 to 61 years old) participated in two studies. Study 1 was a cross-sectional study that involved 9 lean (BMI = 23.0 ± 0.5 kg m⁻², seven women and two men) and nine obese (BMI = 45.6 ± 1.6 kg m⁻², seven women and two men) subjects. Study 2 was a longitudinal study that involved nine obese subjects (BMI = 48.3 ± 3.2 kg m⁻², eight women and one man), who were studied before and 1 year after Roux-en-Y gastric bypass (RYGB) surgery. All subjects provided written informed consent before participating in this study, which was approved by the Institutional Review Board of Washington University School of Medicine in St. Louis, MO. All subjects completed a comprehensive medical evaluation, including a detailed history, physical examination, blood tests, and a 2-h oral glucose tolerance test. No subject had type 2 diabetes, consumed more than 20 g of alcohol/day, smoked cigarettes, engaged in regular exercise (>1 h week⁻¹), or were taking medications known to alter glucose or lipid metabolism.

Experimental procedures and analyses
Hyperinsulinemic-euglycemic clamp procedure and adipose tissue biopsy. Insulin sensitivity was assessed by using the hyperinsulinemic-euglycemic clamp (HEC) procedure, in conjunction with stable isotopically labeled glucose tracer infusion. Subjects were admitted to the Washington University School of Medicine Clinical Research Unit in the evening and consumed a standard dinner. The next morning, after subjects fasted for 12 h overnight, a catheter was inserted into a forearm vein for infusion of tracers, dextrose, and insulin, and a second catheter was inserted into a radial artery for blood sampling. A primed-continuous infusion of [6,6-²H₂]glucose (priming dose: 22.0 µmol kg⁻¹ body weight; infusion rate: 0.22 µmol kg⁻¹ body weight/min; Cambridge Isotope Laboratories, Andover, MA) was started and maintained until the end of the clamp procedure. After a basal period of 3.5 h, insulin was infused at a rate of 50 mU m⁻² body surface area/min for 4 h. During insulin infusion, euglycemia (plasma glucose concentration ~100 mg dL⁻¹) was maintained by infusing 20% dextrose solution enriched to 2.5% with [6,6-²H₂]glucose. Blood samples were obtained immediately before starting the glucose tracer infusion and during the final 30 min of the basal period and the insulin clamp to determine plasma glucose concentration and tracer-to-tracee ratio (TTR). Subcutaneous abdominal tissue biopsies were obtained from the periumbilical area 60-90 min after starting the glucose tracer infusion during the basal period of the clamp procedure, as previously described (19).

After the HEC procedure was completed, the nine obese subjects who participated in Study 2 had RYGB surgery. This procedure involved constructing a small (~20 mL) proximal gastric pouch by stapling across the stomach. A 150-cm Roux-Y limb was constructed by transpylecting the jejunum 30 cm distal to the ligament of Treitz and creating a jejunojejunal anastomosis, 150 cm distal to the transaction. The Roux limb was then anastomosed in a retrocolic fashion to the proximal gastric pouch by using either a hand-sewn or circular stapled technique. No subject experienced serious postoperative complications. The HEC procedure and abdominal fat biopsy were repeated 1 year after RYG surgery, when subjects had lost a significant amount of weight and had maintained their body weight stable (<2% change) for at least 2 weeks before repeat studies were performed.

Assessment of insulin sensitivity. Plasma glucose concentration was determined by using an automated glucose analyzer (YSI 2300 STAT plus; Yellow Springs Instruments, Yellow Springs, OH). Glucose TTR in plasma was determined by using gas chromatography-mass spectrometry (GC-MS) (20,21). Glucose rate of appearance (Ra) in plasma was calculated by dividing the glucose tracer infusion rate by the average plasma glucose TTR during the last 30 min of the basal and insulin infusion periods (22). Glucose rate of disappearance (Rd) from plasma was equal to endogenous glucose Ra plus the rate of exogenously infused dextrose and glucose tracer. Insulin-stimulated glucose Rd in µmol per kg of body weight per minute and the percent increase above basal were used as indices of skeletal muscle insulin sensitivity (21).

Adipose tissue fatty acid analyses. Adipose tissue fatty acids (including mmBCFA) were determined by using GC-MS (Hewlett-Packard 6890N series GC interfaced to an Agilent 5973N mass spectrometer with DB-5 MS 30 m × 0.25 mm × 0.25 µm column) in electron ionization mode, as previously described (23). Both the intra- and inter-assay coefficients of variation for these analyses are below 5%.

Isolation of adipose tissue mRNA and quantitative polymerase chain reaction (qPcr). Frozen adipose tissue samples were homogenized in TRIzol® reagent (Life Technologies, Foster City, CA) and the total RNA was isolated according to the manufacturer’s protocol. cDNA was synthesized and gene expression was determined with real time qPCR by using SybrGreen reagent and the ABI 7500 thermal cycler (Life Technologies), as previously described (24). The primer sequences used are listed in Supporting Information Table S1.
Statistical analyses
All datasets were tested for normality according to the Shapiro–Wilks criteria, and appropriate statistical tests were used for normally and non-normally distributed variables (parametric and non-parametric, respectively). Differences between lean and obese subjects were evaluated by using the Student’s independent $t$ test or the Mann–Whitney $U$ test (for normally and non-normally distributed variables, respectively), and the effects of weight loss were evaluated by using the Student’s paired $t$ test or Wilcoxon’s signed-rank test (for normally and non-normally distributed variables, respectively). The relationship between adipose tissue mmBCFA and insulin sensitivity was assessed by calculating Pearson’s or Spearman’s correlation coefficients (for normally and non-normally distributed variables, respectively). A $P$-value $< 0.05$ was considered statistically significant. Results are presented as means $\pm$ SEM. All analyses were conducted with SPSS version 20 for Windows (IBM SPSS, Chicago, IL).

Results

Insulin sensitivity

**Study 1.** Glucose Rd increased from $11.7 \pm 0.5 \mu$mol kg$^{-1}$ min$^{-1}$ in the basal state to $48.9 \pm 3.3 \mu$mol kg$^{-1}$ min$^{-1}$ during insulin infusion (a $320 \pm 30\%$ increase) in the lean subjects, and from $8.2 \pm 0.5 \mu$mol kg$^{-1}$ min$^{-1}$ in the basal state to $18.0 \pm 2.1 \mu$mol kg$^{-1}$ min$^{-1}$ during insulin infusion in the obese subjects (a $123 \pm 27\%$ increase). Both glucose Rd and the percent increase in glucose Rd during insulin infusion were significantly lower in obese than lean subjects ($P < 0.001$).

**Study 2.** Body weight decreased by $38\% \pm 1\%$, from $132 \pm 10$ kg before to $82 \pm 7$ kg, 1 year after RYGB surgery ($P < 0.001$). Glucose Rd increased from $7.9 \pm 0.3 \mu$mol kg$^{-1}$ min$^{-1}$ in the basal state to $10.5 \pm 0.8 \mu$mol kg$^{-1}$ min$^{-1}$ during insulin infusion before weight loss (a $35\% \pm 11\%$ increase), and from $11.1 \pm 1.7$ in the basal state to $20.2 \pm 2.3 \mu$mol kg$^{-1}$ min$^{-1}$ during insulin infusion after weight loss (a $92\% \pm 18\%$ increase). Both glucose Rd and the percent increase in glucose Rd during insulin infusion were significantly greater after than before RYGB surgery ($P < 0.005$).

Adipose tissue mmBCFA levels in lean and obese subjects

Adipose tissue mmBCFA content as a molar percentage of total adipose tissue fatty acids was $\sim 30\%$ lower in obese than lean subjects ($P = 0.02$) (Figure 1A), but the percentages of major fatty acids (palmitic acid, C16:0 and oleic acid, C18:1(n-9)) were not significantly different between groups (Figure 1B). Individual adipose tissue mmBCFA in lean and obese subjects are shown in Table 1.

De novo synthesis of mmBCFA in adipose tissue requires both BCAA catabolism and lipogenesis catalyzed by FAS, whose gene

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**TABLE 1 Individual adipose tissue mmBCFA content in lean and obese subjects**

| mmBCFA  | Lean ($n = 9$) | Obese ($n = 9$) |
|---------|----------------|-----------------|
|         | Mean $\pm$ SEM | Mean $\pm$ SEM  | $P$ value |
| C15ISO  | 0.0204 $\pm$ 0.0013 | 0.0131 $\pm$ 0.0017 | 0.0588 |
| C15antelSO | 0.0182 $\pm$ 0.0012 | 0.0103 $\pm$ 0.0018 | 0.0223 |
| C17ISO  | 0.0850 $\pm$ 0.0044 | 0.0570 $\pm$ 0.0070 | 0.0470 |
| C17antelSO | 0.1354 $\pm$ 0.0073 | 0.1014 $\pm$ 0.0103 | 0.1665 |
| C19ISO  | 0.0646 $\pm$ 0.0055 | 0.0353 $\pm$ 0.0090 | 0.0090 |
| C21ISO  | 0.0019 $\pm$ 0.0001 | 0.0009 $\pm$ 0.0003 | 0.0179 |
| Total   | 0.3255 $\pm$ 0.0274 | 0.2357 $\pm$ 0.0232 | 0.0235 |

Units are percentages of total adipose tissue fatty acids.
expression was lower in adipose tissue from obese than lean subjects ($P = 0.02$) (Figure 1C).

**Effect of bariatric surgery-induced weight loss on adipose tissue mmBCFA levels**

The percentage of total adipose tissue mmBCFA increased by ~65% after marked weight loss ($P = 0.01$) (Figure 2A). The percentages of individual adipose tissue mmBCFA are shown in Table 2. Adipose tissue FAS gene expression increased after weight loss ($P = 0.05$) (Figure 2B).

**Relationship between adipose tissue mmBCFA and skeletal muscle insulin sensitivity**

There was a positive correlation between adipose tissue mmBCFA content and insulin sensitivity (i.e., insulin-stimulated glucose Rd) among the group of 18 lean and obese subjects in Study 1 (Spearman’s $r = 0.591$, $P = 0.010$) (Figure 3A). There was also a trend toward a positive correlation between the change in adipose tissue mmBCFA content and the change in insulin sensitivity induced by weight loss in the nine obese subjects who had RYGB surgery, but this association did not reach statistical significance (Pearson’s $r = 0.591$, $P = 0.010$) (Figure 3B).

**Discussion**

In the present study, we tested the hypothesis that BCAA-derived lipids (i.e., saturated fatty acids with one methyl branch near the terminal end of the carbon chain) are associated with insulin action. Insulin sensitivity in skeletal muscle (the major site of insulin-mediated glucose disposal) and mmBCFA content in adipose tissue (presumably a major site for de novo synthesis of mmBCFA) were determined in lean and obese subjects and in obese subjects before and after marked weight loss. We found that adipose tissue mmBCFA content was lower in obese than in lean subjects, and increased in extremely obese subjects after bariatric surgery-induced weight loss. Moreover, adipose tissue mmBCFA content correlated directly with skeletal muscle insulin sensitivity, assessed by using the HEC procedure, in conjunction with stable isotopically-labeled glucose tracer infusion. These results indicate that adipose tissue mmBCFA could be involved in the pathogenesis of obesity-related insulin resistance and provide a novel potential mechanistic link between BCAA metabolism and insulin action.

The exact biochemical pathway(s) for de novo synthesis of adipose tissue mmBCFA from BCAA is not clear and requires additional studies to evaluate the incorporation of labeled BCAA into mmBCFA. However, data from a series of studies suggest de novo synthesis of mmBCFA could occur in mammalian cells. First, the enzymatic machinery needed for the conversion of BCAA to mmBCFA exists. The generation of short branched-chain acyl-CoAs by deamination and decarboxylation of BCAA occurs within the mitochondrial matrix (7,8). To utilize the conventional cytosolic FAS I pathway for chain length extension, these branched-chain acyl-CoAs are converted to acyl carnitines prior to transport out of the mitochondria (9). Branched-chain acyl-CoAs can then be recovered from their carnitine esters in the cytosol either by carnitine acyltransferases (25) or via consecutive reactions catalyzed by short-chain acylcarnitine hydrolase (26) and acyl CoA synthetase. Alternatively, the corresponding short-chain branched acyl-acyl carrier protein (ACP) could be used by the mitochondrial FAS II for fatty acyl chain extension (11). Second, it has been shown that radiolabeled BCAA can be converted into fatty acids in adipose tissue (12). Finally, we recently found that the amount of C17ISO in 3T3-L1 cells before differentiation was below the detection limit.

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**Table 2** Individual adipose tissue mmBCFA content in obese subjects before and 1 year after bariatric surgery-induced weight loss

| mmBCFA | Before weight loss ($n = 9$) | After weight loss ($n = 9$) | $P$ value |
|--------|----------------------------|---------------------------|----------|
|        | Mean          | SEM          | Mean          | SEM          |          |
| C15 ISO| 0.0191        | 0.0024       | 0.0844        | 0.0131       | 0.0002    |
| C15 anteISO| 0.0162   | 0.0022       | 0.0761        | 0.0123       | 0.0002    |
| C17 ISO| 0.0865        | 0.0093       | 0.1099        | 0.0133       | 0.1686    |
| C17 anteISO| 0.1524 | 0.0148       | 0.1910        | 0.0227       | 0.1730    |
| C19 ISO| 0.0020        | 0.0003       | 0.0028        | 0.0015       | 0.5921    |
| C21 ISO| 0.0006        | 0.0001       | 0.0011        | 0.0003       | 0.1152    |
| Total  | 0.2767        | 0.0281       | 0.4652        | 0.0581       | 0.0100    |

Units are percentages of total adipose tissue fatty acids.
Obesity

circles) subjects (45x438), and relationship between weight loss-induced changes in adipose tissue total mmBCFA content and insulin-stimulated glucose Rd in a separate group of nine extremely obese subjects (B).

whether there is a causal association between adipose tissue mmBCFA and muscle insulin sensitivity. O

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