

Overnight Lowering of Free Fatty Acids With Acipimox Improves Insulin Resistance and Glucose Tolerance in Obese Diabetic and Nondiabetic Subjects

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Obesity is commonly associated with elevated plasma free fatty acid (FFA) levels, as well as with insulin resistance and hyperinsulinemia, two important cardiovascular risk factors. What causes insulin resistance and hyperinsulinemia in obesity remains uncertain. Here, we have tested the hypothesis that FFAs are the link between obesity and insulin resistance/hyperinsulinemia and that, therefore, lowering of chronically elevated plasma FFA levels would improve insulin resistance/hyperinsulinemia and glucose tolerance in obese nondiabetic and diabetic subjects. Acipimox (250 mg), a long-acting antilipolytic drug, or placebo was given overnight (at 7:00 P.M., 1:00 A.M., 7:00 A.M.) to 9 lean control subjects, 13 obese nondiabetic subjects, 10 obese subjects with impaired glucose tolerance, and 11 patients with type 2 diabetes. Euglycemic-hyperinsulinemic clamps and oral glucose tolerance tests (75 g) were performed on separate mornings after overnight Acipimox or placebo treatment. In the three obese study groups, Acipimox lowered fasting levels of plasma FFAs (by 60–70%) and plasma insulin (by ~50%). Insulin-stimulated glucose uptake during euglycemic-hyperinsulinemic clamping was more than twofold higher after Acipimox than after placebo. Areas under the glucose and insulin curves during oral glucose tolerance testing were both ~30% lower after Acipimox administration than after placebo. We conclude that lowering of elevated plasma FFA levels can reduce insulin resistance/hyperinsulinemia and improve oral glucose tolerance in lean and obese nondiabetic subjects and in obese patients with type 2 diabetes. *Diabetes* 48:1836–1841, 1999

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AUC, area under the curve; FFA, free fatty acid; GIR, glucose infusion rate; IGT, impaired glucose tolerance; ISGU, insulin-stimulated glucose uptake; NA, nicotinic acid; npRQ, nonprotein respiratory quotient.

Obesity is commonly associated with insulin resistance and hyperinsulinemia (1,2), two important cardiovascular risk factors (3). The fact that insulin resistance and hyperinsulinemia increase with weight gain and decrease with weight loss (4–6) suggests that the two have a cause-and-effect relationship. It has recently been suggested that free fatty acids (FFAs) are the link between obesity and insulin resistance/hyperinsulinemia (7), based on evidence showing that plasma FFA levels are commonly elevated in obesity (8,9) and that acute elevations of plasma FFA levels produce insulin resistance in healthy and diabetic subjects (10–15). This alone does not prove, however, that the chronically elevated plasma FFAs are responsible for the insulin resistance in obese subjects. Causal links among insulin resistance, hyperinsulinemia, and FFAs might be more convincingly established by the demonstration that lowering of plasma FFAs also lowers insulin resistance and hyperinsulinemia. So far, this has not been shown. Fulcher et al. (16) have failed to see a beneficial effect on insulin resistance of overnight lowering of plasma FFAs in patients with type 2 diabetes. We were similarly unable to demonstrate a statistically significant reduction in insulin resistance after acutely lowering plasma FFAs for 6 h in four healthy volunteers (11). It appears likely, however, that these failures were caused by problems related to the experimental designs (low clamp insulin infusion rates in the Fulcher study, insufficient number of experiments in our study). It was, therefore, the first objective of the present study to test the hypothesis that overnight lowering of plasma FFA concentrations with Acipimox, a long-acting antilipolytic drug, would reduce insulin resistance in obese subjects with varying degrees of insulin resistance.

FFAs not only produce insulin resistance (10–15), but also stimulate insulin secretion (7). Hence, an improvement in insulin resistance produced by lowering of plasma FFAs may not necessarily result in an improvement in glucose tolerance, which depends primarily on the amount of insulin released in response to a glucose challenge and on target tissue sensitivity to the released insulin. To our knowledge, there are presently no published data relating FFA-induced changes in insulin resistance to glucose tolerance. A second objective of this study was, therefore, to evaluate the relationship between FFA-induced changes in insulin resistance and secretion and oral glucose tolerance in the same obese subjects.

TABLE 1
Clinical characteristics of the study subjects

Group	Sex (F/M)	Age (years)	Race (n)			Weight (kg)	Body surface area (m ²)	BMI (kg/m ²)
			White	Black	Mulatto			
Lean nondiabetic	7/2	39.1 ± 2.3	5	2	2	59.9 ± 2.2	1.61 ± 0.4	22.9 ± 0.26
Obese nondiabetic	10/3	39.4 ± 1.3	7	3	3	83.8 ± 2.6*	1.82 ± 0.03*	34.1 ± 0.8*
Obese IGT	8/2	43.5 ± 1.6	6	2	2	81.6 ± 4.0*	1.83 ± 0.06*	32.3 ± 1.1*
Obese diabetic	9/2	43.6 ± 1.4	6	2	3	80.5 ± 3.9*	1.79 ± 0.05*	32.4 ± 1.0*

Data are means ± SE or *n*. **P* < 0.01 vs. lean nondiabetic control subjects.

RESEARCH DESIGN AND METHODS

Subjects. We have studied 9 healthy nonobese control subjects without a family history of diabetes, hypertension, dyslipidemia, or other known endocrine or metabolic disease and 34 obese subjects divided into three groups according to the degree of their glucose tolerance. Their clinical characteristics are shown in Table 1. There were no significant differences between the four groups with respect to age, sex, or race. Body weight and body surface area were significantly greater in the obese subjects than the lean control subjects (*P* < 0.01). The obese nondiabetic subjects with impaired glucose tolerance (IGT) and the patients with recently diagnosed type 2 diabetes had similar weights, body surface areas, and BMIs.

Patients with clinically significant micro- or macrovascular complications and patients taking lipid-lowering agents or insulin were excluded. None of the volunteers had taken any drugs for at least 3 weeks before the study, and weight had been stable in all subjects for at least 3 months before the study. All subjects consumed a diet containing at least 200 g of carbohydrate for 3 days before the study began. No subject participated in strenuous physical activity. This study was approved by the Ethical Committee of The Hospital das Clinicas, and all subjects gave written informed consent before participation.

Study protocol. All study subjects ate a standard supper at ~7:00 P.M. Acipimox, a potent long-acting antihypertensive nicotinic acid (NA) analog (17), or a placebo was administered in doses of 250 mg at 7:00 P.M. the night before and at 1:00 and 7:00 A.M. on the day of study. The sequence of placebo and Acipimox administration was randomized. At 7:00 A.M. on the day of study, an intravenous cannula was inserted into an antecubital vein, which was kept open with a slow saline drip, and the arm was heated to 50°C in a Plexiglas box to arterialize the blood. A second cannula was inserted into a contralateral antecubital vein for infusion of insulin and glucose. After an equilibration period of 30 min, basal samples were collected for determination of plasma glucose, insulin, and FFA concentrations. Then, euglycemic-hyperinsulinemic clamps were performed by infusing insulin (Novolin R; Novo-Nordisk, Bagsvaerd, Denmark) for 180 min at a rate of 7 pmol · kg⁻¹ · min⁻¹. Euglycemia (~5 mmol/l) was maintained with a variable-rate infusion of 50% glucose. Blood glucose levels were determined at 10-min intervals, and glucose infusion rates (GIRs) were adjusted as needed. Urinary glucose excretion was measured during the clamps and used to correct calculation of peripheral glucose uptake.

On a different day, oral glucose tolerance tests were started at ~7:00 A.M.; 75 g of glucose was administered as a 25% glucose solution over 15 min, and blood samples were drawn at -45, -30, 0, 30, 60, 90, 120, and 180 min for measurement of plasma glucose, insulin, and FFA concentrations.

Analytical methods. Carbohydrate and fat oxidation rates were determined by indirect calorimetry with a computerized flow-through canopy gas analyzer system (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland). Rates of protein oxidation were calculated from urinary nitrogen (N) excretion. Rates of protein oxidation were used to determine the nonprotein respiratory quotient (npRQ) using the tables of Lusk, which are based on an npRQ of 0.707 for 100% fat oxida-

tion and 1.00 for 100% carbohydrate oxidation. It was assumed that for each gram of N excreted in the urine, 6.02 liters of O₂ were consumed and 4.75 liters of CO₂ were produced.

Plasma and urine glucose concentrations were determined with the glucose oxidase method. Plasma FFAs were measured by the method of Chromy et al. (18) as modified by Demacker et al. (19). FFA measurements were corrected for background absorbance in hyperlipemic sera. Plasma insulin was determined by radioimmunoassay with a double antibody, using a modification of the method of Desbuquois and Aurbach (20). The anti-insulin serum cross-reacted completely with proinsulin.

Statistical analysis. All data are presented as means ± SE. Statistical comparisons between placebo and Acipimox experiments were performed using paired Student's *t* test, and a one-way analysis of variance was used for comparison of different groups of subjects. The incremental areas (above baseline) for glucose and insulin during the glucose tolerance tests were calculated using the trapezoidal rule. Correlations between variables were performed using least-squares regression analysis.

RESULTS

Basal plasma FFA, insulin, and glucose levels. Basal data from all subjects undergoing oral glucose tolerance testing and hyperinsulinemic clamping were pooled and analyzed together.

After placebo treatment, basal (after an overnight fast) plasma FFA levels were significantly lower in the lean nondiabetic control subjects (329 ± 28 μmol/l) than in the three obese groups (560 ± 52, 566 ± 83, and 584 ± 39 μmol/l, respectively). After Acipimox administration, basal plasma FFA levels were 60.4 ± 3.0, 57.9 ± 4.1, 56.5 ± 6.8, and 70.4 ± 3.4% lower than after placebo in lean control, obese nondiabetic, obese IGT, and obese diabetic subjects, respectively (Table 2).

After placebo, basal insulin levels were significantly higher in obese IGT and diabetic subjects than in lean nondiabetic control subjects. After Acipimox administration, insulin levels were ~50% lower than after placebo in all four groups (Table 2).

After placebo, basal plasma glucose concentrations were moderately elevated in diabetic patients (6.8 ± 0.4 vs. 4.98 ± 0.1 mmol/l, *P* < 0.01). After Acipimox, basal plasma glucose was lower than that after placebo in all groups (*P* < 0.01). This

TABLE 2
Effect of placebo and Acipimox treatment on basal plasma glucose, insulin, and FFAs

	Lean nondiabetic subjects		Obese nondiabetic subjects		Obese IGT subjects		Obese diabetic subjects	
	Placebo	Acipimox	Placebo	Acipimox	Placebo	Acipimox	Placebo	Acipimox
Glucose (mmol/l)	4.98 ± 0.09	4.63 ± 0.09*	5.03 ± 0.07	4.70 ± 0.09*	5.85 ± 0.24	5.09 ± 0.20*	6.83 ± 0.44†	5.78 ± 0.23*
Insulin (pmol/l)	82 ± 10	38 ± 6*	96 ± 8	49 ± 7*	114 ± 11‡	60 ± 11*	149 ± 20†	78 ± 10*
FFAs (μmol/l)	329 ± 28	128 ± 13*	560 ± 52†	221 ± 21*	566 ± 83‡	242 ± 55*	584 ± 39†	170 ± 21*

Data are means ± SE. **P* < 0.01 for placebo vs. Acipimox; †*P* < 0.01, ‡*P* < 0.05 compared with lean control subjects.

TABLE 3
Effect of placebo and Acipimox on basal carbohydrate and fat oxidation rates

Group	Carbohydrate oxidation ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)		Fat oxidation ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)	
	Placebo	Acipimox	Placebo	Acipimox
Lean nondiabetic	373 \pm 22.2	568 \pm 26*	41 \pm 2	22 \pm 3*
Obese nondiabetic	410 \pm 36	531 \pm 31*	35 \pm 3	26 \pm 3
Obese IGT	386 \pm 36	494 \pm 26*	34 \pm 3	23 \pm 3*
Obese diabetic	424 \pm 42	565 \pm 64*	36 \pm 5	26 \pm 4*

Data are means \pm SE. * $P < 0.01$ for placebo vs. Acipimox.

decrease, although statistically significant, was small in the two nondiabetic groups (~ 0.4 mmol/l or $\sim 7\%$), but larger in the diabetic subjects (~ 1.1 mmol/l or $\sim 15\%$) (Table 2).

Basal carbohydrate and fat oxidation. After placebo, rates of carbohydrate oxidation and fat oxidation were similar in all four groups (Table 3). After Acipimox, fat oxidation was $\sim 50\%$ lower and carbohydrate oxidation $\sim 50\%$ higher than after placebo in the lean nondiabetic control subjects. In the three obese groups, fat oxidation was between 26–28% lower and carbohydrate oxidation was between 29–34% higher after Acipimox than after placebo.

Euglycemic-hyperinsulinemic clamps. Plasma glucose concentrations were clamped at 5.0–5.6 mmol/l, and plasma insulin levels were increased to and maintained at 660–720 pmol/l in all studies (Fig. 1).

After placebo, GIRs, reflecting insulin-stimulated glucose uptake (ISGU), were 50% lower in obese than in lean nondiabetic subjects (1.3 ± 0.06 vs. 2.6 ± 0.1 $\text{mmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, $P < 0.001$) and $\sim 70\%$ lower in obese subjects with IGT or diabetes

compared with lean nondiabetic control subjects (0.7 ± 0.1 or 0.8 ± 0.1 vs. 2.6 ± 0.1 $\text{mmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, $P < 0.001$) (Fig. 2).

After Acipimox administration, GIRs were higher than those after placebo treatment in lean control subjects ($+23 \pm 4\%$, $P < 0.001$) and obese nondiabetic ($+131 \pm 13\%$, $P < 0.0001$), IGT ($+111 \pm 21\%$, $P < 0.001$) and diabetic ($+103 \pm 27\%$, $P < 0.001$) subjects.

There was a linear relationship between changes in GIR (GIR at 180 min – GIR at 0 min) and basal plasma FFAs, such that a decrease in FFAs by 100 $\mu\text{mol/l}$ was associated with an increase in GIR of 0.31 $\text{mmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in nondiabetic (lean and obese) subjects and of 0.12 $\text{mmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in obese subjects with IGT or type 2 diabetes (Fig. 3). In the current study, no glucose tracers were used, and, thus, true rates of glucose uptake could not be determined. At the insulin levels attained during hyperinsulinemic clamp (600–700 pmol/l), endogenous glucose production was probably not completely suppressed, and, therefore, true rates of glucose uptake could have been 10–20% higher than the measured GIR.

Oral glucose tolerance. Acipimox treatment lowered plasma glucose and insulin levels in all four groups (Fig. 4).

Areas under the glucose curves (glucose AUC) decreased by a mean of 25, 29, 26, and 21%, respectively, while insulin AUC decreased by a mean of 42, 19, 41, and 26%, respectively, in lean control, obese nondiabetic, IGT, and diabetic subjects (Table 4).

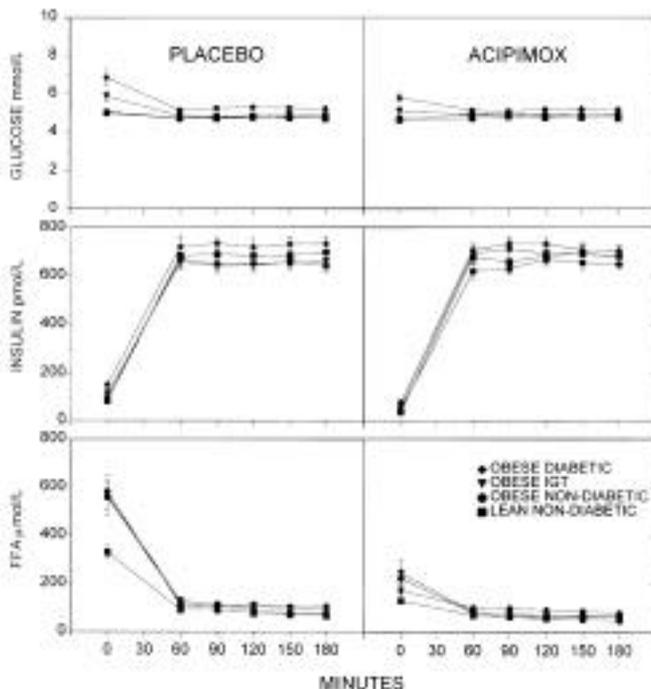


FIG. 1. Plasma glucose, insulin, and FFA levels before and during euglycemic-hyperinsulinemic clamping in lean and obese nondiabetic subjects and in subjects with IGT and type 2 diabetes after overnight treatment with placebo (left) or Acipimox (right).

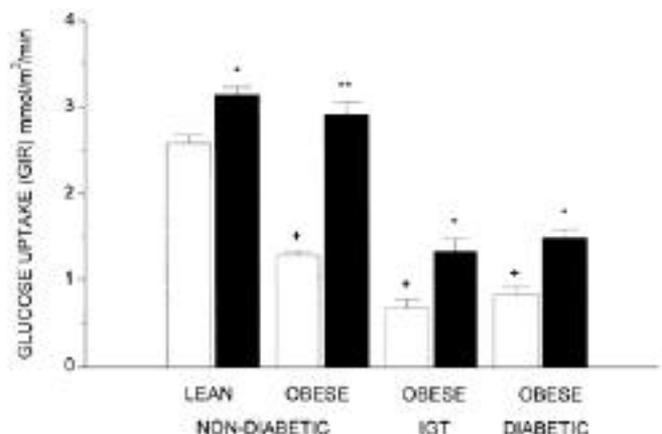


FIG. 2. Rates of glucose infusion needed to maintain euglycemia during hyperinsulinemic clamping (GIR) in lean and obese nondiabetic subjects and in subjects with IGT and type 2 diabetes after overnight treatment with placebo (□) or Acipimox (■). Statistical analysis: * $P < 0.001$, ** $P < 0.0001$ for placebo vs. Acipimox treatment; + $P < 0.001$ compared with lean nondiabetic control subjects.

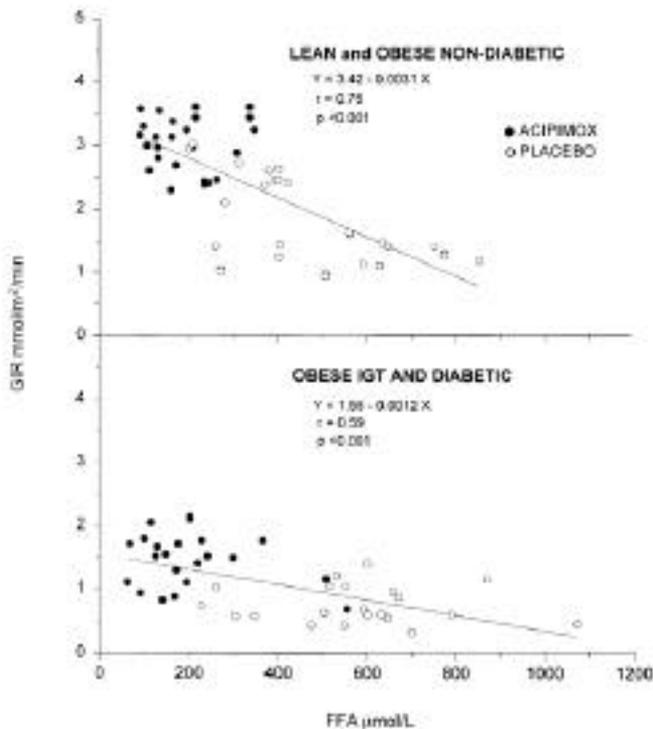


FIG. 3. Correlation between basal FFA levels after overnight treatment with placebo or Acipimox and GIR needed to maintain euglycemia after 150 and 180 min of euglycemic-hyperinsulinemic clamping in lean and obese nondiabetic subjects and in subjects with IGT and type 2 diabetes.

In the IGT group, mean fasting and 2-h glucose concentrations decreased from 5.7 ± 0.2 and 8.8 ± 0.3 mmol/l, respectively, after placebo to 5.5 ± 0.2 and 7.4 ± 0.3 mmol/l, respectively, after Acipimox ($P < 0.01$). After placebo treatment, 3 of the 10 patients had fasting plasma glucose concentrations 6.1–7.0 mmol/l (110–126 mg/dl), and 8 had 2-h plasma glucose concentrations 7.8–11.1 mmol/l (140–200 mg/dl). After Acipimox administration, only one patient had fasting plasma glucose concentrations 6.1–7.0 mmol/l and only two had 2-h glucose concentrations 7.8–11.1 mmol/l. Thus, according to the new American Diabetes Association guidelines (21) Acipimox improved glucose tolerance from impaired to normal in 8 of 10 subjects.

In the diabetic group, mean fasting and 2-h glucose concentrations decreased from 6.9 ± 0.5 and 13.7 ± 0.7 mmol/l, respectively, after placebo to 5.8 ± 0.4 and 10.3 ± 1.0 mmol/l, respectively, after Acipimox ($P < 0.01$). After placebo treatment, 3 of the 11 patients had fasting plasma glucose concentrations >7.0 mmol/l and 10 of 11 had 2-h glucose concentrations >11.1 mmol/l. After Acipimox administration, all 11 patients had fasting plasma glucose <7.0 mmol/l, and only 4 had 2-h glucose concentrations >11.1 mmol/l. Thus, Acipimox improved glucose tolerance from diabetic to impaired in 7 of 11 subjects.

DISCUSSION

Acipimox, FFAs, and insulin resistance. It was the main objective of this study to examine whether overnight lowering of plasma FFA levels with the potent long-acting NA analogue Acipimox could improve insulin resistance in obese subjects exhibiting a wide spectrum of insulin sensitivities rang-

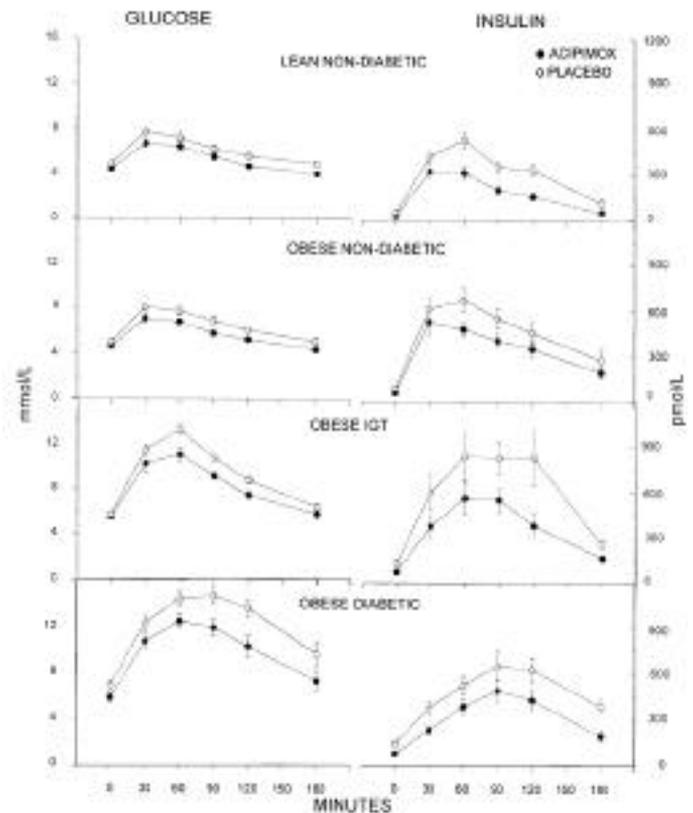


FIG. 4. Plasma glucose (left) and insulin (right) levels during oral glucose tolerance testing (75 g of glucose) after overnight treatment with placebo or Acipimox.

ing from normal to diabetic. Plasma FFA levels were lowered effectively, as evidenced by the fact that at 7:00 A.M. on the day of study, i.e., 6 h after the last dose of Acipimox, and at a time when FFA breakthroughs were most likely to occur (17), plasma FFA concentrations were lower (by an average of ~60%) than after placebo treatment in every one of the 43 study subjects. The decrease in FFAs was associated with an increase in ISGU in all subjects (Fig. 2). The increase was relatively small ($23 \pm 4\%$) in the lean nondiabetic control subjects, in whom Acipimox produced only a modest decrease in plasma FFAs (from 329 ± 28 to 128 ± 13 $\mu\text{mol/l}$). In contrast, in the three obese groups, Acipimox lowered plasma FFA levels from ~600 to between 170 and 240 $\mu\text{mol/l}$ and increased ISGU more than twofold. Lowering of basal plasma FFAs from an elevated to a normal concentration (instead of lowering it to a very low concentration, as was done here) would presumably have had a lesser effect on ISGU. The 131% increase in ISGU was sufficient to normalize insulin sensitivity in the obese nondiabetic subjects. This suggested that elevated plasma FFA levels had been responsible for most of their insulin resistance. On the other hand, in obese subjects with IGT or diabetes, doubling of ISGU was not sufficient to normalize their insulin sensitivity, which remained ~50% below that of the lean nondiabetic control subjects. This suggested that elevated FFA levels were responsible for much, but not all, of the insulin resistance in type 2 diabetes, confirming previous findings from our laboratory (13). The relationship between basal plasma FFAs and GIR (at the end of the clamps) appeared to be linear for nondiabetic and diabetic subjects (Fig. 3). ISGU was zero (i.e., insulin resistance was maximal)

TABLE 4
Effects of placebo and Acipimox on glucose and insulin during oral glucose tolerance tests

Group	Glucose Δ AUC ($\text{mmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$)			Insulin Δ AUC ($\text{mmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$)		
	Placebo	Acipimox	<i>P</i>	Placebo	Acipimox	<i>P</i>
Lean nondiabetic	214 \pm 46	157 \pm 26	0.12	48.8 \pm 4.3	28.7 \pm 3.9	<0.001
Obese nondiabetic	275 \pm 30	192 \pm 24	<0.02	69.3 \pm 10.2	54.0 \pm 7.0	<0.03
Obese IGT	701 \pm 28	512 \pm 31	<0.002	87.5 \pm 15.0	52.3 \pm 9.1	<0.001
Obese diabetic	1,017 \pm 83	785 \pm 86	<0.01	62.4 \pm 7.7	44.5 \pm 7.4	<0.01

Data are means \pm SE of Δ AUC (total – basal AUC).

at a plasma FFA level of \sim 1,100 or \sim 1,300 $\mu\text{mol/l}$, respectively, and a decrease in plasma FFAs of 100 $\mu\text{mol/l}$ resulted in a 9.1 or 7.7% reduction in insulin resistance, respectively, in nondiabetic subjects and in subjects with IGT or type 2 diabetes.

The failure of Fulcher et al. (16) to observe significant improvement in ISGU after overnight Acipimox administration in type 2 diabetic patients may have been, at least in part, due to their use of low clamp insulin infusion rates (0.25 $\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). This produced only small increments in ISGU and was likely to obscure an effect of FFAs on ISGU. Despite these methodological problems, however, their data (12) showed that ISGU increased from 99.6 ± 11 to $124 \pm 18 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ after Acipimox, whereas there was no change after placebo (from 117.3 ± 9.1 to $117 \pm 16 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$). Thus, there was at least a trend toward improved insulin sensitivity with Acipimox, even though these differences may not have been statistically significant.

It has been well established that acute elevations of plasma FFAs produce insulin resistance (10–15). In the present study, we have shown the opposite, namely, that lowering of plasma FFAs improved insulin resistance. It was assumed that Acipimox exerted its effect on insulin resistance via lowering of plasma FFAs and not by direct, i.e., not FFA-related, action. This assumption is supported by the close correlation between plasma FFAs and ISGU (Fig. 3), by the observation of Vaag et al. (22) that Acipimox had no direct effect on basal glucose disposal, and by the report of Saloranta et al. (23) that Acipimox had no direct effect on hepatic glucose production. Confirming many previous reports, we found that lowering of plasma FFA concentrations was associated with a decrease in fat oxidation and an increase in carbohydrate oxidation (7). It should be pointed out, however, that decreasing carbohydrate oxidation is not the mechanism by which FFAs inhibit ISGU. FFAs cause insulin resistance through inhibition of insulin-stimulated glucose transport and/or phosphorylation, as well as by inhibition of glycogen synthesis, processes that require 3–6 h to develop (10,11). FFA-induced inhibition of carbohydrate oxidation, on the other hand, develops almost instantaneously but does not interfere with ISGU for several hours (10). The molecular/biochemical events leading to FFA-induced insulin resistance remain unknown. It has recently been proposed that FFA activation of the hexosamine pathway may play a role in the pathogenesis of insulin resistance (24). This work, however, was performed with rats and needs to be confirmed in human subjects.

FFAs and oral glucose tolerance. Glucose tolerance is a complex process in which the amount of insulin secreted in response to the rising plasma glucose levels and peripheral and hepatic insulin sensitivity play major roles. In the current

study, lowering of plasma FFAs with Acipimox reduced insulin levels (insulin AUC) and glucose levels (glucose AUC) both by \sim 30% during the oral glucose tolerance tests. This indicated that the Acipimox-mediated improvement in insulin sensitivity (\sim 100% during the clamps) was greater than the Acipimox-induced decrease in plasma insulin levels. As a result, 8 of 10 obese subjects with IGT improved to normal glucose tolerance after Acipimox treatment, whereas 7 of 11 obese subjects with type 2 diabetes improved to IGT.

Fasting plasma insulin and glucose. Fasting plasma insulin levels were \sim 50% lower after Acipimox administration than after placebo in all four study groups. We have recently reported a slightly smaller decrease (\sim 30%) in basal plasma insulin after plasma FFA levels were acutely decreased with NA (25). Taken together, the data suggest that plasma FFAs can support up to almost one-half of basal insulin levels.

Fasting plasma glucose also decreased slightly but significantly in all four groups after Acipimox administration, from \sim 7% in lean and obese nondiabetic subjects to \sim 15% in obese patients with IGT or mild type 2 diabetes. Fulcher et al. (16), using the same protocol, also found a \sim 15% decrease in fasting plasma glucose in eight obese patients with mild type 2 diabetes. Similarly, Worm et al. (26) reported a decrease in blood glucose after 3 days of treatment with Acipimox. Others, however, found no effect of Acipimox on blood glucose (22,27–29). These discrepant results are not surprising. Fasting plasma glucose concentrations are primarily determined by the rate of endogenous glucose production (30). A fall in plasma FFAs can be expected to result in a decrease in the rate of gluconeogenesis. This decrease, however, is more or less compensated for by an increase in the rate of glycogenolysis (31), via a process known as hepatic autoregulation (32). Hence, whether Acipimox will lower blood sugar or not probably depends to a large extent on the hepatic glycogen content. If liver glycogen is low, during fasting, for instance, glycogenolysis will be unable to balance the decrease in gluconeogenesis, and the blood sugar will fall (31,32).

Summary and clinical relevance. NA is an excellent lipid-lowering agent (33). Its use in diabetic patients, however, is not recommended because NA frequently causes deterioration of glucose tolerance (34). This is probably due to the short half-life of NA, which produces frequent breakthrough FFA rebounding (17). Acipimox, like NA, lowers lipids effectively, but unlike NA, it is longer acting and therefore much less prone to produce FFA rebounding (17). In fact, none of the 43 subjects in this study had a demonstrable FFA rebound within 6 h after the last Acipimox dose. Lowering of overnight plasma FFA levels with Acipimox markedly improved insulin resistance, oral glucose tolerance, and basal insulin levels in

obese subjects, regardless of the degree of their preexisting insulin resistance. These findings add to a growing body of evidence showing that elevated plasma FFA levels are an important link between obesity and insulin resistance (25,35). Nevertheless, more and longer studies are needed to demonstrate that long-term inhibition of lipolysis is feasible and effective in the treatment of type 2 diabetes, a disease that is characterized by insulin resistance, dyslipidemia, and a two- to fivefold increase in cardiovascular mortality.

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REFERENCES

- Rabinowitz D, Zierler KL: Forearm metabolism in obesity and its response to intra-arterial insulin: characterization of insulin resistance and evidence for adaptive hyperinsulinism. *J Clin Invest* 12:2173-2181, 1962
- Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G, on behalf of the European Group for the Study of Insulin Resistance (EGIR): Insulin resistance and hypersecretion in obesity. *J Clin Invest* 100:1166-1173, 1997
- Zavaroni I, Bonora E, Pagliara M, Dall'Aglio E, Luchetti L, Buonanno G, Bonati PA, Bergonzani M, Gnudi L, Passeri M, Reaven G: Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. *N Engl J Med* 320:702-706, 1989
- Sims EAH, Danforth E Jr, Horton ES, Bray GA, Glennon JA, Salans LB: Endocrine and metabolic effects of experimental obesity in man. *Rec Prog Horm Res* 29:457-496, 1973
- Goto Y, Nakayama Y, Yagi T: Influence of the World War II food shortage on the incidence of diabetes mellitus in Japan. *Diabetes* 7:133-135, 1958
- Schliack V: Mangelernahrung und Diabetes Morbiditat (in German). *Z Klin Med* 151:382-396, 1954
- Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3-10, 1997
- Gorden ES: Non-esterified fatty acids in blood of obese and lean subjects. *Am J Clin Nutr* 8:740-747, 1960
- Reaven GM, Hollenbeck C, Jeng C-Y, Wu MS, Chen Y-D: Mean plasma glucose, free fatty acid, lactate and insulin for 24 h in patients with NIDDM. *Diabetes* 37:1020-1024, 1988
- Boden G, Jadali F, White J, Liang Y, Mozzoli M, Coleman E, Smith C: Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. *J Clin Invest* 88:960-966, 1991
- Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438-2446, 1994
- Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI: Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 97:2859-2865, 1996
- Boden G, Chen X: Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. *J Clin Invest* 96:1261-1268, 1995
- Kelley DE, Mintun MA, Watkins SC, Simoneau JA, Jadali F, Frederickson A, Beattie J, Theriault R: The effect of non-insulin-dependent diabetes mellitus and obesity on glucose transport and phosphorylation in skeletal muscle. *J Clin Invest* 97:2705-2713, 1996
- Bonadonna RC, Zycg K, Boni C, Ferrannini E, DeFronzo RA: Time dependence of the interaction between lipid and glucose in humans. *Am J Physiol* 257:E49-E56, 1989
- Fulcher GR, Walker M, Catalano C, Agius L, Alberti KGMM: Metabolic effects of suppression of nonesterified fatty acid levels with Acipimox in obese NIDDM subjects. *Diabetes* 41:1400-1408, 1992
- Fuccella LM, Goldaniga G, Lovislo P, Maggi E, Musatti L, Mandelli V, Sirtori CR: Inhibition of lipolysis by nicotinic acid and by acipimox. *Clin Pharmacol Ther* 28:790-795, 1980
- Chromy V, Gergel J, Voznincek J, Krombholzová L, Musil J: Assay of serum free fatty acids by extraction-photometric procedure. *Clin Chim Acta* 80:327-332, 1977
- Demacker PNM, Hijmans AGM, Jansen AP: Enzymic and chemical-extraction determinations of free fatty acids in serum compared. *Clin Chem* 28:1765-1768, 1982
- Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free from antibody-bound peptide hormones in radioimmunoassay. *J Clin Endocrinol* 33:732-738, 1971
- The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 29:1183-1197, 1997
- Vaag A, Skott P, Damsbo P, Gall MA, Richter EA, Beck-Nielsen H: Effect of the antilipolytic nicotinic acid analogue acipimox on whole-body and skeletal muscle glucose metabolism in patients with non-insulin dependent diabetes mellitus. *J Clin Invest* 88:1282-1290, 1991
- Saloranta C, Franssila-Kallunki A, Ekstrand A, Taskinen M-R, Groop L: Modulation of hepatic glucose production by non-esterified fatty acids in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 34:409-415, 1991
- Hawkins M, Angelov I, Liu R, Barzilai N, Rossetti L: The tissue concentration of UDP-N-acetylglucosamine modulates the stimulatory effect of insulin on skeletal muscle glucose uptake. *J Biol Chem* 272:4889-4895, 1997
- Boden G, Chen X, Iqbal N: Acute lowering of plasma fatty acids lowers basal insulin secretion in diabetic and nondiabetic subjects. *Diabetes* 47:1609-1612, 1998
- Worm D, Henriksen J, Vaag A, Thyse-Ronn P, Melander A, Beck-Nielsen H: Pronounced blood glucose-lowering effect of the antilipolytic drug acipimox in non-insulin-dependent diabetes mellitus patients during a 3-day intensified treatment period. *J Clin Endocrinol Metab* 78:717-721, 1994
- Johnston P, Hollenbeck C, Sheu W, Chen Y-DI, Reaven G: Acute changes in plasma non-esterified fatty acid concentration do not change hepatic glucose production in people with type 2 diabetes. *Diabet Med* 7:871-875, 1990
- Fulcher G, Farrer M, Thow JC, Johnson AB, Davis SJ, Miller M, Orskov H, Alberti KGMM: The glucose-fatty acid cycle in non-insulin dependent diabetes mellitus: the acute effects of inhibition of lipolysis overnight with acipimox. *Diab Nutr Metab* 4:285-293, 1990
- Berrish T, Elliot C, Cooper B: The role of plasma non-esterified fatty acids during exercise in type 2 diabetes mellitus. *Diabet Med* 10:152-158, 1993
- DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP: The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30:1000-1007, 1981
- Chen X, Iqbal N, Boden G: Effect of FFA on gluconeogenesis and glycogenolysis in normal subjects. *J Clin Invest* 103:365-372, 1999
- Clore JN, Glickman PS, Nestler JE, Blackard WG: In vivo evidence for hepatic autoregulation during FFA-stimulated gluconeogenesis in normal humans. *Am J Physiol* 24:E425-E429, 1991
- Carlson LA, Oro L: The effect of nicotinic acid on the plasma free fatty acids: demonstration of a metabolic type of sympathicolysis. *Acta Med Scand* 172:641-645, 1962
- Garg A, Grundy SM: Nicotinic acid as therapy for dyslipidemia in non-insulin-dependent diabetes mellitus. *JAMA* 246:723-726, 1990
- Boden G: Free fatty acids, insulin resistance and type 2 diabetes mellitus. *Proc Assoc Am Physicians* 3:241-248, 1999