Effect of Decreasing Plasma Free Fatty Acids by Acipimox on Hepatic Glucose Metabolism in Normal Rats

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Increased availability of free fatty acids (FFA) may play a role in the pathogenesis of insulin resistance in the liver. We examined the effects of an antilipolytic nicotinic acid analog (acipimox) on hepatic glucose metabolism in basal and hyperinsulinemic states in normal rats. Acipimox decreased plasma FFA levels profoundly and enhanced the ability of insulin to suppress hepatic glucose production (HGP) and to stimulate peripheral glucose utilization. In the basal state, acipimox inhibited hepatic gluconeogenesis. However, this inhibition was not associated with the change in overall HGP due to the compensatory increase in hepatic glycogenolysis that might occur as a consequence of decreased hepatic glucose-6-phosphate (G-6-P) and/or plasma insulin levels with acipimox. These results support the contention that FFA are an important determinant of insulin action in the liver, and suggest the existence of intrahepatic autoregulatory and/or hormonal regulatory processes for constant HGP in the basal state.

IT WAS MORE THAN 30 years ago that Randle et al[1] introduced the concept of substrate competition between glucose and free fatty acids (FFA), ie, the glucose–fatty acid cycle. According to their hypothesis, increased lipid oxidation with elevated FFA suppresses glucose oxidation in skeletal muscles at the level of pyruvate dehydrogenase and glycolysis at the level of phosphofructokinase.[2] Decreased glycolysis consequently increases glucose-6-phosphate (G-6-P) concentration and decreases glucose uptake.[3] It is now generally agreed that FFA reduce oxidative and nonoxidative glucose disposal in the whole body and in skeletal muscle.[4-6]

Although the effect of FFA on the liver was not included in the original Randle hypothesis, FFA have been shown to stimulate gluconeogenesis in perfused rat liver.[7] FFA oxidation has been shown to provide reducing equivalents (NADH) and adenosine triphosphate for gluconeogenic reactions and to stimulate pyruvate carboxylase via increased acetyl coenzyme A.[2] However, in vivo studies have yielded conflicting results regarding the effects of FFA on hepatic glucose metabolism. Although several studies demonstrated that elevating or decreasing plasma FFA affects hepatic glucose production (HGP) in the hyperinsulinemic state,[8-11] this effect was usually not demonstrable in the basal state.[12-15] Intrahepatic autoregulatory mechanisms that include reciprocal changes in hepatic glycogenolysis and gluconeogenesis have been proposed to explain the lack of effect of FFA on basal HGP,[13-15] but direct evidence for this is lacking.

Acipimox (5-methyl-pyrazine carboxylic acid 4-oxide), a derivative of nicotinic acid, is a powerful inhibitor of lipolysis that significantly decreases plasma FFA.[16] With this drug, we investigated the effects of decreased plasma FFA on hepatic glucose metabolism in the basal state and during insulin infusion. In particular, we directly quantified hepatic gluconeogenesis and glycogenolysis to examine the effects of decreasing FFA on these individual fluxes.

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MATERIALS AND METHODS

Experiment A: Effects of Acipimox on Glucose Turnover During Hyperinsulinemic-Euglycemic Clamps in Normal Conscious Rats

Male Sprague-Dawley rats weighing 300 to 350 g were used for the experiment. The study protocol was approved by the institutional ethics committee for animal experiments. Animals were prepared for glucose clamps according to the method of Buchanan et al.[17,18] At least 4 days before the clamp studies, animals were placed in individual cages. To adapt the animals to tail restraint, which was required for access to tail blood vessels during glucose clamp procedures, the distal one third of each animal's tail was drawn through a hole at the bottom of the cage and secured with a rubber stopper. This arrangement allowed animals to move about freely and did not restrict access to food or water.

Five hours before the insulin infusion, food was removed from the cages and catheters (PE-10, Intramedic; Clay Adams, Parsippany, NJ) were placed in two tail veins and a tail artery of the rats for infusion and blood sampling, respectively. Catheters were placed percutaneously under local anesthesia with lidocaine while the animals were briefly restrained in a towel. Animals were returned to the cages after catheter placement with tails secured as described. Patency of the arterial catheter was maintained by a slow (0.015 mL/min) infusion of saline. Animals were free to move about during the clamp studies, with mobility restricted only slightly compared with unrestrained rats.

Rats were randomly divided into an acipimox group and a placebo group. The acipimox group (n = 8) received acipimox 50 mg/kg body weight[16] 5 hours before and just before initiation of the insulin infusion (time 0). Acipimox (Farmitalia Carlo Erba, Milano, Italy) dispersed in saline (10 mg/mL) was administered using a gastric tube. The placebo group (n = 8) received the same volume of saline.

Insulin (Velosulin; Novo-Nordisk, Gentofte, Denmark) was infused at a rate of 9 pmol/kg/min for 2 hours starting at time 0. A primed (20 μCi) and continuous (0.2 μCi/min) infusion of [3-^{3}H]glucose (New England Nuclear, Boston, MA; 13.2 Ci/mmol) was initiated at −120 minutes and continued throughout the experiment. Blood samples were taken for glucose measurement at 10-minute intervals, and 25% dextrose solution was infused at variable rates to clamp plasma glucose at basal levels.

Blood samples for determination of plasma FFA and insulin concentrations were obtained at 0, 60, and 120 minutes. Blood samples for FFA determination were collected in prechilled tubes containing EDTA and Paroxon (diethyl *p*-nitrophenyl phosphate, a lipoprotein lipase inhibitor, 0.275 mg/mL blood; Sigma, St Louis, MO), immediately centrifuged, and stored at −70°C until analyzed. Blood samples for determination of [^{3}H]glucose specific activity were obtained at −30, −15, 0, 30, 60, 80, 100, and 120 minutes relative to the start of insulin infusion.

Experiment B: Effects of Acipimox on Basal Hepatic Gluconeogenesis and Glycogenolysis in Anesthetized Rats

Rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally and then 6 mg/h intravenously) 3 hours after food removal and placed on a small electric heating pad. A rectal temperature probe was inserted, and body temperature was maintained near 37°C throughout the experiment. Catheters were placed in a tail vein and a tail artery. The rats then received a primed-continuous infusion of [^{14}C]alanine (5 μCi, 0.15 μCi/min; New England Nuclear, Boston, MA; 157 mCi/mmol) and [3-^{3}H]glucose (20 μCi, 0.2 μCi/min) for the measurement of HGP and alanine conversion to glucose. After allowing 2 hours for isotopic equilibration (time 0), the acipimox group (n = 8) received acipimox 50 mg/kg through the gastric tube. The control group (n = 8) received the same volume of saline. Blood samples for determination of plasma FFA and insulin concentrations were obtained at 0, 30, 60, 90, and 120 minutes. Blood samples for determination of glucose, alanine, [^{3}H]glucose, [^{14}C]glucose, and [^{14}C]alanine specific activities were obtained every 15 minutes during the final 30 minutes of equilibration and every 30 minutes thereafter.

A midline incision was made, and it was kept closed except when obtaining a snip-biopsy of the liver.[19] Liver biopsies from the periphery of the medial lobe were performed every 30 minutes from −30 to 120 minutes, and blood loss was prevented by brief tamponade after each biopsy. Each biopsy weighed approximately 30 mg and was immediately digested in hot 30% KOH. At the end of each study, a portion of liver (~1 g) was quickly freeze-clamped using liquid nitrogen, and frozen liver samples were kept at −70°C until analysis. The entire residual liver was then excised and weighed. The weights of biopsy samples, freeze-clamped sample, and residual liver were added to calculate the total liver weight. The rate of glycogen breakdown was estimated from the change in glycogen content of the liver biopsies (milligrams per gram liver wet weight) over a given time interval. The formula is as follows: rate of glycogen breakdown (milligrams per kilogram per minute) = [change in glycogen content (milligrams per gram liver) × total liver weight (grams per kilogram body weight)] ÷ time interval (minutes).

Assay Procedures

Plasma glucose level was measured by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Palo Alto, CA). Plasma FFA and alanine levels were measured by enzymatic assay.[20,21] Plasma insulin was determined by radioimmunoassay using kits for rat insulin (basal; Linco, St Charles, MO) and human insulin (clamp; Dainabott, Tokyo, Japan). Liver glycogen level was measured as glucose after amyloglucosidase digestion, as previously described.[22] G-6-P was extracted from frozen liver powder and determined by enzymatic assay as described by Michal.[23]

Plasma [^{3}H]glucose specific activity was measured in duplicate from the supernatants of saturated $Ba(OH)_2$ and 5.5% $ZnSO_4$ precipitates of plasma samples after evaporation to dryness to eliminate tritiated water.[24] For measurement of the rate of conversion of alanine to circulating glucose, deproteinized plasma was subjected to chromatography over sequential anion-exchange (Dowex-1, chloride form; BioRad, Richmond, CA) and cation-exchange (Dowex-50W, hydrogen form) resins to separate [^{14}C]glucose and [^{14}C]alanine,[25] and the radioactivities were measured in a liquid scintillation counter (Beckman) equipped with appropriate software for dual-label determination and correction for quenching.

Isotopic Determination of Glucose Turnover and Gluconeogenesis

The rate of glucose turnover was assessed isotopically using the steady-state (experiment A) and non–steady-state (experiment B) equations of Steele as modified by DeBodo et al.[26] Values obtained during the basal state and during the final 40 minutes (experiment A) and 60 minutes (experiment B), respectively, were used for statistical analysis. For determination of the rate of conversion of alanine to circulating glucose, the dual-isotope technique of Chiasson et al[27] was used. The rate of gluconeogenic conversion of alanine to glucose was calculated by dividing the production rate of [^{14}C]glucose by the specific activity of [^{14}C]alanine. The estimated gluconeogenic conversion rates were expressed as a percentage of baseline values.[28]

Statistical Analysis

The data are presented as the mean ± SEM. Differences between groups were tested by unpaired Student's *t* test, and differences within groups by paired *t* test.

RESULTS

Experiment A: Effects of Acipimox on Glucose Turnover During Hyperinsulinemic-Euglycemic Clamps in Normal Conscious Rats

The basal plasma FFA level in the acipimox group (180 ± 30 μmol/L) was significantly lower than in the control group (540 ± 50 μmol/L, $P < .005$) and remained low during glucose clamps (Fig 1A). Basal plasma insulin levels were not statistically different between the two groups: 120 ± 24 pmol/L for the control group and 108 ± 18 pmol/L for the acipimox group. Plasma insulin levels were increased similarly in the two groups, with values of 216 ± 18 (control) and 210 ± 18 pmol/L (acipimox) during the second hour of the clamps (Fig 1B).

Plasma glucose concentrations during the basal state and during clamp studies were similar in the two groups (Fig 1C). The basal glucose turnover rate was also similar in the two groups (Table 1). In contrast, the glucose infusion rate to maintain euglycemia during the glucose clamps was significantly higher in the acipimox group compared with the control group (84 ± 6 *v* 38 ± 5 μmol/kg/min, $P < .005$; Fig 1D). Isotopic determination of glucose turnover showed that the endogenous glucose production rate was significantly lower and the glucose utilization rate significantly higher in the acipimox group than in the control group (Table 1).

Experiment B: Effects of Acipimox on Basal Hepatic Gluconeogenesis and Glycogenolysis in Anesthetized Rats

Plasma FFA, insulin, glucose, and alanine levels were similar in acipimox and control groups before administration of acipimox (Fig 2). Acipimox treatment decreased

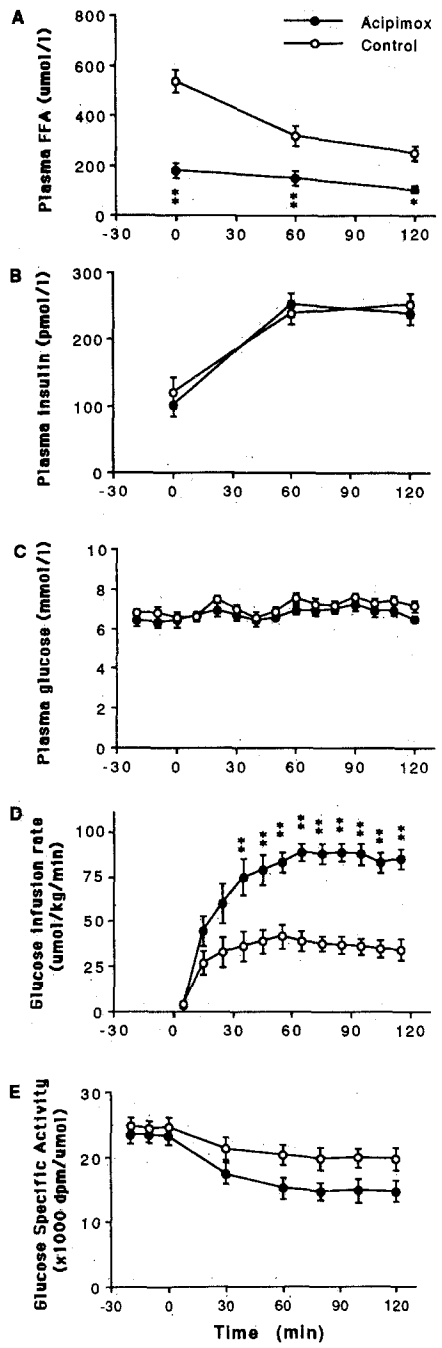

Fig 1. Plasma FFA (A), insulin (B), and glucose (C) concentrations, glucose infusion rate (D), and plasma [^{3}H]glucose specific activity (E) during the hyperinsulinemic glucose clamp in the control and acipimox groups. Acipimox was administered twice, 5 hours before and immediately before initiation of the insulin infusion. *P < .05, **P < .005.

plasma FFA and insulin levels significantly (Fig 2A and B), but did not change plasma glucose levels (Fig 2C). Plasma alanine concentrations tended to be higher after acipimox treatment than after saline, but the difference was not significant (Fig 2D).

Acipimox showed a profound inhibitory effect on hepatic gluconeogenesis. The rate of conversion of alanine to glucose in the second hour of the experiment was 46% of the basal rate (Fig 3A). However, the rate of overall HGP (Fig 3B) and glucose utilization (data not shown) did not change significantly after acipimox treatment.

The basal hepatic glycogen content of the acipimox group (32.1 ± 1.4 mg/g liver wet weight) was similar to that of the control group (31.8 ± 1.6 mg/g liver, nonsignificant; Fig 3C). The rate of glycogenolysis before administration of acipimox was similar in the two groups (19.4 ± 1.7 *v* 17.2 ± 2.2 μmol/kg/min, nonsignificant). In contrast, hepatic glycogen content of the acipimox group (12.6 ± 2.0 mg/g liver) was significantly lower than that of the control group (23.0 ± 1.5 mg/g liver, P < .005) at the end of the experiment (Fig 3C). The rate of glycogenolysis in the second hour of the experiment was significantly higher in the acipimox group compared with the control group (40.5 ± 3.3 *v* 18.9 ± 2.2 μmol/kg/min, P < .005). Hepatic G-6-P concentration at the end of the experiment was significantly lower in the acipimox group than in the control group (39 ± 4 *v* 74 ± 7 μmol/g liver, P < .005).

Pentobarbital anesthesia has been reported to reduce basal HGP and to induce hepatic insulin resistance.[29,30] To further validate our data, separate groups of conscious rats were given acipimox or saline (n = 8 each) at time 0, and were killed at 120 minutes for measurement of hepatic glycogen content. Hepatic glycogen content of conscious rats given acipimox (11.0 ± 0.9 mg/g liver) was also significantly lower than that of control rats (21.8 ± 1.5 mg/g liver, P < .005), and was comparable to that of anesthetized rats given acipimox.

DISCUSSION

The present study demonstrates that insulin sensitivity of the liver and of peripheral tissues is significantly increased by decreasing plasma FFA with acipimox. In the present study, we chose a 9-pmol/kg/min insulin infusion rate to achieve mild hyperinsulinemia, at which changes in HGP can be assessed with higher sensitivity.[31,32] The current data indicating enhanced insulin-mediated suppression of HGP with acipimox are consistent with our previous results showing an opposite effect of increased plasma FFA with Intralipid (Green Cross, Seoul, Korea)-heparin infusion at similar mild hyperinsulinemia in man.[9] On the other hand, inhibition of peripheral glucose utilization was the main mechanism of FFA-induced insulin resistance at higher insulin levels.[9] Similarly, it has been reported that stimulation of peripheral glucose utilization is the main mechanism

Table 1. Rates of Endogenous Glucose Production and Glucose Utilization (both μmol/kg/min) in the Basal State and During Hyperinsulinemic Glucose Clamps in the Control and Acipimox Groups

	Control		Acipimox	
Parameter	Basal	Clamp	Basal	Clamp
Glucose production	53.9 ± 3.9	29.7 ± 1.7	57.8 ± 2.8	8.6 ± 2.2†
Glucose utilization	53.9 ± 3.9	67.0 ± 6.0	57.8 ± 2.8	90.9 ± 6.6*

*P < .05 *v* control group.
†P < .005 *v* control group.

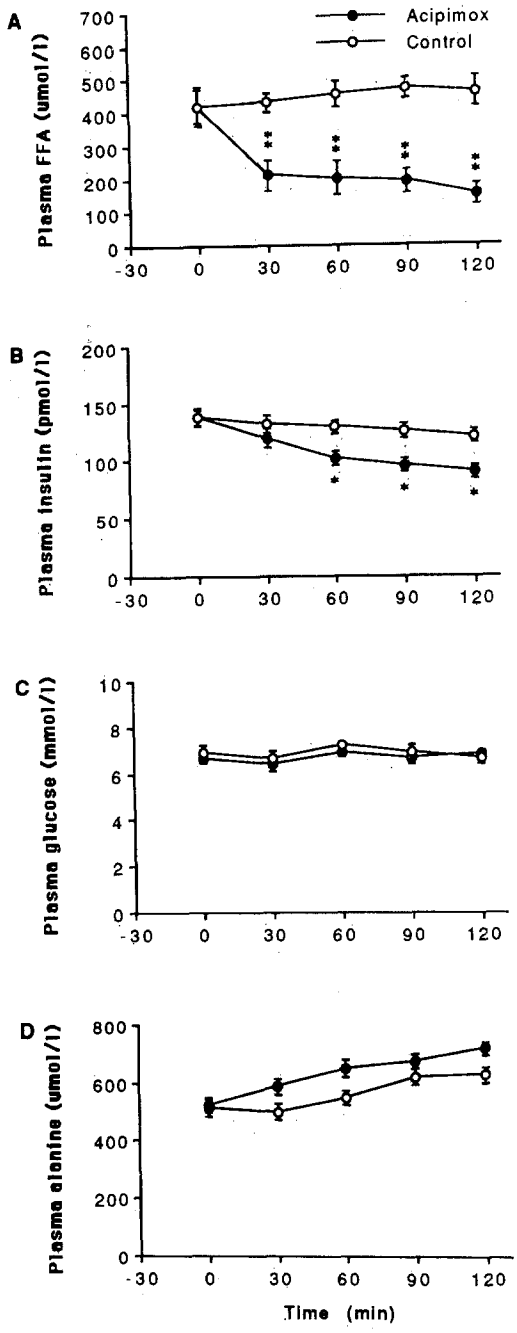

Fig 2. Changes in plasma FFA (A), insulin (B), glucose (C), and alanine (D) concentrations following administration of acipimox or saline at time 0. *$P < .05$, **$P < .005$.

of increased insulin sensitivity with acipimox at higher insulin levels.[33]

Our study confirms that acipimox treatment reduces hepatic gluconeogenesis (as assessed by ^{14}C-alanine incorporation into glucose) significantly.[13] Since acipimox treatment markedly reduces plasma FFA levels, this reduction in plasma FFA levels would be responsible for the reduction in hepatic gluconeogenesis. Although a recent study reported a direct effect of acipimox independent of plasma FFA on peripheral glucose uptake and nonoxidative glucose disposal,[34] such an effect in the liver was negated.[10] Our results are compatible with earlier studies showing that FFA stimulate gluconeogenesis in the perfused rat liver.[7] Decreased availability of FFA and consequent inhibition of lipid oxidation in the liver can suppress gluconeogenesis by several mechanisms[35]: (1) limitation of NADH required for the glyceraldehyde-3-phosphate dehydrogenase reaction, (2) reduction of adenosine triphosphate required to support gluconeogenesis, and (3) suppression of acetyl coenzyme A and other thioesters required to activate pyruvate carboxylase.

Although decreasing plasma FFA with acipimox decreased hepatic gluconeogenesis, this decrease was not associated with the change in overall HGP in the basal

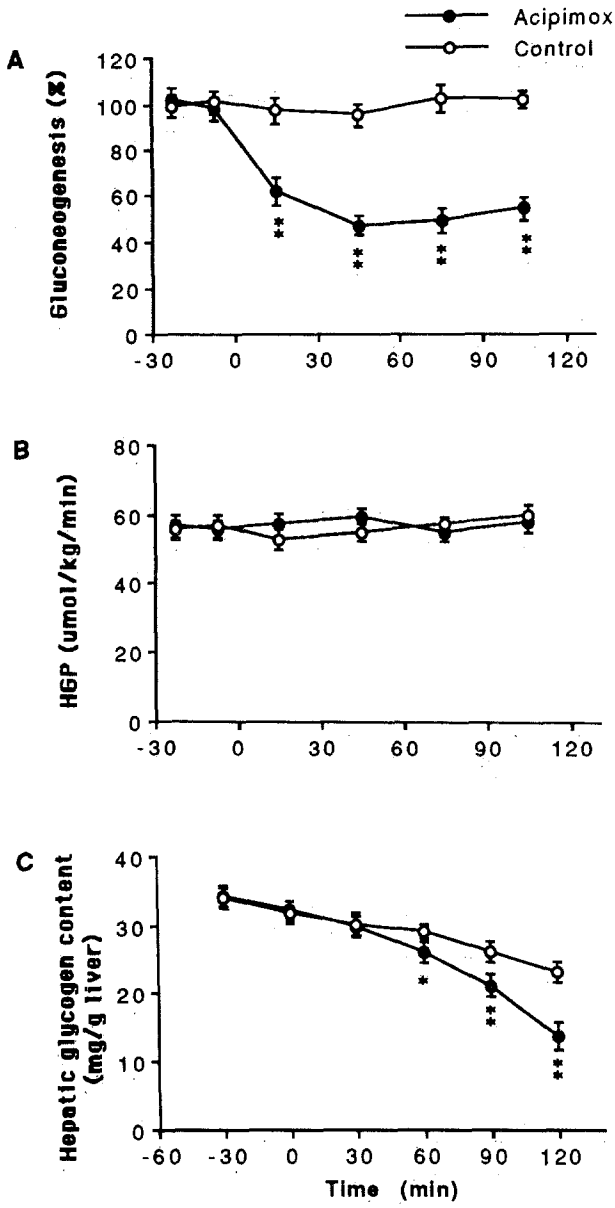

Fig 3. Changes in the rate of gluconeogenesis (A), HGP (B), and hepatic glycogen content (C) following administration of acipimox or saline at time 0. *$P < .05$, **$P < .005$.

state. Previous studies also showed that inhibition of gluconeogenesis by either acipimox or ethanol did not change overall HGP and fasting plasma glucose levels in man.[13,36] Furthermore, increasing the availability of FFA and gluconeogenic substrates increased gluconeogenesis but failed to increase HGP.[15,37] Kubota et al[38] showed that the liver glycogen content of rats given ethanol was significantly lower than that of the controls, and suggested that there were compensatory increases in hepatic glycogenolysis during inhibition of gluconeogenesis with ethanol. However, to our knowledge, the present study is the first direct demonstration of the reciprocal changes in gluconeogenesis and glycogenolysis using serial measurement of hepatic glycogen content.

The mechanism underlying the reciprocal changes in hepatic glycogenolysis and gluconeogenesis remains to be determined. In our study, hepatic G-6-P concentration was lower in the acipimox group than in the control group. This suggests that inhibition of gluconeogenesis by acipimox induced decreases in hepatic G-6-P concentration. Since G-6-P is an inhibitor of glycogen phosphorylase,[39] the decreased G-6-P concentration may have resulted in stimulation of glycogen phosphorylase and glycogen breakdown.

Another possible mechanism underlying the reciprocal changes of glycogenolysis and gluconeogenesis is the change in plasma insulin concentrations. Stimulation of insulin secretion by FFA is well documented,[40] but the effect of acipimox on plasma insulin concentration is controversial.[34,41] In the present study, we noted a small but significant decrease in plasma insulin concentrations following acipimox treatment, which may have contributed to the increase in hepatic glycogenolysis. The finding that inhibition of insulin secretion by somatostatin augments the hypoglycemic effect of nicotinic acid[42] or the hyperglycemic effect of Intralipid-heparin infusion[8] also supports this possibility, but further studies are required to determine how much of the change in hepatic glycogenolysis after acipimox treatment is attributable to the changes in plasma insulin concentration.

In contrast to the basal state, acipimox significantly decreased HGP during the hyperinsulinemic-euglycemic clamps. These results are in agreement with a recent study in man that demonstrated an inhibitory effect of acipimox on HGP during insulin infusion.[43] It is also noteworthy that an elevation of plasma FFA concentrations increased HGP during hyperinsulinemic clamp studies in man.[9] One possible explanation for these findings would be that the compensatory increase in glycogenolysis with acipimox in the basal state did not occur during the hyperinsulinemic clamps because the elevated plasma insulin suppressed glycogenolysis.[44] Alternatively, if the compensatory increase in hepatic glycogenolysis with acipimox in the basal state is due to decreases in plasma insulin (see above), it is obvious that such a mechanism would be masked during hyperinsulinemic clamps wherein plasma insulin levels were similarly elevated by exogenous insulin infusion.

Although the present study clearly demonstrates inhibition of tracer-determined gluconeogenesis with acipimox treatment, the magnitude of this inhibition may not represent the true rate because of the technical problem inherent to the tracer methodology.[27,28] Note that our measurement assessed gluconeogenesis from only one precursor (alanine). The degree of inhibition with acipimox might be different for gluconeogenesis from different precursors (ie, alanine, lactate, and glycerol). In addition, the measured rate of gluconeogenic conversion is an underestimate of the true rate, because of the dilution of the oxaloacetate pool by unlabeled carbons from acetyl coenzyme A.[45] The validity of this qualitative index for gluconeogenesis from alanine depends on the degree of precursor dilution under the experimental conditions. Conditions associated with increasing FFA concentrations slightly increase dilution of the oxaloacetate pool.[46] Therefore, rates of gluconeogenesis probably will be underestimated to a greater extent in the control group than in the acipimox group, and the real difference between the two groups would be greater. However, it is important to point out that the decrease in tracer-determined gluconeogenesis with acipimox treatment (ie, 54%) was similar to the decrease (57%) in gluconeogenesis independently estimated as HGP minus glycogenolysis. These data suggest that the estimated changes in gluconeogenesis using ^{14}C-alanine in the present study well represented the changes in total gluconeogenic flux.

In summary, decreasing plasma FFA levels with acipimox treatment enhanced insulin-mediated suppression of HGP in normal rats. In the basal state, acipimox inhibited hepatic gluconeogenesis, but this inhibition was not associated with the change in overall HGP due to the compensatory increase in hepatic glycogenolysis that presumably occurred as a consequence of decreased plasma insulin and/or hepatic G-6-P levels with acipimox. These results support the contention that FFA are an important determinant of insulin action in the liver, and suggest the existence of intrahepatic autoregulatory and/or hormonal regulatory processes for constant HGP in the basal state.

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