Acute inhibition of lipolysis does not affect postprandial suppression of endogenous glucose production

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Acute inhibition of lipolysis does not affect postprandial suppression of endogenous glucose production. Am J Physiol Endocrinol Metab 289: E941–E947, 2005. First published July 5, 2005; doi:10.1152/ajpendo.00195.2005.—To test the hypothesis that intrahepatic availability of fatty acid could modify the rate of suppression of endogenous glucose production (EGP), acipimox or placebo was administered before and during a test meal. We used a modified isotopic methodology to measure EGP in 11 healthy subjects, and $^1$H magnetic resonance spectroscopic measurement of hepatic triglyceride stores was also undertaken. Acipimox suppressed plasma free fatty acids markedly before the meal (0.05 ± 0.01 mmol/l at −10 min, $P = 0$) and throughout the postprandial period (0.03 ± 0.01 mmol/l at 150 min). Mean peak plasma glucose was significantly lower after the meal on acipimox days (8.9 ± 0.4 vs. 10.1 ± 0.5 mmol/l, $P < 0.01$), as was mean peak serum insulin (653.1 ± 99.9 vs. 909 ± 118 pmol/l, $P < 0.01$). Fasting EGP was similar (11.15 ± 0.58 μmol·kg$^{-1}$·min$^{-1}$ placebo vs. 11.17 ± 0.89 μmol·kg$^{-1}$·min$^{-1}$ acipimox). The rate of suppression of EGP after the meal was almost identical on the 2 test days (4.36 ± 1.52 vs. 3.69 ± 1.21 μmol·kg$^{-1}$·min$^{-1}$ at 40 min). There was a significant negative correlation between the acipimox-induced decrease in peak plasma glucose and liver triglyceride content ($r = −0.827$, $P = 0.002$), suggesting that, when levels of liver fat were low, inhibition of lipolysis was able to affect glucose homeostasis. Acute pharmacological sequestration of fatty acids in triglyceride stores improves postprandial glucose homeostasis without effect on the immediate postprandial suppression of EGP.

METHODS

Subjects. Eleven normal, healthy subjects were recruited. The group consisted of six male and five female subjects with the anthropometric and metabolic characteristics shown in Table 1. Subjects were chosen to represent a wide range of body mass indexes (BMI). Athletes in training were excluded, as were any subjects with metabolic disease or any family history of type 2 diabetes. Subjects had no history of hepatic or renal disease. The nature, purpose, and potential risks of the study were explained to all the subjects, and their informed, voluntary, written consent was obtained before their participation. The study protocol was reviewed and approved by the Joint Ethics Committee, University of Newcastle upon Tyne.

Study protocol. The study protocol is summarized in Fig. 1. For 3 days before the study, subjects consumed their normal weight-maintaining diet. Subjects were studied after an overnight fast. On the day of the study, subjects arrived at 0700, and anthropometric measurements were recorded. An intravenous cannula for infusion was placed in an antecubital fossa vein, and a second cannula was placed in a distal forearm vein, this hand being placed in a heated box at 50°C to allow sampling of arterialized blood. Baseline blood samples were taken, as well as blood for HbA1c, urea and electrolytes, liver function tests, and lipid profile. After the subjects had been resting semireclining for ≥30 min, they were given either 250 mg of acipimox or methylcellulose placebo of identical appearance in a single-blind fashion. Ten minutes later, subjects were given a priming dose of 300 μg of 6,6-dideuterated glucose in 7.5 ml of saline and the infusion of 6,6-dideuterated glucose (0.04 mg·ml$^{-1}$·min$^{-1}$) was commenced. A rapid increase in insulin secretion that acutely suppresses hepatic glucose production and enhances glucose storage and oxidation.

We (25) have previously shown, using novel isotopic methodology, that endogenous glucose production (EGP) was almost completely suppressed within 30 min postprandially in young normal subjects. However, a subsequent study on older, more obese normal subjects showed a slower, less complete postprandial suppression of EGP (21). Given that the major metabolic difference between our two groups of normal subjects was degree of obesity, we postulated that intrahepatic availability of fatty acid could modify the rate of suppression of EGP. Such an effect could explain the slower, incomplete suppression of EGP in subjects with type 2 diabetes. Conversely, if the fatty acid supply was curtailed, it would be expected that postprandial EGP suppression would be more rapid and more complete.

The present study was designed to test this hypothesis using acipimox, a nicotinic acid derivative that suppresses both peripheral tissue lipolysis (7, 23) and hepatic triglyceride release (20). To clarify more fully the relationship between fatty acid supply and EGP, nondiabetic subjects with a wide range of body weights were studied.

PLASMA GLUCOSE CONCENTRATION is determined by the interaction of two processes, the rate of release of glucose into the bloodstream and the rate of uptake of glucose by cells. In the fasted state, glucose is produced by the breakdown of glycogen stores or gluconeogenesis. In the postprandial state, absorption of glucose and other substrates from the intestine exceeds the body’s energy requirements. Homeostasis is maintained by a rapid increase in insulin secretion that acutely suppresses hepatic glucose production and enhances glucose storage and oxidation.

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period of 180 min was allowed for equilibrium of D-glucose; the end of this period was taken to be time 0. A further dose of either acipimox or placebo was given at –10 min. At 0 min, a standard liquid mixed meal (100 g carbohydrate, 12.5 g fat, 16 g protein) containing 2 g of 2-deuterated glucose was provided and subjects were asked to consume it over a 5-min period. The rate of infusion of 6,6-dideuterated glucose was adjusted in a stepwise fashion to reproduce the anticipated pattern of endogenous glucose release after the meal. The protocol was determined iteratively in preliminary studies and was as follows: basal period, 100% of basal infusion rate; 0–10 min, 85%; 10–20 min, 75%; 20–25 min, 65%; 25–30 min, 50%; 30–40 min, 40%; 40–260 min, 30%; 260–280 min, 40%; 280–300 min, 55%; 300–320 min, 70%; 320–340 min, 85%; and 340–450 min, 100%. At 170 min, the subjects were given a third dose of either acipimox or placebo. The study was concluded at 450 min.

Fuel oxidation rates were determined by means of constant flow hood calorimetry (Deltra Trac 17) using equations of Frayn (8). Measurements were made over 20-min periods every hour. Frequent blood samples were taken for the determination of plasma glucose, 2-deuterated glucose-glucose, 6,6-dideuterated glucose, lactate, triglycerides, nonesterified fatty acids, insulin, C-peptide, and glucagon.

Each subject was studied twice, once receiving acipimox and once receiving placebo in a blinded fashion and random order.

**Metabolite and hormone assays.** Plasma glucose was measured on a Yellow Springs glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma free fatty acids (FFA) were measured on a Roche Cobas centrifugal analyzer using a colorimetric assay (ABX Diagnostics, Montpellier, France). Plasma glucagon concentration was measured by radioimmunoassay. Plasma lactate was measured on perchloric acid extracts on a Roche Cobas Bio centrifugal analyzer.

Plasma glucagon concentration was measured by radioimmunoassay (ABX Diagnostics, Montpellier, France). Serum insulin and glucagon were measured using a Roche Cobas centrifugal analyzer, using a colorimetric assay (Wako Chemicals, Neuss, Germany). Plasma triglycerides were also measured on a Roche Cobas centrifugal analyzer by using a Wako kit (Wako Chemicals, Neuss, Germany). Plasma free fatty acids (FFA) were measured on a Yellow Springs glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Plasma glucose was determined by gas chromatography-mass spectroscopy, using a Hewlett Packard GC fitted with a 60 m mol/2 capillary column and a FID detector. Spectra were analyzed using an MR user interface (13). Automatic phase correction was performed using the water peak in the spectra, and spectra peak amplitudes estimated by fitting Lorentzian curves to the spectra. To calculate molar density of triglycerides from the amplitudes of water and intramyocellular lipid estimated in the spectral fitting, we used the formulae described by Szczepaniak et al. (24).

**Calculations of EGP.** The profile of exogenous concentration, i.e., the component of total glucose concentration due to endogenous glucose ingestion, was initially calculated. Because this is proportional to the 6,6-dideuterated glucose, its calculation is straightforward and model independent (6). We then calculated the time course of the endogenous glucose concentration, i.e., the component of total glucose due to EGP only, by subtracting the calculated exogenous component and the 6,6-dideuterated glucose concentration from the measured total glucose concentration. The steady-state values of plasma clearance rate (PCR) and basal EGP (basal EGP = PCR × basal glucose concentration) were estimated from the 6,6-dideuterated glucose decay curve after the prime dose of 6,6-dideuterated glucose given 3 h before the meal (5). Subsequently, the time course of EGP was calculated from endogenous glucose concentration and 6,6-dideuterated glucose data. Because glucose had been infused mimicking the expected behavior of EGP, the ratio of 6,6-dideuterated glucose to endogenously produced glucose was steady, thus allowing a more reliable estimation of EGP. EGP was calculated using the two-compartment model of Radziuk et al. (15). The concentration of 6,6-dideuterated glucose and the ratio between 6,6-dideuterated glucose and endogenous glucose were smoothed by using an algorithm based on stochastic nonparametric deconvolution (22).

**Insulin sensitivity.** The homeostatic model assessment index of insulin resistance (HOMA-IR) was calculated using fasting insulin and glucose concentrations (12).

**3 H magnetic resonance spectroscopy.** Localized 3 H-nuclear magnetic resonance (MR) spectra of liver and muscle were obtained in a 1.5 Tesla MR scanner with a 3H transmitter/receiver coil placed over the measured tissue. Liver spectra were acquired by applying the breath hold-triggered stimulation echo acquisition sequence. Spectra were collected without water suppression, using a point-resolved spectroscopy sequence (4), with a 2 × 2 × 2-cm voxel in the soleus muscle and a 3 × 3 × 3-cm voxel in the liver, using an echo time of 25 ms and repetition time of 5,000 ms with 32 acquisitions and a spectral resolution of 1 Hz.

Spectra were analyzed using an MR user interface (13). Automatic phase correction was performed using the water peak in the spectra, and the water peak was assigned to 4.68 ppm. Lipid spectral peaks were assigned as described in Boesch et al. (3) and spectra peak amplitudes estimated by fitting Lorentzian curves to the spectra. To calculate molar density of triglycerides from the amplitudes of water and intramyocellular lipid estimated in the spectral fitting, we used the formulae described by Szczepaniak et al. (24).

**Statistical methods.** Data are presented as means ± SE unless otherwise stated. Comparisons between groups were performed using Student’s paired t-test. Relationships were tested by linear correlation analysis. A prior power calculation indicated that 10 subjects were required to provide a 90% power to detect a 20% difference in suppression of EGP.

**RESULTS**

**Plasma glucose.** There was no difference in fasting mean plasma glucose on the acipimox study days compared with placebo study days (5.2 ± 0.1 at –195 min). During the

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Table 1. Clinical characteristics of the subjects

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Values are means ± SE. BMI, body mass index; FFA, fasting blood glucose; Chol, cholesterol; TG, triglyceride.

Fig. 1. Schema of study protocol. Timing of acipimox or placebo administration is indicated together with timing of indirect calorimetry. The 3-h run-in period (dark gray) and the period of variable 6,6-dideuterated (D)-glucose infusion (light gray) are illustrated.
prolonged fast of the 3-h run-in period, mean plasma glucose fell slightly on both study days (5.1 ± 0.1 vs. 5.0 ± 0.1 mmol/l, respectively, at 0 min).

After the consumption of the mixed meal, mean arterialized plasma glucose levels rose sharply on both study days, reaching a peak of 8.3 ± 0.4 mmol/l at 60 min on acipimox study days and a peak of 9.3 ± 0.6 mmol/l at 80 min on placebo study days (P = 0.01; Fig. 2). Mean peak plasma glucose was significantly lower on acipimox compared with placebo days (8.9 ± 0.4 vs. 10.1 ± 0.5 mmol/l, P < 0.01). The difference in postprandial mean plasma glucose was significant after 80 min (8.1 ± 0.6 vs. 9.3 ± 0.6 mmol/l, P < 0.01) and remained significant until after 120 min (7.2 ± 0.6 vs. 9.0 ± 0.6, P < 0.01).

After 120 min, mean plasma glucose levels on the 2 study days followed a similar pattern, falling to a nadir (4.5 ± 0.2 mmol/l at 280 min on acipimox days, 4.3 ± 0.2 mmol/l at 280 min on placebo days) before rising slightly toward the end of the study (5.1 ± 0.1 mmol/l acipimox, 5.0 ± 0.2 mmol/l placebo).

Serum insulin. Fasting mean serum insulin levels were not significantly different on the 2 study days (59.5 ± 12.3 vs. 66.2 ± 17.7 pmol/l), and serum insulin levels fell slightly but not significantly during the 3-h run-in period before the meal (35.3 ± 8.1 vs. 56.2 ± 14 pmol/l at −10 min). After the meal, mean serum insulin on the acipimox days reached a peak of 505 ± 104 pmol/l at 60 min and remained approximately at this level until 150 min, falling thereafter to basal levels. On the placebo days, mean serum insulin was significantly higher 60 min after the meal (658 ± 111 pmol/l, P < 0.05) and peaked later (757 ± 143 pmol/l at 80 min; Fig. 2) before returning to basal levels. Mean peak serum insulin was significantly lower on acipimox days (653.1 ± 99.9 vs. 909 ± 118 pmol/l, P < 0.01). Mean serum insulin levels remained significantly lower on the acipimox days until the end of the study (26.4 ± 7.8 vs. 58.9 ± 17.9 pmol/l at 480 min, P < 0.05).

C-peptide. C-peptide levels on the 2 study days were not significantly different in the fasted state (0.8 ± 0.1 vs. 0.7 ± 0.1 mmol/l at −195 min) during the run-in period (0.5 ± 0.0 vs. 0.7 ± 0.1 nmol/l at −10 min) or in the early postprandial period (2.7 ± 0.3 vs. 2.4 ± 0.4 nmol/l at 40 min). By 80 min, mean C-peptide levels were significantly lower after the administration of acipimox compared with placebo (3.6 ± 0.4 vs. 4.7 ± 0.4 nmol/l, P = 0.02) and remained significantly lower at 150 min, when levels on both days reached a maximum (3.6 ± 0.4 vs. 5.3 ± 0.5 nmol/l, P < 0.01). Toward the end of the study, mean C-peptide levels returned to basal but remained lower on the acipimox days (0.46 ± 0.07 vs. 0.88 ± 0.16 nmol/l at 480 min, P < 0.01).

Glucagon. Initial mean fasting glucagon levels were 92.9 ± 12.4 pg/ml on the placebo day and 86.2 ± 11.6 pg/ml on the acipimox day (P = 0.1). Glucagon levels reached a nadir at 150 min after the mixed meal on both days (72.4 ± 7.4 vs. 82.1 ± 6.4 pg/ml, P = 0.07). Thereafter, glucagon levels rose to a peak of 107.5 ± 16.9 pg/ml at 340 min on the acipimox day compared with 106.5 ± 13.7 pg/ml at 390 min on the placebo day (P = 0.9).

FFA and glycerol. On the placebo study days, the mean fasting FFA level was 0.47 ± 0.05 mmol/l. During the extended fast of the run-in period, FFA rose to 0.6 ± 0.05 mmol/l. After ingestion of the meal, FFA levels fell to 0.05 ± 0.02 mmol/l at 150 min before rising to 0.8 ± 0.03 mmol/l at the end of the study. On the acipimox study days, mean fasting plasma FFA level was not significantly different from placebo (0.52 ± 0.05 mmol/l). However, after the ingestion of acipimox, plasma FFA were markedly suppressed (0.05 ± 0.01

![Fig. 2. Changes in plasma glucose, serum insulin, C-peptide, and glucagon during the study period after administration of acipimox (●) and placebo (○). Data are shown as means ± SE.](Image 346x157 to 540x721)
mmol/l at −10 min, \( P < 0.0001 \) and remained suppressed throughout the postprandial period (0.03 ± 0.01 mmol/l at 150 min) until 340 min, when FFA levels began to rise (Fig. 3). At the end of the study, levels were still significantly lower than those seen on the placebo days (0.31 ± 0.1 mmol/l, \( P < 0.0001 \)).

Mean fasting glycerol level on the acipimox day was 44 ± 7 \( \mu \)mol/l. After the ingestion of acipimox, glycerol levels fell to <10 \( \mu \)mol/l and remained suppressed until 390 min. On the placebo day, a slight decrease was observed during the run-in period (50 ± 8 \( \mu \)mol/l at −195 min, 45 ± 5 \( \mu \)mol/l at −10 min). Postprandially, levels fell to a nadir of 12 ± 3 \( \mu \)mol/l at 150 min. Levels subsequently rose on the placebo day, reaching 67 ± 9 \( \mu \)mol/l by the end of the study.

Fasting 3-hydroxybutyrate (3-OHB) was initially higher on the acipimox day (68 ± 15 vs. 55 ± 11 \( \mu \)mol/l, \( P = 0.4 \)). However, after the administration of acipimox, levels were suppressed <20 \( \mu \)mol/l throughout most of the study, recovering to 68 ± 27 \( \mu \)mol/l at 450 min. On the placebo day, levels rose during the extended part of the run-in period (147 ± 24 \( \mu \)mol/l at 0 min) but fell after the meal (7 ± 2 \( \mu \)mol/l at 150 min). A marked rise in 3-OHB was observed towards the end of the study (385 ± 84 \( \mu \)mol/l at 450 min).

**Triglycerides.** Mean plasma triglyceride levels were similar on the 2 days in the fasting state (1.13 ± 0.13 vs. 1.0 ± 0.11 mmol/l at −195 min, \( P = 0.8 \)), although triglyceride levels were lower in the acipimox group throughout the study. Triglyceride levels decreased in both groups during the run-in period (1.04 ± 0.11 vs. 0.89 ± 0.11 mmol/l at −10 min, \( P < 0.05 \)). Mean triglyceride levels were significantly higher on placebo days just after the meal was ingested (1.15 ± 0.12 vs. 0.82 ± 0.12 mmol/l at 10 min, \( P < 0.01 \)). In both groups, triglyceride levels rose after the meal, reaching an initial peak in the placebo group at 80 min (1.26 ± 0.16 mmol/l) and in the acipimox group at 260 min (1.06 ± 0.15 mmol/l).

**Metabolites.** Fasting lactate levels were similar on the 2 study days (0.82 ± 0.08 mmol/l acipimox studies vs. 0.81 ± 0.08 mmol/l placebo studies, \( P = 0.9 \)). Levels fell on both days during the run-in period, with significantly lower levels observed on the placebo day (0.69 ± 0.04 vs. 0.48 ± 0.04 mmol/l, \( P < 0.01 \)). After the meal, mean plasma lactate rose on both days, with a similar peak observed on the placebo day (0.97 ± 0.07 mmol/l at 80 min) compared with the acipimox day (0.92 ± 0.01 mmol/l at 60 min). Thereafter, levels fell similarly in both groups.

Fasting alanine levels in the 2 days were similar (290 ± 27 \( \mu \)mol/l acipimox vs. 273 ± 24 \( \mu \)mol/l placebo, \( P = 0.4 \)). Levels on both days fell in the run-in period, with a more marked fall being observed on the placebo day (193 ± 23 vs. 261 ± 21 \( \mu \)mol/l at −10 min, \( P < 0.01 \)). After the mixed meal, alanine levels rose sharply on the placebo day so that, at 80 min, levels were again similar (252 ± 19 vs. 280 ± 22 \( \mu \)mol/l, \( P = 0.1 \)).

**EGP.** From Fig. 4, it can be seen that the infusion of 6,6-dideuterated glucose was closely matched on both study days. The appearance of 2-deuterated glucose-glucone from the mixed meal in plasma was similar on both days.

Steady-state rates of EGP were similar (11.15 ± 0.58 \( \mu \)mol·kg⁻¹·min⁻¹ placebo vs. 11.17 ± 0.89 mg·kg⁻¹·min⁻¹ acipimox). The initial suppression of EGP after the meal was not significantly different on the 2 study days (4.36 ± 1.52 vs. 0.08 mmol/l placebo studies, \( P < 0.0001 \)).
fasting state (1.7 ± 0.2 vs. 1.1 ± 0.2 mg·kg⁻¹·min⁻¹, P < 0.03). After the meal there was no significant difference between the 2 study days (2.7 ± 0.2 vs. 2.5 ± 0.2 mg·kg⁻¹·min⁻¹ at 180 min, P = 0.4). Toward the end of the study, the mean glucose oxidation rate fell on both days but declined more slowly on the acipimox days (1.7 ± 0.2 vs. 0.9 ± 0.3 mg·kg⁻¹·min⁻¹ at 420 min, P = 0.11).

A lower mean lipid oxidation rate was observed during the run-in period on acipimox study days compared with placebo study days (0.3 ± 0.1 vs. 0.6 ± 0.1 mg·kg⁻¹·min⁻¹, P = 0.07). There was no significant difference in rates of lipid oxidation during the postprandial period (0.1 ± 0.1 vs. 0.2 ± 0.1 mg·kg⁻¹·min⁻¹ at 180 min), although the subsequent recovery was delayed on acipimox days (0.4 ± 0.1 vs. 0.8 ± 0.2 mg·kg⁻¹·min⁻¹ at 420 min, P = 0.12).

1H-MR spectroscopy. The mean soleus intramyocellular and hepatic triglyceride content is shown in Table 1. There was a positive correlation between soleus triglyceride content and BMI (r = 0.70, P = 0.02) and between soleus and liver triglyceride content (r = 0.70, P < 0.02). The range of liver triglyceride content is reflected by the range of BMI in the subjects selected. There was a general trend for liver triglyceride to be higher in individuals with a high BMI, but this relationship did not achieve significance (r = 0.53, P = 0.1). Insulin sensitivity as measured by HOMA-IR correlated significantly with liver triglyceride content (r = 0.77, P < 0.01) and soleus triglyceride content (r = 0.77, P < 0.01).

Although there was no relationship between the change in EGP in the first 60 min postprandially and hepatic triglyceride content on either study day (r = −0.239, P = 0.51 placebo day; r = −0.01, P = 0.979 acipimox day), there was a strong negative correlation between the acipimox-induced difference in peak plasma glucose and liver triglyceride content (r = −0.827, P = 0.002; Fig. 5).

DISCUSSION

The experimental protocol successfully achieved suppression of whole body lipolysis so that plasma FFA decreased before the test meal and remained almost undetectable for 6 h postprandially. This was associated with a 25% reduction in peak plasma glucose levels in these normal subjects. However, acipimox suppression of lipolysis had no effect upon the early

3.69 ± 1.21 μmol·kg⁻¹·min⁻¹ at 40 min). However, EGP reached a nadir of 2.07 ± 1.28 μmol·kg⁻¹·min⁻¹ at 60 min on the acipimox day compared with a nadir of 2.70 ± 0.62 μmol·kg⁻¹·min⁻¹ at 150 min on the placebo day (Fig. 4). It is noteworthy that the rapid fall in plasma glucose from 60 to 120 min on the acipimox day (8.3 ± 0.4 vs. 7.2 ± 0.6 mmol/l, P < 0.05) was associated with a rise in EGP (2.07 ± 1.32 vs. 5.19 ± 2.02 μmol·kg⁻¹·min⁻¹). The subsequent increases in EGP toward the end of the study produced similar rates at 450 min (10.05 ± 1.47 vs. 9.94 ± 0.79 μmol·kg⁻¹·min⁻¹).

Substrate oxidation. Mean glucose oxidation rate was significantly higher 120 min after acipimox administration in the

Fig. 4. Changes in 2-deuterated (D)-glucose (top), 6,6-dideuterated glucose (middle), and endogenous glucose production (EGP, bottom) during the study period after administration of acipimox (●) and placebo (○). Isotopic data are shown as atoms percent excess (APE). Data are shown as means ± SE.

Fig. 5. Relationship between liver TG content and the difference (Δ) in peak plasma glucose between acipimox and placebo studies.
postprandial rate of suppression of EGP, this being 33% compared with 39% at 40 min after the test meal. A nonsignificantly greater suppression in the acipimox group at 60 min was followed by a rebound associated with a sharp fall in plasma glucose levels. The time to maximum suppression of EGP was similar after acipimox or placebo administration (95 ± 15 vs. 102 ± 15 min, P = 0.65), and the overall degree of suppression of EGP over the postprandial hours did not differ between the test days (0.5 ± 1.04 vs. 0.71 ± 0.77 μmol·kg⁻¹·min⁻¹, P = 0.88).

While the present studies have been underway, more information has been accumulated to suggest that excess triglyceride stores in liver play an important role in glucose homeostasis. Ryyysy et al. (16) demonstrated that the dose requirements of people with type 2 diabetes on insulin therapy were closely related to hepatic triglyceride content. They also observed that hepatic triglyceride content was related to the extent of suppression of hepatic glucose production by intravenous insulin. A subsequent study (27) from the same group showed that hepatic triglyceride content was more closely related to indexes of insulin resistance than was obesity itself. Longer-term modulation of hepatic triglyceride content has been reported to improve postprandial suppression of EGP in rodents (11), and fatty acids inhibit insulin suppression of glycogenolysis in both rodents and humans (2). Furthermore, a very low fat diet-induced chronic decrease in hepatic triglyceride in patients with type 2 diabetes has been shown to bring about a marked improvement of suppression of hepatic glucose production during insulin infusion (29 ± 22 vs. 99 ± 3% decrease) (14).

In the light of this growing body of knowledge linking excess hepatic fat with decreased insulin sensitivity, the demonstrated lack of effect upon rate of suppression of EGP during acute acipimox administration must be considered. One possible explanation for the lack of effect upon hepatic glucose production, the major component of EGP, might be that the acipimox effect upon lipolysis is entirely expressed in adipose tissue and muscle without a measurable effect upon intrahepatic triglyceride. In rodents, acipimox suppresses the release of fatty acids from the liver (20). In humans it is conceivable that the drug might not act upon intrahepatic lipolysis, or the effect upon this process is insufficient to change hepatic glucose production. In one of the earliest studies in humans, acipimox was observed not to change skeletal muscle activity of pyruvate dehydrogenase or phosphofructokinase even though glucose metabolism was promoted, and this was taken to imply a predominantly hepatic effect (28). EGP during steady-state hyperinsulinemia was suppressed considerably more (18 vs. 50 μg·m⁻²·min⁻²) in obese type 2 diabetic subjects given acipimox compared with placebo (9). No previous studies on rate of suppression of EGP are available.

The original hypothesis postulated that the effect of acipimox on rate of suppression of EGP would be greatest in those subjects with increased levels of intrahepatic triglyceride because of removal of the inhibitory effect of local fatty acid release. This hypothesis was comprehensively refuted by the study, with a significantly negative correlation between the degree of effect upon peak plasma glucose levels and the liver triglyceride concentration being observed (Fig. 4). Above a liver triglyceride concentration of 150 μmol/g, it appeared that acipimox had no effect whatsoever upon peak postprandial plasma glucose levels. A possible explanation might be that the higher the concentration of intrahepatic triglyceride, the less likely it is that the degree of inhibition of pharmacologically induced lipolysis could achieve a sufficient suppression of fatty acid supply within the hepatocyte. Hence, in subjects with low-normal intrahepatic triglyceride stores, suppression of lipolysis in the liver may be achieved by acipimox, whereas in those with a gross oversupply of fatty acid the drug is unable to achieve a change sufficient to alter carbohydrate metabolism. Such a mechanism has previously been postulated in respect of the effect of visceral fat upon hepatic glucose metabolism (10). If this supramaximal supply concept is correct, hepatic glycogen synthesis might be affected to account for the lower postprandial plasma glucose levels. Direct evidence for this is lacking, although when hepatic fat content is decreased by pioglitazone, insulin-stimulated hepatic glucose uptake increases substantially (1).

In addition to possible effects on hepatic glycogen synthesis, other mechanisms for this postprandial benefit on plasma glucose must be considered. During sustained hyperinsulinemia in type 2 diabetic subjects, acipimox has been observed to bring about a decrease of about one-third in glucose uptake (9, 17). Under the conditions of these studies, it is likely that insulin sensitivity of muscle glycogen synthesis explains the acipimox-induced change. It is not possible to estimate the relative contributions of changes in either liver or muscle glycogen synthesis to the results of the present study, although it is known that at peak postprandial levels, liver and muscle glycogen stores account for ~30 and 20%, respectively, of meal carbohydrate (25, 26). Change in oxidative glucose disposal also has to be considered. Rates of glucose oxidation were significantly enhanced by acipimox. During the second and third postprandial hours, when the difference in plasma glucose levels was greatest, glucose oxidation averaged 2.6 and 2.2 mg·kg⁻¹·min⁻¹ (P = 0.017). During this period, ~60% of the 100-g carbohydrate content of the meal would be absorbed, giving a mean rate of appearance of exogenous glucose of 6 mg·kg⁻¹·min⁻¹. The difference in oxidation rates would account for less than 10% of the glucose flux and, hence, would be insufficient by itself to explain the 25% decrease in peak blood glucose levels. Other studies have observed a substantial effect of acipimox upon glucose oxidation rates and only a small effect on glucose storage, but this is seen only during low-dose insulin infusions achieving much lower plasma insulin levels than seen postprandially (18, 19).

In summary, acipimox-induced inhibition of lipolysis brought about no change in initial rates of postprandial suppression of EGP, although the degree of effect on peak plasma glucose levels was inversely correlated with basal hepatic triglyceride concentration. In the presence of low levels of liver fat, inhibition of lipolysis appeared able to affect glucose homeostasis, whereas high levels of liver fat were associated with a lack of effect of acipimox on plasma glucose despite adequate suppression of plasma fatty acid concentrations. Acute pharmacological sequestration of fatty acids in triglyceride stores improves postprandial glucose homeostasis without a major effect on glucose output from the liver.

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REFERENCES


