

Effect of a Sustained Reduction in Plasma Free Fatty Acid Concentration on Intramuscular Long-Chain Fatty Acyl-CoAs and Insulin Action in Type 2 Diabetic Patients

Mandeep Bajaj,¹ Swangjit Suraamornkul,¹ Anthony Romanelli,² Gary W. Cline,² Lawrence J. Mandarino,¹ Gerald I. Shulman,² and Ralph A. DeFronzo¹

To investigate the effect of a sustained (7-day) decrease in plasma free fatty acid (FFA) concentrations on insulin action and intramyocellular long-chain fatty acyl-CoAs (LCFA-CoAs), we studied the effect of acipimox, a potent inhibitor of lipolysis, in seven type 2 diabetic patients (age 53 ± 3 years, BMI 30.2 ± 2.0 kg/m², fasting plasma glucose 8.5 ± 0.8 mmol/l, HbA_{1c} $7.5 \pm 0.4\%$). Subjects received an oral glucose tolerance test (OGTT) and 120-min euglycemic insulin (80 mU/m² per min) clamp with 3-[³H]glucose/vastus lateralis muscle biopsies to quantitate rates of insulin-mediated whole-body glucose disposal (R_d) and intramyocellular LCFA-CoAs before and after acipimox (250 mg every 6 h for 7 days). Acipimox significantly reduced fasting plasma FFAs (from 563 ± 74 to 230 ± 33 μ mol/l; $P < 0.01$) and mean plasma FFAs during the OGTT (from 409 ± 44 to 184 ± 22 μ mol/l; $P < 0.01$). After acipimox, decreases were seen in fasting plasma insulin (from 78 ± 18 to 42 ± 6 pmol/l; $P < 0.05$), fasting plasma glucose (from 8.5 ± 0.8 to 7.0 ± 0.5 mmol/l; $P < 0.02$), and mean plasma glucose during the OGTT (from 14.5 ± 0.8 to 13.0 ± 0.8 mmol/l; $P < 0.05$). After acipimox, insulin-stimulated R_d increased from 3.3 ± 0.4 to 4.4 ± 0.4 mg \cdot kg⁻¹ \cdot min⁻¹ ($P < 0.03$), whereas suppression of endogenous glucose production (EGP) was similar and virtually complete during both insulin clamp studies (0.16 ± 0.10 vs. 0.14 ± 0.10 mg \cdot kg⁻¹ \cdot min⁻¹; $P > 0.05$). Basal EGP did not change after acipimox (1.9 ± 0.2 vs. 1.9 ± 0.2 mg \cdot kg⁻¹ \cdot min⁻¹). Total muscle LCFA-CoA content decreased after acipimox treatment (from 7.26 ± 0.58 to 5.64 ± 0.79 nmol/g; $P < 0.05$). Decreases were also seen in muscle palmitoyl CoA (16:0; from 1.06 ± 0.10 to 0.75 ± 0.11 nmol/g; $P < 0.05$), palmitoleate CoA (16:1; from 0.48 ± 0.05 to 0.33 ± 0.05 nmol/g; $P = 0.07$), oleate CoA (18:1; from 2.60 ± 0.11 to 1.95 ± 0.31 nmol/g; $P < 0.05$), linoleate CoA (18:2; from 1.81 ± 0.26 to 1.38 ± 0.18 nmol/g; $P = 0.13$), and linolenate CoA (18:3; from 0.27 ± 0.03 to 0.19 ± 0.02 nmol/g; $P < 0.03$) levels after acipimox treatment. Muscle stearate CoA (18:0) did not decrease after acipimox treat-

ment. The increase in R_d correlated strongly with the decrease in muscle palmitoyl CoA ($r = 0.75$, $P < 0.05$), oleate CoA ($r = 0.76$, $P < 0.05$), and total muscle LCFA-CoA ($r = 0.74$, $P < 0.05$) levels. Plasma adiponectin did not change significantly after acipimox treatment (7.9 ± 1.8 vs. 7.5 ± 1.5 μ g/ml). These data demonstrate that the reduction in intramuscular LCFA-CoA content is closely associated with enhanced insulin sensitivity in muscle after a chronic reduction in plasma FFA concentrations in type 2 diabetic patients despite the lack of an effect on plasma adiponectin concentration. *Diabetes* 54:3148–3153, 2005

Multiple disturbances in free fatty acid (FFA) metabolism, including daylong elevated plasma FFA levels and accelerated rates of lipolysis, are a characteristic feature of type 2 diabetes (1–4). Elevated plasma FFA concentrations impair glucose metabolism by inhibiting the more proximal steps of insulin action in muscle (5–11) as well by augmenting basal hepatic gluconeogenesis and impairing the suppression of hepatic glucose production by insulin (7,12,13). In addition to having FFAs circulating in plasma in increased amounts, type 2 diabetic and obese patients have increased stores of triglycerides in muscle (14) and liver (15), which correlates closely with the presence of insulin resistance in these tissues. It is now recognized that the triglycerides in liver and muscle are in a state of constant turnover and that the metabolites of intracellular FFA metabolism (i.e., cytosolic long-chain fatty acyl-CoAs [LCFA-CoAs]) can impair insulin action in both liver and muscle (16,17). Cytosolic LCFA-CoA esters are intermediates in lipid synthesis/oxidation and are primarily derived from circulating fatty acids or intramuscular lipid sources such as triglycerides and phospholipids. With respect to insulin action, rodents fed a high-fat diet manifest increased intramuscular LCFA-CoA content, which is associated with insulin resistance (18). In contrast, weight loss in morbidly obese humans is associated with a reduction in intramuscular LCFA-CoA levels and enhanced insulin action (14,19).

Adipocytes function not only as fat depots that release FFAs but also as endocrine organs that release hormones and cytokines in response to specific extracellular stimuli or changes in metabolic status. These secreted proteins, which include tumor necrosis factor- α , interleukin 6, leptin, resistin, adiponectin, and others, perform a variety of diverse functions and have been referred to collectively as “adipocytokines.” Plasma levels of adiponectin are re-

From the ¹Diabetes Division, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas; and the 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 Mandeep Bajaj, MD, Associate Professor, Endocrinology Division, Department of Medicine, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-1060. E-mail: mandeepbajaj@hotmail.com.

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EGP, endogenous glucose production; FFA, free fatty acid; LCFA-CoA, long-chain fatty acyl-CoA; OGTT, oral glucose tolerance test.

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duced in obese humans and type 2 diabetic patients (20). Some (21) but not all (22) studies have demonstrated that plasma adiponectin levels are reduced in the offspring of type 2 diabetic patients. It has been suggested that adiponectin functions as an adipostat to regulate energy balance, and that adiponectin deficiency might contribute to the development of insulin resistance and type 2 diabetes (23). The injection of recombinant adiponectin in mice increases fatty acid oxidation in muscle, reduces triglyceride content in muscle, improves muscle sensitivity to insulin, and decreases basal hepatic glucose output (23–25). Increased FFA transport/oxidation in muscle and a reduction in plasma FFA and muscle triglyceride concentrations also have been reported after adiponectin administration in rodents (23). However, the relation between plasma adiponectin and intramuscular LCFA-CoA levels in type 2 diabetic patients has not been previously studied.

Vaag et al. (26) demonstrated that acipimox, a long-acting analog of nicotinic acid, acutely improves insulin action in type 2 diabetic patients by increasing glucose oxidation as well as nonoxidative glucose disposal. Acipimox had a stimulatory effect on glycogen synthase activity in skeletal muscle. Santomauro et al. (27) demonstrated that overnight administration of acipimox inhibits lipolysis, lowers plasma FFA levels, reduces insulin resistance, increases carbohydrate oxidation, and improves oral glucose tolerance in lean and obese nondiabetic subjects and in subjects with impaired glucose tolerance and type 2 diabetes. Worm et al. (28) administered acipimox for 3 days to eight patients with poorly controlled type 2 diabetes who were on insulin treatment and demonstrated that mean diurnal plasma FFA levels were significantly decreased after acipimox treatment and were associated with a decrease in mean plasma glucose and insulin levels. Stein et al. (29) demonstrated a reduction in intramyocellular triglyceride content after a 6-h infusion of nicotinic acid in healthy, nondiabetic subjects. However, the effect of a sustained (>72-h) reduction in plasma FFA concentrations on intramuscular LCFA-CoAs has not been previously studied. The current study was designed to test the hypothesis that the improvement in insulin sensitivity after a sustained reduction in the plasma FFA concentration after 7 days of acipimox treatment is associated with a decrease in intramuscular LCFA-CoA levels in type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS

We studied seven type 2 diabetic patients (five men, two women; mean age 53 ± 3 years, mean BMI 30.2 ± 2.0 kg/m²). Their fasting plasma glucose concentration and HbA_{1c} were 8.5 ± 0.8 mmol/l and $7.5 \pm 0.4\%$, respectively. The mean plasma lipid concentrations were as follows: total cholesterol 195 ± 14 mg/dl, LDL cholesterol 120 ± 12 mg/dl, HDL cholesterol 38 ± 3 mg/dl, and triglycerides 177 ± 33 mg/dl. Of the seven subjects, three were taking a stable dosage of a sulfonylurea drug (glipizide) for at least 3 months before the study (a dosage that was maintained during the study) and four subjects were treated with diet alone. Patients who had ever received insulin, metformin, or a thiazolidinedione were excluded. Apart from their diabetes, all subjects were in good general health based on their medical history, a physical examination, a screen blood chemistry and hematologic tests, a urinalysis, and an electrocardiogram. Weight was stable in all subjects (± 2 lb) for at least 3 months before the study. No subject was taking any medication known to affect glucose metabolism other than the sulfonylureas. None of the subjects participated in any heavy exercise, and they were instructed not to engage in vigorous exercise for at least 3 days before the study. The purpose, nature, and potential risks of the study were explained to all subjects, and written consent was obtained before their participation. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

All subjects met with a dietitian 3 weeks before the study and were

instructed to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat (10% saturated fat, 10% polyunsaturated fat, and 10% monounsaturated fat), and 20% protein. During the week before the start of acipimox treatment, all subjects received a 75-g oral glucose tolerance test (OGTT) and a euglycemic insulin clamp (30) study in combination with 3-[³H]glucose to examine hepatic and peripheral tissue sensitivity to insulin and vastus lateralis muscle biopsy. The euglycemic insulin clamp study was started at 0600, and the OGTT was begun at 0800 after a 10- to 12-h overnight fast.

After completion of these studies, subjects were started on acipimox, 250 mg p.o. q6h (0600, 1200, 1800, and 2400) for 7 days. The last dose of acipimox was administered at 0600 on day 8. Adherence to the weight-maintaining diet was reinforced on a second meeting with the dietitian on day 1 of the study. At 1600 on day 5, all subjects were admitted to the General Clinical Research Center, where they remained until completion of the study on day 8. While in the center, subjects received a weight-maintaining diet of the same composition and were encouraged to ambulate freely. There was no change in body weight in any subject between the pre- and postacipimox studies. Blood samples for determining fasting plasma FFA concentrations were drawn at 0600 immediately before the administration of acipimox on days 6–8. The OGTT was repeated on day 7, and the euglycemic-hyperinsulinemic clamp/vastus lateralis muscle biopsy study was repeated on day 8 after patients fasted overnight for 10–12 h.

Oral glucose tolerance test. Baseline blood samples for determining plasma glucose, adiponectin, FFA, and insulin concentrations were drawn at -30 , -15 , and 0 min. At 0 min (0830), subjects ingested 75 g of glucose in 300 ml of orange-flavored water. Plasma glucose, FFA, and insulin concentrations were then measured at 15-min intervals for 2 h.

Hyperinsulinemic-euglycemic clamp. Insulin sensitivity was assessed with a euglycemic insulin clamp, as previously described (30). At 0600 (-180 min), a primed (25 μ Ci)-continuous (0.25 μ Ci/min) infusion of 3-[³H]glucose was started via a catheter placed into an antecubital vein and continued throughout the study. A second catheter was placed retrogradely into a vein on the dorsum of the hand, which was then placed in a heated box (60°C). Baseline arterialized venous blood samples for determining plasma 3-[³H]glucose radioactivity and plasma glucose, FFA, and insulin concentrations were drawn at -30 , -20 , -10 , -5 , and 0 min. At 0 min (0900), a primed-continuous infusion of human regular insulin (Novolin; Novo Nordisk, Princeton, NJ) was started at a rate of 80 mU/min per m² of body surface area and continued for 120 min. After the start of insulin, no glucose was infused until the plasma glucose concentration declined to 100 mg/dl, the level at which it was maintained. During the insulin clamp, arterialized blood samples were collected every 5 min to determine plasma glucose levels and a 20% glucose infusion was adjusted based on the negative feedback principle to maintain the plasma glucose concentration at ~ 5.6 mmol/l. Blood samples for determining plasma insulin and 3-[³H]glucose specific activity were collected every 10–15 min during the insulin clamp. Continuous indirect calorimetry using a ventilated hood system (Deltatrac II; Sensor Medics, Yorba Linda, CA) was performed during the last 40 min of the basal period and during the last 30 min of the insulin clamp, as previously described (31).

Intramyocellular LCFA-CoA determination: liquid chromatography-tandem mass spectrometry. A needle biopsy of the vastus lateralis was obtained under local anesthesia in the fasted condition immediately before the hyperinsulinemic-euglycemic clamp (0845). Visible fat and/or connective tissue was removed from the sample (50–100 mg) and frozen in liquid nitrogen for subsequent analyses. LCFA-CoAs were extracted from the biopsy sample by solid-phase extraction, and C17 CoA was added as an internal standard, as previously described (19,32,33). A tandem mass spectrometer (API 3000; PerkinElmer Sciex) interfaced with a TurboIonSpray ionization source was used for mass spectrometry/mass spectrometry analysis (33). Fatty acyl-CoAs were ionized in a negative electrospray mode, and the transition pairs $[M-2H]^-/[M-H-80]^-$ 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 (33). Total LCFA-CoA content was calculated as the sum of the LCFA-CoA species measured (19).

Analytic determinations. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Plasma insulin concentrations were measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Tritiated glucose specific activity was determined on deproteinized barium/zinc plasma samples, as previously described (2). Plasma FFA concentrations were determined by an enzymatic calorimetric quantification method (Wako, Nuess, Germany). Plasma adiponectin concentrations were measured by radioimmunoassay (Linco Research, St. Charles, MO).

Calculations. Under steady-state postabsorptive conditions, the rate of endogenous glucose appearance (R_{eG}) was calculated as the 3-[³H]glucose infusion rate (dpm/min) divided by the steady-state plasma 3-[³H]glucose specific activity (dpm/mg). In the postabsorptive state, the hepatic insulin

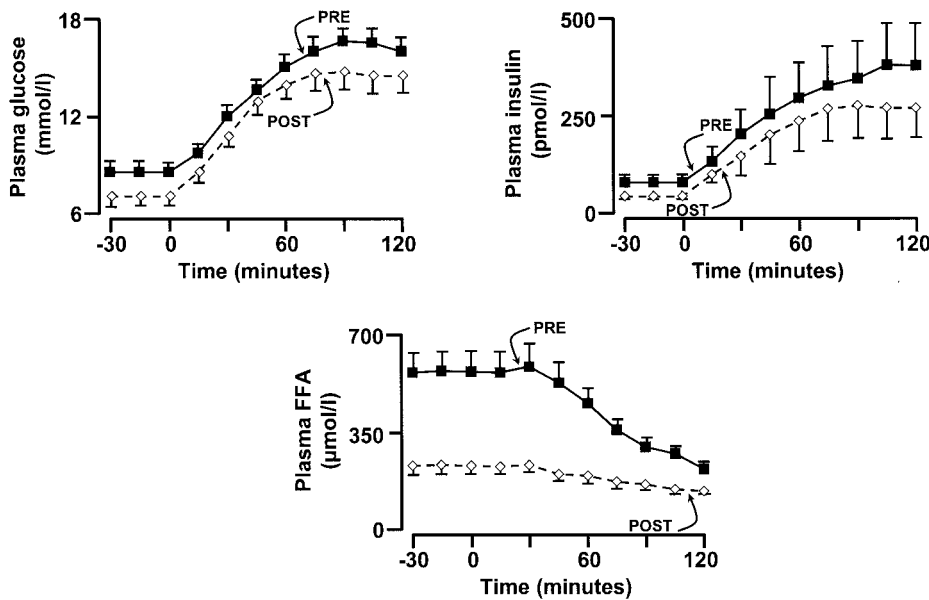


FIG. 1. Plasma glucose, insulin, and FFA concentrations during the 75-g OGTT before (PRE; ■) and after (POST; ◇) acipimox treatment.

resistance index was calculated as the product of endogenous glucose production (EGP) and the fasting plasma insulin concentration. During the euglycemic insulin clamp, the rate of whole-body glucose appearance (R_a) was calculated using Steele's equation (34) using a distribution volume of 250 ml/kg. EGP was calculated by subtracting the exogenous glucose infusion rate from R_a . The rate of insulin-mediated whole-body glucose disposal (R_d) was determined by adding the rate of residual EGP to the exogenous glucose infusion rate.

Statistical analysis. Statistical calculations were performed with StatView for Windows, Version 5.0 (SAS Institute, Cary, NC). Values before and after acipimox treatment were compared using paired t tests. Data are presented as means \pm SE. $P < 0.05$ was considered to be statistically significant. Linear regression analysis was used to examine the relation between intramuscular LCFA-CoA concentrations and peripheral (muscle) insulin sensitivity.

RESULTS

Fasting plasma FFAs. After acipimox treatment, fasting plasma FFA levels measured at 0600 (i.e., before the morning acipimox dose) were significantly reduced, dropping from 563 ± 74 to 233 ± 32 $\mu\text{mol/l}$ ($P < 0.01$) on day 6, 241 ± 46 $\mu\text{mol/l}$ ($P < 0.01$) on day 7, and 285 ± 42 $\mu\text{mol/l}$ ($P < 0.03$) on day 8.

Oral glucose tolerance test. After acipimox treatment, the baseline fasting plasma FFA concentration was reduced from 563 ± 74 to 230 ± 33 $\mu\text{mol/l}$ ($P < 0.01$) (Fig. 1). Significant reductions were seen in fasting plasma insulin (from 78 ± 18 to 42 ± 6 pmol/l; $P < 0.05$) and fasting plasma glucose (from 8.5 ± 0.8 to 7.0 ± 0.5 mmol/l; $P < 0.02$) concentrations after acipimox treatment, as well as in mean plasma glucose (from 14.5 ± 0.8 to 13.0 ± 0.8 mmol/l; $P < 0.05$) and FFA (409 ± 44 to 184 ± 22 $\mu\text{mol/l}$; $P < 0.01$) concentrations during the OGTT after acipimox treatment. The mean plasma insulin concentration during the OGTT decreased slightly (from 302 ± 92 to 220 ± 60 pmol/l; $P < 0.10$) after acipimox treatment.

Euglycemic-hyperinsulinemic clamp. The steady-state plasma glucose concentrations during the 120-min euglycemic insulin clamp were similar before and after acipimox treatment (5.8 ± 0.2 vs. 5.7 ± 0.2 mmol/l). The steady-state plasma insulin concentrations during the insulin clamp studies were similar before and after acipimox treatment (902 ± 66 vs. 840 ± 70 pmol/l). The fasting plasma FFA levels before the start of the euglycemic insulin clamp were significantly lower after acipimox treatment (300 ± 78 vs. 670 ± 73 $\mu\text{mol/l}$; $P < 0.02$).

Basal rates of EGP were similar before and after acipimox treatment (1.9 ± 0.2 vs. 1.9 ± 0.2 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). However, the basal hepatic insulin resistance index was significantly reduced after acipimox treatment (from 148 ± 33 to 79 ± 12 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \times \text{pmol/l}$; $P < 0.05$). Insulin-mediated suppression of EGP, determined during the 90- to 120-min period of the euglycemic insulin clamp, was similar and almost complete before and after acipimox treatment (0.16 ± 0.10 vs. 0.14 ± 0.10 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively).

In the basal state, the total body lipid oxidation rate was decreased after acipimox treatment (from 0.82 ± 0.05 to 0.64 ± 0.07 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$), whereas glucose oxidation was increased (from 0.8 ± 0.1 to 1.3 ± 0.2 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$). After acipimox treatment, the whole-body glucose disposal rate (R_d) during the insulin clamp was significantly increased (4.4 ± 0.4 vs. 3.3 ± 0.4 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.03$) (Fig. 2), due entirely to an increase in insulin-stimulated nonoxidative glucose disposal (from 1.0 ± 0.2 to 2.0 ± 0.4 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.02$). The increase in R_d correlated strongly with the decrease in the fasting plasma FFA concentration after acipimox treatment ($r = -0.78$, $P < 0.03$).

Intramyocellular LCFA-CoAs. Total muscle LCFA-CoA content decreased significantly (from 7.26 ± 0.58 to 5.64 ± 0.79 nmol/g; $P < 0.05$) after acipimox treatment (Fig. 2). Muscle palmityl CoA also decreased after acipimox treatment (16:0; from 1.06 ± 0.10 to 0.75 ± 0.11 nmol/g; $P < 0.05$), as did oleate CoA (18:1; from 2.60 ± 0.11 to 1.95 ± 0.31 nmol/g; $P < 0.05$) and linolenate CoA (18:3; from 0.27 ± 0.03 to 0.19 ± 0.02 nmol/g; $P < 0.03$). There was a trend for a decrease in muscle palmitoleate CoA (16:1; from 0.48 ± 0.05 to 0.33 ± 0.05 nmol/g; $P = 0.07$) and linoleate CoA (18:2; from 1.81 ± 0.26 to 1.38 ± 0.18 nmol/g; $P = 0.13$) after acipimox treatment. Muscle stearate CoA did not decrease after acipimox treatment (18:0; 1.04 ± 0.11 vs. 1.01 ± 0.12 nmol/g; NS). The increase in R_d correlated with the decrease in muscle palmityl CoA ($r = 0.75$, $P < 0.05$), oleate CoA ($r = 0.76$, $P < 0.05$), and total muscle LCFA-CoA ($r = 0.74$, $P < 0.05$) (Fig. 3).

Plasma adiponectin concentration. The plasma adiponectin did not change significantly after acipimox treatment (7.9 ± 1.8 vs. 7.5 ± 1.5 $\mu\text{g/ml}$) (Fig. 2).

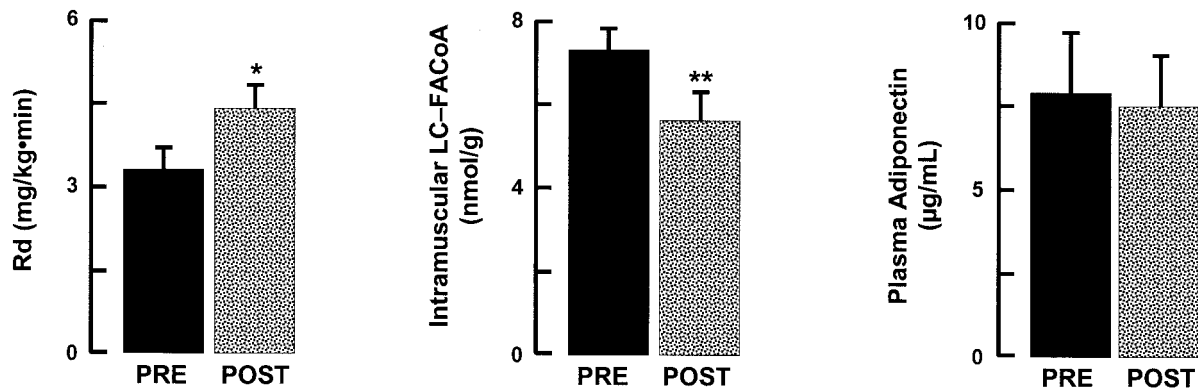


FIG. 2. Effect of acipimox on insulin-stimulated glucose disposal (R_d), intramuscular LCFA-CoA concentrations, and plasma adiponectin levels before (PRE) and after (POST) treatment. * $P < 0.03$; ** $P < 0.05$.

DISCUSSION

In the present study, we examined the effect of a sustained (7-day) reduction in plasma FFA concentrations achieved with acipimox on intramyocellular LCFA-CoAs and peripheral insulin sensitivity in type 2 diabetic patients. The results demonstrated that a sustained reduction in plasma FFAs is associated with a reduction in intramyocellular LCFA-CoAs and enhanced peripheral tissue (muscle) insulin sensitivity, due entirely to an increase in nonoxidative glucose disposal, which primarily reflects muscle glycogen synthesis (35). The reduction in muscle LCFA-CoAs and improved peripheral insulin sensitivity with acipimox treatment were not associated with any change in plasma adiponectin levels. These results indicate that the beneficial effects of reduced plasma FFA levels on insulin sensitivity and glucose tolerance in type 2 diabetic patients are mediated by changes in intramyocellular LCFA-CoAs and are independent of any changes in the circulating adiponectin concentration.

Results from most (36–40) but not all (41) studies have suggested that increases in plasma FFA concentrations cause insulin resistance in skeletal muscle by inhibiting the insulin signal transduction pathway at the level of insulin receptor substrate 1 tyrosine phosphorylation. An increase in the delivery of fatty acids to muscle or a decrease in intracellular metabolism of fatty acids leads to an increase in intracellular fatty acid metabolites such as diacylglycerol, fatty acyl-CoAs, and ceramides. In vitro and

in vivo studies in rodents have shown that these metabolites activate serine/threonine kinases via a process involving protein kinase C θ (42,43). Subsequent phosphorylation of serine/threonine sites on insulin receptor substrates (1 and 2), in turn, reduces the ability of the insulin receptor substrates to activate phosphatidylinositol 3-kinase (16,37, 44). As a consequence, glucose transport activity and other events downstream of insulin receptor signaling are diminished (37). A major defect in insulin-stimulated glycogen synthesis is a characteristic finding in all insulin-resistant states, including obesity and type 2 diabetes (6,45). Vaag et al. (26) have shown that an acute reduction in plasma FFA concentrations with acipimox stimulates skeletal muscle glycogen synthase activity in type 2 diabetic patients. The results of the present study have demonstrated for the first time that a sustained reduction in plasma FFAs with acipimox administration reduces intramyocellular LCFA-CoAs and augments insulin-stimulated nonoxidative glucose disposal in type 2 diabetic patients. The importance of the acipimox-induced decrease in the intramyocellular LCFA-CoA concentration is underscored by its strong correlation with improvement in whole-body (primarily muscle) glucose disposal during the hyperinsulinemic-euglycemic clamp. Other possible mechanisms for the improvement in insulin sensitivity after a decrease in circulating FFA concentrations include an increase in intramyocellular lipid oxidation, a direct membrane effect, and/or a reduction in other intramyocellular lipotoxic (ceramides and diacylglycerol) metabolites (46).

Although muscle stearate CoA (18:0) did not decrease with acipimox treatment, the three major and most abundant long-chain acyl-CoA species in skeletal muscle (palmityl CoA [16:0], oleate CoA [18:1], and linoleate CoA [18:2]) decreased significantly after acipimox treatment. Stein et al. (47) reported that saturated fatty acids (e.g., palmitic, stearic) promote the hypersecretion of insulin more effectively than unsaturated fatty acids of similar chain lengths (e.g., linoleic, oleic) and speculated that saturated fatty acids and their intracellular fatty acyl-CoA derivatives may play a central role in the development of insulin resistance (47). In support of this concept, the consumption of saturated fats has been shown to be associated with hyperinsulinemia and the subsequent development of type 2 diabetes, hypertension, and obesity (48). In the present study, we did observe an improvement in insulin sensitivity despite the lack of a reduction in muscle stearate CoA (18:0), suggesting that insulin sensitivity is independent of changes in intramyocellular stear-

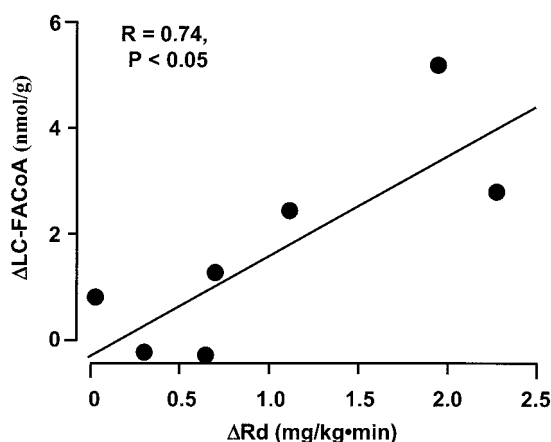


FIG. 3. Relation between the decrease in intramuscular LCFA-CoA concentrations (Δ LC-FACoA) and increase in whole-body glucose disposal (ΔR_d) during the euglycemic insulin clamp after acipimox treatment.

ate CoA in type 2 diabetic patients. However, we also observed a highly significant relation between the reduction in both palmityl and oleate CoA in human skeletal muscle and the improvement in insulin sensitivity after acipimox, indicating that both saturated (palmityl CoA) and unsaturated (oleate CoA) fatty acyl-CoAs of similar chain length are associated with FFA-induced insulin resistance in type 2 diabetic patients.

Our results also demonstrated that a prolonged reduction in plasma FFA concentrations after acipimox treatment, although associated with a reduction in intramyocellular LCFA-CoAs, does not alter plasma adiponectin concentrations in type 2 diabetic patients. Low plasma adiponectin concentrations have been consistently observed in patients with obesity and type 2 diabetes (20). Previous studies from our laboratory have shown a strong relation between reduced plasma adiponectin levels and hepatic and peripheral tissue insulin resistance in type 2 diabetic patients (49). One of the primary effects of adiponectin is to increase fatty acid oxidation in muscle, leading to a decrease in intracellular fatty acid metabolites (i.e., fatty acyl-CoAs, diacylglycerol, and ceramides) and enhanced insulin signal transduction (23). This observation points to adiponectin as the possible link between LCFA-CoAs and insulin resistance in patients with obesity and type 2 diabetes. However, after acipimox treatment, the plasma adiponectin concentration did not change despite an improvement in peripheral insulin sensitivity and a reduction in intramyocellular LCFA-CoAs. These results indicate that changes in plasma adiponectin levels cannot explain the chronic reduction in plasma FFA/intramyocellular LCFA-CoA concentrations and improved insulin sensitivity in the current study. In a previous study from our laboratory (50), plasma adiponectin levels did not change significantly in insulin-resistant offspring of type 2 diabetic patients after 7 days of acipimox treatment. Similar to the results of the present study, plasma FFA levels decreased and insulin sensitivity improved after acipimox therapy in that study. Taken together, these data suggest that circulating plasma adiponectin and FFA concentrations are independent predictors of insulin sensitivity.

Fulcher et al. (51) demonstrated a small (13%) improvement in insulin sensitivity in nondiabetic healthy subjects independent of changes in plasma FFA levels after the administration of acipimox (1 g) in combination with an intralipid/heparin infusion; these results suggested a direct drug effect. However, in the present study, the enhanced insulin sensitivity of type 2 diabetic patients after acipimox treatment strongly correlated with decreases in plasma FFA and intramyocellular LCFA-CoA concentrations, suggesting that the reduction in plasma FFA levels is the predominant mechanism for the acipimox-induced improvement in insulin sensitivity. Consistent with the results of the present study, Qvistad et al. (52) recently demonstrated that the acipimox-induced enhancement in insulin sensitivity was reversed with the infusion of intralipid/heparin in type 2 diabetic patients.

Vaag and Beck-Nielsen (53) studied the effect of prolonged (3-month) acipimox treatment in type 2 diabetic patients and demonstrated the lack of an effect of acipimox on glycemic control and circulating insulin levels. They also noted a twofold increase in fasting plasma FFA levels after 3 months of acipimox treatment and stated that the rebound lipolysis that is associated with the long-term administration of acipimox limits the use of acipimox as a possible therapeutic option for type 2 diabetic

patients. In our study, we measured fasting plasma FFA concentrations at 0600 immediately before the ingestion of acipimox on days 6–8 and demonstrated a sustained suppression of plasma FFA concentrations. Therefore, the improvement in insulin sensitivity cannot be attributed to an acute acipimox-induced suppression of lipolysis/plasma FFA levels in individuals who had experienced a rebound from the antilipolytic effect of acipimox.

In summary, the results of the present study demonstrate that a sustained reduction in plasma FFA concentrations after 7 days of acipimox therapy in type 2 diabetic patients 1) enhances insulin-mediated whole-body glucose disposal, 2) decreases intramyocellular LCFA-CoA levels, 3) reduces fasting and postprandial plasma glucose concentrations, 4) improves the basal hepatic insulin resistance index, and 5) does not significantly alter the circulating adiponectin concentration.

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