Effects of Acute Changes of Plasma Free Fatty Acids on Intramyocellular Fat Content and Insulin Resistance in Healthy Subjects

Guenther Boden,1 Brett Lebed,1 Melanie Schatz,1 Carol Homko,1 and Susan Lemieux2

The reason for the 3- to 4-h delay between a rise in plasma free fatty acid (FFA) levels and the development of insulin resistance remains unknown. In the current study, we have tested the hypothesis that the delay may be caused by the need for plasma FFAs to first enter muscle cells and to be re-esterified there before causing insulin resistance. To this end, we have determined intramyocellular triglyceride (IMCL-TG) content with proton nuclear magnetic resonance (1H-NMR) spectroscopy in healthy volunteers before and 4 h after lowering of plasma FFAs (with euglycemic-hyperinsulinemic clamping) or after increasing plasma FFAs (with lipid plus heparin infusions). Increasing plasma FFAs (from 516 to 1,207 μmol/l or from 464 to 1,857 μmol/l, respectively) was associated with acute increases in IMCL-TG from 100 to 109 ± 5% (P < 0.05) or to 133 ± 11% (P < 0.01), respectively, and with a significant increase in insulin resistance (P < 0.05 after 3.5 h). Lowering of plasma FFAs from 560 to 41 μmol/l was associated with a tendency for IMCL-TG to decrease (from 100 to 95 ± 3%). Changes in plasma FFAs correlated linearly with IMCL-TG (r = 0.74, P < 0.003). The demonstration that acute changes in plasma FFAs were accompanied by corresponding changes in IMCL-TG and with the development of insulin resistance, taken together with previous reports of a close correlation between IMCL-TG and insulin resistance, supported the notion that accumulation of IMCL-TG is a step in the development of FFA-induced insulin resistance. Diabetes 50:1612–1617, 2001

Other, which has reached epidemic proportions in the U.S. (1), is associated with insulin resistance (2), a well-established risk factor not only for type 2 diabetes but also for atherosclerotic vascular disease (3). Plasma free fatty acids (FFAs), which are generally elevated in obese subjects (4,5), have been demonstrated to be a major link between obesity and insulin resistance (rev. in 6). Thus, lowering plasma FFAs decreases insulin resistance (7), whereas raising plasma FFAs increases insulin resistance (after a delay of 3–4 h) in nondiabetic and diabetic subjects (8–10). The mechanism by which FFAs cause insulin resistance, however, is not completely understood. The long (3–4 h) delay between the rise in plasma FFAs and the onset of inhibition of insulin-stimulated glucose uptake makes a direct effect of FFAs on insulin action unlikely. On the other hand, an explanation for the delayed onset of insulin resistance may be that FFAs need to accumulate first as triglycerides (TGs) inside muscle fibers. In support of this notion, several studies in animals and humans have demonstrated a close relationship between muscle fat content and insulin resistance (11–16). Most of these studies, however, were unable to differentiate between extramyocellular (EMCL) fat, i.e., fat located in adipocytes between muscle fibers, and intramyocellular (IMCL) fat, i.e., fat located within muscle fibers. It has recently been shown that IMCL fat content can be quantitated with proton-nuclear magnetic resonance (1H-NMR) spectroscopy (17,18). In this study, we have used 1H-NMR spectroscopy to test the hypothesis that FFA-induced inhibition of insulin-stimulated glucose uptake is accompanied by IMCL accumulation of fat by assessing effects of acutely raising of plasma FFA levels on insulin resistance and on IMCL-TG content in soleus muscles of young healthy volunteers.

RESEARCH DESIGN AND METHODS

Subjects. A total of 15 healthy young volunteers (8 males and 7 females) participated in three different studies. One subject participated in all three studies; 4 participated in two studies (3 in studies 1 and 2 and 1 in studies 2 and 3); 10 participated in one study (3 in study 1, 2 in study 2, and 5 in study 3). The subjects’ ages, weights, heights, and body compositions are shown in Table 1. None of the subjects had a family history of diabetes or any other endocrine disorder, and none was taking any medication. Their weights were stable for at least 2 months, and their diets contained a minimum of 250 g/day of carbohydrate for at least 2 days before the studies. Informed written consent was obtained from each participant after explanation of the nature, purpose, and potential risks of these studies. The study protocol was approved by the Institutional Review Board of Temple University Hospital. Subjects were admitted to Temple University Hospital’s General Clinical Research Center on the evening before the studies. The studies began at 8:00 a.m. after an overnight fast with the subjects reclining in bed. A short polyethylene catheter was inserted into an antecubital forearm vein for blood sampling. This arm was wrapped with a heating blanket (−70°C) to arterialize venous blood.

Study design. Study 1 was a 4-h euglycemic-hyperinsulinemic clamp. Plasma FFAs decreased to very low levels because of insulin-induced inhibition of
lipolysis. 1H-NMR spectroscopy was performed within 15 min before and after the clamps.

Study 2 was a 4-h euglycemic-hyperinsulinemic clamp with simultaneous IV infusion of lipid plus heparin. Plasma FFA levels rose because hepatic-mediated lipolysis from the infused fat exceeded insulin-mediated antilipolysis in adipose tissue. 1H-NMR spectroscopy was performed as in study 1.

Study 3 was a 4-h infusion of lipid plus heparin (as in study 2) without infusion of insulin. Plasma FFA levels rose to higher levels than those in study 2, presumably because FFAs released from adipose tissue, which continued uninhibited, were added to FFAs released from the infused fat. 1H-NMR spectroscopy was performed as in studies 1 and 2.

Studies were performed in random order separated by 1–2 months.

Euglycemic-hyperinsulinemic clamping with and without lipid/heparin. Regular human insulin (Humulin R; Eli Lilly, Indianapolis, IN) was infused intravenously at a rate of 7 pmol/kg per min for 4 h, and plasma glucose concentrations were clamped at ~5 mmol/l by a feedback-controlled glucose infusion (study 1).

In study 2, euglycemic-hyperinsulinemic clamping was performed as described above. In addition, Liposyn II (Abbott Laboratories, North Chicago, IL), a 20% triglyceride emulsion (10% safflower and 10% soybean oil), plus heparin (0.4 U/kg per min) were infused at a rate of 1.5 ml/min for 4 h, and plasma glucose concentrations were clamped at ~5 mmol/l by a feedback-controlled glucose infusion (study 1).
versus the insulin alone group starting at 180 min ($P < 0.01$) (Fig. 2). Fat oxidation was not determined in the lipid only group.

**Changes in IMCL-TG and plasma FFAs.** In the insulin only group, hyperinsulinemic-euglycemic clamping for 4 h tended to decrease IMCL-TG (100 ± 0 vs. 95 ± 3%, NS). In the insulin plus lipid group, IMCL-TG rose from 100 ± 0 to 109 ± 5% ($P < 0.04$). Individually, IMCL-TG rose in six of seven subjects (range 5–38%). In the lipid only group, IMCL-TG rose from 100 ± 0 to 133 ± 11% ($P < 0.02$). Individually, IMCL-TG rose in six of seven subjects (range 5–50%) (Fig. 3).

These increases in IMCL-TG content were associated with similar increases in plasma FFA levels (during the preceding 3 h), whereas decreasing IMCL-TG content was associated with decreasing plasma FFA levels (Fig. 3B). This resulted in a highly significant and close linear correlation between plasma FFA levels and IMCL-TG content ($r = 0.74$, $P < 0.0003$) (Fig. 4).

**EMCL-TG.** There were no significant changes in EMCL-TG in response to insulin alone (100 ± 0 vs. 1.22 ± 34%), to insulin plus lipid (109 ± 43%), or to lipid alone (146 ± 23%).

**DISCUSSION**

It was the objective of this study to test whether changes in insulin resistance produced by acute changes in plasma FFA levels involved corresponding changes in IMCL-TG. We found that IMCL-TG changed acutely, in what appeared to be a dose-dependent way, with changes in plasma FFA concentration ($r = 0.74$). Increases in plasma FFA levels of ~700 and ~1,400 μmol/l were accompanied by increases in IMCL-TG of 9% ($P < 0.04$) and 33% ($P < 0.02$), respectively, over the 4-h study period. A decrease in plasma FFAs by ~500 μmol/l was associated with a 5% decrease in IMCL-TG, which, however, was not statistically significant. There may be several reasons why it was easier to demonstrate an increase, rather than a decrease, in IMCL-TG. First, the increases in plasma FFA levels were larger than the decrease (~700 and ~1,400 μmol/l vs. ~500 μmol/l). Second, it is possible, in fact it is likely, that a decrease in IMCL-TG takes more time than an increase because the decrease depends on the rate of TG consumption in muscle, which, in resting muscle, is probably quite low.
It is worth noting that the increase in IMCL-TG in response to a rise in plasma FFAs (during lipid/heparin plus insulin infusions) was associated with a 40% increase in whole-body insulin resistance, confirming previous reports (8–10). On the other hand, it remains to be shown whether a decrease in IMCL-TG is associated with a decrease in insulin resistance.

The validity of the IMCL-TG measurements is supported by evidence demonstrating that the proton resonances at 1.3 ppm (used here to represent IMCL-TG) are produced by methylene groups of acyl chains of IMCL-TG (17,18). This evidence includes the demonstration that tissues that did not have adipocytes, including liver and heart muscle, had single resonances at 1.3 ppm (22–24), whereas adipose tissue had single resonances at ~1.6 ppm (17,18,24). Further support that the proton resonances at 1.3 ppm represented IMCL-TG was provided by findings that 1) the resonances at 1.3 ppm correlated with markers of muscle mass (for instance, creatine), whereas the resonances at 1.6 ppm did not (17); 2) the resonances at 1.3 ppm decreased after exercise and recovered after resting and after eating (17); and 3) patients with generalized lipodystrophy, a disorder characterized by almost complete absence of adipose tissue, had abundant signals at 1.3 but none at 1.6 ppm (18). It also needs to be pointed out that the methylene groups of circulating TGs or FFAs do not contribute to the generation of the IMCL-TG resonances, as only stationary tissues or fluid is detected by 1H-NMR spectroscopy (25).

The acute changes in IMCL-TG observed here were compatible with results of two previous studies in which FFA turnover was determined (26,27). In one study, the rate of FFA release after 4 h of hyperinsulinemia (comparable with the insulin only group in the current study), was 1.6 μmol/kg per min, and the rate of FFA oxidation was 1.7 μmol/kg per min; hence, the rate of FFA re-esterification was slightly negative (26). This was entirely compatible with the trend in the current study toward a decrease in IMCL-TG. In a second study, when lipid/heparin was infused (comparable with the insulin plus lipid group in the current study), the rate of FFA release by lipolysis from the infused lipid was ~17 μmol/kg per min, while FFA oxidation was ~3 μmol/kg per min; hence, the rate of whole-body FFA re-esterification was ~14 μmol/kg per min (27), which is compatible with the 9% increase in IMCL-TG observed in the current study.

The demonstration that the IMCL-TG content and the level of insulin resistance were both regulated by the level of plasma FFAs does not alone prove that there was a cause-and-effect relationship between changes in IMCL-TG and changes in insulin resistance. Nevertheless, the close association between IMCL-TG and insulin resistance, reported in several recent studies (15,28,29), supported the hypothesis that plasma FFA levels first needed to enter muscle cells and to be re-esterified to IMCL-TG before they caused insulin resistance. This may also explain the 3- to 4-h delay in the onset of FFA-mediated insulin resistance (4,8,9). Presumably, IMCL-TG started to increase at the beginning of the insulin plus fat infusions. The rather sudden development of insulin resistance after ~2 h, therefore, suggested that IMCL-TG (or unidentified factors associated with IMCL-TG) may need to reach certain threshold concentrations before they can interfere with insulin action.

IMCL-TG accumulation may not, however, be the only mechanism by which FFAs cause insulin resistance. For
instance, it seems quite possible that lipid/heparin infusion could also induce changes in fatty acid composition in muscle membrane phospholipids, which could then lead to changes in membrane fluidity and insulin resistance (30).

How could IMCL-TG cause insulin resistance? IMCL-TG has been shown to be a metabolically active pool of fat (31–33), consisting of small oil droplets that are located in close proximity to mitochondria, thereby providing fuel for oxidation (34,35). There is, however, good evidence indicating that changes in fat oxidation are not the immediate reason for the development of insulin resistance. As shown before (8,9) and again in this study, lipid/heparin infusion inhibited fat oxidation long before it inhibited insulin-stimulated glucose uptake. This makes it unlikely that the decrease in fat oxidation was directly responsible for the insulin resistance, as originally postulated by Randle et al. (36). In addition, Han et al. (37) have shown that insulin resistance produced by high-fat feeding in rats was unrelated to changes in fat oxidation. A more likely cause for insulin resistance may have been an increase in cytosolic long-chain acyl-CoA (LC-CoA) concentration associated with the synthesis or the lipolysis of IMCL-TG. LC-CoA is known to increase diacylglycerol and protein kinase C (PKC). The latter can inhibit insulin action via serine-threonine phosphorylation of insulin receptor substrate-1 (IRS-1) (38). In support of this notion, muscle concentration of LC-CoA have been reported to correlate well with insulin resistance and to be increased in the high-fat feeding rat model (39). Moreover, Griffin et al. (40) have recently shown blunting of insulin-stimulated IRS-1 tyrosine phosphorylation and a fourfold increase in inactive (membrane-bound) PKC in lipid/heparin–infused rats.

In summary, we have previously demonstrated that acute increases of plasma FFAs cause insulin resistance with a 3- to 4-h delay (8,9,41). Here, we show that IMCL-TG content and insulin resistance change together within 4 h in response to changes in plasma FFA concentrations. Taken together with studies demonstrating that IMCL-TG content correlated well with insulin resistance, our findings support the claim that an increase in IMCL-TG content comprises a step in the FFA-induced development of insulin resistance.

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REFERENCES


