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**Research Article**

In vitro studies indicate that FFA compete with glucose as an oxidative fuel in muscle and, in addition, stimulate gluconeogenesis in liver. During counterregulation of hypoglycemia, plasma FFA increase and this is associated with an increase in glucose production and a suppression of glucose utilization. To test the hypothesis that FFA mediate changes in glucose metabolism that occur during counterregulation, we examined the effects of acipimox, an inhibitor of lipolysis, on glucose production and utilization ([3-3H]glucose), and incorporation of [U-14C]-alanine into glucose during insulin-induced hypoglycemia. Eight normal volunteers were infused with insulin for 8 h to produce modest hypoglycemia (approximately 3 mM) on two occasions, first without acipimox (control) and then with acipimox administration (250 mg per os at 60 and 240 min). Despite identical plasma insulin concentrations, glucose had to be infused in the acipimox experiments (glucose-clamp technique) to maintain plasma glucose concentrations identical to those in control experiments. Acipimox completely prevented counterregulatory increases in lipolysis so that during the last 4 h plasma FFA were below baseline values and averaged 67 +/- 13 vs. 725 +/- 65 microM in control experiments, $P < 0.001$. Concomitantly, overall glucose production was reduced by 40% (5.5 +/- 11 vs. 9.3 +/- 0.7 mumol/kg per min, $P < 0.001$), and gluconeogenesis from alanine was reduced by nearly 70% (0.32 +/- 0.09 vs. […]



# Demonstration of a Critical Role for Free Fatty Acids in Mediating Counterregulatory Stimulation of Gluconeogenesis and Suppression of Glucose Utilization in Humans

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## Abstract

**In vitro studies indicate that FFA compete with glucose as an oxidative fuel in muscle and, in addition, stimulate gluconeogenesis in liver. During counterregulation of hypoglycemia, plasma FFA increase and this is associated with an increase in glucose production and a suppression of glucose utilization. To test the hypothesis that FFA mediate changes in glucose metabolism that occur during counterregulation, we examined the effects of acipimox, an inhibitor of lipolysis, on glucose production and utilization ([3-$^3$H]glucose), and incorporation of [U-$^{14}$C]-alanine into glucose during insulin-induced hypoglycemia. Eight normal volunteers were infused with insulin for 8 h to produce modest hypoglycemia (~ 3 mM) on two occasions, first without acipimox (control) and then with acipimox administration (250 mg per os at 60 and 240 min). Despite identical plasma insulin concentrations, glucose had to be infused in the acipimox experiments (glucose-clamp technique) to maintain plasma glucose concentrations identical to those in control experiments. Acipimox completely prevented counterregulatory increases in lipolysis so that during the last 4 h plasma FFA were below baseline values and averaged 67±13 vs. 725±65 μM in control experiments, $P < 0.001$. Concomitantly, overall glucose production was reduced by 40% (5.5±11 vs. 9.3±0.7 μmol/kg per min, $P < 0.001$), and gluconeogenesis from alanine was reduced by nearly 70% (0.32±0.09 vs. 1.00±0.18 μmol/kg per min, $P < 0.001$), while glucose utilization increased by 15% (10.8±1.4 vs. 9.3±0.7 μmol/kg per min). We conclude that FFA play a critical role in mediating changes in glucose metabolism during counterregulation, and that under these conditions, FFA exert a much more profound effect on hepatic glucose production than on glucose utilization. (*J. Clin. Invest.* 1993. 92:1617–1622.) Key words: free fatty acids • gluconeogenesis • counterregulation • hypoglycemia**

## Introduction

In 1963, Randle et al. (1) postulated a glucose/fatty acid cycle in which FFA compete with glucose as a physiological substrate. Over the years, considerable attention has been paid to the interaction between fat and glucose metabolism in cardiac and skeletal muscle (2). It is now generally accepted that FFA compete with glucose as an oxidative fuel (3), and this has been demonstrated to reduce skeletal muscle glucose uptake in humans (4, 5).

An additional effect of FFA, its increase of hepatic glucose output, was postulated shortly after Randle's original proposal (6–9). Most in vitro studies have found that FFA augment gluconeogenesis (6–13). However, in vivo studies have yielded conflicting results concerning the effects of FFA on overall hepatic glucose output (14–27). Thus, the effect of FFA on hepatic glucose output and gluconeogenesis in vivo remain in doubt.

During counterregulation of hypoglycemia, plasma FFA concentrations and rates of lipid oxidation increase (23, 26). This is temporally associated with an increase in glucose production and a suppression of glucose utilization (23, 26). Therefore, it has been postulated recently that FFA may mediate some of the changes in glucose metabolism that occur during counterregulation of hypoglycemia (23).

To test this hypothesis and to determine the relative impact of alterations in FFA availability on glucose production and glucose utilization, we assessed the effect of acipimox, an inhibitor of lipolysis (19, 24), on glucose production, gluconeogenesis, and glucose utilization, as well as plasma alanine fluxes during counterregulation of insulin-induced hypoglycemia in normal volunteers.

## Methods

*Subjects.* Informed consent was obtained from eight healthy volunteers (four males and four females) aged 25±1 yr who were all within 10% of their ideal body weight (101±2%, Metropolitan Life Insurance Tables; body mass index of 24.4±1.0 kg/m$^2$) and had no family history of diabetes mellitus or other endocrine disease. Each subject was studied on two different occasions: control experiments without acipimox were performed first, followed by experiments with acipimox 7–10 d later. For 3 d before each experiment, all subjects consumed a weight maintenance diet containing ≥ 250 g carbohydrate.

*Protocol.* Institutional Review Board approval was obtained for these studies. In both experiments, subjects were admitted to the Clinical Research Center between 6:30 and 7:00 a.m., after overnight fasting (10–12 h). They were placed in bed rest and maintained supine positions throughout the experiments. To obtain arterialized venous blood samples, a dorsal vein of a hand was cannulated in a retrograde position with a 21-gauge butterfly needle, and the hand was maintained at 60–65°C in a thermoregulated Plexiglass box. An antecubital vein of the contralateral arm was cannulated with an 18-gauge catheter for infusion of [3-$^3$H]glucose, [$^{14}$C]alanine, insulin, and, when required, glucose (20% solution) by separate syringe pumps (Harvard Apparatus, Inc., The Ealing Co., South Natick, MA). Both forearm and venous lines were kept patent by 0.9% NaCl infused by separate peristaltic

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pumps (VM 8000 M; Vial Medical, St. Martin-Le-Vinoux, Grenoble, France), at a rate 30 ml/h. Between 7:00 and 7:30 a.m. primed continuous infusions of [3-$^3$H]glucose (22 $\mu$Ci, 0.22 $\mu$Ci/min) (New England Nuclear, Boston, MA) and [$^{14}$C]alanine (28 $\mu$Ci, 0.28 $\mu$Ci/min) (Amersham Corp., Arlington Heights, IL) were begun. 3 h were allowed for isotopic equilibration, after which baseline blood samples were taken. Lipid and carbohydrate oxidation expenditure were measured in all subjects by indirect calorimetry (28). 90 min before beginning experiments, a transparent plastic ventilated hood was placed over the subject's head and made airtight around the neck. Air flow and $O_2$ and $CO_2$ concentrations in the expired and inspired air were measured by a computerized continuous open-circuit system (Deltatrac; Datex Instruments Co., Helsinki, Finland) (29) that has a precision of 2.5% for oxygen consumption and 1.0% for carbon dioxide production. Protein oxidation was estimated from urinary excretion of urea before and during insulin hypoglycemia. After the subjects had adapted to the hood and stabilized their breathing pattern, gas-exchange measurements were taken during a 45-min basal period and were continued throughout the studies.

In control experiments, insulin (regular U-40; Eli Lilly & Co., Indianapolis, IN), diluted to 1 U/ml in 100 ml of 0.9% NaCl containing 2 ml of the subject's blood, was infused at the rate of 0.35 mU · Kg$^{-1}$ · min$^{-1}$ for 8 h to induce moderate hypoglycemia. In acipimox experiments, insulin was infused as in the control experiments and acipimox (5-methyl-pyrazene-carboxylic acid 4-oxide) (Olbetam, Farmitalia Carlo Erba, Milan, Italy) (250 mg p.o.) was given 60 min after starting the insulin infusion and again at 240 min to inhibit lipolysis. Because we anticipated from previous studies (23) that suppression of lipolysis might impair glucose counterregulation, glucose was infused when needed to maintain plasma glucose concentrations identical to those in the control experiments using the glucose-clamp technique as previously described (23).

*Analyses.* Blood samples were collected at 30-min intervals and assayed for glucose (Beckman glucose analyzer; Beckman Instruments, Fullerton, CA), alanine (30), [$^3$H]- and [$^{14}$C]glucose radioactivity (31), [$^{14}$C]alanine radioactivity (31), insulin (23), glucagon (23), cortisol (23), growth hormone (23), epinephrine, and norepinephrine (23), FFA (Wako NEFA C test kit, Wako Chemicals GmbH, Neuss, Germany), 3-$\beta$-OH-butyrate (30), and glycerol (30). For FFA determination, blood (2 ml) was collected in tubes containing 50 $\mu$l of the lipoproteinlipase inhibitor diethyl-*p*-nitrophenyl-phosphate (Paraoxon; Sigma Chemical Co., St. Louis, MO) diluted to 0.04% in diethyl ether (32). Urine was collected from the onset to the end of each study period to determine nitrogen excretion using the Kjeldahl method (33).

*Calculations.* Oxidation rates for carbohydrate, fat, and protein were calculated from the measured $O_2$ consumption, $CO_2$ production, and urinary nitrogen excretion before and during the insulin infusion adjusted for changes in serum urea during insulin infusion (34, 35). Rates of glucose and alanine appearance (Ra) and disappearance (Rd) were calculated using the non-steady-state equations of De Bodo et al. (36); a pool size of 0.65 and a distribution of 200 mg/kg were used. When glucose was infused, endogenous glucose appearance was calculated by subtracting the exogenous glucose infusion rate from the overall glucose appearance. Gluconeogenesis was estimated as the rate of appearance of glucose from alanine calculated using the non-steady-state double isotope technique of Chiasson et al. (37) in which the appearance of $^{14}$C in glucose is traced by [$^3$H]glucose. Data in text and figures are given as mean±SEM, and the statistical significance was evaluated using Student's paired *t* test. A $P < 0.05$ was considered significant.

## Results

*Plasma insulin and glucose concentrations, and glucose infusion rates (Fig. 1).* Baseline and subsequent plasma insulin concentrations were comparable in the control and acipimox

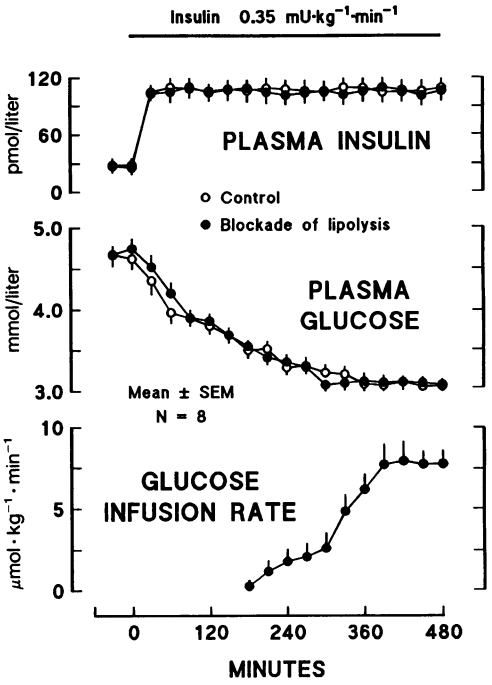


*Figure 1.* Plasma insulin and glucose concentrations and glucose infusion rates.

experiments. During infusion of insulin, plasma glucose levels decreased similarly in both experiments to a plateau of ~ 3.0 mM. However, in the acipimox experiments, glucose had to be infused to maintain the plasma glucose concentrations comparable.

*Plasma counterregulatory hormone concentrations (Fig. 2).* Baseline plasma concentrations of all counterregulatory hormones were comparable in control and acipimox experiments. During the insulin-induced hypoglycemia, plasma glucagon, epinephrine, and norepinephrine responses were not significantly different in both experiments. After an initial delay, plasma growth hormone responses were greater in the acipimox experiments (21.5±2.3 vs. 4.3±1.1 ng/ml at 480 min, $P < 0.001$). Although initially plasma cortisol responses were

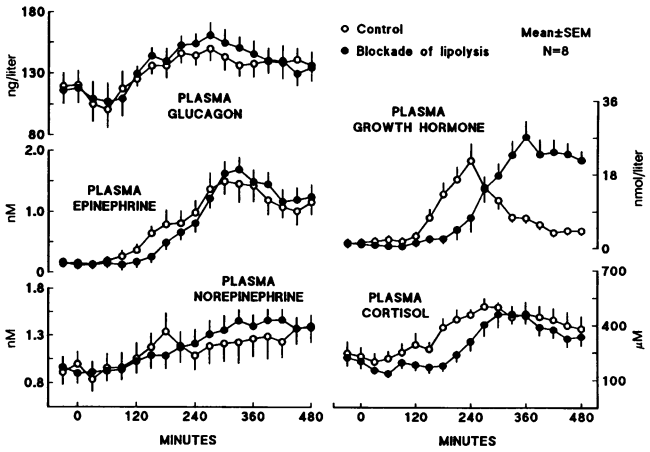


*Figure 2.* Plasma glucagon, epinephrine, norepinephrine, growth hormone, and cortisol concentrations.

also delayed in the acipimox experiments, ultimately responses were comparable to those in the control experiments.

*Plasma FFA, glycerol, and β-OH-butyrate concentrations and rates of lipid oxidation (Fig. 3).* Baseline measurements were comparable in control and acipimox experiments. In control experiments, plasma FFA, glycerol, and β-OH-butyrate were initially suppressed for 120–240 min after starting the insulin infusion but subsequently increased markedly above baseline values. Lipid oxidation followed a similar pattern. In the acipimox experiments, plasma FFA, glycerol, and β-OH-butyrate as well as rates of lipid oxidation remained suppressed below basal values throughout the study (all $P < 0.001$ vs. control after 240 min).

*Hepatic glucose output and gluconeogenesis from alanine (Figs. 4 and 5).* Baseline hepatic glucose output was comparable in both experiments. In control experiments, it decreased transiently after starting the insulin infusion and then increased to a peak rate of 12.1±1.1 μmol/kg per min at 210 min. In the acipimox experiments, hepatic glucose output was similar to that in control experiments through 240 min but afterwards was consistently less. Thus during the last 240 min hepatic glucose output was only 5.5±1.1 μmol/kg per min in acipimox experiments compared to 9.3±0.7 μmol/kg per min in control experiments, $P < 0.01$.

Baseline rates of gluconeogenesis from alanine were comparable in both experiments. In control experiments, it increased progressively after 240 min, reaching values more than threefold above baseline. In the acipimox experiments, gluconeogenesis did not increase at all, and during the last 240 min, averaged only 0.32±0.09 μmol/kg per min compared to 1.00±0.18 μmol/kg per min in control experiments, $P < 0.001$. As shown in Fig. 5, rates of gluconeogenesis during the last 240 min of both experiments were positively correlated with rates of lipid oxidation ($r = 0.91$, $P < 0.001$).

*Plasma alanine concentration, appearance, and disappearance (Fig. 6).* Baseline plasma alanine concentrations were comparable in both experiments. In the control experiments during infusion of insulin, plasma alanine gradually decreased by ~ 130 μmol/liter over the course of the study. In the acipimox experiments, plasma alanine decreased significantly less (by 90 μmol/liter), so that at the end of the study it averaged 250±36 vs. 190±22 μM in control experiments, $P < 0.01$.

Baseline rates of plasma alanine appearance and disappearance were comparable in both experiments. In control experiments, after a transient decrease after starting the insulin infusion, plasma alanine appearance returned to basal rates after 120 min. In the acipimox experiments, plasma alanine appearance remained suppressed throughout so that during the last hour of the experiment, it averaged 5.0±1.2 vs. 6.8±1.1 μmol/kg per min in control experiments, $P < 0.05$. In control experiments, the proportion of alanine appearing in plasma that was converted to plasma glucose increased from 13±3% at baseline to 32±2% by the end of the experiment ($P < 0.001$), whereas in the acipimox experiments, it did not increase (12±3% at baseline vs. 10±2% at the end of the experiment).

Rates of plasma alanine disappearance in control experiments transiently decreased after start of the insulin infusion and then rose to slightly above basal values (7.8±1.1 μmol/kg per min at 480 min vs. 6.7±1.3 μmol/kg per min at baseline, $P < 0.05$). In contrast in the acipimox experiments, after a transient decrease and return to rates comparable to those in control experiments, plasma alanine disappearance progressively decreased, so that at the end of the experiment, it averaged only 4.7±1.2 μmol/kg per min, $P < 0.01$ vs. the control experiment.

*Glucose utilization and oxidation (Figs. 4 and 7).* Rates of glucose utilization and oxidation were comparable in both experiments at baseline and for the initial 150–180 min. Subsequently both glucose utilization and glucose oxidation were consistently greater in the acipimox experiments. During the last 240 min of the study, glucose utilization and oxidation averaged 10.8±1.4 and 9.5±1.3 μmol/kg per min, respectively, vs. 9.3±0.7 and 4.8±1.0 μmol/kg per min in control experiments, both $P < 0.01$. As shown in Fig. 7, rates of glucose utilization and oxidation during the last 240 min of both exper-

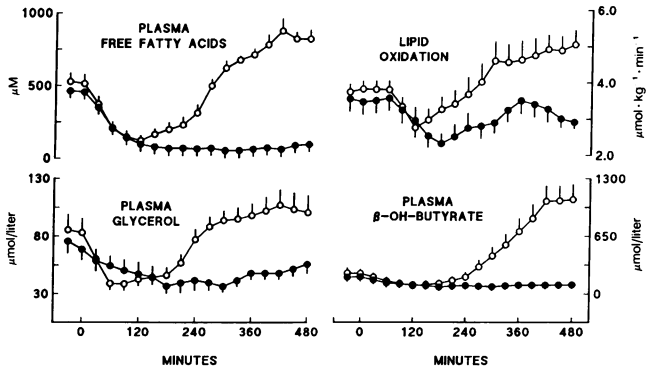


*Figure 3.* Plasma FFA, glycerol and β-OH-butyrate concentrations and rates of lipid oxidation. ○, Control; ●, blockade of lipolysis. Data are mean±SEM. $n = 8$.

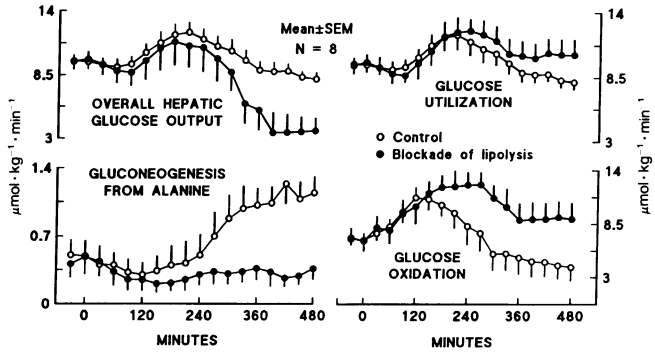


*Figure 4.* Rates of overall hepatic glucose output, gluconeogenesis from alanine, glucose utilization, and glucose oxidation.

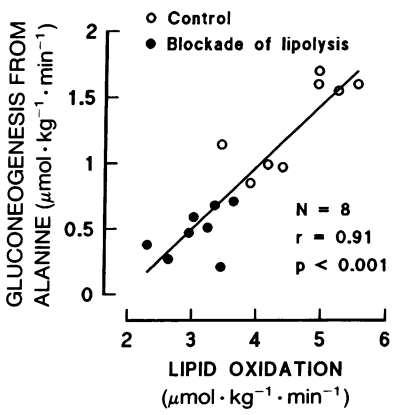


*Figure 5.* Correlation between rates of lipid oxidation and gluconeogenesis from alanine.

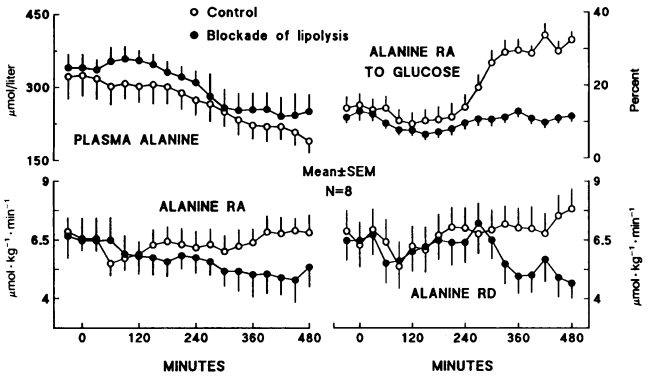


*Figure 6.* Plasma alanine concentrations, rates of alanine appearance and disappearance, and fractional conversion of alanine to glucose.

iments were inversely correlated with rates of lipid oxidation ($r = -0.71$, $P < 0.001$ and $r = -0.80$, $P < 0.001$).

## Discussion

The present studies were undertaken to test the hypothesis that alterations in FFA mediate changes in glucose production and utilization that occur during counterregulation of hypoglycemia. For this purpose, we assessed the effect of acipimox, an inhibitor of lipolysis, on overall hepatic glucose output, gluconeogenesis from alanine, glucose utilization, and glucose oxidation during counterregulation of insulin-induced hypoglycemia in normal volunteers.

Administration of acipimox suppressed lipolysis (as reflected by plasma glycerol and FFA levels) throughout the experiments and markedly reduced lipid oxidation. During the last 4 h of the experiments, overall hepatic glucose output was reduced by 40% (5.5±1.1 vs. 9.3±0.7 μmol/kg per min), gluconeogenesis from alanine was reduced by nearly 70% (0.32±0.09 vs. 1.00±0.18 μmol/kg per min), glucose utilization was increased by ~ 15% (10.8±1.4 vs. 9.3±0.7 μmol/kg per min), and glucose oxidation was doubled (9.5±1.3 vs. 4.8±1.0 μmol/kg per min). These results thus indicate that alterations in FFA availability markedly influence glucose metabolism during counterregulation and that under these conditions FFA exert a much more profound effect on hepatic glucose production than on glucose utilization. Moreover, to the best of our knowledge, these results provide the first clear cut evidence for a dependency of an increase in gluconeogenesis on an increase in FFA availability in vivo.

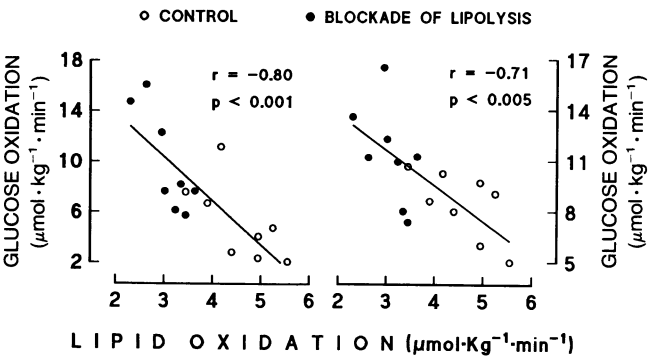


*Figure 7.* Correlations between rates of lipid oxidation and glucose oxidation and utilization.

It has been proposed that most of the compensatory increase in hepatic glucose output during prolonged counterregulation is due to gluconeogenesis (38, 39). Our results support this view. Alanine usually accounts for ~ 15% of overall gluconeogenesis (40). The reduction in conversion of alanine to glucose (0.7 μmol/kg per min glucose equivalents) observed in the present study during inhibition of lipolysis suggests that suppression of gluconeogenesis accounted for substantially all of the associated reduction in hepatic glucose output (3.8 μmol/kg per min).

As expected (11) (see below), the mechanism for the suppression of gluconeogenesis during inhibition of lipolysis by acipimox appeared to be intrahepatic since the fractional conversion of alanine to glucose by the liver was reduced despite the fact that alanine availability, as reflected by plasma alanine concentrations, was increased. This increase in plasma alanine occurred because its rate of disappearance from plasma decreased more than its rate of appearance decreased. The latter might reflect the fact that more glucose was being oxidized during the inhibition of lipolysis, and, therefore, less glucose was available for conversion to alanine. The decrease in alanine disappearance from plasma might simply reflect less alanine being used for gluconeogenesis.

Presumably the effects of acipimox on gluconeogenesis and glucose utilization were mediated by suppression of lipid oxidation secondary to inhibition of lipolysis. This is supported by the correlations that we found between rates of lipid oxidation and rates of both gluconeogenesis ($r = 0.91$) and glucose utilization ($r = -0.71$) and by the temporal relationship between changes in lipid oxidation and those of gluconeogenesis and glucose utilization. Inhibition of lipid oxidation within the liver could suppress gluconeogenesis by several mechanisms (11): (*a*) limitation of NADH needed for the glyceraldehyde 3-phosphate dehydrogenase reaction; (*b*) reduction of ATP needed to support gluconeogenesis; and (*c*) suppression of acetyl–coenzyme A and other thio-esters needed to activate pyruvate carboxylase. Some other action of acipimox on hepatic glucose output not involving FFA oxidation appears unlikely since Saloranta et al. (19) were able to completely reverse the effects of acipimox on hepatic glucose production by restoration of FFA availability. Moreover, nicotinic acid, of which acipimox is a derivative, has no direct effect on gluconeogenesis by the perfused rat liver (13). Regarding glucose utilization, inhibition of lipid oxidation could increase glucose uptake and oxidation by a complex set of mechanisms recently reviewed by Randle et al. (2). The observation by Piatti et al. (5) that acipimox did not further increase forearm glucose uptake when plasma FFA were already markedly suppressed by insulin argues against an effect of acipimox on glucose utilization not involving changes in FFA availability.

Although most in vitro studies have found that FFA increase gluconeogenesis (6–13), a consistent effect on overall hepatic glucose output in vivo has not been demonstrated (14–26). In addition to the present experiments, only one other study (22) has directly examined the effect of FFA on gluconeogenesis and overall hepatic glucose output in humans. In that study, Clore et al. (22) found that increasing FFA availability increased gluconeogenesis from alanine consistent with our

finding that a reduction in FFA availability reduced gluconeogenesis from alanine. However, Clore et al. (22) found no change in overall hepatic glucose output whereas in our study overall hepatic glucose output was markedly reduced.

An explanation for this difference may relate to two factors. First of all, there appears to be some autoregulatory mechanism, probably involving inhibition of glycogenolysis, by which an increase in gluconeogenesis does not necessarily increase overall hepatic glucose output (41, 42). This may explain why Clore et al. (22) observed no increase in overall hepatic glucose output. Secondly, suppression of gluconeogenesis in our experiments probably decreased overall hepatic glucose output because under our experimental conditions gluconeogenesis was responsible for most of hepatic glucose output (38, 39). Moreover, a compensatory increase in glycogenolysis would have been minimal because of the ongoing insulin infusion and because of previous glycogen depletion caused by glycogenolysis earlier during counterregulation and nearly 24 h of fasting. Support for this explanation is provided by studies (42, 43) in which inhibition of gluconeogenesis by ethanol in overnight fasted volunteers who would have had glycogen reserves did not affect hepatic glucose output, whereas it did reduce hepatic glucose output in 69-h-fasted volunteers whose glycogen would have been depleted (44).

In summary, the present studies demonstrate that inhibition of lipolysis by acipimox during counterregulation of insulin-induced hypoglycemia reduced overall hepatic glucose output by 40% and gluconeogenesis from alanine by 70% while increasing glucose utilization by 15%. These results indicate that FFA play a role in mediating changes in glucose metabolism that occur during counterregulation and that, under these conditions, FFA exert a much more profound effect on hepatic glucose production than on glucose utilization. Finally, our results suggest that increased FFA availability/oxidation may be a prerequisite for increased gluconeogenesis.

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## References

1. Randle, P. J., P. B. Garland, C. N. Hales, and E. A. Newsholme. 1963. The glucose-fatty acids cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* 1:785–789.

2. Randle, P. J., A. L. Kerbey, and J. Espinal. 1988. Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones. *Diabetes Metab. Rev.* 4:623–638.

3. Thiebaud, D., D. A. DeFronzo, E. Jacot, A. Golay, K. Acheson, E. Maeder, E. Jequier, and J. P. Felber. 1985. Effect of long-chain triglyceride infusion on glucose metabolism in man. *Metabolism.* 31:1128–1136.

4. Walker, M., G. R. Fulcher, C. F. Sum, H. Orskov, and K. G. M. M. Alberti. 1991. Effect of glycemia and nonesterified fatty acids on forearm glucose uptake in normal humans. *Am. J. Physiol.* 261:E304–E311.

5. Piatti, P. M., L. D. Monti, M. Pacchioni, A. E. Pontiroli, and G. Pozza. 1991. Forearm insulin- and noninsulin-mediated glucose uptake and muscle metabolism in man: role of free fatty acids and blood glucose levels. *Metabolism.* 40:926–933.

6. Struck, E., J. Ashmore, and O. Wieland. 1966. Effects of glucagon and long chain fatty acids on glucose production by isolated perfused rat liver. *Adv. Enzy. Reg.* 4:219–224.

7. Herrera, M. G., D. Kamm, N. Ruderman, and G. F. Cahill. 1966. Non-hormonal factors in the control of gluconeogenesis. *Adv. Enzyme. Regul.* 4:225–235.

8. Friedman, B., E. H. Goodman, Jr., and S. Weinhouse. 1967. Effects of insulin and fatty acids on gluconeogenesis in the rat. *J. Biol. Chem.* 242:3620–3627.

9. Williamson, J. R., R. A. Kreisberg, and P. W. Felts. 1966. Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc. Natl. Acad. Sci. USA.* 56:247–254.

10. Frohlich, J., and O. Wieland. 1971. Glucagon and the permissive action of fatty acids in hepatic gluconeogenesis. *Eur. J. Biochem.* 19:557–562.

11. Blumenthal, S. A. 1983. Stimulation of gluconeogenesis by palmitic acid in rat hepatocytes: evidence that this effect can dissociated from the provision of reducing equivalents. *Metabolism.* 32:971–976.

12. Hems, R., B. D. Ross, and M. N. Berry. 1966. Gluconeogenesis in the perfused rat liver. *Biochem. J.* 101:284–292.

13. Exton, J. H., S. B. Lewis, and C. R. Park. 1971. Examination of in vitro effects of nicotinic acid on basal and hormone-stimulated glycogenolysis, gluconeogenesis, ureogenesis and ketogenesis in the isolated perfused rat liver. *In* Metabolic Effects of Nicotinic Acid and Its Derivatives. K. F. Gay and L. A. Carlson, editors. Hans Huber, Toronto, Ontario, Canada. 853–859.

14. Bougneres, P. F., L. Castano, F. Rocchiccioli, H. P. Gia, B. Leluyer, and P. Ferre. 1989. Medium-chain fatty acids increase glucose production in normal and low birth weight newborns. *Am. J. Physiol.* 256:E692–E697.

15. Ratheiser, K., B. Schneeweib, W. Waldhausl, P. Fasching, A. Korn, P. Nowotny, M. Rohac, and H. P. O. Wolf. 1991. Inhibition by etomoxir of carnitine palmitoyltransferase I reduces hepatic glucose production and plasma lipids in noninsulin-dependent diabetes mellitus. *Metabolism.* 40(11):1185–1190.

16. Bevilacqua, S., R. Bonadonna, G. Buzzigoli, C. Boni, D. Ciociaro, F. Maccari, M. A. Giorico, and E. Ferrannini. 1987. Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. *Metabolism.* 36:502–506.

17. Groop, L. C., R. C. Bonadonna, M. Shank, A. S. Petrides, and R. A. DeFronzo. 1991. Role of free fatty acids and insulin in determining free fatty acid and lipid oxidation in man. *J. Clin. Invest.* 87:83–89.

18. Johnston, P., C. Hollenbeck, W. Sheu, Y-D. I. Chen, and G. M. Reaven. 1990. Acute changes in plasma non-esterified fatty acid concentration do not change hepatic glucose production in people with type 2 diabetes. *Diabetic Med.* 7:871–875.

19. Saloranta, C., A. Franssila-Kallunki, A. Ekstrand, M.-R. Taskinen, and L. Groop. 1991. Modulation of hepatic glucose production by non-esterified fatty acids in type 2 (noninsulin-dependent) diabetes mellitus. *Diabetologia.* 34:409–415.

20. Clore, J. N., P. S. Glickman, S. T. Helm, J. E. Nestler, and W. G. Blackard. 1991. Evidence for dual control mechanism regulating hepatic glucose output in nondiabetic man. *Diabetes.* 40:1033–1040.

21. Tutwiler, G. F., J. Joseph, and N. Wallace. 1985. Effect of methyl palmoxirate, an oral hypoglycemic agent, on epinephrine-induced hyperglycemia in the rat. *Biochem. Pharmacol.* 34:2217–2220.

22. Clore, J. N., P. S. Glickman, J. E. Nestler, and W. G. Blackard. 1991. In vivo evidence for hepatic autoregulation during FFA-stimulated gluconeogenesis in normal humans. *Am. J. Physiol.* 261:E425–E429.

23. Fanelli, C., P. De Feo, F. Porcellati, G. Perriello, E. Torlone, F. Santeusanio, P. Brunetti, and G. B. Bolli. 1992. Adrenergic mechanisms contribute to the late phase of hypoglycemic glucose counterregulation in humans by stimulating lipolysis. *J. Clin. Invest.* 89:2005–2013.

24. Vaag, H., P. Skott, P. Damsho, M.-A. Gall, E. A. Richter, and H. Beck-Nielsen. 1991. Effect of the antilipolytic nicotinic acid analogue acipimox on whole body acid skeletal muscle glucose metabolism in patients with noninsulin-dependent diabetes mellitus. *J. Clin. Invest.* 88:1282–1290.

25. Baron, A. D., G. Brechtel, and S. V. Edelman. 1989. Effects of free fatty acids and ketone bodies on in vivo noninsulin-mediated glucose utilization and production in humans. *Metabolism.* 38:1056–1061.

26. Caprio, S., R. A. Gelfand, W. V. Tamborlane, and R. S. Sherwin. 1989. Oxidative fuel metabolism during mild hypoglycemia: critical role of free fatty acids. *Am. J. Physiol.* 256:E413–E419.

27. Fulcher, G., M. Walker, C. Catalano, L. Agius, and K. Alberti. 1992. Metabolic effects of suppression of nonesterfied fatty acid levels with acipimox in obese NIDDM subjects. *Diabetes.* 41:1400–1408.

28. Jequier, E. 1985. Direct and indirect calorimetry in man. *In* Substrate and Energy Metabolism. J. S. Garrow and D. Halliday, editors. Wiley & Sons, Ltd., London. 82–92.

29. Merilainen, P. T. 1987. Metabolic monitor. *Int. J. Clin. Monit. Comput.* 4:167–177.

30. Lowry, O., and J. Passonneau. 1972. Typical fluorometric procedures for metabolite assays. *In* A Flexible System for Enzymatic Analysis. O. Lowry and J. Passoneau, editors. Academic Press, Inc., New York. 89–92.

31. Kreisberg, R. A., A. M. Siegal, and W. C. Owen. 1972. Alanine and gluconeogenesis in man: effect of ethanol. *J. Clin. Endocrinol.* 34:876–883.

32. Miles, J., R. O. Glassock, J. Aikens, J. Gerich, and M. Haymond. 1983. A microfluorimetric method for the determination of free fatty acids on plasma. *J. Lipid Res.* 24:96–99.

33. Hawk, P. B. 1947. Kjeldhal method. *In* Practical Physiological Chemistry. P. Hawk, B. Oser, and W. Summerson, editors. Blakiston, Philadelphia. 814–822.

34. Ferrannini, E. 1988. The theoretical basis of indirect calorimetry: a review. *Metabolism.* 37:287–301.

35. Tappy, L., O. E. Owen, and G. Boden. 1988. Effect of hyperinsulinemia on urea pool size and substrate oxidation rates. *Diabetes.* 37:1212–1216.

36. DeBodo, R., R. Steele, N. Altzuler, A. Dunn, and J. Bishop. 1963. On the hormonal regulation of carbohydrate metabolism: studies with 14C glucose. *Recent Prog. Horm. Res.* 19:445–448.

37. Chiasson, J. L., J. E. Lilienquist, W. W. Lacy, A. S. Jennings, and A. D. Cherrington. 1977. Gluconeogenesis: methodological approaches in vivo. *Fed. Proc.* 36:229–235.

38. Lecavalier, L., G. Bolli, P. Cryer, and J. Gerich. 1989. Contributions of gluconeogenesis and glycogenolysis during glucose counterregulation in normal humans. *Am. J. Physiol.* 256:E844–E851.

39. Frizzell, R., G. Hendrick, D. Biggers, D. Lacy, D. Donahue, D. Green, R. Carr, P. Williams, R. Stevenson, and A. Cherrington. 1988. Role of gluconeogenesis in sustaining glucose production during hypoglycemia caused by continuous insulin infusion in conscious dog. *Diabetes.* 37:749–759.

40. Gerich, J., M. Haymond, R. Rizza, C. Verdonk, and J. Miles. 1981. Hormonal and substrate determinants of hepatic glucose production in man. *In* The Regulation of Carbohydrate Formation and Utilization in Mammals. C. Veneziale, editor. University Park Press, Baltimore. 419–457.

41. Jenssen, T., N. Nurjhan, A. Consoli, and J. Gerich. 1990. Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. Demonstration of hepatic autoregulation without a change in plasma glucose concentrations. *J. Clin. Invest.* 86:489–497.

42. Jahoor, F., E. Peters, and R. Wolfe. 1990. The relationship between gluconeogenic substrate and glucose production in humans. *Am. J. Physiol.* 258:E288–E296.

43. Puhakainen, I., V. A. Koivisto, and H. Yki-Jarvinen. 1991. No reduction in total hepatic glucose output by inhibition of gluconeogenesis with ethanol in NIDDM patients. *Diabetes.* 40:1319–1327.

44. Wolfe, B. M., J. R. Havel, E. B. Marliss, J. P. Kane, J. Seymour, and S. P. Ahuja. 1976. Effects of a 3-day fast and of ethanol on splanchnic metabolism of FFA, amino acids, and carbohydrate in healthy young men. *J. Clin. Invest.* 57:329–340.