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Chronic Reduction of Plasma Free Fatty Acid Improves Mitochondrial Function and Whole-Body Insulin Sensitivity in Obese and Type 2 Diabetic Individuals

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Insulin resistance and dysregulation of free fatty acid (FFA) metabolism are core defects in type 2 diabetic (T2DM) and obese normal glucose tolerant (NGT) individuals. Impaired muscle mitochondrial function (reduced ATP synthesis) also has been described in insulin-resistant T2DM and obese subjects. We examined whether reduction in plasma FFA concentration with acipimox improved ATP synthesis rate and altered reactive oxygen species (ROS) production. Eleven NGT obese and 11 T2DM subjects received *1*) OGTT, *2*) euglycemic insulin clamp with muscle biopsy, and *3*) ^{1}H-magnetic resonance spectroscopy of tibialis anterior muscle before and after acipimox (250 mg every 6 h for 12 days). ATP synthesis rate and ROS generation were measured in mitochondria isolated from muscle tissue ex vivo with chemoluminescence and fluorescence techniques, respectively. Acipimox *1*) markedly reduced the fasting plasma FFA concentration and enhanced suppression of plasma FFA during oral glucose tolerance tests and insulin clamp in obese NGT and T2DM subjects and *2*) enhanced insulin-mediated muscle glucose disposal and suppression of hepatic glucose production. The improvement in insulin sensitivity was closely correlated with the decrease in plasma FFA in obese NGT (r = 0.81) and T2DM (r = 0.76) subjects (both $P < 0.001$). Mitochondrial ATP synthesis rate increased by >50% in both obese NGT and T2DM subjects and was strongly correlated with the decrease in plasma FFA and increase in insulin-mediated glucose disposal (both $r > 0.70$, $P < 0.001$). Production of ROS did not change after acipimox. Reduction in plasma FFA in obese NGT and T2DM individuals improves mitochondrial ATP synthesis rate, indicating that the mitochondrial defect in insulin-resistant individuals is, at least in part, reversible.

Insulin resistance is a core defect in type 2 diabetes (T2DM), and it is strongly associated with obesity (1). Although the etiology of insulin resistance is not fully understood, it is well recognized that dysregulation of fat metabolism plays a pivotal role in the development of insulin resistance, and this has been referred to as "lipotoxicity" (2). Insulin-resistant individuals manifest an increase in the day-long plasma free fatty acid (FFA) concentration (3) and increased fat content in insulin-responsive tissues, skeletal muscle, and liver (4–6).

In vivo and ex vivo studies (7–9) have demonstrated impaired mitochondrial function in skeletal muscle in insulin-resistant individuals. However, the relationship between impaired mitochondrial function, insulin resistance, and dysregulation of fat metabolism is a subject of debate (10–12). It has been suggested that the mitochondrial defect in skeletal muscle is primary and could lead to an increase in intramyocellular (IMCL) fat content,

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PATHOPHYSIOLOGY

thereby impairing insulin signaling and resulting in insulin resistance (7). Others have suggested that impaired mitochondrial function in skeletal muscle in insulin-resistant individuals results secondary to the defect in insulin action and is not the cause of the insulin resistance. We and others (13–15) previously have shown that lowering the plasma FFA concentration with acipimox improves insulin sensitivity in T2DM individuals. The aim of the current study was to examine the effect of lowering the plasma FFA concentration with acipimox on mitochondrial ATP synthesis rate and reactive oxygen species (ROS) production in obese insulin-resistant individuals with *1*) normal glucose tolerance (NGT) and *2*) T2DM.

We hypothesize that if the mitochondrial defect associated with insulin resistance is a primary disturbance in insulin-resistant individuals, improving insulin sensitivity in skeletal muscle by lowering plasma FFA concentration with acipimox will not be associated with an improvement in muscle mitochondrial function.

RESEARCH DESIGN AND METHODS

Subjects

We studied 11 healthy obese NGT individuals and 11 T2DM patients. Insulin-resistant individuals manifest impaired mitochondrial function, and insulin resistance is a core defect in both T2DM and obese nondiabetic individuals. Because both T2DM and obese NGT individuals have been reported to manifest impaired mitochondrial function, we included both of these insulin-resistant groups in the study to examine the effect of lowering plasma FFA on insulin sensitivity and mitochondrial function. Of the 11 T2DM patients, 2 were drug naïve, while the other 9 subjects were treated with metformin (n = 5), sulfonylurea (n = 1) or a combination of the two (n = 3). Inclusion criteria were *1*) age 18–65 years; *2*) BMI 30–37 kg/m^2, *3*) normal oral glucose tolerance test (OGTT) according to American Diabetes Association criteria for obese nondiabetic subjects, and *4*) drug-naïve or sulfonylurea and/or metformin T2DM treatment. Exclusion criteria included *1*) previous treatment with insulin or thiazolidinediones (>2 weeks within the previous year); *2*) blood pressure >140/90 mmHg; *3*) serum creatinine >1.6 mg/dL; *4*) hematocrit <35%; *5*) evidence of major organ system disease as determined by medical history, physical exam, and routine screening blood chemistries; and *6*) medications known to affect glucose metabolism, other than metformin and sulfonylurea.

The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and informed written consent was obtained from all subjects prior to their participation.

Study Design

After screening, eligible subjects underwent a 2-h 75-g OGTT, a 4-h hyperinsulinemic-euglycemic clamp (60 mU/m^2 · min) with two vastus lateralis muscle biopsies before and at the end of the insulin clamp, a dual-energy X-ray absorptiometry (DEXA) scan, and ^{1}H-magnetic resonance spectroscopy (MRS) to quantitate tibialis anterior muscle lipid content. After completing the baseline studies, subjects received acipimox (Olbetam, U.K.), 250 mg orally every 6 h (at 0600, 1200, 1800, and 2400 h), for 12 days. On day 11, subjects received a repeat OGTT, DEXA, and MRS studies, and on day 12 the hyperinsulinemic-euglycemic clamp was repeated. Before the initiation of acipimox, all participants received dietary counseling and were asked to consume a standard American Diabetes Association, weight-maintaining diet and to maintain the same physical activity throughout the study. A daily call was made to all participants to encourage compliance with the treatment recommendations.

OGTT

Baseline blood samples for determination of plasma glucose, FFA, and insulin concentrations were drawn at −30, −15, and 0 min. At time zero (0830 h), subjects ingested 75 g glucose in 300 mL orange-flavored water. Plasma glucose, FFA, and insulin concentrations were measured at 15-min intervals for 2 h.

DEXA

DEXA (Hologic, Inc., Waltham, MA) was performed to determine fat and lean body mass before and after acipimox treatment.

Hyperinsulinemic-Euglycemic Clamp

At 0600 h (−180 min), after a 10-h overnight fast, a prime (25 mCi × fasting plasma glucose [FPG]/100) continuous (0.25 μCi/min) infusion of [3-^{3}H]-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 determination of plasma [3-^{3}H]-glucose radioactivity and plasma glucose, insulin, FFA, and glycerol concentrations were drawn at −30, −20, −10, −5, and 0 min. A needle biopsy of the vastus lateralis muscle was obtained under local anesthesia before the start (−60 min) and at the end (240 min) of the hyperinsulinemic-euglycemic clamp. At time zero, insulin was infused at 60 mU/kg · min. Arterialized blood samples were collected every 5 min for plasma glucose determination, and a 20% glucose infusion was adjusted to maintain the plasma glucose concentration at 100 mg/dL. Throughout the insulin clamp, blood samples for determination of plasma glucose concentration were drawn every 10–15 min for determination of plasma insulin, FFA, and glycerol concentrations and [3-^{3}H]-glucose specific activity. Continuous indirect calorimetry using a ventilated hood system (Deltatrac II; SensorMedics, Yorba Linda, CA) was performed during the last 40 min of the basal period and during the last 30 min of the insulin clamp.

Mitochondrial ATP Synthesis

Mitochondrial ATP synthesis rate was measured ex vivo with a chemiluminescence technique as previously described

(16). Briefly, mitochondria were isolated from fresh muscle tissue with differential centrifugation. Mitochondrial protein (4 μg) was aliquoted to each reaction well. Substrates were added as follows: 2.5 mmol/L pyruvate, 2.5 mmol/L glutammate, 5 mmol/L succinate plus 0.001 mmol/L rotenone, and palmitoyl-L-carnitine. Malate (2.5 mmol/L) was added to complex I substrates. Luciferine/luciferase was added to monitor ATP production. After a 5-min incubation at 37°C, the substrates were added and the reaction was started by the addition of ADP.

Mitochondrial ROS Production

The rate of mitochondrial ROS production was measured by quantification of the release of mitochondrial H_2O_2 with the fluorescent dye Amplex Red (Molecular Probes, Eugene, OR) as previously described (8). ROS production rate was performed in mitochondria under state II (with substrate and without the addition of ADP) conditions. The substrate concentrations were the same as with the measurement of ATP synthesis. Fluorescence was observed at 530 nm excitation and 590 nm emission for 5 min. The slope in fluorescence was converted to the H_2O_2 production rate using a standard curve.

In Vivo ^{1}H-Nuclear Magnetic Resonance Spectroscopy

Experiments were performed in a 3 Tesla magnetic resonance imaging scanner (TIM Trio; Siemens Medical Solutions, Malvern, PA) using a standard birdcage volume extremity coil with the subject laying in a supine position with the most extended part of the left calf in the center of the coil. Multislice T1-weighted spin-echo images were acquired to facilitate the positioning of a parallelepiped volume of interest of 15 mm × 15 mm × 25 mm in the tibialis anterior muscle. A PRESS single-voxel MRS technique was used with repetition time/echo time = 3,000 ms/250 ms. Two spectra were acquired: one with spectral suppression of the water signal (number of signals acquired = 128) and the second without the water signal suppressed (number of signals acquired = 4), which served as a reference scan for lipid concentration estimates.

The position of the water peak was used to shift the water-suppressed spectrum to ensure that the suppressed water line was exactly at 4.77 ppm. Spectral data were analyzed using the jMRUI software (17) using prior knowledge values for the AMARES quantitation package (18) as developed by Weis et al. (19) to distinguish the extramyocellular (EMCL)-CH_2 spectral line from the IMCL-CH_2 line. Baseline correction was performed by truncating the first two points of the free induction decay and by applying the time domain Hankel-Lanczos Singular Value Decomposition filter to remove the underlying tails from the residual water resonance. The total lipid content in the musculature was computed from the ratio of EMCL and IMCL methylenes (EMCL-CH_2 + IMCL-CH_2) and the unsuppressed water line. Spectral lines were corrected for relaxation effects using the following expression: exp(−echo time/T2) [1 − exp(−repetition time/T1)], applying the values T1(IMCL) = 413 ms, T1(EMCL) = 420 ms, T1(H_2O) = 1,387 ms, T2(IMCL) = 90.9 ms, T2(EMCL) = 77.5 ms, and T2(H_2O) = 28.4 ms, as reported by Krssák et al. (20) for tibialis muscle. The equation of Szczepaniak et al. (21) was used to calculate the absolute concentrations expressed as millimoles per kilogram of wet weight from the methylene-to-water spectral intensity ratio (Z). If LC is the lipid content in millimoles per kilogram wet weight, then $LC = (ZW \times 10^6) \div [885.4\ D\ (ZW + P)]$, where W = 0.76 represents the tissue water content relative to total weight (kg/kg) of the normal muscle tissue, T = 1.024 is the weighted density of the fat (triglyceride fatty acids) relative to the triolein standard (molecular weight 885.4) (21), D = 1.05 kg/L is the density of lean muscle tissue, and P = 0.61 is the relative methylene proton density (mol/mol) of tissue fat versus water (19).

Analytical Determinations

Plasma glucose was measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentration was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Tritiated glucose specific activity was determined on deproteinized barium/zinc plasma samples. Plasma FFA concentration was determined by an enzymatic colorimetric quantification method (Wako Chemicals, Nuess, Germany). Plasma glycerol was determined by an enzymatic colorimetric quantification method (Cayman Chemical Company, Ann Arbor, MI).

Calculations

Under steady-state postabsorptive conditions, the rate of endogenous glucose appearance (R_a) was calculated as the [3-^{3}H]-glucose infusion rate (DPM/min) divided by the steady-state plasma [3-^{3}H]-glucose specific activity (DPM/mg). During the euglycemic insulin clamp, the R_a was calculated with the Steele equation (22), using a distribution volume of 250 mL/kg. Endogenous (primarily reflects hepatic) glucose production (EGP) was calculated by subtracting the exogenous glucose infusion rate from R_a. The rate of insulin-mediated total body glucose disposal (R_d) was determined by adding the rate of residual EGP to the exogenous glucose infusion rate. ATP synthesis rate was calculated as nmol/mg protein · min, and H_2O_2 production was expressed as pmol/mg protein · min.

Statistical Analyses

All statistical analyses were performed with SPSS, version 20 (SPSS, Chicago, IL). Values are expressed as means ± SEM. Paired Student *t* test was used to compare differences between the means before and after acipimox treatment. Statistical significance was considered at $P < 0.05$. Simple Pearson correlation analysis was used to evaluate the correlation between variables. Fisher *r*-to-*z* transformation was used to convert each correlation coefficient into a *z* score. The *z* critical value for the 90% level of confidence was 1.645. The *z* score calculation was used to test the difference between correlation coefficients in NGT and T2DM participants.

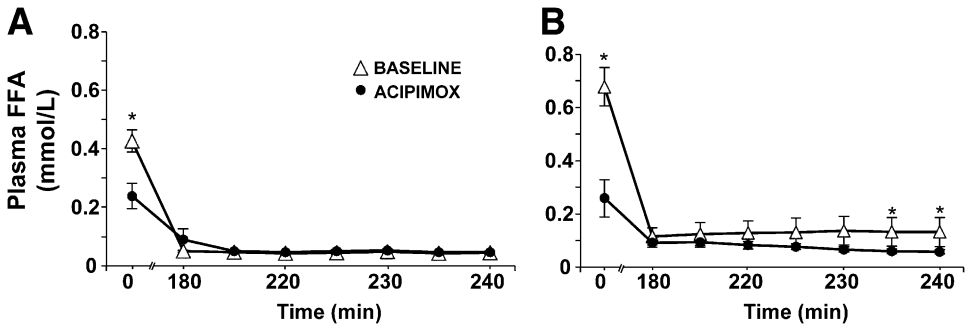

Figure 1—Plasma FFA concentrations during the hyperinsulinemic-euglycemic clamp at baseline and after acipimox treatment. *A*: Obese NGT group. *B*: T2DM group. *$P \leq 0.05$.

RESULTS

Effect of Acipimox on Plasma FFA Concentration

Fasting plasma FFA levels were 0.67 ± 0.08 in T2DM and was suppressed to 0.13 ± 0.054 mmol/L during the last hour of the clamp. In obese NGT individuals, the fasting plasma FFA was 0.45 ± 0.03 mmol/L and suppressed to 0.046 ± 0.005 mmol/L during the clamp (Fig. 1). At the end of 2 weeks of treatment with acipimox, there was a marked decrease in plasma FFA concentration in both obese NGT and T2DM individuals. In T2DM, the fasting plasma FFA was 0.25 ± 0.05 mmol/L ($P < 0.01$ vs. pre-acipimox) and suppressed to 0.057 ± 0.008 mmol/L ($P < 0.05$ vs. pre-acipimox), while in obese NGT individuals the fasting plasma FFA was 0.22 ± 0.04 ($P < 0.01$ vs. pre-acipimox) and suppressed to 0.043 ± 0.003 mmol/L (*P* = not significant vs. pre-acipimox) (Table 1).

Effect of Plasma FFA Reduction on Insulin Sensitivity in Muscle, Liver, and Adipose Tissue

Whole-Body (Primarily Reflects Muscle) Insulin Sensitivity

Total body insulin-mediated glucose disposal (TGD) divided by steady-state plasma insulin concentration (SSPI) during the insulin clamp was significantly greater in obese NGT subjects at baseline (TGD = 6.4 ± 0.5 mg/kg/min and SSPI = 90.7 ± 0.6 μU/mL) compared with T2DM individuals (TGD = 4.1 ± 0.4 mg/kg/min and SSPI = 91.0 ± 0.2 μU/mL; $P < 0.05$ vs. obese NGT group), and it was significantly increased after acipimox treatment in obese NGT (TGD = 7.2 ± 0.6 mg/kg/min and SSPI = 86.8 ± 0.8 μU/mL; *P* = 0.01) and T2DM (TGD = 5.1 ± 0.4 mg/kg/min and SSPI = 95.7 ± 0.1 μU/mL; *P* = 0.04) groups (Fig. 2*A*). Thus, acipimox caused a similar increase in insulin-mediated whole-body (muscle) glucose uptake in both groups (by 16% and 18% in obese and T2DM individuals, respectively). Moreover, the increase in insulin-mediated glucose disposal produced by acipimox strongly correlated with the decrease in fasting plasma FFA concentration in both groups ($r = -0.76$, $P < 0.001$, in NGT obese and $r = -0.81$, $P < 0.001$, in T2DM) (Supplementary Fig. 1). The resting respiratory quotient was higher in obese NGT compared with T2DM subjects (0.81 ± 0.01 vs. 0.75 ± 0.02, $P < 0.02$), but it did not change after acipimox treatment in either obese NGT (0.81 ± 0.02, *P* = 0.80) or T2DM (0.78 ± 0.01, *P* = 0.07) individuals. Energy expenditure was slightly higher in T2DM compared with NGT individuals (1,569 ± 76 vs. 1,313 ± 130 kcal/day; *P* = 0.12), and it decreased significantly after acipimox treatment in obese NGT individuals (1,214 ± 120 kcal/day, *P* = 0.01) and remained unchanged in T2DM individuals (1,446 ± 86 kcal/day, *P* = 0.23). In the basal state, total body lipid oxidation rate was decreased after acipimox treatment in T2DM individuals (2.2 ± 0.01 to 1.9 ± 0.01 mg/kg · min, $P < 0.05$) but did not change in obese NGT individuals (2.1 ± 0.02 to 2.1 ± 0.01 mg/kg · min, *P* = 0.50). Glucose oxidation was increased after acipimox treatment in T2DM individuals (0.5 ± 0.001 to 0.7 ± 0.002 mg/kg · min, $P < 0.05$) and did not change in obese NGT individuals (1.1 ± 0.002 to 1.2 ± 0.001 mg/kg · min, *P* = 0.52). Insulin-stimulated nonoxidative glucose disposal increased in T2DM (4.4 ± 0.3 to 5.7 ± 0.6 mg/kg · min, $P < 0.05$) and obese NGT (7.5 ± 0.3 to 8.2 ± 0.8 mg/kg · min, $P < 0.05$) individuals after acipimox treatment.

Liver

Acipimox did not affect the basal rate of EGP, which was similar before and after acipimox treatment in both

Table 1—Clinical characteristics of study participants

	Obese NGT	T2DM	*P*
Age (years)	41 ± 9	55 ± 9	0.006
Sex (male/female)	3/8	8/3	0.008
BMI (kg/m^2)	33.2 ± 1.1	32.5 ± 1.6	0.55
HbA_{1c}, % (mmol/mol)	5.4 ± 0.7 (36 ± 5)	7.0 ± 0.3 (53 ± 2)	<0.001
FPG (mmol/L)	5.8 ± 0.1	7.9 ± 0.7	0.001
FPI (μU/mL)	6.5 ± 1.2	10.8 ± 1.9	0.02
2-h PG (mmol/L)	7.0 ± 0.2	15.2 ± 1.5	<0.001
Fasting plasma FFA (mmol/L)	0.45 ± 0.03	0.67 ± 0.08	0.01

Data are means ± SEM unless otherwise indicated. 2-h PG, 2-h plasma glucose during OGTT.

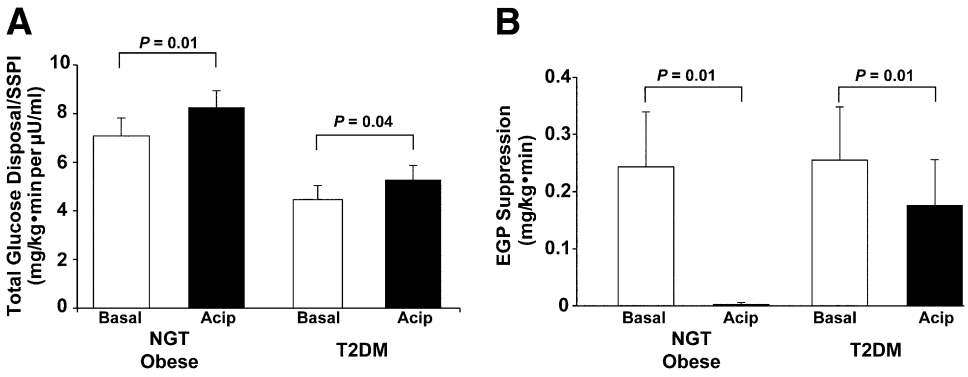

Figure 2—Whole-body insulin sensitivity and EGP suppression during hyperinsulinemic-euglycemic clamp at baseline and after acipimox (Acip) treatment. *A*: Total R_d/SSPI in obese NGT and T2DM groups. *B*: EGP suppression in obese NGT and T2DM groups.

groups (1.96 ± 0.07 vs. 1.99 ± 0.08 mg/kg · min in obese NGT and 1.94 ± 0.12 vs. 2.00 ± 0.11 mg/kg · min in T2DM). However, the fasting plasma insulin (FPI) concentration significantly decreased after acipimox treatment. Thus, the product of basal EGP × FPI, which represents the hepatic insulin resistance index under postabsorptive conditions, was significantly reduced after acipimox in T2DM patients (13.3 ± 2.1 vs. 16.2 ± 2.0, P = 0.02), while it did not change in the obese NGT group (Supplementary Fig. 2*A*). Insulin-mediated suppression of EGP during the euglycemic insulin clamp was significantly increased after acipimox treatment in both groups. The residual EGP during the last hour of the insulin clamp was significantly decreased in both groups (0.243 ± 0.09 to 0.003 ± 0.003 mg/kg · min [P = 0.01] in NGT obese and 0.255 ± 0.0.09 to 0.176 ± 0.07 mg/kg · min [P = 0.01] in T2DM) (Fig. 2*B*).

Adipose Tissue Insulin Sensitivity

The product of fasting plasma FFA and FPI, which represents the adipose tissue insulin resistance index, was significantly higher in T2DM versus obese NGT individuals (4.9 ± 1.0 vs. 2.1 ± 0.3, respectively, P = 0.04), and it was significantly decreased by acipimox treatment in both groups (2.1 ± 0.3 to 1.3 ± 0.3 [P = 0.01] in obese NGT and 4.9 ± 1.0 to 1.6 ± 0.5 [P = 0.03] in T2DM) (Supplementary Fig. 2*B*).

Mitochondrial ATP Synthesis

Complex I– (pyruvate, glutamate, and palmitoyl-L-carnitine) and complex II– (succinate) supported ATP synthesis rates were similar in obese NGT and T2DM individuals. After acipimox treatment, mitochondrial ATP synthesis rate increased by >50% in both obese NGT and T2DM individuals (Fig. 3). Moreover, the increase in ATP synthesis rate after acipimox strongly correlated with both the decrease in plasma FFA concentration (Fig. 4) and the increase in insulin-mediated glucose disposal (Fig. 5).

The Fisher *r-z* transformation was performed to test for differences in the correlation coefficient for each group and substrates. The correlation coefficients for mitochondrial ATP synthesis rate with FFA and TGD/SPPI were similar in obese NGT and T2DM groups.

ROS Production

The rate of mitochondrial ROS generation was similar in obese NGT and T2DM individuals, and there was no physiologically significant change in the rate of mitochondrial ROS generation after acipimox treatment in either group (Supplementary Fig. 3).

Acipimox and Muscle Fat Content

Baseline IMCL (33 ± 6 vs. 12 ± 03 mmol/kg wet wt, P = 0.01) and EMCL (288 ± 22 vs. 251 ± 27 mmol/kg wet wt, P = 0.02) fat content were significantly higher in T2DM compared with obese NGT individuals (Fig. 6). After acipimox treatment, IMCL fat content did not change in either group. EMCL fat content was significantly reduced in T2DM (from 288 ± 22 to 246 ± 24 mmol/kg wet wt, P = 0.01), while it did not significantly change in the obese NGT group.

DISCUSSION

The role of dysregulation of FFA metabolism in the pathogenesis of insulin resistance in skeletal muscle is well recognized (1). We (23) and others (24) have shown that chronic physiologic elevation of the plasma FFA concentration impairs insulin signaling and decreases insulin-mediated glucose disposal in lean healthy individuals. Conversely, reduction of plasma FFA concentration improves insulin-mediated glucose disposal in obese nondiabetic and in T2DM individuals (13–15). The results of the current study confirm these observations and extend them to demonstrate that lowering the plasma FFA concentration with acipimox not only enhances insulin sensitivity but also improves mitochondrial ATP synthesis rate in insulin-resistant obese nondiabetic and T2DM individuals. Of note, acipimox also improved suppression of EGP both in obese NGT and in T2DM individuals, suggesting an improvement in hepatic insulin sensitivity. Although the improvement in hepatic insulin sensitivity was greater in the obese NGT group (Fig. 2), T2DM

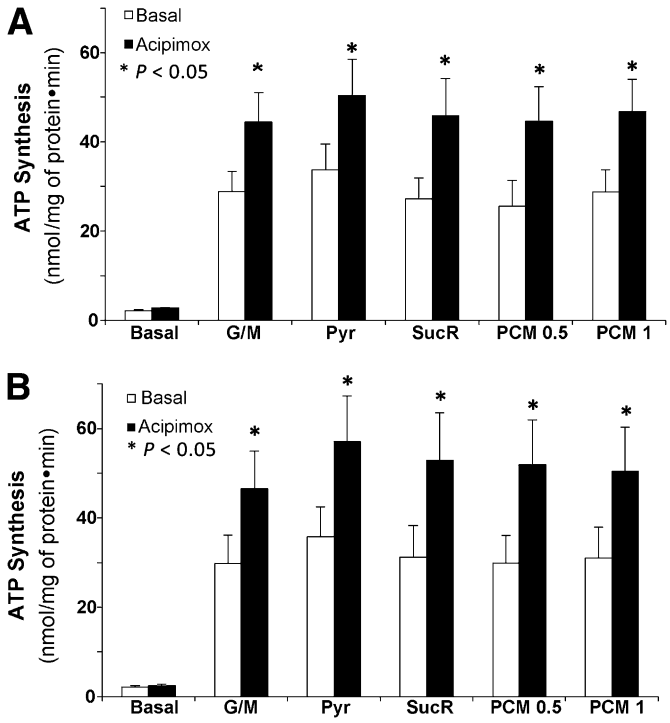

Figure 3—Mitochondrial ATP synthesis rate at baseline and after acipimox treatment. *A*: Obese NGT group. *B*: T2DM groups. G/M, glutamate/malate; Pyr, pyruvate; SucR, succinate plus rotenone; PCM 0.5, 0.5 mmol/L palmitoyl-L-carnitine; PCM 1, 1 mmol/L palmitoyl-L-carnitine. *$P \leq 0.05$.

individuals had well-controlled diabetes mellitus (FPG 145 mg/dL) and the rate of EGP was not elevated. Previous studies have shown that the EGP does not increase until the FPG exceeds 160–180 mg/dL (25,26).

Lowering the plasma FFA concentration caused >50% increase in mitochondrial ATP synthesis rate in both obese NGT and T2DM individuals without altering mitochondrial ROS generation. Moreover, the improvement in

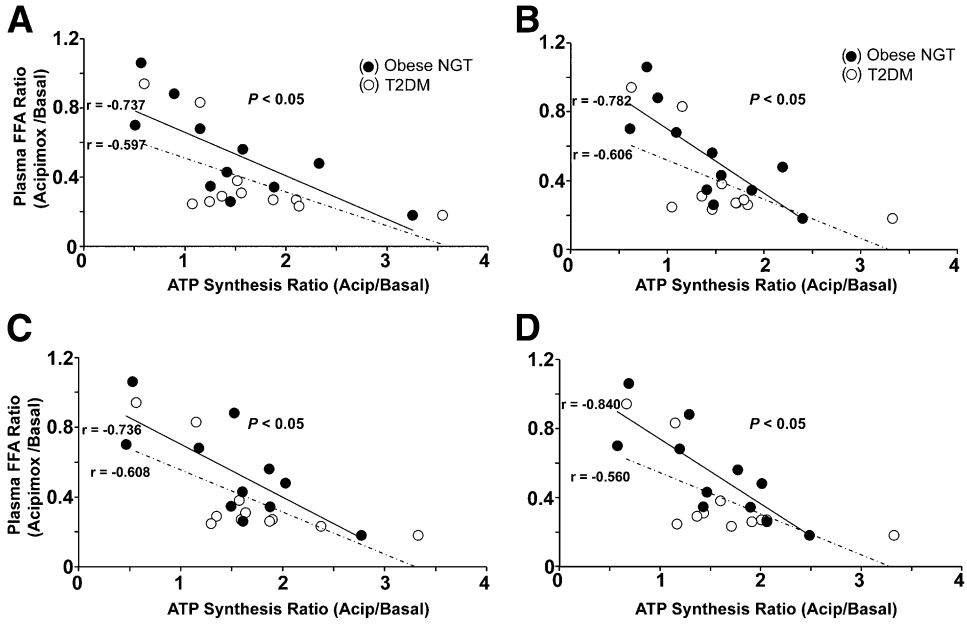

Figure 4—Pearson correlation between plasma FFA ratio (acipimox [Acip]/basal) and ATP synthesis ratio (acipimox/basal) in obese NGT and T2DM groups. *A*: Glutamate/malate. *B*: Pyruvate. *C*: Succinate plus rotenone. *D*: 1 mmol/L palmitoyl-L-carnitine.

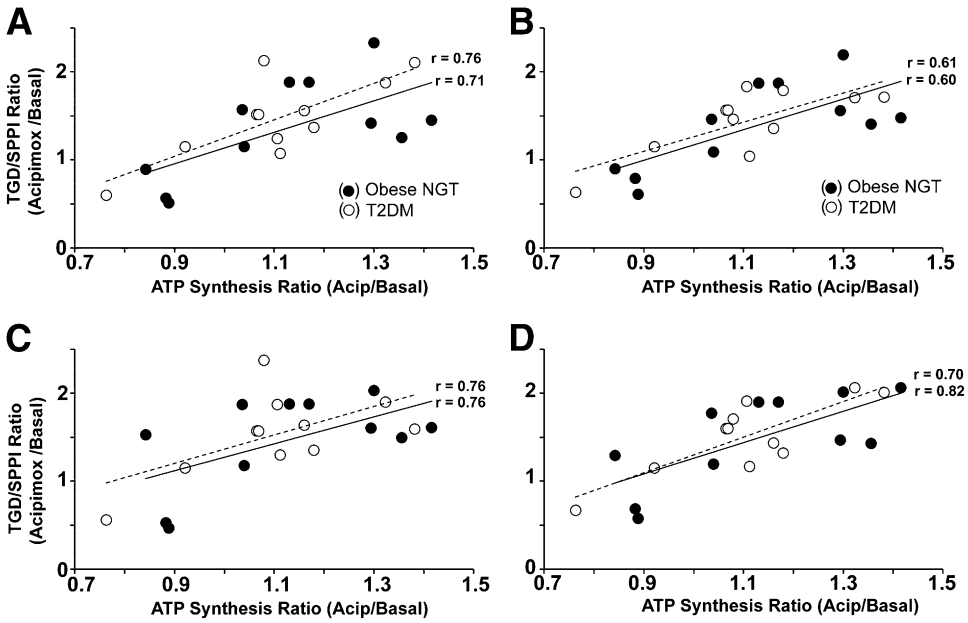

Figure 5—Pearson correlation between TGD-to-SPPI ratio (acipimox [Acip]/basal) and ATP synthesis ratio (acipimox/basal) in obese NGT and T2DM groups. *A*: Glutamate/malate. *B*: Pyruvate. *C*: Succinate plus rotenone. *D*: 1 mmol/L palmitoyl-L-carnitine.

mitochondrial ATP synthesis strongly correlated with the decrease in plasma FFA levels and with the improvement in insulin sensitivity (Figs. 4 and 5). The improvement in insulin sensitivity and mitochondrial function brought about acipimox treatment was comparable in obese NGT individuals and in T2DM individuals. These results suggest that impaired mitochondrial function in T2DM is associated with insulin resistance and that hyperglycemia per se plays little role in the development of mitochondrial dysfunction in T2DM individuals.

Previous studies (7–12) consistently have demonstrated that insulin resistance is associated with impaired mitochondrial ATP synthesis rate in skeletal muscle, and the impairment in mitochondrial ATP synthesis has been suggested to represent a primary defect in skeletal muscle that contributes to the increase in IMCL fat content and development of insulin resistance. The reduction in EMCL fat in T2DM individuals most likely is explained by the decrease in plasma FFA concentration leading to decreased FFA flux into muscle. Although it is widely accepted that insulin-resistant individuals manifest an impairment in mitochondrial function in skeletal muscle, the causal relationship between insulin resistance and mitochondrial dysfunction is unclear. An association between insulin resistance and impaired mitochondrial function has been observed in cross-sectional studies (7–9); however, this does not prove causality. Moreover, other studies have suggested that impaired mitochondrial function is the result, not the cause, of insulin resistance (10–12,27). In the current study, we demonstrate that lowering the plasma FFA concentration reverses, at least in part, the defect in mitochondrial ATP synthesis. We (16,28) and others (29,30) previously demonstrated that physiologic elevation of fatty acid metabolites, e.g., palmitoyl-L-carnitine, markedly impaired mitochondrial function, i.e., "mito-toxicity." Thus, it is possible that the increase in mitochondrial ATP synthesis rate observed with acipimox represents a direct effect of lowering the plasma FFA concentration with resultant decrease in muscle content of FFA metabolites. If insulin resistance were the cause of impaired mitochondrial ATP synthesis, it is possible that the improvement in insulin sensitivity brought about by reduction in plasma FFA concentration contributed to the improvement in mitochondrial function. Nonetheless, whether the improvement in mitochondrial function is due to removal of the toxic effects of IMCL FFA metabolites (not measured in the current study) or due to the improvement in insulin sensitivity, it indicates that the mitochondrial defect observed in insulin-resistant individuals is reversible and is unlikely to be the primary defect in ATP synthesis, e.g., inherited, responsible for the development of insulin resistance. Future studies will be required to determine the molecular mechanisms by which lowering plasma FFA concentration improves mitochondrial function.

Mitochondrial ROS generation is a by-product of normal mitochondrial metabolism. In a previous study (8), we demonstrated that NGT insulin-sensitive individuals manifest an increased rate of ATP synthesis rate and an increased rate of ROS generation compared with insulin-resistant individuals. In the current study, the improvement in insulin sensitivity and increase in mitochondrial ATP synthesis rate observed with lowering the plasma FFA concentration were not accompanied by an

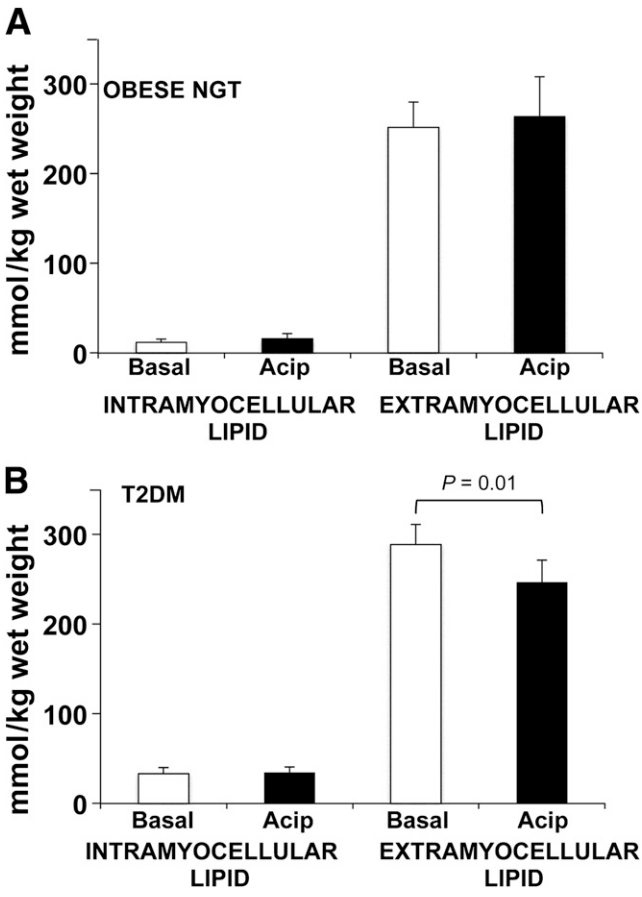

Figure 6—Tibialis anterior muscle lipid content at baseline and after acipimox (Acip) treatment. *A*: IMCL and EMCL lipid content in obese NGT group. *B*: IMCL and EMCL lipid content in T2DM group.

increase in the rate of ROS generation. This observation indicates that the increase in mitochondrial ATP synthesis rate brought about by lowering the plasma FFA is likely due to removal of an inhibitory process on mitochondrial ATP synthesis.

In summary, lowering the plasma FFA concentration in obese NGT and T2DM individuals improves mitochondrial ATP synthesis rate, indicating that the mitochondrial defect observed in insulin-resistant individual is, at least in part, reversible.

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