Original Articles

Inhibition of Lipolysis Decreases Lipid Oxidation and Gluconeogenesis From Lactate But Not Fasting Hyperglycemia or Total Hepatic Glucose Production in NIDDM

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We determined whether overnight inhibition of lipolysis by a long-acting nicotinic acid derivative (acipimox) decreases gluconeogenesis from lactate in NIDDM patients. For this purpose, 250 mg of acipimox or placebo was administered in a double-blind crossover study at 2400, 0400, and 0800 to 8 NIDDM patients (54 ± 4 yr of age, body mass index 29.5 ± 1.3 kg/m^2, fasting plasma glucose 11 ± 1 mM). The next morning, total hepatic glucose production (glucose R_a) and gluconeogenesis from lactate were determined using primed, continuous infusions of [3-^{3}H]glucose and [U-^{14}C]lactate. Glucose and lipid oxidation rates were measured using indirect calorimetry. Mean overnight serum free fatty acid concentrations averaged 242 ± 8 μM after acipimox and 721 ± 30 μM after placebo ($P < 0.001$). Inhibition of lipolysis decreased lipid oxidation from 33 ± 3 to 22 ± 2 J · kg^{-1} · min^{-1} ($P < 0.001$) and increased carbohydrate oxidation from 15 ± 3 to 23 ± 2 μmol · kg^{-1} · min^{-1} ($P < 0.005$). Gluconeogenesis from lactate decreased by ~40%, from 6.2 ± 0.6 to 3.8 ± 0.5 μmol · kg^{-1} · min^{-1} ($P < 0.005$); lactate oxidation increased from 5.6 ± 0.8 to 7.9 ± 1.1 μmol · kg^{-1} · min^{-1} ($P < 0.005$), with no change in plasma lactate concentrations or total lactate R_d. Fasting plasma glucose concentrations were comparable at 2400 (10.0 ± 1.1 vs. 10.6 ± 1.3 mM, acipimox vs. placebo) and between 0900 and 1000 (10.6 ± 1.3 and 11.3 ± 1.3 mM, respectively). Also, total glucose production rates remained unchanged (14.0 ± 1.2 vs. 14.9 ± 1.3 mol · kg^{-1} · min^{-1}, respectively). Serum growth hormone (2.8 ± 0.9 vs. 0.5 ± 0.2 μg/l, $P < 0.05$) and cortisol (444 ± 19 vs. 352 ± 28 nM, $P < 0.05$) concentrations were higher after acipimox than placebo administration. We conclude that inhibition of lipolysis decreases lipid oxidation and gluconeogenesis from lactate, but fails to lower fasting plasma glucose concentrations and total hepatic glucose production in NIDDM patients.
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Fasting hyperglycemia in NIDDM patients is a consequence of increased HGP (1). Studies using [2-^{14}]acetate (2), [3-^{14}C]lactate (3), and [U-^{14}C]glycerol (4,5) tracers have indicated that gluconeogenesis accounts for all of the increase in HGP. Studies with NMR spectroscopy to quantitate gluconeogenesis have indicated that tracer techniques may even have underestimated gluconeogenesis (6). These data make inhibition of gluconeogenesis an attractive target for treatment of hyperglycemia in NIDDM patients.

Plasma FFA concentrations (7–11), as well as their turnover rate, frequently are elevated in NIDDM patients. Furthermore, fasting (12) and overnight (13,14) FFA concentrations correlate with HGP rates in NIDDM. Studies performed using isolated, glycogen-depleted rat liver have shown that oxidation of FFA provides energy for gluconeogenesis and stimulates it in an FFA-concentration–dependent manner (15). These data have led to the suggestion that increased FFAs may contribute to the excessive rates of gluconeogenesis in NIDDM patients (16). Inhibition of FFA oxidation, either via FFA oxidation or antilipolytic agents, should therefore decrease gluconeogenesis (16). However, human data demonstrating an inhibitory effect of antilipolytic agents on hepatic gluconeogenesis are still lacking.

Previously, we used ethanol to block gluconeogenesis in overnight fasted NIDDM patients (17). Infusion of

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NIDDM, non-insulin-dependent diabetes mellitus; R_a, rate of appearance; R_d, rate of disappearance; HGP, hepatic glucose production; NMR, nuclear magnetic resonance; FFA, free fatty acid; FPG, fasting plasma glucose; BMI, body mass index; SA, specific activity; GH, growth hormone; HPLC, high-performance liquid chromatography; RIA, radioimmunoassay; ANOVA, analysis of variance.

ethanol decreased gluconeogenesis from lactate and glycerol by ~70%. However, despite significant inhibition of gluconeogenesis, no decrease in overall HGP or FPG occurred (17). Identical experiments performed in fed rats demonstrated that the lack of a hypoglycemic effect of ethanol probably was caused by accelerated glycogenolysis (18). Indeed, liver glycogen content was 75% lower after ethanol than after saline treatment (18). In normal subjects, Jenssen et al. (19) found no change in overall HGP despite a threefold increase in lactate gluconeogenesis, which was induced by infusing lactate. These data have been interpreted to suggest that a reciprocal regulatory mechanism exists between hepatic gluconeogenesis and glycogenolysis (17,19), which might complicate attempts to inhibit HGP via inhibition of gluconeogenesis. It is, however, unknown whether a comparable regulatory mechanism is operative when attempts are made to inhibit gluconeogenesis by antilipolytic agents.

This study was undertaken to determine whether overnight inhibition of lipolysis in NIDDM patients decreases gluconeogenesis, and, if so, whether this decreases overall HGP or the FPG concentration.

RESEARCH DESIGN AND METHODS

Eight male NIDDM patients participated in the study. Patients with clinically significant nephropathy, retinopathy, neuropathy, hypertension, or coronary heart disease, and those taking hypolipemic agents, were excluded from the study. The mean age of the patients was 54 ± 4 yr, BMI of 29.5 ± 1.3 kg/m^2, HBA_{1c} of 8.1 ± 0.5%, FPG of 10.9 ± 1.3 mM, serum C-peptide of 0.87 ± 0.1 nM, and serum insulin of 66 ± 23 pM. None of the patients had been treated with insulin. All of the patients were taking oral antidiabetic agents (glyburide or glipizide), which were discontinued 3 days before the study.

The purpose, nature, and potential risks of the study were explained to the patients before their informed consent was obtained. The experimental protocol was approved by the Ethical Committee of the Helsinki University Hospital.

The study was performed in a randomized, double-blind, placebo-controlled fashion. The patients were admitted to the hospital 1 day before the study and placed on a weight-maintaining diet that contained 25 kcal/kg, with 50% of calories from carbohydrate, 30% from fat, and 20% from protein. The metabolic measurements were performed after an overnight (from 2000) fast. Infusions of [3-^{3}H]glucose and [U-^{14}C]lactate were used to determine glucose R_a, glucose R_d, lactate R_a, lactate R_d, lactate oxidation, and gluconeogenesis from lactate, as detailed below. Substrate oxidation rates were calculated from indirect calorimetry measurements.

At 2000 on the evening before the study, an indwelling catheter, equipped with an obturator, was placed in an antecubital vein. Acipimox (250 mg) or similar-looking placebo capsules were given at −360 min (at 2400), at 0 min (at 0600), and at 120 min (at 0800).

To determine total HGP rate and lactate kinetics, and gluconeogenesis from lactate, a primed, continuous-infusion mixture of [3-^{3}H]glucose (bolus 40 μCi in 100 ml of saline, infusion rate of 0.20 μCi/min) (Amersham, Buckinghamshire, England) and [U-^{14}C]lactate (bolus 5 μCi, infusion rate of 0.15 μCi/min) (Amersham) was started and continued for 240 min (17). The priming doses of [3-^{3}H]glucose and [U-^{14}]lactate were given between 0600 and 0610. To prime the bicarbonate pool, a bolus of $Na^{14}HCO_3$ was given at the same time (20). The bicarbonate bolus was calculated as bolus (μCi/min) = fraction oxidized precursor × infusion rate of labeled precursor (μCi/min) × 85, assuming a priming dose:isotope infusion rate ratio of 85:1 and 40% lactate oxidation (20,21). CO_2 SA was determined in breath samples collected at 180, 210, and 240 min. CO_2 production was measured by indirect calorimetry.

At 0730, another catheter was placed in a heated hand vein to obtain arterialized venous blood for sampling. At 0800, a basal blood sample was taken for measurement of plasma glucose, serum C-peptide, and insulin. Glucose and lactate SAs were measured at 120, 150, 180, 210, and 240 min to determine glucose and lactate kinetics, and gluconeogenesis from lactate. Serum GH, cortisol, and glucagon concentrations were measured at 120, 180, and 240 min.

Respiratory exchange measurements. Indirect calorimetry measurements were performed with a computerized flow-through canopy gas analyzer system (Deltatrac Metabolic Monitor, Datex, Helsinki, Finland) (17). Samples of expired air, which were suctioned at 40 l/min, were analyzed for O_2 and CO_2 concentration differences using paramagnetic O_2 and infrared CO_2 analyzers, respectively. The hood was placed on the subject's head 10 min before the measurements were started at 0830. The protein oxidation rate was estimated from urinary urea nitrogen excretion. The following constants were used for calculation of substrate oxidation rates (22): oxidation of 6.25 g of protein produces 1 g of urea nitrogen, 1 g of protein requires 966 ml of O_2 and produces 782 ml of CO_2, 1 g of glucose requires 746 ml of O_2 and produces 746 ml of CO_2, oxidation of lipid requires 2029 ml of O_2 and produces 1430 ml of CO_2. Energy production rates ($J \cdot kg^{-1} \cdot min^{-1}$) were calculated assuming that oxidation of 1 mg of carbohydrate produces 15.65 J; 1 mg of lipid produces 39.75 J; and 1 mg of protein produces 17.15 J (22).

[^{3}H]glucose SA. Plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and evaporated as described previously (17). The dried glucose residue was resuspended and counted in a double-channel liquid scintillation counter (Rackbeta 1215, Wallac, Turku, Finland) after adding 10 ml Aquasol liquid scintillation fluid (NEN-DuPont, Boston, MA), and corrected for quenching. The [^{3}H]glucose SA (dpm/μmol) was calculated by dividing the disintegrations per minute in 0.3 ml plasma by the plasma glucose concentration (μmol/ml). The infusate was diluted 1:100 and 1:1000, and duplicates were counted to determine the infusate [3-^{3}H] concentration.

[^{14}C]lactate and [^{14}C]glucose SAs. Plasma samples were processed with ion-exchange chromatography, as

described previously in detail (17). Our previous studies have shown that the SA of [^{14}C]lactate is identical when determined using HPLC chromatography after ion-exchange chromatography, compared with the SA of the weakly bound anionic fraction eluted by 0.2 N formic acid from ion-exchange columns (23).

CO_2 SA. To determine CO_2 SA, breath samples were collected into a 5-L anesthesia bag, and CO_2 was trapped by bubbling through a hyamine:phenolphthalein:ethanol (3:1:5 vol:vol:vol) solution (3 ml traps 1 mmol CO_2). The CO_2 trapping capacity was checked by titration with 0.15 M HCl (20).

Other measurements. Plasma FFAs were measured by a fluorometric method (24). Serum-free insulin concentrations were determined after precipitation with polyethylene glycol (25) by RIA with the Phadeseph insulin RIA kit (Pharmacia, Uppsala, Sweden). Serum glucagon was measured by RIA with the double-antibody glucagon kit (Diagnostic, Los Angeles, CA) (26); serum GH was measured by the human GH RIA kit (Oris, Git-Sur-Yvetta Cedex, France) (27); and serum cortisol was measured by RIA with the [^{125}I]cortisol kit (Farmos Diagnostica, Turku, Finland) (28). Blood HbA_{1c} was determined by ion-exchange chromatography (29). Reference values for HbA_{1c} in our laboratory are 4.0–6.0%. Plasma C-peptide concentration was determined by RIA (30).

Calculation of lactate and glucose kinetics. Glucose and lactate R_a and R_d were calculated using the Steele equation (31), assuming a pool fraction of 0.65 for glucose (32) and lactate (33), and a distribution volume of 200 ml/kg for glucose (31) and 500 ml/kg for lactate (33). The rate of glucose formation from lactate was calculated from 180 to 240 min, according to Chiasson et al. (34). The percentage of glucose R_a from plasma lactate was calculated as (glucose SA ^{14}C) × 100/(lactate SA × 2). Glucose R_a from plasma lactate (μmol · kg^{-1} · min^{-1}) was calculated from (R_a glucose) × glucose from plasma lactate (%) × 0.01. Note that this rate is an underestimate of the true rate because of dilution of the oxaloacetate pool by unlabeled carbons from acetyl-CoA (35). The validity of this qualitative index for gluconeogenesis from lactate depends on the degree of precursor dilution under the experimental conditions. In humans (36) and dogs (37), conditions associated with increasing FFA concentrations, such as fasting (36,37) and pancreatectomy (37), slightly increase dilution of the oxaloacetate pool. Therefore, rates of gluconeogenesis probably will be more underestimated in the saline than in the acipimox study. The proportion of lactate R_a disposed by gluconeogenesis was calculated by dividing the rate of lactate gluconeogenesis by lactate R_a.

Statistical analysis. ANOVA for repeated measurements followed by Student's *t* test were used where appropriate for statistical analysis, using BMDP computer program 4V (BMDP Statistical Software, Los Angeles, CA). Correlation analyses were performed using Spearman's rank correlation coefficient. All results represent means ± SE during the last h of the infusions (180–240 min).

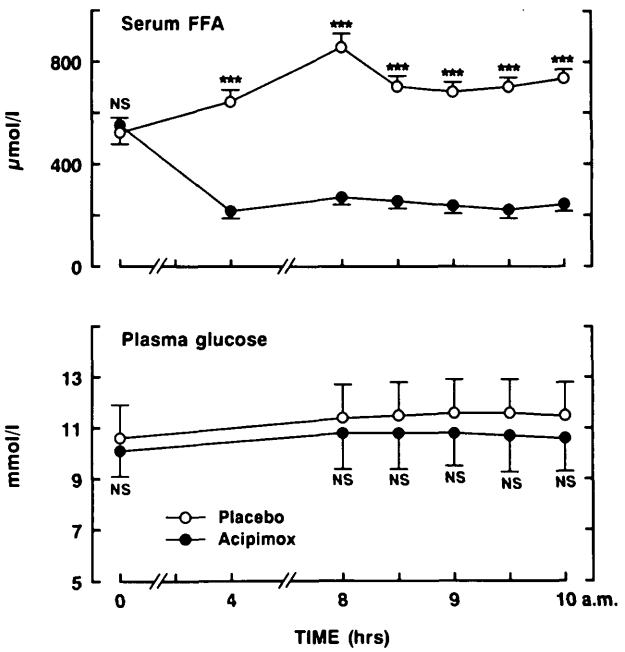

FIG. 1. Serum FFA and plasma glucose concentrations during acipimox and placebo studies. ***$P < 0.001$ for acipimox vs. placebo.

RESULTS

FFA and substrate oxidation rates. At 2400, baseline serum FFA averaged 552 ± 38 and 523 ± 45 μM in the acipimox and placebo studies (NS). The mean overnight serum FFA concentrations were significantly lower after acipimox (242 ± 8 μM) than after placebo (721 ± 30 μM, ($P < 0.001$) (Fig. 1). Carbohydrate oxidation rates were significantly higher after acipimox (23 ± 2 J · kg^{-1} · min^{-1} or 8.3 ± 0.7 μmol glucose · kg^{-1} · min^{-1}) than after placebo (15 ± 3 J · kg^{-1} · min^{-1} or 5.3 ± 1.0 μmol glucose · kg^{-1} · min^{-1}, $P < 0.005$) (Fig. 2). The lactate oxidation rate also was significantly higher after acipimox (7.9 ± 1.1 μmol lactate · kg^{-1} · min^{-1}) than after placebo (5.6 ± 0.8 μmol lactate · kg^{-1} · min^{-1}, $P < 0.01$). Rates of lipid oxidation decreased after acipimox (33 ± 3 J · kg^{-1} · min^{-1}) compared with placebo (22 ± 2 J · kg^{-1} · min^{-1}, $P < 0.001$). Rates of protein oxidation (10 ± 1 vs. 11 ± 1 J · kg^{-1} · min^{-1}, acipimox

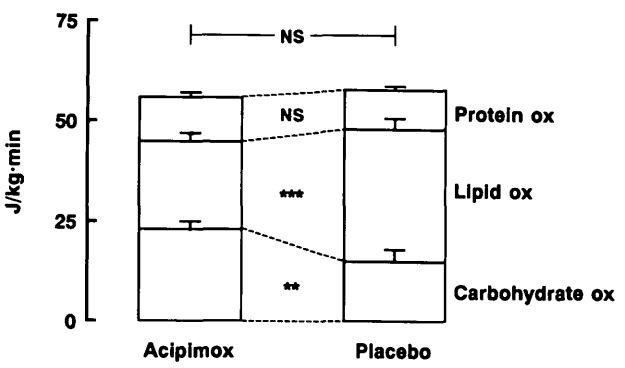

FIG. 2. Rates of energy expenditure (total height of three stacked columns), and rates of protein (upper), lipid (middle), and carbohydrate (lower) oxidation in acipimox and placebo studies. **$P < 0.01$, ***$P < 0.001$ acipimox vs. placebo.

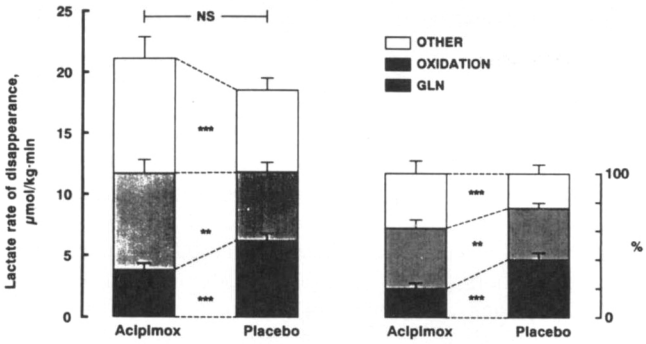

FIG. 3. Lactate R_d (total height of three stacked columns), gluconeogenesis from lactate (■), lactate oxidation (▨), and other fate of lactate R_d (□) in placebo and acipimox studies. Left, absolute rates of lactate disposal; Right, the percentage of lactate disposed via different pathways.

vs. placebo) and total energy expenditure (56 ± 1 vs. 58 ± 1 J · kg^{-1} · min^{-1}, respectively) were similar in both studies.

Glucose metabolism. Plasma glucose concentrations were comparable at 2400 (10.0 ± 1.1 vs. 10.6 ± 1.3 mM, acipimox vs. placebo study) before any drugs and between 0900 and 1000 (10.6 ± 1.3 and 11.3 ± 1.3 mM, respectively) (Fig. 1). Glucose R_a and R_d were identical during acipimox (R_a of 14.0 ± 1.2 and R_d of 14.4 ± 1.2 μmol · kg^{-1} · min^{-1}) and placebo administration (14.9 ± 1.3 and 15.0 ± 1.4 μmol · kg^{-1} · min^{-1}, respectively).

Lactate metabolism. The blood lactate concentrations were comparable after acipimox (949 ± 95 μM) and placebo (880 ± 88 μM). Both lactate R_a and R_d tended to be higher after acipimox (19.1 ± 1.8 and 19.2 ± 1.8 μmol · kg^{-1} · min^{-1}) than after placebo (15.3 ± 0.9 and 15.3 ± 1.0 μmol · kg^{-1} · min^{-1}), but the difference was not significant (Fig. 3).

Glucose production from lactate. Gluconeogenesis from lactate was ~40% lower after acipimox (3.8 ± 0.5 μmol · kg^{-1} · min^{-1}) than after placebo (6.2 ± 0.6 mol · kg^{-1} · min^{-1}, $P < 0.002$). The percentage of glucose derived from lactate also was significantly lower after acipimox (13 ± 2%) than after placebo (20 ± 1%, $P < 0.001$).

Fate of lactate. The percentage of lactate R_d diverted toward glucose was significantly lower after acipimox than after placebo 20 ± 3 vs. 40 ± 5% ($P < 0.001$) (Fig. 3). Lactate oxidation rates were higher after acipimox than after placebo (42 ± 6 [7.9 ± 1.1] vs. 36 ± 4% [5.6 ± 0.8 μmol · kg^{-1} · min^{-1}], $P < 0.005$), and the proportion of lactate R_d used for other metabolic pathways was significantly higher after acipimox than after placebo (37 ± 9 vs. 23 ± 7%, $P < 0.001$).

Counterregulatory hormone concentrations. Serum GH, cortisol, and glucagon concentrations are shown in Table 1. The mean GH (2.8 ± 0.9 vs. 0.5 ± 0.2 μg/L, acipimox vs. placebo, $P < 0.05$) and cortisol (444 ± 19 vs. 352 ± 28 nM, respectively, $P < 0.02$) concentrations were significantly higher after acipimox than after placebo administration. Serum glucagon concentrations (94 ± 9 vs. 93 ± 9, acipimox vs. placebo) were similar in both studies.

TABLE 1
Counterregulatory hormone concentrations after acipimox and placebo administration

	Time (min)	Placebo	Acipimox
Serum GH (μg/L)	120	0.4 ± 0.2	1.6 ± 0.6
	180	0.9 ± 0.5	3.6 ± 1.1
	240	0.3 ± 0.1	3.3 ± 1.0
	Mean	0.5 ± 0.2	2.8 ± 0.9*
Serum corticol (nM)	120	440 ± 54	581 ± 36
	180	314 ± 24	388 ± 30
	240	302 ± 24	363 ± 40
	Mean	352 ± 28	444 ± 19†
Serum glucagon (ng/L)	120	98 ± 9	95 ± 11
	180	94 ± 9	96 ± 10
	240	86 ± 8	90 ± 9
	Means	93 ± 9	94 ± 9

Data are means ± SE.
*$P < 0.05$ acipimox vs. placebo.
†$P < 0.02$ acipimox vs. placebo.

DISCUSSION

These data demonstrate that overnight inhibition of lipolysis decreases gluconeogenesis in NIDDM patients. This decrease, however, changes neither FPG concentrations nor overall HGP.

The observed decrease in gluconeogenesis by acipimox is based on the assumption that diminished FFA availability and lipid oxidation either does not change or does not decrease dilution of the gluconeogenic precursor label in the oxaloacetate pool. The latter assumption seems reasonable because increased FFA availability slightly increases the fraction of carbons in the oxaloacetate pool, which originates from acetyl CoA in the rat (38), and human (36). Consequently, rates of gluconeogenesis were more likely to be underestimated in the present placebo than in the acipimox study.

The inhibition of gluconeogenesis achieved by acipimox is compatible with previous animal data showing that FFAs regulate gluconeogenesis. In the isolated, glycogen-deplete rat liver, the rate of phosphoenolpyruvate gluconeogenesis depends on FFA availability (15). FFA oxidation provides reducing equivalents (NADH) and ATP for gluconeogenic reactions and stimulates, via increased acetyl CoA, pyruvate carboxylase (16). The exact magnitude by which acipimox decreased gluconeogenesis in our patients cannot be determined with certainty. Studies attempting to quantitate phosphoenolpyruvate gluconeogenesis with [2-^{14}C]acetate (2) or, indirectly, by subtracting glycogenolysis, as measured with NMR spectroscopy (6), from overall glucose production, have indicated that gluconeogenesis is responsible for all of the increase in glucose production in NIDDM patients after an overnight fast. Gluconeogenesis has been estimated to account for ~50 (2) of overall glucose production in NIDDM patients at a blood glucose concentration of ~10 mM. The rate of gluconeogenesis from lactate alone, when determined quantitatively using [3-^{14}C]lactate, accounts for ~40% of the increase in overall glucose production in NIDDM patients (3). In the latter study, dilution of labeled lactate because of carbon exchange in the oxaloacetate pool was similar in normal

subjects and NIDDM patients (3). These considerations indicate that even if the decrease in FFA availability by acipimox only inhibited lactate gluconeogenesis, this alone should have measurably decreased overall glucose production. On the other hand, in vitro, FFAs inhibit all of phosphoenolpyruvate gluconeogenesis (15), not just that from lactate.

Although gluconeogenesis was significantly inhibited by acipimox, we found no change in overall HGP and no decrease in the FPG concentration. The latter finding is compatible with some (39) but not all (41) previous studies in NIDDM patients. Fulcher et al. (41) determined HGP rates 14 h after acipimox administration and observed a significant (1.5 mM) decrease in the FPG concentration. The authors hypothesized that the failure of other investigators to demonstrate a significant decrease in plasma glucose probably is related to the short duration of antilipolysis before plasma glucose measurements (41). Antilipolysis of sufficient length could, in turn, be necessary because of the time lag between decreases in FFAs and HGP (42), and because FFAs may be hypoglycemic only when the liver is glycogen deplete. The latter possibility probably explains at least some of the discrepancies found in various studies on the effect of FFAs on gluconeogenesis.

In fed mice, inhibition of lipolysis with nicotinic acid leads to depletion of glycogen in both the liver and in skeletal muscle (43). Such data suggest the existence of a compensatory mechanism in the liver and in skeletal muscle, which switches the energy source from oxidation of lipid (FFA) to glucose (glycogen) under conditions of diminished FFA availability. If this fuel-switch mechanism also operates in humans, antilipolytic agents would be expected to have a hypoglycemic effect only under conditions when the liver is glycogen deplete, such as after prolonged hypoglycemia (44), exercise, or fasting. Thus, it is possible that hepatic glycogen stores were deplete after 14 h of antilipolysis in the study of Fulcher et al. (41), and that they were not deplete after acute administration of acipimox in the studies of Vaag et al. (45) and Saloranta et al. (39,40). In our study, no tendency toward a lowering of blood glucose concentrations was evident 10 h after the first acipimox dose, a finding similar to that reported in an earlier study using a 10-h infusion of nicotinic acid (46).

Although the antilipolytic effect of acipimox lasts longer than that of nicotinic acid, a rebound increase in FFA is observed after 10–12 h, even when acipimox is used (39). Because GH secretion increases in response to a decrease in FFA (47), and GH is lipolytic (48), it is possible that the rebound phenomenon is mediated, in part, by GH. The effect of the increases in GH or cortisol, before any rebound of FFA, on HGP in this study is unclear. Both hormones stimulate glucose output and lipolysis (48,49). It is possible that a hypoglycemic effect of antilipolysis was abolished by increases in GH and cortisol. The mechanism of counterregulation could involve both an increase in glucose production (gluconeogenesis from substrates other than lactate, acceleration of glycogenolysis) and a decrease in glucose utilization (48,49). Although the increases in GH and cortisol concentrations might complicate the use of antilipolytic agents as tools to treat diabetes, they can be regarded as a physiologically meaningful protective mechanism against fuel deficiency under conditions where the availability of lipid fuel has been artificially suppressed. Indeed, if FFA levels remained suppressed beyond the time when liver glycogen stores were depleted, severe hypoglycemia probably would develop.

Although inhibition of gluconeogenesis is considered an important target for control of HGP by various drugs in NIDDM, these data indicate that unless compensatory glycogenolysis can be prevented, inhibition of gluconeogenesis alone by antilipolytic agents does not lower the blood glucose concentration. Fortunately, and in contrast to nicotinic acid (50), acipimox does not appear to have unfavorable effects on plasma glucose, which makes it a useful drug in the treatment of dyslipidemic NIDDM patients (51).

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