

Mediation of the Hepatic Effects of Growth Hormone by Its Lipolytic Activity

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ABSTRACT

The aim of the study was to investigate the acute effect of GH *per se*, independent from its lipolytic activity, on glucose and lipid oxidation and glucose turnover in seven healthy subjects. Five tests lasting 360 min were performed. Each test consisted of a 4-h equilibration period followed by a euglycemic hyperinsulinemic (25 mU/kg·h) clamp lasting 2 h. In test 1 (control experiment) saline was infused, leaving GH and FFA at basal levels. In tests 2, 3, and 4, GH was infused (80 ng/kg·min) to increase GH levels. Whereas in test 2 FFA levels were free to increase due to GH lipolytic activity, in test 3 FFA elevation was prevented by using an antilipolytic compound (Acipimox) that allowed evaluation of the effect of GH at low FFA levels. In test 4 (GH+Acipimox+heparin) GH infusion was associated with the administration of Acipimox and heparin to maintain FFA at the basal level to evaluate the effect of GH *per se* independent from

GH lipolytic activity. In test 5 Acipimox and a variable heparin infusion were given to evaluate possible effects of Acipimox other than the inhibition of lipolysis.

During the euglycemic hyperinsulinemic clamp in the presence of high GH and FFA levels (test 2), glucose oxidation was significantly lower and lipid oxidation was significantly higher than in tests 1, 3, 4, and 5. During the same period, hepatic glucose production was completely suppressed in the control study (test 1; 94%) and in test 5 (99.6%), whereas it was significantly less inhibited (65%, 74%, and 73%) when GH was administered in tests 2, 3, and 4.

In conclusion, these results suggest that GH directly mediates the reduction of insulin's effect on the liver. In addition, the effect of GH on glucose and lipid oxidation is not direct, but is mediated by its lipolytic activity. (*J Clin Endocrinol Metab* 84: 1658–1663, 1999)

MANY STUDIES have shown that GH can induce insulin resistance (1–3), although the mechanism by which an acute increase in its levels can decrease glucose uptake is still debated. *In vitro* studies have shown that GH decreases glucose uptake without affecting glucose oxidation in human erythrocytes (4, 5), fat tissue (6), or diaphragm cultures (7). *In vivo*, hepatic glucose seems to reduce forearm glucose uptake, glucose oxidation, and glycogen synthase activity and to impair insulin suppression of hepatic glucose production (8–10) in favor of increased lipid oxidation. Recently, Neely *et al.* (11) have shown that after an overnight GH infusion in normal subjects, both GH and free fatty acid (FFA) levels were positively correlated with the increase in peripheral and hepatic insulin resistance. In addition, in most of the previous studies, high levels of GH were invariably associated with high circulating levels of glycerol, FFA, and β -hydroxybutyrate (8–10, 12, 13). Therefore, it remains unclear whether the effect of GH on glucose metabolism is direct (10) or mediated by GH-induced lipolytic action through the well known glucose-fatty acid cycle or Randle cycle, as hypothesized by Davidson *et al.* (14). Indeed, an increase in FFA levels can compete with glucose utilization

on the oxidative pathway in muscle and induce insulin resistance (15–18).

The purpose of this study was to investigate the acute effect of a systemic elevation of GH levels independent from its lipolytic action on glucose and lipid oxidation and hepatic glucose production (HGP) in normal man. Our results indicate that GH directly decreases the insulin suppressibility of HGP. By contrast, GH influences glucose and lipid oxidation only when its ability to stimulate lipolysis is maintained.

Subjects and Methods

The experimental protocol was approved by the local ethical committee, and informed consent was obtained from each volunteer. Seven healthy male subjects (mean age, 25 \pm 3 yr; weight, 72.4 \pm 4 kg; fat-free mass, 55.9 \pm 2.8 kg) underwent three different tests (lasting 360 min) in random order, with at least a 15-day interval between two consecutive tests. In each test a 4-h equilibration period (0–240 min) was followed by a euglycemic hyperinsulinemic (25 mU/kg·h) clamp lasting 2 h (240–360 min), with blood glucose maintained at basal values by means of a variable glucose (20%) infusion (19).

Test 1 was the control experiment. During this test saline was infused, leaving GH and FFA at their basal levels.

Test 2 was designed to study the effect of increased GH and FFA levels. GH was infused (80 ng/kg·min) to achieve circulating GH levels similar to those seen in major surgery (20, 21) and severe trauma (22). A concomitant elevation of FFA levels was induced by the lipolytic effect of GH.

Test 3 allowed evaluation of the effect of GH in the presence of low FFA levels. GH was infused as in test 1, whereas lipolysis was inhibited using Acipimox (250 mg at 0 and 120 min). Acipimox is a nicotinic acid analog that blocks the spontaneous as well as noradrenaline-, theophylline-, and GH-induced lipolysis from the adipose tissue.

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In the same subjects two additional tests (tests 4 and 5) were carried out. Test 4 was performed to evaluate the effect of GH *per se* independent from the effect of FFA. GH was infused as in test 2, and FFA were kept at the basal levels observed in test 1 despite the administration of Acipimox (250 mg at 0 and 120 min) by giving a variable heparin infusion.

Test 5 was performed to evaluate whether Acipimox could show metabolic effects other than inhibition of lipolysis. Also in this case, FFA were kept at the basal levels as in test 1 despite the administration of Acipimox (250 mg at 0 and 120 min) by giving a variable heparin infusion.

In tests 4 and 5 heparin infusion was modified according to the plasma measurement of FFA levels performed using COBAS FARA every 20 min. To achieve this goal, the following changes were performed in the enzymatic colorimetric method usually used for FFA assay. First, it was automated on a centrifugal analyzer (COBAS FARA II). The total time of the procedure was reduced to 8 min, allowing us to perform measurements of FFA every 20 min during both tests. The present method correlated with a standard manual procedure ($r = 0.93$; slope = 0.85; intercept = 0.14 mmol/L; $P < 0.0001$). Intra- and interassay coefficients of variation were 3.01% and 4.24%, respectively.

On the morning of each test, a 20-gauge plastic cannula (Abbot T, Abbot, Ireland Ltd., Sling, Ireland) was inserted in a dorsal vein of one hand in a retrograde position, and the hand was maintained at 55°C for intermittent sampling of arterialized blood (glucose, FFA, insulin, GH, and glucagon). A 20-gauge plastic cannula was inserted into a large antecubital vein of the same arm for the administration of different infusions. The total glucose disposal rate was evaluated isotopically by means of a prime (5 mg/kg) continuous (0.05 mg/kg·min) infusion of [6,6- $^2\text{H}_2$]glucose. During the clamp period, blood glucose was kept constant by means of a variable infusion of 20% dextrose. A fixed amount of dideuterated glucose was also added to the glucose bag (~1.4% cold glucose) to maintain isotopic enrichment constant (23). Both the rate of appearance (Ra) and the rate of disappearance (Rd) of unlabeled glucose were calculated with Steele's model (24), taking into account the non-negligible stable isotope mass as indicated previously (25). HGP was derived by subtracting the known constant glucose infusion rate from the Ra.

For 30 min immediately before and during the last 30 min of the euglycemic clamp, the rates of glucose and lipid oxidation were calculated at 1-min intervals using indirect calorimetry. Oxygen uptake and carbon dioxide production were calculated as previously described (26). Protein oxidation was calculated multiplying urinary N excretion by 6.25 (26) and was normalized by urea clearance (27). The urinary nitrogen excretion rate was calculated by collecting urine throughout the test.

Assays

Changes in glucose infusion rates were made according to measurements obtained every 5 min using a glucose analyzer (YSI, Inc., Yellow Springs, OH). All samples were assayed for insulin, GH, and glucagon in a single assay. The methods to measure hormones and isotopic enrichment of [6,6- $^2\text{H}_2$]glucose have been reported previously (28, 29).

Statistical analysis

All values are expressed as the mean \pm SE at each time interval. Multiple comparisons among tests were performed by Scheffe's F test when appropriate.

Results

Metabolic and hormone levels

Figure 1 shows the insulin, glucose, GH, and FFA profiles observed during the five studies. For the sake of clarity, we will discuss the preclamp and clamp periods separately.

Preclamp period (0–240 min). Glucose and insulin levels were similar at the baseline in the five tests (Fig. 1).

GH levels remained at basal levels during the control study (test 1). GH levels increased to a similar extent (range,

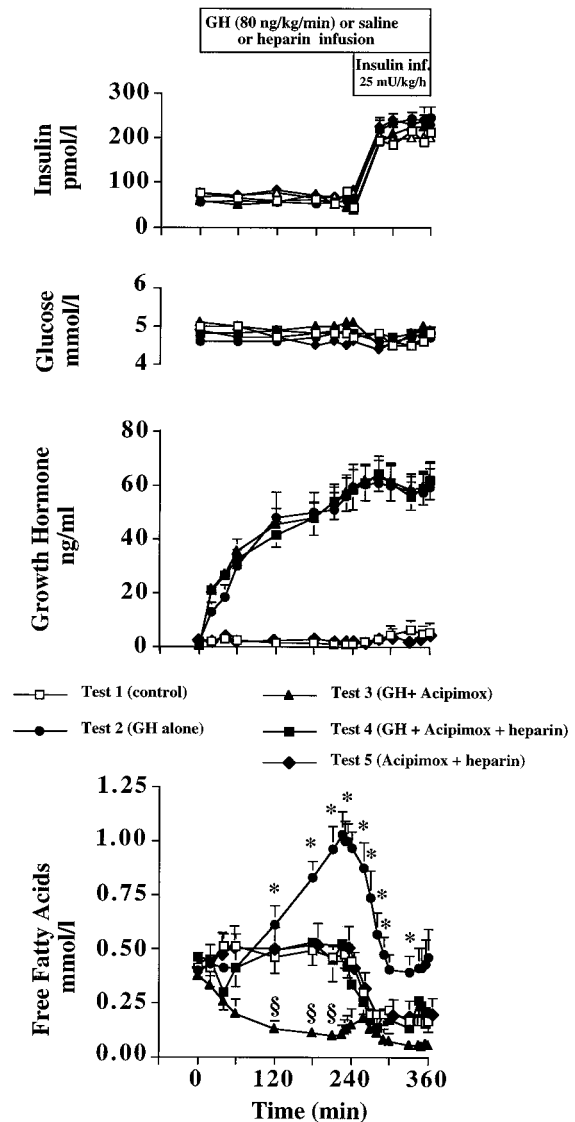


FIG. 1. Blood glucose, serum insulin, serum GH, and plasma FFA profiles (mean \pm SE) during the different tests. Test 1 was the control experiment; saline was infused, leaving GH and FFA at their basal levels. Test 2 was designed to study the effects of increased GH and FFA levels; GH was infused alone. Test 3 allowed evaluation of the effect of GH in the presence of low FFA levels; GH was infused as in test 2, and Acipimox was administered orally to inhibit lipolysis. Test 4 was carried out to evaluate the effect of GH *per se* independent from the effect of FFA; GH was infused as in test 2, and FFA was kept at the basal level by the administration of Acipimox and variable heparin infusion. Test 5 was performed to evaluate whether Acipimox could have metabolic effects other than inhibition of lipolysis; FFA were kept at basal levels by the administration of Acipimox and variable heparin infusion. *, $P < 0.05$, test 2 vs. tests 1, 3, 4, and 5; §, $P < 0.05$, test 3 vs. tests 1, 4, and 5.

55–70 ng/mL) during tests 2, 3, and 4. During the infusion of GH alone (test 2), FFA increased significantly, achieving levels significantly higher than those observed in the other four tests. In contrast, during test 3, FFA levels were suppressed ($P < 0.05$ vs. tests 1, 2, 4, and 5). During tests 4 and 5, FFA levels were successfully clamped at the basal levels observed in the control study (Fig. 1).

Insulin-like growth factor I levels before the start of the

study were similar in all tests (test 1, 195.3 ± 17.7 ; test 2, 185.8 ± 20.9 ; test 3, 230.3 ± 20.3 ; test 4, 203.5 ± 21.6 ; test 5, 210.8 ± 12.8 ng/mL). Glucagon levels remained unchanged in all tests (Table 1).

Clamp period (240–360 min). Glucose levels were successfully clamped in all tests, with a coefficient of variation of less than 5% (Fig. 1). Insulin levels reached a plateau between 210–260 pmol/L (Fig. 1). GH levels remained at the plateau values achieved before the clamp period in all tests (Fig. 1). FFA decreased in all tests, remaining higher in test 2 with respect to the other four tests. No significant differences were found for FFA among tests 1, 3, 4, and 5. Insulin-like growth factor I levels remained unchanged during the study in all tests (test 1, 202.1 ± 17.9 ; test 2, 189.9 ± 15.1 ; test 3, 215.1 ± 22.6 ; test 4, 193.8 ± 17.3 ; test 5, 200.7 ± 15.9 ng/mL).

No significant differences were found in glucagon levels at the end of the study in all tests (Table 1).

Glucose metabolism and indirect calorimetry results

In Table 2 are reported the glucose (atom percent excess; APE) enrichment during the last 30 min of the preclamp and clamp periods.

Table 3 reports the glucose turnover results, *i.e.* Rd and HGP. Figure 2 shows the glucose and lipid oxidation results. Again, for the sake of clarity we will discuss the preclamp and clamp periods separately.

Preclamp period (0–240 min). Rd and HGP were not significantly different among the tests, although Rd and HGP tended to be higher during tests 2, 3, and 4 (*i.e.* when GH was infused) than during tests 1 and 5. When pooling the data from the five studies, a positive significant correlation between GH levels and HGP was found ($r = 0.49$; $P < 0.01$; data not shown). Glucose oxidation was significantly lower during test 2 than during the other four tests (Fig. 2). Lipid oxidation was significantly higher during test 2 than during tests 3 and 4 ($P < 0.05$; Fig. 2). In addition, lipid oxidation was significantly lower during test 3 than during tests 1 and 5 ($P < 0.05$). The protein oxidation rate was similar during all tests (data not shown).

Clamp period (240–360 min). At the end of the clamp period, the glucose infusion rate (M value) was significantly lower during test 2 than during the other four tests (Table 3). Rd was not significantly different among the tests, whereas HGP was significantly lower in tests 1 and 5 than in the other tests. If the HGP results are expressed in terms of percent inhibition with respect to values during the preclamp period, HGP

inhibition was 94% in test 1 and 99.6% in test 5 compared to 65%, 74%, and 73% in tests 2, 3, and 4, respectively ($P < 0.05$, tests 1 and 5 *vs.* tests 2–4; Table 3). Once again, GH levels remained significantly correlated with HGP ($r = 0.53$; $P < 0.01$; data not shown).

Glucose oxidation increased significantly in all tests, but remained significantly lower during test 2 than during the other tests (Fig. 2). Lipid oxidation remained significantly higher during test 2 than during the other tests. Protein oxidation was similar during all tests (data not shown).

Discussion

The results of the present study demonstrate the existence of a direct effect of GH on HGP independent of a simultaneous increase in FFA levels. This result was obtained with the measurement of total body glucose metabolism using an isotope tracer methodology. The possibility of determining total body glucose disposal (Rd) and the amount of the glucose infusion rate (M value) during the clamp allowed us to correctly measure the HGP during the clamp period. During both preclamp and clamp periods, Rd was not different among tests, although it tended to be decreased during the clamp period in test 2. These data were due to the fact that during the infusion of GH alone (test 2), the M value was significantly decreased and HGP was significantly increased compared to those during the control study (test 1).

In the period before the beginning of the glucose clamp, although HGP during GH infusion (with or without Acipimox) did not significantly increase with respect to the control test with saline infusion, a significant positive correlation between GH and HGP steady state levels was found. Such an action of GH is consistent with the results of previous studies showing hepatic receptors for GH (30–32) and with the presence of a direct inhibitory effect of GH on insulin binding in the liver (30–32) but not in muscle (8). Previous studies have been shown that after receptor binding, multiple signaling events occur that are mediated by the GH, including the tyrosine phosphorylation and activation of several cellular proteins (33, 34). In cultured cells, GH determines an activation of JAK2 (Janus kinase-2) and several members of the STAT (signal transducer and activator of transcription) family of proteins, STAT1, -3, and -5 (35–37). The phosphorylated STAT proteins translocate into the nucleus, bind to DNA, and can activate the transcription of specific genes. The administration of GH *in vivo* to hypophysectomized rats induced a stimulation of STAT1, -3, and -5 in liver nuclear fraction (38–40), whereas this effect has been shown in nonhypophy-

TABLE 1. Glucagon levels (picograms per mL) at baseline and at the end of the preclamp and clamp periods

	Test 1	Test 2	Test 3	Test 4	Test 5
Baseline	125.3 ± 33.7	131.5 ± 27.6	181.9 ± 48.6	135.3 ± 13.2	138.3 ± 17.9
Preclamp period	149.8 ± 61.0	125.5 ± 23.2	141.4 ± 29.2	140.0 ± 7.8	143.0 ± 16.5
Clamp period	109.0 ± 29.3	107.9 ± 17.5	121.3 ± 33.5	134.2 ± 5.8	140.0 ± 15.1

Test 1 was the control experiment; saline was infused, leaving GH and FFA at their basal levels. Test 2 was designed to study the effects of increased GH and FFA levels; GH was infused alone. Test 3 allowed evaluation of the effect of GH in the presence of low FFA levels; GH was infused as in test 2, and Acipimox was administered orally to inhibit lipolysis. Test 4 was carried out to evaluate the effect of GH *per se* independent from the effect of FFA; GH was infused as in test 2, and FFA was kept at the basal level by the administration of Acipimox and variable heparin infusion. Test 5 was performed to evaluate whether Acipimox could have metabolic effects other than inhibition of lipolysis; FFA was kept at the basal level by the administration of Acipimox and variable heparin infusion. Values are the mean \pm SE.

TABLE 2. Glucose enrichment (APE) during the last 30 min of the preclamp and clamp periods

	Test 1	Test 2	Test 3	Test 4	Test 5
Preclamp period					
210 min	2.50 ± 0.07	2.27 ± 0.11	2.28 ± 0.06	2.16 ± 0.15	2.55 ± 0.15
220 min	2.51 ± 0.11	2.23 ± 0.10	2.26 ± 0.08	2.15 ± 0.13	2.60 ± 0.10
230 min	2.56 ± 0.14	2.22 ± 0.12	2.24 ± 0.07	2.14 ± 0.13	2.55 ± 0.15
240 min	2.52 ± 0.12	2.22 ± 0.12	2.24 ± 0.06	2.18 ± 0.14	2.58 ± 0.17
Clamp period					
330 min	2.55 ± 0.09	2.73 ± 0.14	2.51 ± 0.09	2.55 ± 0.09	2.66 ± 0.16
340 min	2.58 ± 0.10	2.73 ± 0.14	2.50 ± 0.10	2.53 ± 0.12	2.66 ± 0.13
350 min	2.62 ± 0.11	2.69 ± 0.12	2.48 ± 0.10	2.53 ± 0.12	2.69 ± 0.15
360 min	2.55 ± 0.10	2.72 ± 0.11	2.52 ± 0.10	2.54 ± 0.11	2.66 ± 0.13

Test 1 was the control experiment; saline was infused, leaving GH and FFA at their basal levels. Test 2 was designed to study the effects of increased GH and FFA levels; GH was infused alone. Test 3 allowed the evaluation of the effect of GH in the presence of low FFA levels; GH was infused as in test 2, and Acipimox was administered orally to inhibit lipolysis. Test 4 was carried out to evaluate the effect of GH *per se* independent from the effect of FFA; GH was infused as in test 2, and FFA was kept at the basal level by the administration of Acipimox and variable heparin infusion. Test 5 was performed to evaluate whether Acipimox could have metabolic effects other than inhibition of lipolysis; FFA was kept at the basal level by the administration of Acipimox and variable heparin infusion. Values are the mean ± SE.

TABLE 3. Glucose turnover results during preclamp and clamp periods

	Test 1	Test 2	Test 3	Test 4	Test 5
Preclamp period					
Rd (μmol/kg FFM/min)	14.14 ± 0.54	15.55 ± 0.52	15.39 ± 0.23	17.13 ± 1.43	12.78 ± 1.44
HGP (μmol/kg/min)	10.83 ± 0.51	12.16 ± 0.53	12.01 ± 0.28	12.79 ± 0.92	10.19 ± 0.46
Clamp period					
M value (μmol/kg FFM/min)	24.44 ± 1.26	14.96 ± 1.62 ^a	23.25 ± 2.89	19.94 ± 1.51	24.89 ± 3.24
Rd (μmol/kg FFM/min)	25.24 ± 1.52	20.46 ± 1.52	27.29 ± 2.07	24.59 ± 1.84	23.84 ± 3.25
HGP (μmol/kg/min)	0.60 ± 0.46 ^b	4.32 ± 0.57	3.11 ± 0.83	3.45 ± 0.57	0.02 ± 0.25 ^b
% Inhibition HGP	94.8 ± 4.1 ^b	65.1 ± 3.9	73.7 ± 7.2	73.3 ± 3.3	99.6 ± 2.4 ^b

Test 1 was the control experiment; saline was infused, leaving GH and FFA at their basal levels. Test 2 was designed to study the effects of increased GH and FFA levels; GH was infused alone. Test 3 allowed the evaluation of the effect of GH in the presence of low FFA levels; GH was infused as in test 2, and Acipimox was administered orally to inhibit lipolysis. Test 4 was carried out to evaluate the effect of GH *per se* independent from the effect of FFA; GH was infused as in test 2, and FFA was kept at the basal level by the administration of Acipimox and variable heparin infusion. Test 5 was performed to evaluate whether Acipimox could have metabolic effects other than inhibition of lipolysis; FFA was kept at the basal level by the administration of Acipimox and variable heparin infusion. Values are the mean ± SE.

^a $P < 0.05$ vs. tests 1, 3, 4, and 5.

^b $P < 0.05$ vs. tests 2, 3, and 4.

sectomized rats for JAK2 and STAT5 (41). Recently, the measurement of tyrosine phosphorylation of insulin receptor substrate-1 in liver and muscle of normal rats treated with GH showed a 10% decreased of this expression in liver compared to no effect in muscle (41).

At the end of the euglycemic hyperinsulinemic clamp, in the presence of mild hyperinsulinemia, HGP was completely suppressed only during tests 1 and 5, when GH was not administered. When GH was infused, HGP was significantly less inhibited compared to that in the control study. This effect of GH on HGP was virtually the same when FFA were suppressed by Acipimox or maintained at the basal levels by using Acipimox and heparin. These data suggest that GH is able to acutely mediate the reduction of hepatic sensitivity to insulin independent of FFA levels. They also provide a possible interpretation of previous results in IDDM patients with GH deficiency, in whom an infusion of heparin to increase FFA levels did not increase HGP when performed during the early hours of the morning (42).

Our results indicate that the decreases in the M value and glucose oxidation after GH administration are mediated by GH-increased FFA levels rather than by a direct effect of GH *per se*. This can be appreciated by comparing the results obtained during the preclamp period of the five tests performed in the present study. In keeping with the findings of

Moller *et al.* (9), glucose oxidation was significantly decreased, and lipid oxidation was significantly enhanced during an acute infusion of GH (test 2). However, when lipolysis and lipid oxidation were completely blocked by Acipimox (test 3) or were maintained at the basal levels by the administration of Acipimox and heparin (test 4), the effect of GH on glucose oxidation were similar to that found during saline infusion (test 1). Of note is that a similar pattern of results was found at the end of the euglycemic clamp. These findings suggest that the effect of GH on glucose and lipid oxidation is not direct, but is mediated by its lipolytic activity. This conclusion is supported by previous *in vitro* studies showing that 4- to 6-h exposure to GH did not affect either glucose oxidation by adipose tissue from hypoxic rats (43) or glucose conversion to glycogen in soleus muscle in rats (44).

As in the present study Acipimox was used to inhibit lipolysis, it could be argued that the effect on glucose metabolism seen in this study not only was due to an acute decrease in FFA levels but, to some extent, was also related to the drug. Fulcher *et al.* (45) administered 1000 mg Acipimox (twice the amount used in the present study) and simultaneously clamped FFA levels (at 0.4 mmol/L) by intralipid infusion. They found that glucose oxidation during an euglycemic hyperinsulinemic clamp was unaffected by the drug, and the increase in glucose disposal was only due

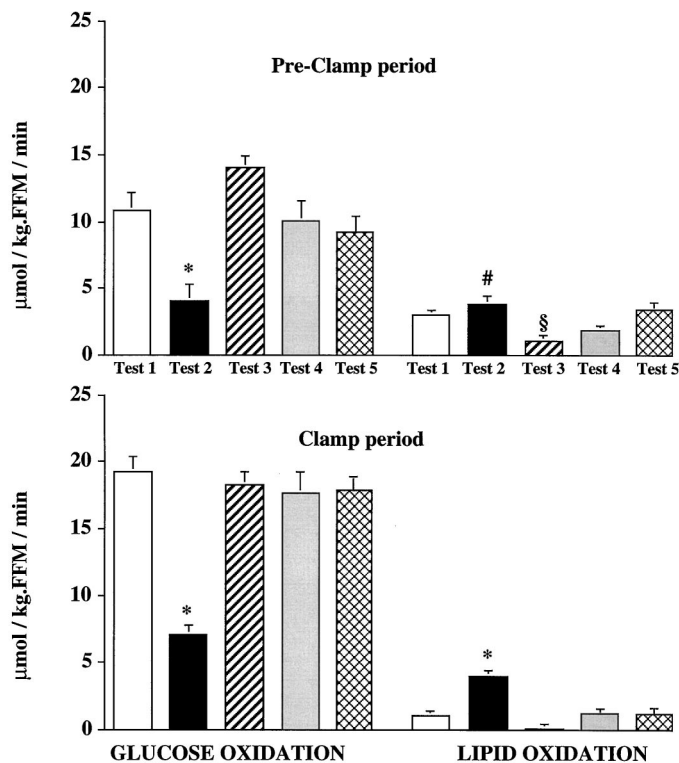


FIG. 2. Glucose and lipid oxidation (mean \pm SE) during preclamp (upper part) and clamp (lower part) periods. Test 1 was the control experiment; saline was infused, leaving GH and FFA at their basal levels. Test 2 was designed to study the effects of increased GH and FFA levels; GH was infused alone. Test 3 allowed evaluation of the effect of GH in the presence of low FFA levels; GH was infused as in test 2, and Acipimox was administered orally to inhibit lipolysis. Test 4 was carried out to evaluate the effect of GH *per se* independent from the effect of FFA; GH was infused as in test 2, and FFA were kept at basal levels by the administration of Acipimox and variable heparin infusion. Test 5 was performed to evaluate whether Acipimox could have metabolic effects other than inhibition of lipolysis; FFA were kept at the basal level by the administration of Acipimox and variable heparin infusion. #, $P < 0.05$, test 2 vs. tests 3 and 4; *, $P < 0.05$, test 2 vs. tests 1, 3, 4, and 5; §, $P < 0.05$, test 3 vs. tests 1 and 5.

to the enhancement of nonoxidative glucose disposal. The results of our study are in agreement with these findings, as glucose oxidation was similar when Acipimox was administered (tests 3–5) and during saline infusion (test 1). Thus, it is most likely that in our study Acipimox did not appreciably influence glucose metabolism. These conclusions are also supported by previous studies in which Acipimox was unable to enhance either the insulin-mediated forearm glucose uptake during euglycemic hyperinsulinemic clamp in healthy subjects (46) or the suppression of HGP by insulin in noninsulin-dependent diabetic subjects when it was administered in association with heparin to maintain high FFA levels (47).

As chronic therapy with Acipimox seems to increase glucagon levels (48), acute changes in this hormone after administration of the drug could explain some of the differences in HGP among tests. However, in the present study the measurement of glucagon levels at the end of the preclamp and clamp periods did not show any statistical differences

among tests. This suggests that Acipimox does not acutely influence glucagon levels in these particular conditions.

In conclusion, our results indicate that GH directly mediates the reduction of insulin's effect on the liver. In addition, under acute GH administration, GH influences glucose and lipid oxidation only when its ability to stimulate lipolysis is maintained.

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