Acute effect of growth hormone to induce peripheral insulin resistance is independent of FFA and insulin levels in rats

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Kim, Jason K., Cheol S. Choi, and Jang H. Youn. Acute effect of growth hormone to induce peripheral insulin resistance is independent of FFA and insulin levels in rats. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E742–E749, 1999.—To examine whether growth hormone (GH) induces peripheral insulin resistance by altering plasma free fatty acid (FFA) or insulin levels, the effects of GH infusion on insulin-stimulated glucose fluxes were studied in conscious rats under two protocols. In study 1, either saline (n = 7) or human recombinant GH (21 µg·kg⁻¹·h⁻¹; n = 8) was infused for 300 min, and insulin-stimulated glucose fluxes were estimated during the final 150-min period of hyperinsulinemic euglycemic clamps. In study 2, hyperinsulinemic euglycemic clamps were first conducted for 150 min (to raise plasma insulin and suppress FFA levels), and saline or GH (n = 7 for each) was subsequently infused for the following 300-min clamp period. In study 1, GH infusion in the basal state did not significantly alter plasma FFA or insulin levels. In contrast, GH infusion decreased insulin-stimulated glucose uptake, glycolysis, and glycogen synthesis by 32, 27, and 40%, respectively (P < 0.05). In study 2, GH infusion during hyperinsulinemic euglycemic clamps did not alter plasma FFA or insulin levels (P > 0.05). GH infusion had no effect on insulin-stimulated glucose uptake during the initial 150 min but eventually decreased insulin-stimulated glucose uptake by 37% (P < 0.05), similar to the results in study 1. These data indicate that GH induces peripheral insulin resistance independent of plasma FFA and insulin levels. The induction of insulin resistance was preceded by suppression of glycogen synthesis, consistent with the hypothesis that metabolic impairment precedes and causes development of peripheral insulin resistance.

glycolysis; glycogen synthesis; skeletal muscle; glucose 6-phosphate; growth hormone; free fatty acid

SINCE THE INITIAL FINDINGS of glucose intolerance and insulin resistance in acromegalic patients (4, 11, 12, 30), the effects of growth hormone (GH) on insulin action have been extensively studied. Although it is now well recognized that acute or chronic elevation of plasma GH levels causes insulin resistance (6, 13, 20, 26), the underlying mechanisms remain controversial.

Because GH promotes lipolysis in adipose tissues (8, 21) and chronic elevation of GH levels is associated with increased plasma free fatty acid (FFA) levels (8, 13, 29), increased lipid availability has been suggested to be responsible for insulin resistance with elevated GH levels (13, 29). However, previous studies have also shown that GH infusion decreased peripheral insulin action without or preceding changes in plasma lipid levels, suggesting that acute GH induction of peripheral insulin resistance is independent of its lipolytic effects (6, 20). Despite these findings, however, there is still a possibility that GH stimulates lipolysis in insulin-sensitive cells and induces insulin resistance by increasing intracellular FFA levels (in the absence of changes in plasma levels). On the other hand, some studies (1, 13, 24) have demonstrated that GH increases circulating insulin levels. Because sustained hyperinsulinemia decreases insulin sensitivity in normal subjects (9, 18, 25), hyperinsulinemia also has been suggested to be responsible for GH-induced insulin resistance (13, 26).

One goal of the present study was to examine whether acute GH induction of insulin resistance is influenced by altering circulating FFA or insulin levels. To achieve this goal, GH was infused for 5 h in basal and in hyperinsulinemic states, which represent two conditions of vastly different plasma FFA and insulin levels. If the effects of GH infusion on insulin action are similar under these experimental conditions, such data would indicate that lipid and insulin levels do not play a major role in GH-induced insulin resistance. On the other hand, if the GH effects are significantly different under the two conditions, such data would suggest the importance of insulin and/or FFA levels in GH-induced insulin resistance.

We recently put forth the hypothesis that during the development of insulin resistance in skeletal muscle, impairment of intracellular glucose metabolism precedes and causes development of insulin resistance (i.e., decrease in insulin’s action on glucose uptake) (15, 16). To support this hypothesis, we demonstrated that metabolic impairment (i.e., suppression of glycolysis) precedes the development of insulin resistance during high-fat feeding in rats (15). In addition, we demonstrated that suppression of intracellular glucose metabolism (glycolysis or glycogen synthesis) during hyperinsulinemic euglycemic clamps causes insulin resistance in skeletal muscle (16). In an effort to extend or generalize this hypothesis, in the present study we tested it in the development of insulin resistance with GH infusion. Thus another goal of the present study was to examine whether there are changes in glucose metabolic fluxes preceding the development of insulin resistance during GH infusion.

METHODS

Animals

Male Wistar rats weighing 250–275 g were obtained from Charles River (Wilmington, MA) and studied ≥5 days after arrival. Animals were housed under controlled temperature conditions...
(22 ± 2°C) and lighting (12:12-h light-dark cycle; 0600–1800 light, 1800–0600 dark) with free access to water and standard rat chow. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California.

Catheterization

At least 4 days before experiments, animals were placed in individual cages with wired floors. The distal one-third of each rat's tail was drawn through a hole placed low on the side of the cage and secured there with a rubber stopper. This arrangement was required to protect tail blood vessel catheters during experiments (14, 16). Animals were free to move about and allowed unrestricted access to food and water. Two tail vein infusion catheters and one tail artery blood sampling catheter were placed the day before the experiment and 2–4 h before (i.e., ~0800) the start of experiments, respectively. Catheters were placed percutaneously during local anesthesia with lidocaine while rats were restrained in a towel. Animals were returned to their cages after catheter placement, with tails secured as described above and free to move about during the experiments. Patency of the arterial catheter was maintained by a slow (0.016 ml/min) infusion of heparinized saline (10 U/ml).

Experimental Protocols

Two studies were carried out in normal rats (~280 g), starting at 1000–1200 after an overnight fast; food was removed at 1700 on the day before the experiment.

Study 1: effects of GH infusion in the basal state. Experiments began with a constant infusion of saline (n = 7) or human recombinant GH (21 µg·kg⁻¹·h⁻¹; n = 8; Genentech, South San Francisco, CA), which lasted for 300 min. The GH dose was selected because it was previously shown to acutely induce a marked peripheral insulin resistance in vivo in rats (13). During the infusions, blood samples were collected for the determination of plasma glucose, insulin, and FFA concentrations. During the final 150 min of saline or GH infusion, insulin action was assessed using a hyperinsulinemic euglycemic clamp. Porcine insulin (Novo Nordisk, Princeton, NJ) was infused at a rate of 30 pmol·kg⁻¹·min⁻¹ to raise plasma insulin within a physiological range (14, 16), and blood samples (40 µl) were collected at 10- to 20-min intervals for the immediate measurement of plasma glucose. Dextrose (20%) was infused at variable rates to maintain plasma glucose at 5.5 mM, which represents basal glucose concentrations in overnight-fasted rats. To estimate insulin-stimulated whole body glucose fluxes, [3-³H]glucose was infused (0.2 µCi/min) throughout the clamp. Blood samples (60 µl) were taken every 10 min during the last 60 min of clamps. At the end of clamps, animals were anesthetized, and soleus muscles were rapidly dissected out, frozen with liquid N2-cooled aluminum blocks, and stored at −70°C for later analysis. These muscles were used to determine glucose 6-phosphate (G-6-P) concentration.

Study 2: effects of GH infusion in an insulin-stimulated state. A 450-min hyperinsulinemic euglycemic clamp was conducted with a continuous infusion of porcine insulin (30 pmol·kg body weight⁻¹·min⁻¹), as described in study 1. After the initial 150-min clamp (control period) to attain steady-state insulin action (and also elevated insulin and decreased FFA levels), the clamp was continued with a constant infusion of saline (n = 7) or human recombinant GH (21 µg·kg⁻¹·h⁻¹; n = 7) during the remaining 300-min period (experimental period). To estimate insulin-stimulated whole body glucose fluxes, [3-³H]glucose was infused (0.2 µCi/min) throughout the clamps. Blood samples (60 µl) for measurements of plasma [³H]glucose and [³H]2O concentrations were taken every 10 or 20 min, starting at 90 min after the start of insulin infusion. Additional blood samples (60 µl) were taken at 60, 90, 150, 210, 270, 330, 390, and 450 min for the determination of plasma insulin and/or FFA concentrations. To compensate for the blood loss during the clamps due to numerous blood samplings, fresh red blood cells (1.5 ml) from littermates were washed with and suspended in saline and were infused throughout the clamps, as in our previous study (16). At the end of clamps, animals were anesthetized, and soleus muscles were collected as described in study 1.

Analysis

Plasma glucose was analyzed during the clamps with 10 µl of plasma by a glucose oxidase method on a Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Plasma insulin was measured by RIA using kits from Linco Research (St. Charles, MO); basal insulin levels (i.e., without insulin infusion) were measured using a rat insulin RIA kit, and clamp insulin levels were determined using a porcine insulin RIA kit. Plasma FFA was determined using an acyl-CoA oxidase-based colorimetric kit (Wako Pure Chemical Industries, Osaka, Japan). For the determination of plasma [³H]glucose, plasma was deproteinized with ZnSO₄ and Ba(OH)₂, dried to remove [³H]2O, resuspended in water, and counted in scintillation fluid (Ready Safe, Beckman, Fullerton, CA). The plasma concentration of [³H]2O was determined by the difference between [³H] counts without and with drying. Muscle G-6-P concentration was determined using G-6-P dehydrogenase (19).

Calculations

In study 1, steady-state rates of glucose fluxes were determined during the final 40–60 min of hyperinsulinemic clamps. Total glucose appearance and whole body glucose uptake were determined as the ratio of the [³H]glucose infusion rate (dpm/min) to the specific activity of plasma glucose (dpm/µmol) during the final 40 min of the clamps. Hepatic glucose output (HGO) was determined by subtracting the glucose infusion rate from the total glucose appearance. Whole body glycolysis was calculated from the rate of increase in plasma [³H]2O concentration, determined by linear regression of the measurements during the last 60 min of clamps (32). Whole body glycogen synthesis was estimated by subtracting whole body glycolysis from whole body glucose uptake, with the assumption that glycolysis and glycogen synthesis account for the majority of insulin-stimulated glucose uptake (27). In study 2, the rates of whole body glucose appearance and glucose uptake were calculated using the Steele’s nonsteady state equations (31). For this calculation, plasma glucose, [³H]glucose specific activity, and plasma [³H]2O data (from 90 to 450 min) were smoothed using the OOPSEG algorithm (5). HGO was calculated by subtracting glucose infusion rates from the rates of glucose appearance. Whole body glycolysis was calculated from the rate of increase in plasma [³H]2O concentration, which was calculated at each sampling time as the slope of the smoothed [³H]2O curve, divided by the specific activity of plasma [³H]glucose as previously described (32). Whole body glycogen synthesis was estimated by subtracting whole body glycolysis from whole body glucose uptake.

Statistical Analysis

Data are expressed as means ± SE. The significance of the differences in mean values between the saline group (control)
and the GH group was evaluated using the unpaired two-tailed t-test. Differences were considered significant at \( P < 0.05 \).

**RESULTS**

**Effects of GH Infusion in the Basal State (Study 1)**

During the initial 150-min period of saline or GH infusion, plasma glucose, insulin, and FFA concentrations were constant (Fig. 1). GH infusion in this basal state did not significantly affect plasma glucose, insulin, or FFA concentrations (\( P > 0.05 \)).

During the final 150-min clamp period, plasma insulin was raised to \( \sim 0.85 \) nM, and plasma glucose was clamped at \( \sim 5.5 \) mM (Fig. 1). Plasma FFA concentrations were suppressed by 70–80% during the hyperinsulinemic clamps. Again, GH infusion did not affect plasma insulin or FFA concentrations during the clamps. In both groups, glucose infusion rates required to clamp plasma glucose increased rapidly during the initial 60 min of insulin infusion and reached steady-state levels by the end of clamps. However, glucose infusion rates were significantly lower with GH infusion, and such effects of GH were statistically significant at 220 min and thereafter.

GH infusion decreased steady-state (260–300 min) insulin-stimulated glucose uptake by 32% (111 ± 6 vs. 164 ± 10 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \)) in the saline group; Fig. 2). In contrast, GH infusion did not significantly alter HGO during the clamps (27 ± 5 vs. 14 ± 12 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \); \( P > 0.05 \)). Thus these data indicate that the reduced glucose requirement during the clamps was predominantly due to the effects of GH on peripheral insulin action. GH infusion also decreased insulin-stimulated glycolysis by 27% (70 ± 7 vs. 95 ± 8 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \)) and glycogen synthesis by 40% (42 ± 5 vs. 69 ± 6 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \)). Intracellular G-6-P concentration indicates relative activities of glucose transport/phosphorylation and glucose metabolism distal to G-6-P. Despite the changes in glucose fluxes, G-6-P concentrations in soleus muscle, measured at the end of clamps, were not significantly altered by GH infusion (287 ± 32 vs. 275 ± 38 nmol/g in the saline group; \( P > 0.05 \)).

**Effects of GH Infusion in an Insulin-Stimulated State (Study 2)**

In this study, hyperinsulinemic euglycemic clamps were conducted during the initial 150 min to attain a new steady state of elevated plasma insulin and decreased FFA concentrations before GH infusion was...
initiated. After this initial control clamp period, the clamps were continued with saline or GH infusion during the remaining 300 min.

During the entire 450-min clamp period, plasma insulin was raised to and maintained at ~0.8 nM, and plasma glucose was clamped at ~5.5 mM in both saline and GH infusion groups (Fig. 3). Plasma FFA concentrations were suppressed by 70–80% within 60 min of insulin infusion and remained suppressed throughout the clamps in both groups (Fig. 3). Thus GH infusion did not affect the ability of insulin (at a steady-state concentration of 0.8 nM) to suppress plasma FFA concentrations. In both groups, glucose infusion rates increased rapidly during the initial 60 min of clamps and reached steady-state levels within 150 min (Fig. 3). During the experimental period (150–450 min) of saline or GH infusion, glucose infusion rates were relatively constant with saline infusion. In contrast, glucose infusion rates started to decrease at ~150 min after the start of GH infusion and decreased by 37% at the end of clamps (99 ± 8 vs. 157 ± 9 µmol·kg⁻¹·min⁻¹ in the saline group).

Insulin-stimulated whole body glucose uptake, glycolysis, and glycogen synthesis were relatively constant with saline infusion (Fig. 4). In contrast, insulin-stimulated glucose uptake was significantly decreased with GH infusion, starting at ~150 min after the start of GH infusion. At the end of clamps, insulin-stimulated glucose uptake was decreased by 37% with GH infusion (120 ± 12 vs. 191 ± 7 µmol·kg⁻¹·min⁻¹ in the

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**Fig. 3.** Plasma insulin (A) and FFA (B) levels, glucose infusion rate (C), and plasma glucose levels (D) during 450-min hyperinsulinemic euglycemic clamps with saline (○) or GH (●) infusion started at 150 min (study 2). Values are means ± SE for 7 experiments. *P < 0.05 vs. saline infusion (control) group.

**Fig. 4.** Time courses of changes in whole body insulin-stimulated glucose uptake (A), glycolysis (B), and glycogen synthesis (C) during hyperinsulinemic euglycemic clamps with saline (○) or GH (●) infusion started at 150 min (study 2). Data are means ± SE for 7 experiments expressed in µmol·kg⁻¹·min⁻¹. *P < 0.05 vs. saline infusion (control) group.
saline group). GH infusion did not alter HGO throughout the clamps (20 ± 6 vs. 34 ± 6 µmol·kg⁻¹·min⁻¹ in the saline group at the end of clamps; P > 0.05). Again, these data indicate that the effect of GH infusion to reduce glucose infusion rates during the clamps was predominantly due to its effects on peripheral insulin action.

GH infusion significantly suppressed insulin-stimulated glycogen synthesis and increased glycolysis within 60 min after the start of GH infusion (Fig. 4). These effects of GH infusion on intracellular glucose fluxes clearly preceded the effects of GH infusion on glucose uptake. Insulin-stimulated glycogen synthesis and glycolysis returned to preinfusion levels at ~120 min after the start of GH infusion. Subsequently, both fluxes declined as glucose uptake decreased and reached ~60% of control levels (i.e., of the saline infusion group) at the end of clamps (Fig. 4). Thus these data demonstrate that GH infusion had rapid effects on intracellular glucose metabolic fluxes that preceded those on insulin-stimulated glucose uptake.

DISCUSSION

Previous studies have suggested that GH causes insulin resistance by promoting lipolysis and increasing circulating FFA or insulin levels (6, 8, 13, 26, 29). In the present study, we demonstrated that a 5-h infusion of human GH in conscious rats, at a dose (21 µg·kg⁻¹·h⁻¹) that raises plasma GH within a physiological range (13), caused peripheral insulin resistance without altering plasma FFA or insulin levels. In addition, these effects of GH on insulin action were identical whether GH was infused in the basal or insulin-stimulated states, representing two conditions where plasma FFA and insulin concentrations were different by severalfold. These results strongly indicate that GH acutely induces peripheral insulin resistance independent of circulating FFA and insulin levels. Another important finding in the present study was that GH infusion rapidly suppressed glycogen synthesis and increased glycolysis during the hyperinsulinemic clamps (study 2). These effects on GH on intracellular glucose fluxes occurred within 60 min, clearly preceding the changes in glucose uptake. Although we do not have data indicating a causal relationship between these GH effects, these results are consistent with the hypothesis, put forth in our previous publications (15, 16), that impairment of intracellular glucose metabolism precedes and causes development of insulin resistance in skeletal muscle.

Our finding that GH acutely induces peripheral insulin resistance independent of circulating FFA is consistent with some of the previous human studies. Bratusch-Marrain et al. (6) showed that a physiological elevation of plasma GH levels for 12 h induced peripheral insulin resistance without significant elevation of plasma FFA levels. In addition, Møller et al. (20) showed that GH infusion during a hyperinsulinemic euglycemic clamp decreased forearm glucose uptake preceding significant elevation of plasma FFA levels. These studies suggested that the acute effects of GH on glucose metabolism are independent of changes in lipolysis. However, although statistically insignificant, plasma FFA levels were elevated in these studies when GH effects on peripheral insulin action were manifested. Therefore, the possibility could not be excluded that GH stimulates lipolysis in insulin-sensitive cells and induces insulin resistance by increasing intracellular FFA levels (in the absence of significant changes in plasma levels). The present study demonstrates that the effects of 5-h GH infusion on peripheral insulin action were similar whether GH was infused with (hyperinsulinemic state) or without (basal state) a 70–80% suppression of lipolysis (reflected in plasma FFA levels; Fig. 3). These data convincingly exclude the possibility that the acute GH effect to induce peripheral insulin resistance is mediated by altering lipolysis. To our knowledge, we are the first to compare the effects of GH infusion under basal vs. hyperinsulinemic conditions.

The present study was focused on the mechanisms of acute GH induction of insulin resistance. Chronic GH treatment may induce metabolic changes and/or insulin resistance via mechanisms not seen in an acute study (13). The chronic effects of GH treatment have been extensively studied in domestic animals, as recently reviewed by Etherton and Bauman (10). These studies in domestic animals have demonstrated that GH has dramatic effects on adipose tissue and lipid metabolism and that these effects are chronic rather than acute. Therefore, although GH may acutely induce insulin resistance independent of lipid metabolism, as the present data indicate, it is possible that altered lipid metabolism may play a role in the regulation of insulin action with chronic elevation of plasma GH levels.

The effects of GH infusion to decrease glycogen synthesis and to increase glycolysis during the hyperinsulinemic clamps were rather unexpected. In fact, we designed study 2 to examine the possibility that GH antagonizes insulin's antilipolytic action in muscle and suppresses glycolysis by increasing intracellular FFA levels. Thus the direction of change in glycolysis was opposite to what we suspected. It is also important to note that the effects of GH on glycolysis and glycogen synthesis were opposite to those occurred in the absence of changes in glucose uptake. It is likely that GH infusion primarily affected one of these metabolic fluxes and that this primary change was compensated for by the change in the other flux, resulting in no changes in glucose uptake. This concept is supported by our previous finding (16) that a suppression of glycolysis (or glycogen synthesis) caused increases in glycogen synthesis (or glycolysis) without altering glucose uptake during hyperinsulinemic euglycemic clamps similar to those in the present study. The question then arises as to which flux is the primary site of GH action. Bak et al. (3) reported that insulin stimulation of glycogen synthase in skeletal muscle was inhibited by 41% after a 5-h GH infusion in humans. Although this study did not examine whether the effects can occur within 1 h of GH infusion, as the present data indicate, these data support the notion
that the primary effect of GH was to suppress insulin-stimulated glycogen synthesis under our experimental conditions. If so, the increase in glycolysis was likely secondary to this primary effect, presumably due to increased G-6-P as a result of suppressed glycogen synthesis, as we previously observed with amylidine infusion (16). What would be the significance of the rapid effect of GH on glycogen synthesis? Our previous study (16) showed that suppressing glycogen synthesis during hyperinsulinemic euglycemic clamps, to a similar degree as the present study, caused a decrease in insulin-stimulated glucose uptake. Taken together, these data raise the possibility that GH induces insulin resistance acutely by first suppressing insulin-stimulated glycogen synthesis. This intriguing possibility, however, needs to be directly examined in future studies.

The effect of GH on the rate of glycogen synthesis appeared to be transient; the flux rate decreased during the 1st h of GH infusion, increased to reach control levels in the 2nd h, and subsequently decreased again as glucose uptake fell. The reason for the changes in the 2nd h is unclear. Because the rate of glycogen synthesis estimated in the present study represents the rate of net glycogen synthesis, these changes can be accounted for by changes not only in the activity of glycogen synthase but also in the activity of glycogen phosphorylase or in substrate levels, such as G-6-P.

GH promotes lipolysis in adipose tissue, and chronic elevation of GH is associated with increased FFA levels and hyperinsulinemia (8, 13, 21, 29). In the present study, GH infusion in the basal state for 150 min did not increase plasma FFA levels. Previous studies have reported inconsistent findings in regard to the effects of GH on basal (or fasting) plasma FFA levels in humans (6, 21, 29) and in rats (13, 23). Møller et al. (21) showed that a 4-h GH infusion (1.2 µg·kg⁻¹·h⁻¹) raised plasma FFA levels in normal human subjects. In contrast, Bratusch-Marrain et al. (6) did not observe any significant changes in plasma FFA levels after 2 or 12 h of GH infusion (2 µg·kg⁻¹·h⁻¹) in normal subjects. In rats, Hettiarachchi et al. (13) showed that a 5-h infusion of human GH (21 µg·kg⁻¹·h⁻¹) significantly raised plasma FFA levels, whereas Ng et al. (23) reported that a 48-h infusion of human GH (160 µg·kg⁻¹·h⁻¹) did not change basal FFA levels. The reasons for these discrepant findings are unclear. The rate of GH infusion does not appear to explain the discrepancy, because those studies reporting no effects (6, 23) used higher infusion rates than those reporting significant effects of GH infusion on plasma FFA levels (13, 21).

Although the effects of GH on basal FFA levels have been inconsistent, previous human studies convincingly demonstrated the effects of GH to antagonize insulin’s ability to suppress lipolysis and to decrease plasma FFA levels. Bak et al. (3) showed that GH infusion raised plasma FFA levels during 5-h hyperinsulinemic clamps in normal subjects. Similar results were reported by Møller et al. (20, 21). However, in the present study in rats, GH infusion for 5 h did not alter plasma FFA levels during hyperinsulinemic clamps. The discrepancy may be explained by the fact that the clamp insulin levels in the present study (800–850 pM) were, although within a physiological range, much higher than those in the human studies by Bak et al. and Møller et al. (<250 pM), and GH infusion at the present dose could not overcome the antilipolytic effects of insulin. However, Ng et al. (23) reported that GH administration in rats for 48 h at a dose eightfold higher than ours caused increased FFA levels during hyperinsulinemic clamps at insulin levels similar to ours. Thus the effects of GH to antagonize insulin’s antilipolytic action may be observed depending on the dose and the duration of GH administration, as well as on insulin levels used during the clamp.

It is established that chronic elevation of GH is associated with hyperinsulinemia (13, 24, 26). Because sustained hyperinsulinemia causes decreased insulin sensitivity in humans (9, 18, 25), hyperinsulinemia has also been suggested to be responsible for GH-induced insulin resistance (13, 26). However, in contrast with increased insulin secretion during chronic administration of GH (1, 24), short-term GH infusion has been shown to exert no or even inhibitory effects on basal insulin secretion (2, 17, 21, 29). Adamson and Cerasi (2) showed that plasma insulin levels decreased after a 30-min GH infusion at various doses. Also, MacGorman et al. (17) and Møller et al. (21) showed that a 4-h GH infusion did not affect basal plasma insulin levels in normal subjects. Similarly, Sherwin et al. (29) demonstrated that basal plasma insulin levels did not significantly increase until 4 h of GH infusion in normal subjects. Thus the effects of GH to alter basal insulin levels may be observed depending on the dose and the duration of GH administration. In this study, GH was infused in the basal state for only 2.5 h and, consistent with the mentioned human studies, we did not observe any effects of GH infusion on basal plasma insulin levels.

Intracellular G-6-P concentration indicates relative activities of glucose transport/phosphorylation and glucose metabolism distal to G-6-P. Despite substantial changes in insulin-stimulated glucose uptake and intracellular glucose fluxes, muscle G-6-P levels at the end of hyperinsulinemic clamps were not altered with GH infusion. However, these data do not necessarily indicate that muscle G-6-P levels were constant (or unaltered) during GH infusion. It is possible that G-6-P may have been elevated during an initial period of GH infusion as a result of suppressed glycogen synthesis. Such increases in G-6-P may have been normalized at the end of clamps when insulin action on glucose uptake was significantly decreased. In other words, decreases in insulin-stimulated glucose uptake may have balanced the decreases in intracellular glucose metabolism (i.e., glycogen synthesis) at the end of clamps, resulting in normalization of G-6-P levels. Such a temporal pattern of changes in muscle G-6-P levels has been previously observed during the development of insulin resistance with suppression of glycogenesis or glycogen synthesis during hyperinsulinemic euglycemic clamps (16).
The approach of using $^3$H$_2$O production for the estimation of whole body glycolysis would overestimate glycolysis if there were significant futile cycling between G-6-P and triose phosphates. However, there is evidence suggesting that the futile cycling in skeletal muscle, the major site of insulin-mediated glucose uptake, may be insignificant. For example, futile cycling between fructose 6-phosphate and fructose 1,6-bisphosphate was estimated to be only ~10% of the glycolytic rate in the presence of insulin in skeletal muscle isolated from fed rats (7). In addition, the loss of $^3$H from [3-3H]glucose (28) and the randomization of $^{14}$C label from [1-$^{14}$C]glucose (22) during the incorporation of glucose into muscle glycogen in vivo, two phenomena that would result from the futile cycling, appeared to be ~10%. Thus the possible overestimation of glycolysis due to futile cycling may be 10% at the most. However, we cannot exclude the possibility that the apparent effects of GH to suppress glycolysis synthesis and to increase glycolysis (study 2) were artifacts arising from increased futile cycling in skeletal muscle with GH infusion. Therefore, further studies are needed to directly demonstrate the rapid effect of GH on muscle glycogen synthesis.

In conclusion, the acute effects of GH infusion to decrease insulin-stimulated glucose uptake were similar whether GH was infused in the basal or in an insulin-stimulated state, indicating that GH induces peripheral insulin resistance independent of plasma FFA and insulin levels. In addition, GH infusion rapidly (within 60 min) suppressed glycogen synthesis, and this effect of GH clearly preceded its effect on insulin-stimulated glucose uptake. These results are consistent with our hypothesis that impairment of intracellular glucose metabolism precedes and causes the development of insulin resistance in skeletal muscle.

We are grateful to Dr. Marianne Hamilton-Wessler for insightful comments on the manuscript. This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant R-29-DK-47947 and a research grant from the American Diabetes Association. Address for correspondence and reprint requests: J. H. Youn, Dept. of Physiology and Biophysics, Univ. of Southern California School of Medicine, 1333 San Pablo Ave., MMR 626, Los Angeles, CA 90089-9124 (E-mail: youn@syntax.hsc.usc.edu). Received 20 January 1999; accepted in final form 24 May 1999.

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