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Research Article

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Skeletal Muscle Glycogenolysis Is More Sensitive to Insulin than Is Glucose Transport/Phosphorylation

Relation to the Insulin-mediated Inhibition of Hepatic Glucose Production

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Abstract

The effects of minimal increments in plasma insulin concentrations on hepatic glucose production and glucose uptake, skeletal muscle net glycogen synthesis and glycogenolysis, glycogen synthase and phosphorylase activity, glucose-6-phosphate and uridinediphosphoglucose (UDPG) concentrations were examined in 24-h and in 6-h fasted conscious rats. Insulin was infused for 120 min at rates of 1.5, 3, 6, 12, 24, and 108 pmol/kg per min in 24-h fasted rats and at rates of 3, 6, 9, 12, 36, and 108 pmol/kg per min in 6-h fasted rats while endogenous insulin release was inhibited by SRIF infusion and plasma glucose was maintained at the basal level. All rats received an infusion of [3-³H]glucose. The portion of the muscle glucose-6-phosphate (G6P) pool derived from net glycogenolysis was estimated from the ratio of specific activities of muscle UDPG and plasma glucose. Minimal increments in the circulating insulin levels, which did not stimulate glucose uptake, caused: (a) the increase in skeletal muscle glycogen synthase activity and the decrease in the rate of muscle glycogenolysis and in the G6P concentration; (b) the inhibition of hepatic glucose production. Net muscle glycogen synthesis was not stimulated despite submaximal activation of glycogen synthase, and its onset correlated with the rise in muscle G6P levels. Thus, insulin's inhibition of muscle glycogenolysis is the most sensitive insulin action on skeletal muscle and its dose-response characteristics resemble those for the inhibition of hepatic glucose production. These findings indicate that skeletal muscle glycogen synthase may play a major role in carbohydrate homeostasis even under postabsorptive (basal insulin) conditions and support the notion that insulin may exert some of its effects on the liver through an indirect or peripheral mechanism. (*J. Clin. Invest.* 1993; 92:2963–2974.) Key words: insulin action • muscle glycogenolysis • glucose uptake • glycogen synthase • glucose-6-phosphate

Introduction

The decrease in insulin's ability to stimulate nonoxidative glucose disposal (1–5) and to inhibit hepatic glucose production (6, 7) is a major feature of non-insulin-dependent diabetes

mellitus (NIDDM).¹ Glycogen synthase is believed to be the rate-limiting enzyme in the glycogen synthetic pathway (8, 9). In NIDDM, defects have been demonstrated in both the "functional mass" of the enzyme (3) and in its activation state (2–4). Recently, genetic (10, 11) and functional (12, 13) alterations in this enzyme have been reported in nonobese NIDDM subjects and in siblings of NIDDM individuals, suggesting the potential role of muscle glycogen synthase in the pathogenesis of impaired glucose tolerance and diabetes. However, it is commonly believed that muscle glycogen storage does not play a significant role in the postabsorptive glucose homeostasis. Furthermore, several studies have demonstrated that the contribution of muscle glycogen storage to overall glucose fluxes is modest under physiological plasma insulin concentrations and route of carbohydrate administration (14–18). In fact, following meal ingestion the transient and moderate hyperinsulinemia and hyperglycemia leads to the partial inhibition of hepatic glucose production and to the stimulation of skeletal muscle glucose uptake (15, 16). The latter effect accounts for 30–50% (14–16) of the change in glucose metabolism and only a small portion (25–30%) of the glucose taken up by the skeletal muscle is stored into glycogen (15–17).

In skeletal muscle, both plasma-derived glucose and glycogen can be phosphorylated to glucose-6-phosphate and can then enter glycolysis. In turn, glycolysis leads either to lactate formation or pyruvate oxidation in the Krebs cycle. Since the rates of net glycogen synthesis/breakdown reflect the balance of the glycogenic and glycogenolytic fluxes, the effect of insulin on this process can be mediated by either the stimulation of the activity of glycogen synthase as well as the inhibition of the activity of glycogen phosphorylase. Substrate-mediated regulation of glucose uptake and of hepatic glucose production has long been recognized (19). Recent evidence suggest a link between the peripheral and hepatic effects of insulin (20–26). In fact, the inhibition of gluconeogenesis by insulin may be mediated via the intrahepatic diversion of three carbon compounds toward other pathways, namely glycolysis/oxidation and glycogen formation (26, 27), and/or via an insulin-induced reduction in the influx of gluconeogenic substrates and energy to the liver (27, 28).

Thus, it is important to examine the role of glycogen synthase in the regulation of the rates of net glycogenolysis in skeletal muscle and to correlate insulin action in skeletal muscle and liver under basal and physiologically hyperinsulinemic conditions. We investigated this issue by measuring the rate of hepatic glucose production and the contribution of plasma glucose to the muscle glucose-6-phosphate pool at increasing plasma insulin concentrations. Our results indicate that the

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1. Abbreviations used in this paper: G-6-P, glucose-6-phosphate; NIDDM, non-insulin-dependent diabetes mellitus.

muscle glycogen synthase is exquisitely sensitive to insulin's activation and, in the presence of low plasma insulin concentrations, its predominant role is to limit muscle glycogenolysis. The implication of this finding for the *in vivo* hepatic and peripheral sensitivity to insulin is discussed.

Methods

Animals. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in all studies. Rats were housed in individual cages and subjected to a standard light (6 AM to 6 PM)–dark (6 PM to 6 AM) cycle. One week before the *in vivo* study, rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg body wt) and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery. The venous catheter was extended to the level of the right atrium and the arterial catheter was advanced to the level of the aortic arch (29, 30).

Euglycemic clamp study. Studies were performed in awake, unstressed, chronically catheterized rats using the euglycemic clamp technique in combination with [^3H]glucose infusion as previously described (26, 29–31). Rats were fasted for 6 ($n = 43$) or 24 ($n = 42$) h before the *in vivo* studies. These two nutritional states were selected since 24-h fast in rats is associated with marked hepatic glycogen depletion and decreased muscle glycogen concentration, while the 6-h fasted rats more closely resemble the postabsorptive state in humans, with substantial glycogen content in both liver and muscle. Basal glycogen concentrations may affect “glycogen turnover” and the response of glycogen synthase to insulin's activation. Additionally, since in 24-h fasted animals hepatic glucose production is completely due to gluconeogenesis, while in 6-h fasted animals both gluconeogenesis and glycogenolysis contribute to hepatic glucose output, the comparison of these two different metabolic states may add to our understanding of the relation between the effects of insulin on peripheral and hepatic glucose metabolism. Briefly, 5–7 d following the placement of the catheters, primed-continuous infusions of somatostatin (0.8 $\mu\text{g/kg}$ per min) and insulin (1.5–108 pmol/kg per min) or saline were administered, and a variable infusion of a 25% glucose solution was started at time zero and periodically adjusted to clamp the plasma glucose concentration at the basal level. Somatostatin was infused in all studies in the attempt to

minimize variations in the portal vein glucagon and insulin concentration in the various experimental protocols. 24-h fasted rats received an infusion of saline ($n = 6$) or insulin at either 1.5 ($n = 7$), 3 ($n = 5$), 6 ($n = 6$), 12 ($n = 7$), 24 ($n = 2$) or 108 ($n = 9$) pmol/kg per min for 2 h. 6-h fasted rats received an infusion of saline ($n = 4$) or insulin at either 3 ($n = 4$), 6 ($n = 7$), 9 ($n = 6$), 18 ($n = 7$), 36 ($n = 5$), or 108 ($n = 10$) pmol/kg per min for 2 h. At the beginning of the infusion study a prime-continuous infusion of HPLC purified [^3H -3]glucose (New England Nuclear, Boston, MA; 15–40- μCi bolus, 0.4 $\mu\text{Ci/min}$) was initiated and maintained throughout the remainder of the study. Plasma samples for determination of [^3H]glucose specific activity were obtained at 10-min intervals throughout the saline and the insulin/somatostatin infusions. Plasma samples for determination of plasma insulin, lactate, and NEFA concentrations were obtained at time –30, 0, 30, 60, 90, and 120 min during the study. The total volume of blood withdrawn was ~ 3.0 ml/study; to prevent volume depletion and anemia, a solution (1:1 vol/vol) of ~ 4.0 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 U/ml) was infused. At the end of the insulin infusion, rats were anesthetized (pentobarbital 60 mg/kg body wt, *i.v.*), the abdomen was quickly opened, and rectus abdominal muscle was freeze-clamped *in situ* with aluminum tongs precooled in liquid nitrogen (32). The time from the injection of the anesthetic until freeze-clamping of the liver was less than 45 s. All tissue samples were stored at -80°C for subsequent analysis.

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committees of the Albert Einstein College of Medicine.

Contribution of glycogenolysis to muscle UDPglucose *in vivo*. In skeletal muscle, glucose-6-phosphate and UDP glucose can derive from plasma glucose and from prestored glycogen. Under steady-state conditions, the plasma glucose specific activity and the intracellular specific activity of UDPglucose should be equal if all of the UDPglucose is from plasma, *i.e.*, net glycogenolysis is completely suppressed. However, in the presence of low plasma insulin concentrations, it is conceivable that a substantial portion of the glucose-6-phosphate and UDPglucose pools is derived from preexisting unlabeled glycogen, which would dilute the intracellular specific activity and decrease the ratio with the plasma specific activity. Thus, the relative net contribution of plasma glucose and glycogen to the composition of the skeletal muscle UDPg-

Table I. Mean Plasma Glucose, Insulin, FFA, and Lactate Concentration during the Last 30 min of Saline Infusions (Basal) and of Euglycemic Clamp Studies Performed in the Presence of Increasing Plasma Insulin Concentrations in 24-h (Upper Panel) and 6-h (Lower Panel) Fasted Conscious Rats

	Insulin infusion rate						
	pmol/kg per min						
	Basal	1.5	3	6	12	24	108
<i>n</i>	6	7	5	6	7	2	9
Glucose, mM	5.7 $\pm$ 0.3	5.7 $\pm$ 0.3	5.6 $\pm$ 0.01	5.7 $\pm$ 0.1	5.7 $\pm$ 0.1	5.7	5.6 $\pm$ 0.1
Insulin, pM	126 $\pm$ 8	111 $\pm$ 4	175 $\pm$ 5	253 $\pm$ 7	460 $\pm$ 18	738	2436 $\pm$ 159
FFA, mM	0.65 $\pm$ 0.06	0.69 $\pm$ 0.05	0.52 $\pm$ 0.04	0.41 $\pm$ 0.03	0.29 $\pm$ 0.04	0.13	0.14 $\pm$ 0.02
Lactate, mM	0.56 $\pm$ 0.05	0.54 $\pm$ 0.04	0.42 $\pm$ 0.04	0.53 $\pm$ 0.04	0.81 $\pm$ 0.04	1.37	1.43 $\pm$ 0.07
	Basal	3	6	9	18	36	108
<i>n</i>	4	4	7	6	7	5	10
Glucose, mM	7.9 $\pm$ 0.3	7.8 $\pm$ 0.3	7.7 $\pm$ 0.2	7.8 $\pm$ 0.1	7.8 $\pm$ 0.1	7.9 $\pm$ 0.2	7.7 $\pm$ 0.2
Insulin, pM	206 $\pm$ 12	190 $\pm$ 4	276 $\pm$ 6	355 $\pm$ 7	525 $\pm$ 24	1284 $\pm$ 84	2672 $\pm$ 119
FFA, mM	0.79 $\pm$ 0.09	0.83 $\pm$ 0.03	0.69 $\pm$ 0.05	0.45 $\pm$ 0.03	0.30 $\pm$ 0.04	0.19 $\pm$ 0.08	0.21 $\pm$ 0.03
Lactate, mM	0.96 $\pm$ 0.03	0.90 $\pm$ 0.01	0.68 $\pm$ 0.03	0.82 $\pm$ 0.06	1.22 $\pm$ 0.12	1.64 $\pm$ 0.09	1.89 $\pm$ 0.05

Values are mean $\pm$ SE.

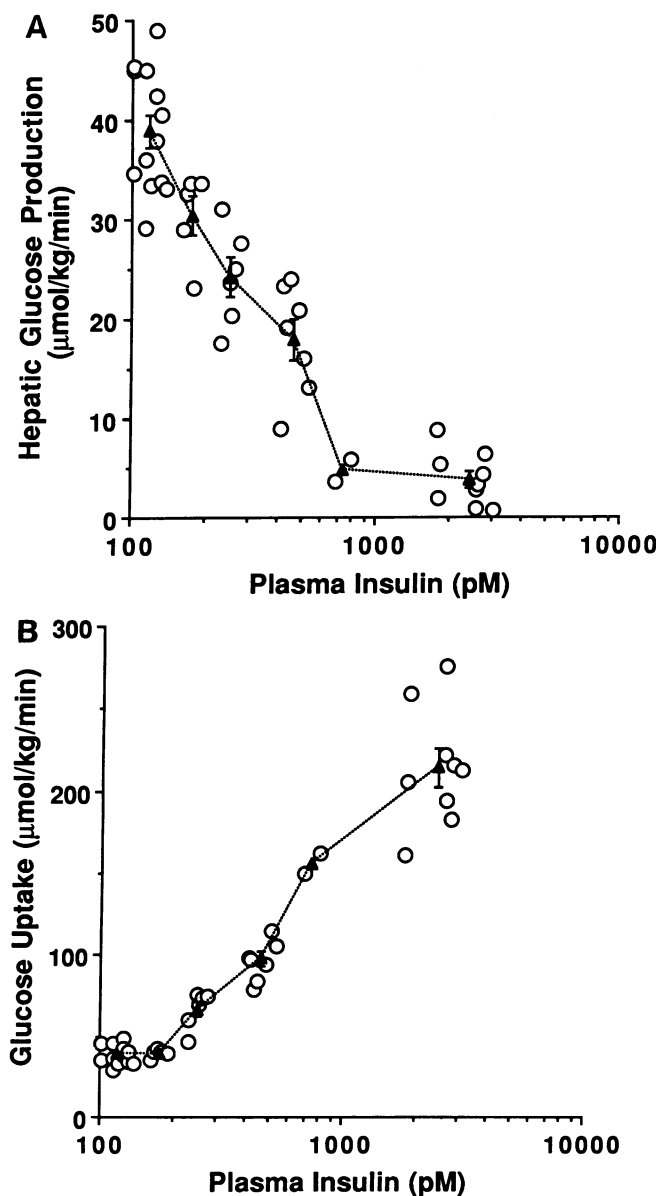


Figure 1. Dose-response for the effects of increasing plasma insulin concentrations on hepatic glucose production (*upper panel*) and peripheral glucose uptake (*lower panel*) in 24-h fasted rats. Experimental points are the mean $\pm$ SE of determination obtained under steady-state conditions during the last 30 min of the *in vivo* studies (90–120 min). Since the results obtained during the saline infusion studies and the combined somatostatin and insulin replacement infusions (1.5 pmol/kg per min) were similar (Tables I–IV) they are graphically presented in relation to the circulating insulin concentrations. Hepatic glucose production was significantly ($P < 0.01$) decreased at all insulin infusion rates. The rate of glucose uptake was increased significantly ($P < 0.01$) over basal values during the 6, 12, 24, and 108 pmol/kg per min insulin infusions only.

lucose can be measured by comparing the [^3H]glucose specific activities in plasma and in muscle UDPglucose. It is important to recognize that this experimental approach, similarly to most tracer techniques, measures net substrate flux. Thus, if bidirectional flux occurs, e.g., glycogen synthesis and glycogen breakdown, this method will provide an assessment of the balance between the two opposite fluxes. For example, if labeled glucose is incorporated into glycogen (glycogen synthesis) and then phosphorylated back into glucose-1-phosphate $\rightarrow$

glucose-6-phosphate (glycogenolysis) the specific activity of the glucose-6-phosphate pool would not change. On the contrary, if pre-formed glycogen (unlabeled) contributes to the glycogenolytic flux, this would dilute the G-6-P specific activity and would be correctly interpreted as net glycogenolysis. However, if glycogen turnover involves exchange of labeled molecules of glucose (accumulating into glycogen) with unlabeled molecules of glucose-1-phosphate (glycogen-

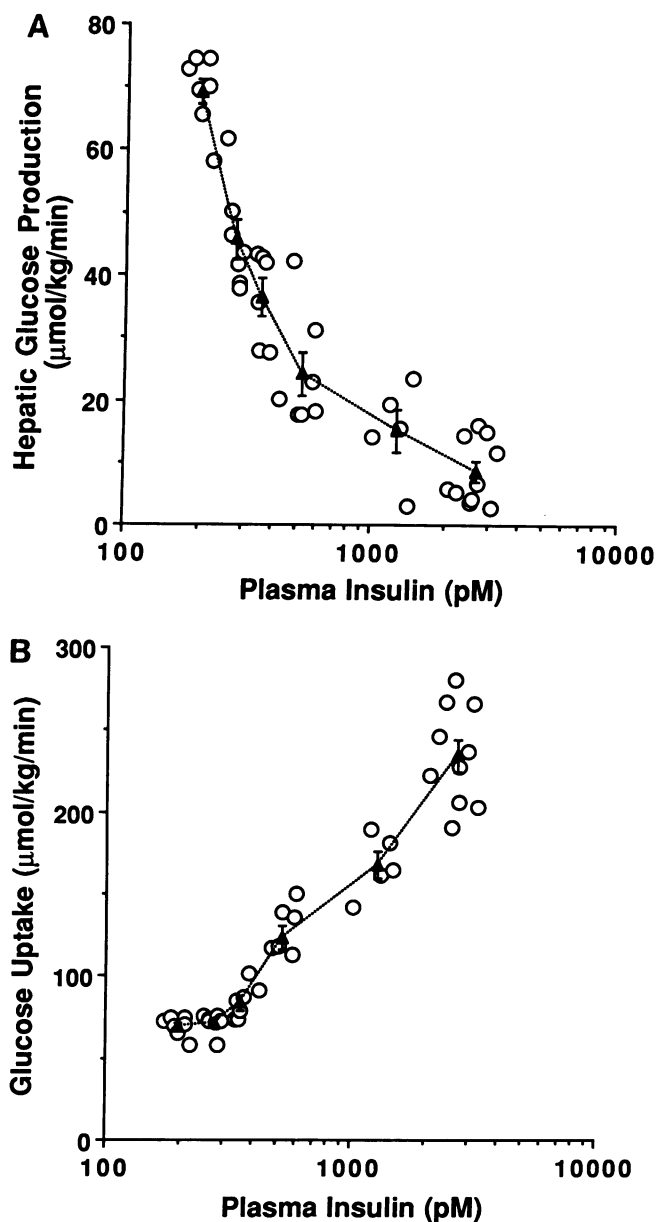


Figure 2. Dose-response for the effects of increasing plasma insulin concentrations on hepatic glucose production (*upper panel*) and peripheral glucose uptake (*lower panel*) in 6-h fasted rats. Experimental points are the mean $\pm$ SE of determination obtained under steady-state conditions during the last 30 min of the *in vivo* studies (90–120 min). Since the results obtained during the saline infusion studies and the combined somatostatin and insulin replacement infusions (3 pmol/kg per min) were similar (Tables I–IV) they are graphically presented in relation to the circulating insulin concentrations. Hepatic glucose production was significantly ($P < 0.01$) decreased at all insulin infusion rates. The rate of glucose uptake was increased significantly ($P < 0.01$) over basal values during the 18, 36, and 108 pmol/kg per min insulin infusions only.

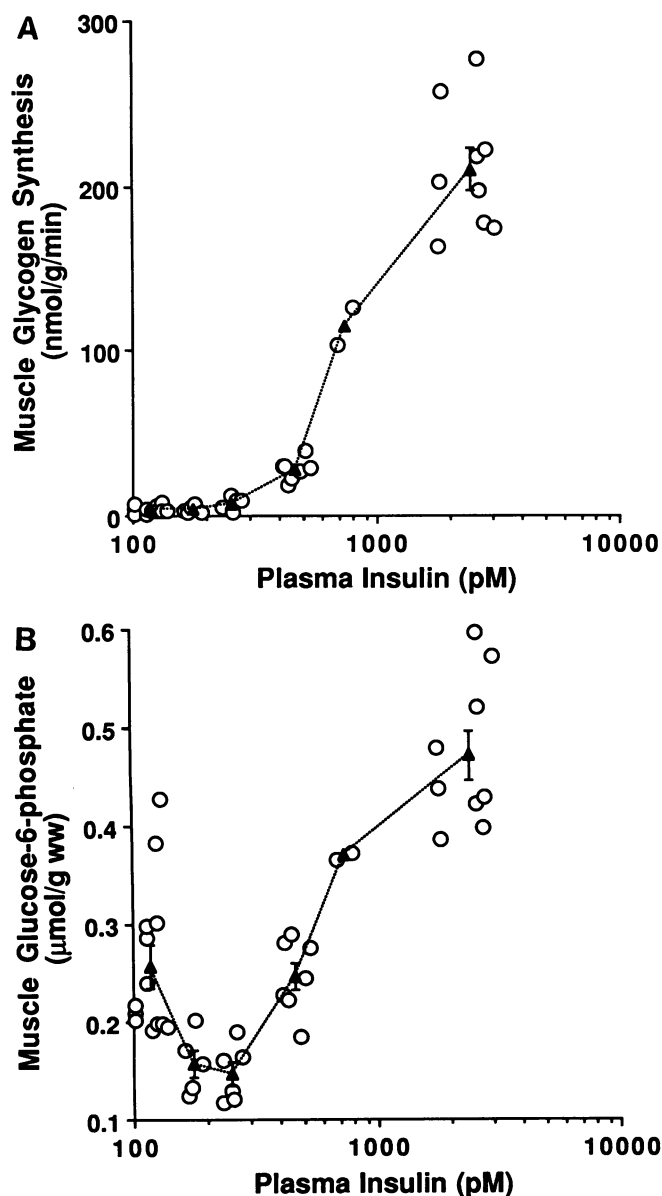


Figure 3. Dose-response for the effects of increasing plasma insulin concentrations on the net rates of muscle glycogen synthesis (*upper panel*) and the skeletal muscle glucose-6-phosphate concentrations (*lower panel*) in 24-h fasted rats. Muscle glycogen synthesis was measured as the UDPglucose specific activity in the same muscle. Since the results obtained during the saline infusion studies and the combined somatostatin and insulin replacement infusions (1.5 pmol/kg per min) were similar (Tables I–IV) they are graphically presented in relation to the circulating insulin concentrations. Muscle glycogen synthesis was significantly ($P < 0.01$) increased at plasma insulin concentrations > 460 pM. The glucose-6-phosphate concentrations were significantly ($P < 0.01$) decreased from basal values during the 3 and 6 pmol/kg per min insulin infusions, and they were significantly increased during the 108 pmol/kg per min insulin infusion.

olysis), this may result in an overestimation of the muscle glycogenolysis at low plasma insulin concentrations. In fact, in the presence of glycogen turnover, i.e., glucose cycling into glycogen with no net contribution of glycogen to the G6P pool, there may be a dilution of the specific activity of G6P. However, several lines of evidence suggest that this potential issue had a minimal impact in our estimate of muscle

glycogenolysis: (a) the most accepted model for glycogen synthesis and degradation, i.e., last in/first out, would predict no change in G-6-P specific activity and according to this model glucose cycling through glycogen would be silent with the present experimental approach; (b) the total amount of glucose label present into muscle glycogen at the

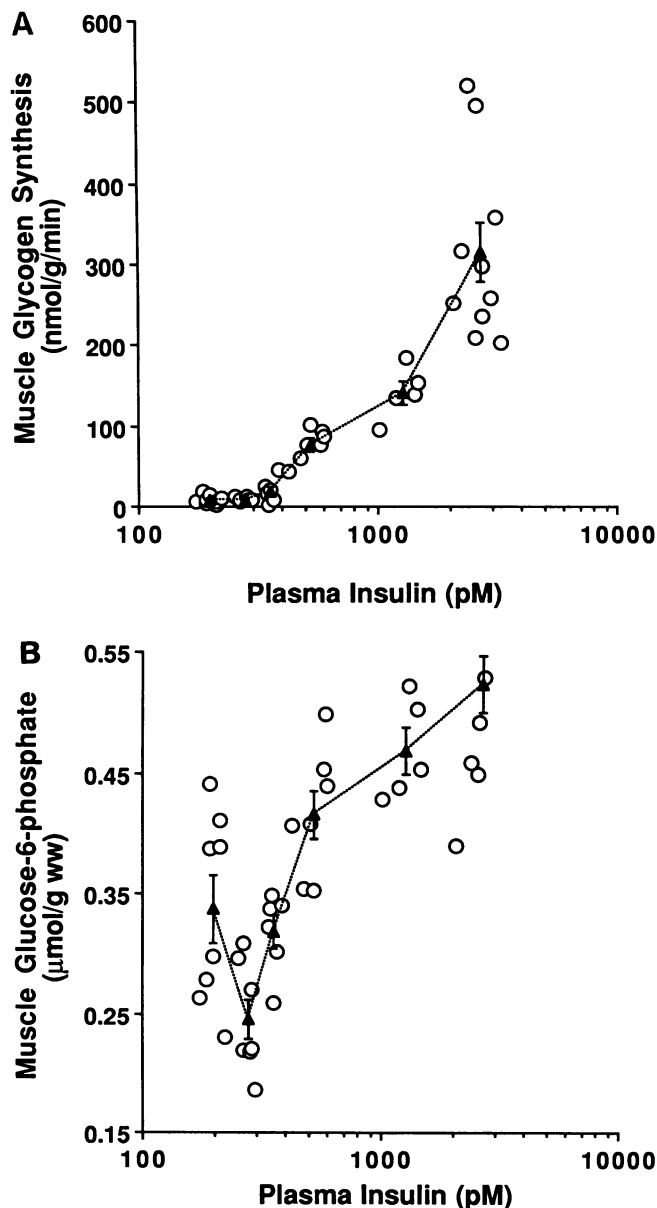


Figure 4. Dose-response for the effects of increasing plasma insulin concentrations on the net rates of muscle glycogen synthesis (*upper panel*) and the skeletal muscle glucose-6-phosphate concentrations (*lower panel*) in 6-h fasted rats. Muscle glycogen synthesis was measured as the UDPglucose specific activity in the same muscle. Since the results obtained during the saline infusion studies and the combined somatostatin and insulin replacement infusions (3 pmol/kg per min) were similar (Tables I–IV) they are graphically presented in relation to the circulating insulin concentrations. Muscle glycogen synthesis was significantly ($P < 0.01$) increased at plasma insulin concentrations > 530 pM. The glucose-6-phosphate concentrations were significantly ($P < 0.01$) decreased from basal values during the 6 pmol/kg per min insulin infusion, and they were significantly increased during the 18, 36, and 108 pmol/kg per min insulin infusions.

end of the 2-h study, which represents a maximal estimate of the exchange of tritium label between glycogen and G6P pools, was negligible at the end of the low insulin infusion protocols (sp act of glucosyl units in muscle glycogen = 0.056 dpm/nmol in the 24-h fasted rats and 0.087 dpm/nmol in the 6-h fasted rats); (c) finally, under hyperinsulinemic conditions, the rates of net glycogen synthesis estimated from the incorporation of tritiated glucose into muscle glycogen were similar to the rates calculated from the increment above basal in the muscle glycogen concentrations. It should also be noted that under conditions of active glycogen synthesis, when net glycogenolysis is absent, the equality of the specific activities of muscle UDPglucose and plasma glucose, though confirming the absence of net glycogenolysis, does not exclude the presence of active cycling through glycogen. In fact, under these conditions it is conceivable that the outer glucosyl units on the glycogen particles will have a specific activity similar to that of plasma glucose and their exchange with plasma-derived glucosyl units would be silent.

In summary, the portion of the skeletal muscle glucose-6-phosphate pool directly derived from plasma glucose can be calculated as the ratio of [^3H]UDPglucose and plasma [^3H]glucose specific activities. Similarly, the portion of the muscle glucose-6-phosphate pool derived from net glycogenolysis can be calculated as $1 - \text{the ratio } [\text{H}]\text{-UDPglucose sp act} / \text{plasma } [\text{H}]\text{-glucose sp act}$ (26, 33). As discussed above, this calculation may modestly overestimate the net contribution of glycogenolysis to the muscle G6P pool in the presence of low plasma insulin concentrations.

Glycogen formation in vivo. Muscle glycogen synthesis was quantitated by measuring the incorporation of [^3H]glucose counts into muscle glycogen divided by the [^3H]UDPglucose specific activity (26). Muscle glycogen concentration was determined after digestion with amyloglucosidase as previously described (31, 32). The intraassay and the interassay coefficients of variation (CV) were < 10% (at 0.250 g% tissue wt) when a liver or muscle homogenate was assayed as multiple aliquots. Aliquots of the tissue homogenate (100 μl) were employed to determine the amount of tritium label in glycogen. Glycogen was precipitated by washing in 10 vol of absolute ethanol and by incubating for 1 h at -20°C . The procedure was repeated three times and then the precipitate was collected, dried down, and dissolved in water before scintillation counting. The recovery of free [^3H]glucose, added to test the procedure, was < 1% of the free glucose radioactivity added to the homogenate in each assay. The glycogen synthetic rate was obtained by dividing the [^3H]glucose radioactivity in glycogen (dpm/g tissue) by the specific activity of [^3H]UDPglucose in the same tissue

sample (dpm/nmol). The rate of net glycogen synthesis is expressed as nmol of glucose in glycogen per gm of tissue.

Glycogen synthase. Muscle glycogen synthase activity was measured by a modification (32, 34, 35) of the method of Thomas et al. (36) and is based on the measurement of the incorporation of radioactivity into glycogen from UDP-[U- ^{14}C]glucose. Tissue samples (20–30 mg) were homogenized in 2.0 ml of Tris/HCl buffer, pH 7.8, containing 10 mmol/liter EDTA, 5 mmol/liter DTT, 50 mmol/liter NaF, and 2.5 g/liter rabbit liver glycogen Type III. The homogenate was centrifuged at 2,000 g for 15 min (at 4°C) and the supernatant used for glycogen synthase assay by measuring the incorporation of UDP-[U- ^{14}C]glucose into glycogen at 30°C . To approximate the in vivo conditions, synthase activity was measured in the presence of physiologic 0.11 mmol/liter glucose-6-P. Total glycogen synthase D activity was measured in the presence of 7.2 mmol/liter glucose-6-P. For the kinetic analysis, the assay was conducted at final concentrations of 0.003, 0.017, 0.033, 0.09, 0.33, and 1.4 mM UDPG; the data were linearized as Eadie-Hofstee plots and fit using linear regression. The K_m for UDPG is the reciprocal of the slope whereas $V_{\max}$ is the y -intercept divided by the slope. To approximate the in vivo glycolytic rates, incubations are also carried out in presence of the UDPG (5–50 μM) and G-6-P (0.11 and 0.25 mM) concentrations within the physiological range for rat muscle.

Glycogen phosphorylase. Muscle glycogen phosphorylase activity was measured as previously described (35). This assay is based on the measurement of the incorporation of ^{14}C into glycogen from labeled glucose-1-phosphate. Glycogen phosphorylase a , the active phosphorylated enzyme, was assayed in the absence of AMP, and phosphorylase b , the total enzyme activity, was assayed in the presence of 5 mM AMP. Tissue homogenates (20–30 mg) were prepared as described above. The supernatant was used for glycogen phosphorylase assay by measuring the incorporation of [^{14}C]glucose-1-phosphate into glycogen at 30°C in a mixture containing 33 mM Mes, 200 mM potassium fluoride, 0.45% mercaptoethanol, 15 mM glucose-1-P (50 $\mu\text{Ci}/\text{mmol}$), and 3.4 mg/ml glycogen. Phosphorylase b was assayed in the same manner except that the mixture contained 100 mM glucose-1-P (6 $\mu\text{Ci}/\text{mmol}$), 13.4 mg/ml glycogen, and 5 mM AMP.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Inc., Palo Alto, CA) and plasma insulin by radioimmunoassay using rat and porcine insulin standards. Plasma [^3H]glucose radioactivity was measured in duplicate on the supernatants of $\text{Ba}(\text{OH})_2$ and ZnSO_4 precipitates of plasma samples after evaporation to dryness to eliminate tri-

Table II. Skeletal Muscle UDP-Glucose Concentration and Specific Activity, Plasma Glucose Specific Activity, and Ratio of UDP-Glucose over Plasma Glucose Specific Activity (U/G sp act) at the End of Saline Infusions (Basal) and at the End of Euglycemic Clamp Studies Performed in the Presence of Increasing Plasma Insulin Concentrations in 24-h (Upper Panel) and 6-h (Lower Panel) Fasted Conscious Rats

	Insulin infusion rate						
	pmol/kg per min						
	Basal	1.5	3	6	12	24	108
UDP-Glucose, nmol/g	31.3 $\pm$ 3.7	29.8 $\pm$ 2.9	24.5 $\pm$ 1.2	19.6 $\pm$ 2.1	17.7 $\pm$ 1.5	20.0	17.6 $\pm$ 1.4
UDPG sp act, dpm/nmol	3.3 $\pm$ 1.1	4.1 $\pm$ 0.5	9.6 $\pm$ 1.8	19.3 $\pm$ 1.4	16.6 $\pm$ 1.7	13.1	11.3 $\pm$ 1.8
Glucose sp act, dpm/nmol	49.5 $\pm$ 7.9	47.2 $\pm$ 6.2	45.8 $\pm$ 1.1	37.1 $\pm$ 2.3	20.1 $\pm$ 3.0	14.4	12.0 $\pm$ 1.6
Ratio U/G sp act	0.07 $\pm$ 0.02	0.09 $\pm$ 0.01	0.21 $\pm$ 0.03	0.52 $\pm$ 0.03	0.83 $\pm$ 0.02	0.91	0.94 $\pm$ 0.02
	Basal	3	6	9	18	36	108
UDP-Glucose, nmol/g	32.6 $\pm$ 3.8	31.6 $\pm$ 2.2	24.0 $\pm$ 2.3	15.8 $\pm$ 2.6	11.9 $\pm$ 0.7	17.6 $\pm$ 1.7	14.9 $\pm$ 3.2
UDPG sp act, dpm/nmol	3.6 $\pm$ 1.2	4.1 $\pm$ 0.8	11.9 $\pm$ 2.2	19.6 $\pm$ 3.1	26.5 $\pm$ 2.7	19.8 $\pm$ 1.0	15.2 $\pm$ 1.4
Glucose sp act, dpm/nmol	44.0 $\pm$ 5.6	43.2 $\pm$ 4.3	42.3 $\pm$ 6.5	39.1 $\pm$ 2.1	31.1 $\pm$ 3.0	20.0 $\pm$ 2.2	15.3 $\pm$ 1.7
Ratio U/G sp act	0.08 $\pm$ 0.02	0.10 $\pm$ 0.01	0.29 $\pm$ 0.03	0.50 $\pm$ 0.03	0.85 $\pm$ 0.02	0.99 $\pm$ 0.02	0.99 $\pm$ 0.02

Values are mean $\pm$ SEM.

tiated water. Muscle glucose-6-phosphate concentrations were measured spectrophotometrically as described by Michal (37). Muscle glycogen was determined as previously described (31, 32). Muscle UDPglucose concentration and specific activity were obtained through two sequential chromatographic separations and UV detection, as previously reported (26, 33). Plasma lactate and muscle G-6-P were measured spectrophotometrically (38, 39). Plasma nonesterified fatty acids concentrations were determined by an enzymatic method with an automated kit according to the manufacturer's specifications (Waco Pure Chemical Industries, Osaka, Japan). Data for total body glucose uptake and suppression of hepatic glucose production represent the mean values during the last 30 min. The hepatic glucose production was calculated as the difference between the tracer derived rate of appearance and the infusion rate of glucose. All values are presented as the mean $\pm$ SEM. Comparisons between groups were made using repeated measures ANOVA where appropriate. Where F-ratios were significant, further comparisons were made using Student's *t* tests.

Results

General characteristics of the animals. The mean body wt was 303 ± 3 g for the 24-h fasted rats and 326 ± 5 g for the 6-h fasted animals. Both the plasma glucose (7.8 ± 0.1 vs. 5.6 ± 0.1 mM; $P < 0.01$) and insulin (204 ± 10 vs. 126 ± 6 pM; $P < 0.01$) concentrations were significantly higher in the 6-h fasted group compared to the control group.

Insulin clamp study (Table I; Figs. 1 and 2). In both groups the results obtained during the saline infusion studies and the combined somatostatin and insulin replacement infusions (1.5 and 3 pmol/kg per min, for the 24-h and 6-h fasted groups, respectively) were similar (Tables I–IV). The plasma glucose concentration was maintained at the basal level during the insulin clamp studies (Table I). Steady state plasma glucose concentrations during the studies were similar during the six insulin doses within the same group (Table I). The coefficients of variation in plasma glucose and insulin levels were less than 5 and 10%, respectively, in all studies. After hyperinsulinemia the absolute plasma FFA concentration (Table I) as well as the decrement in plasma FFA concentration below the correspondent basal level were significantly reduced at the lowest dose insulin clamp study in both 24-h (+64 pM insulin) and 6-h (+86 pM insulin) fasted rats. The plasma lactate concentration was significantly reduced during the lowest dose insulin clamp study and it raised significantly above basal levels with an increase in the plasma insulin concentration of 349 pM in the 24-h fasted group and of 335 pM in the 6-h fasted group (Table I). Fig. 1 shows the relationships between plasma insulin concentration and hepatic glucose production (A) and tissue glucose uptake (B) in 24-h fasted rats. Fig. 2 shows the relationships between plasma insulin concentration and hepatic glucose production (A) and tissue glucose uptake (B) in 6-h fasted rats. After 24-h fast, hepatic glucose production was significantly inhibited by 22% in response to an increment of 64 pM in the plasma insulin concentration; at this plasma insulin concentration tissue glucose uptake was not stimulated. Half-maximal suppression of hepatic glucose production was achieved with an increase in the plasma insulin concentration of 188 pM, while the half-maximal stimulation of tissue glucose uptake required increasing the plasma insulin levels by 543 pM. Similarly, after 6-h fast, hepatic glucose production was significantly inhibited by 34 and 47% at plasma insulin concentrations (276 and 355 pM, respectively) at which tissue glucose uptake was not significantly stimulated. Half-maximal sup-

pression of hepatic glucose production was achieved with an increase in the plasma insulin concentration of 125 pM, while the half-maximal stimulation of tissue glucose uptake required an increase in plasma insulin levels of 614 pM. In 24-h fasted animals, from a basal level of 38.9 ± 1.7 μ mol/kg per min, the hepatic glucose production decreased to 30.4 ± 2.1 , 24.2 ± 2.0 , 17.9 ± 2.1 , 4.8, and 3.9 ± 0.9 μ mol/kg per min and the tissue glucose uptake rose to 39.6 ± 1.3 , 66.6 ± 4.7 , 98.1 ± 4.7 , 156.3, and 214.7 ± 11.9 μ mol/kg per min. In 6-h fasted animals, from a basal level of 69.3 ± 1.9 μ mol/kg per min, the hepatic glucose production decreased to 45.7 ± 3.2 , 36.5 ± 3.0 , 24.2 ± 3.5 , 15.1 ± 3.4 , and 8.5 ± 1.6 μ mol/kg per min and the tissue glucose

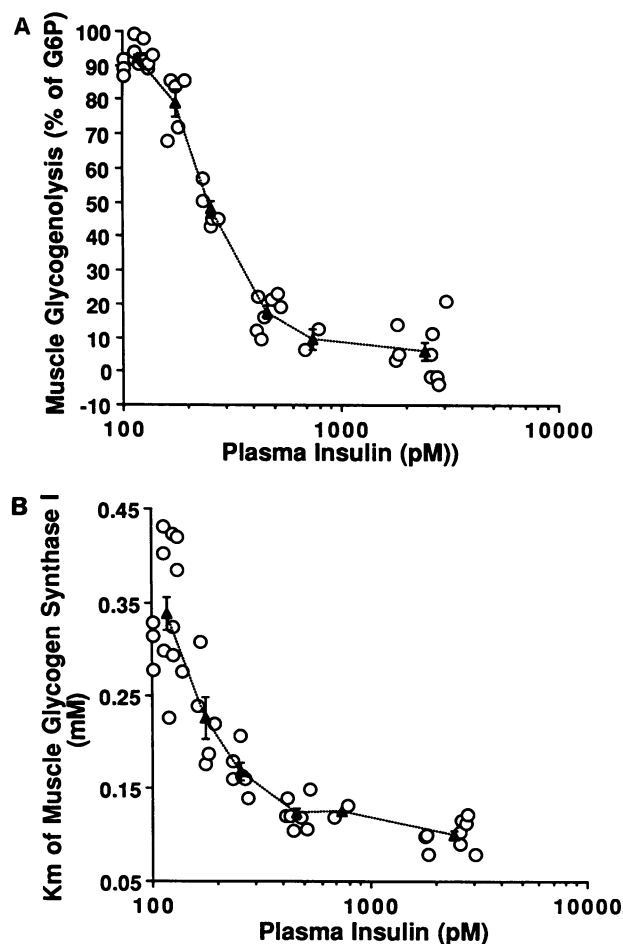


Figure 5. Dose-response for the effects of increasing plasma insulin concentrations on the percent contribution of net skeletal muscle glycogenolysis to the G6P pool (upper panel) and the K_m for UDPglucose of the muscle glycogen synthase (lower panel) in 24-h fasted rats. The portion of the muscle glucose-6-phosphate pool derived from net glycogenolysis was calculated as $1 - \frac{[^3\text{H}]\text{-UDPglucose sp act}}{[^3\text{H}]\text{-glucose sp act}}$. Since the results obtained during the saline infusion studies and the combined somatostatin and insulin replacement infusions (1.5 pmol/kg per min) were similar (Tables I–IV) they are graphically presented in relation to the circulating insulin concentrations. The net rate of muscle glycogenolysis and the K_m of the glycogen synthase (in the presence of 0.11 mM G6P) were significantly ($P < 0.01$) decreased in response to an increase in the plasma insulin concentration as low as 57 pM. Insulin achieved its maximal effect on these parameters with an increment in the plasma concentrations of 342 pM.

uptake rose to 71.2 ± 2.2 , 83.3 ± 4.4 , 123.2 ± 7.4 , 167.6 ± 8.3 , and 234.5 ± 9.6 $\mu\text{mol/kg}$ per min.

Muscle glycogen synthesis (Figs. 3 A and 4 A). The fasting muscle glycogen concentration was higher in 6-h fasted compared to 24-h fasted rats (46.4 ± 4.6 vs. 31.4 ± 2.3 $\mu\text{mol/g}$ wet wt).

At the end of the first two low dose insulin clamp studies the muscle glycogen concentration was not significantly increased in both 6-h (44 ± 3.9 and 47 ± 4.6 $\mu\text{mol/g}$ wet wt) and 24-h fasted rats (31.9 ± 1.7 and 32.9 ± 2.1 $\mu\text{mol/g}$ wet wt) compared to basal levels. In response to the three higher insulin infusions, muscle glycogen concentration was significantly increased in 6-h fasted (54.9 ± 4.3 , 65.3 ± 3.9 , and 84.2 ± 4.7 $\mu\text{mol/g}$ wet wt) rats. In the 24-h fasted rats, the muscle glycogen concentration was significantly increased above basal levels at the end of the highest insulin infusion (56.8 ± 3.9 $\mu\text{mol/g}$ wet wt). However, the measurement of the increment in muscle glycogen concentration may not represent a highly sensitive assay at low rates of net muscle glycogen synthesis. Thus, the rates of net muscle glycogen synthesis were assessed at the end of the insulin clamp studies by dividing the radioactivity accumulated in isolated muscle glycogen by the specific activity of UDPglucose in the same tissue sample (Figs. 3 A and 4 A). During the two lowest insulin doses, there was no increment in muscle glycogen synthesis above basal level in both groups. The first significant increase in the net rates of glycogen synthesis were measured at 525 pM insulin in the 6-h fasted rats (76.5 ± 7.6 vs. 9.2 ± 1.9 nmol/g wet wt per min) and at 460 pM insulin in the 24-h fasted rats (28.3 ± 2.5 vs. 4.0 ± 0.6 nmol/g wet wt per min).

Muscle glucose-6-phosphate concentration (Figs. 3 B and 4 B). Skeletal muscle glucose-6-phosphate concentration was significantly decreased at low plasma insulin concentrations and increased during the highest insulin clamp studies, in both 24-h and 6-h fasted animals. Particularly, in 24-h fasted rats, the muscle glucose-6-phosphate levels were significantly decreased

by increasing the plasma insulin concentration by either 64 pM (0.157 ± 0.014 vs. 0.257 ± 0.022 $\mu\text{mol/g}$ wet wt) and by 142 pM (0.147 ± 0.012 $\mu\text{mol/g}$ wet wt). In 6-h fasted animals, the muscle glucose-6-phosphate concentration significantly declined in response to an increment in the plasma insulin concentration of 86 pM (0.245 ± 0.017 vs. 0.337 ± 0.028 $\mu\text{mol/g}$ wet wt) and returned toward basal level by further increasing the plasma insulin concentration by 165 pM (0.318 ± 0.014 $\mu\text{mol/g}$ wet wt). In both groups, further increases in the circulating insulin levels lead to a progressive increase in the skeletal muscle glucose-6-phosphate concentration.

Contribution of glycogenolysis to muscle UDPglucose in vivo (Figs. 5 A and 6 A; Table II). Under postabsorptive or fasting conditions, the intracellular UDPglucose specific activity in skeletal muscle was much lower than the plasma glucose specific activity, suggesting that the majority of the skeletal muscle UDPglucose was derived from glycogen, while only a small portion, $\sim 10\%$, was derived from plasma glucose (Table II). However, with increasing insulin levels, the specific activity of muscle UDPglucose and plasma glucose progressively came together, suggesting increasing inhibition of net glycogenolysis. A significant inhibition of net glycogenolysis was detected in response to minimal increments in the plasma insulin concentrations ($+64$ – $+86$ pM) in both 24-h and 6-h fasted rats. Figs. 4 A and 5 A display the high sensitivity of this process to insulin, with an almost complete equilibration between plasma glucose and muscle UDPglucose specific activities achieved with an increase in the plasma insulin concentrations of ~ 300 pM. In a subgroup of rats the relation between the muscle UDPglucose and the plasma glucose specific activity was examined after 60 min of moderate hyperinsulinemia to verify the achievement of steady state for the two specific activities at the end of the in vivo studies. In 24-h fasted rats, the ratio between UDPglucose and plasma glucose specific activities was similar during the infusion of 3 pmol/kg per min insulin at 60

Table III. K_m and V_{max} for the Skeletal Muscle Glycogen Synthase at the End of Saline Infusions (Basal) and at the End of Euglycemic Clamp Studies in the Presence of Physiologic (0.11 mM) and Saturating (7.2 mM) Concentrations of G6P

G-6-P	Insulin infusion rate						
	Basal	1.5	3	6	12	24	108
pmol/kg per min							
0.11 mM:							
K_m , mM	0.36 ± 0.04	0.32 ± 0.02	0.23 ± 0.02	0.17 ± 0.01	0.12 ± 0.01	0.12	0.10 ± 0.01
V_{max} , $\mu\text{mol/g}$ wet wt per min	0.42 ± 0.07	0.47 ± 0.03	0.43 ± 0.02	0.40 ± 0.05	0.42 ± 0.05	0.53	0.45 ± 0.06
7.2 mM:							
K_m , mM	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04	0.04 ± 0.01
V_{max} , $\mu\text{mol/g}$ wet wt per min	0.44 ± 0.05	0.49 ± 0.03	0.41 ± 0.03	0.42 ± 0.04	0.42 ± 0.04	0.50	0.48 ± 0.05
G-6-P	Basal	3	6	9	18	36	108
0.11 mM:							
K_m , mM	0.30 ± 0.02	0.28 ± 0.01	0.21 ± 0.01	0.15 ± 0.01	0.13 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
V_{max} , $\mu\text{mol/g}$ wet wt per min	0.68 ± 0.07	0.72 ± 0.06	0.72 ± 0.09	0.81 ± 0.05	0.62 ± 0.05	0.63 ± 0.07	0.72 ± 0.07
7.2 mM:							
K_m , mM	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
V_{max} , $\mu\text{mol/g}$ wet wt per min	0.68 ± 0.07	0.68 ± 0.04	0.71 ± 0.07	0.79 ± 0.06	0.64 ± 0.06	0.62 ± 0.06	0.69 ± 0.06

Insulin clamps were performed at increasing plasma insulin concentrations in 24-h (upper panel) and 6-h (lower panel) fasted conscious rats. Values are mean $\pm$ SEM.

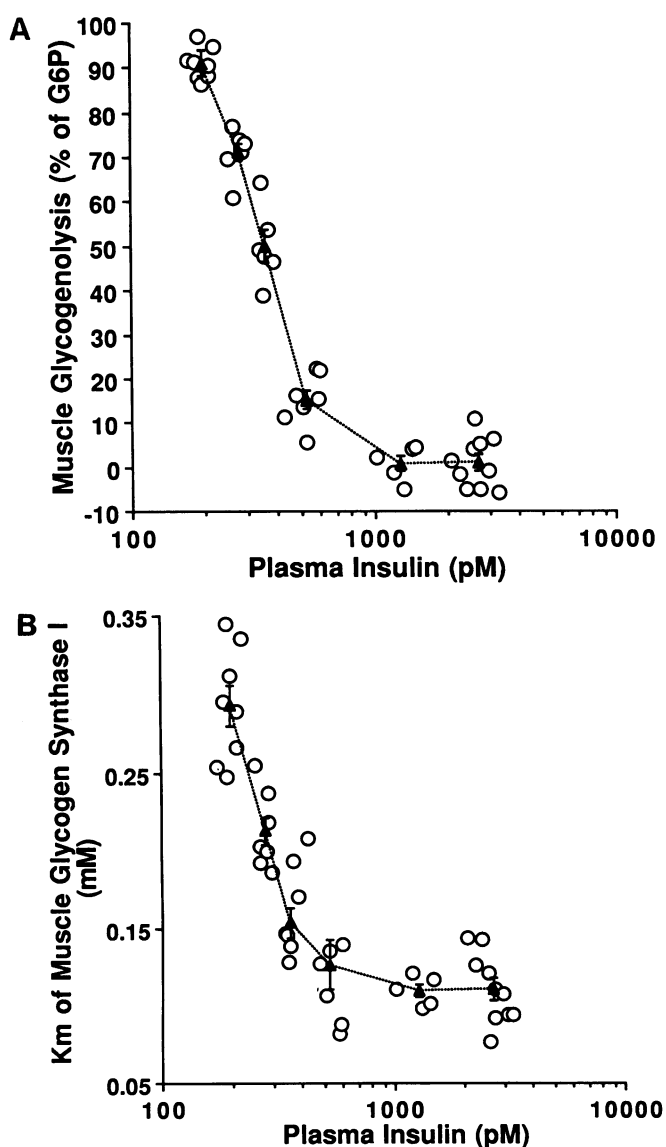


Figure 6. Dose-response for the effects of increasing plasma insulin concentrations on the percent contribution of net skeletal muscle glycogenolysis to the G6P pool (*upper panel*) and the K_m for UDPglucose of the muscle glycogen synthase (*lower panel*) in 6-h fasted rats. The portion of the muscle glucose-6-phosphate pool derived from net glycogenolysis was calculated as $1 - \frac{[^3\text{H}]\text{-UDPglucose sp act}}{\text{plasma } [^3\text{H}]\text{-glucose sp act}}$. Since the results obtained during the saline infusion studies and the combined somatostatin and insulin replacement infusions (3 pmol/kg per min) were similar (Tables I-IV) they are graphically presented in relation to the circulating insulin concentrations. The net rate of muscle glycogenolysis and the K_m of the glycogen synthase (in the presence of 0.11 mM G6P) were significantly ($P < 0.01$) decreased in response to an increase in the plasma insulin concentration as low as 78 pM. Insulin achieved its maximal effect on these parameters with an increment in the plasma concentrations of 327 pM.

min (0.23 ± 0.04) and at 120 min (0.21 ± 0.03). Similarly, during the infusion of 6 pmol/kg per min insulin in 6-h fasted rats, the ratio UDPglucose and plasma glucose specific activities was comparable at 60 min (0.26 ± 0.05) and at 120 min (0.29 ± 0.03).

Kinetic analysis of muscle glycogen synthase (Figs. 5 B and 6 B; Table III). Insulin exerts most of its short-term effects on skeletal muscle glycogen synthase by decreasing the enzyme's phosphorylation, which in turn leads to increased affinity for its substrate, i.e., decreased K_m for UDPglucose. Thus, the effect of increasing plasma insulin concentrations on the K_m of the skeletal muscle glycogen synthase assayed in the presence of physiologic glucose-6-phosphate concentrations is displayed in Figs. 5 B and 6 B. In both experimental groups, insulin caused a progressive activation of the enzyme, which reached its maximum at ~ 500 pM plasma insulin. A significant decrease in K_m was detected with minimal increments in the plasma insulin concentrations, i.e., +64 pM and +86 pM for the 24-h and 6-h fasted rats, respectively. The EC_{50} for the effect of insulin on muscle glycogen synthase K_m was 209 and 298 pM for the 24-h and 6-h fasted rats, respectively. Kinetic analysis of individual dose-response curves (Table IV) revealed that insulin decreased the K_m for UDPG, but did not change the V_{max} . To evaluate the impact of the increased muscle G-6-P concentration on the K_m of the glycogen synthase, the kinetic analysis of the enzyme was also performed in the presence of 0.25 mM G-6-P in tissue samples obtained at the end of the 12 pmol/kg per min insulin clamp studies in 24-h fasted rats. The increase in the G-6-P concentration from 0.11 to 0.25 mM caused a significant decline in the K_m for UDPglucose from 0.12 ± 0.01 mM to 0.07 ± 0.01 mM.

Muscle glycogen phosphorylase (Table IV). The effect of increasing plasma insulin concentrations on glycogen phosphorylase activity is shown in Table IV. Phosphorylase *a* and *b* activities were similar at all the plasma insulin concentrations examined in both groups.

Correlations (Figs. 7 and 8). In both groups (24-h and 6-h fasted rats), the effect of insulin on skeletal muscle glycogenolysis was correlated to the effect on the hepatic glucose production and muscle glycogen synthase.

Discussion

The present studies were undertaken to define the dose-response relationship between insulin action on hepatic glucose production and several parameters of skeletal muscle glucose metabolism. Our results confirm the finding (6, 7, 22, 24, 32) that the hepatic glucose production is more sensitive to insulin than is the peripheral glucose uptake. However, we found that the sensitivity of skeletal muscle glycogenolysis and glycogen synthase to insulin is much higher than that of glucose uptake and similar to that of hepatic glucose production. The activation of the skeletal muscle glycogen synthase by insulin paralleled, and was highly correlated to, the insulin-mediated inhibition of net muscle glycogenolysis and this effect was submaximal at plasma insulin concentrations that did not stimulate net muscle glycogen synthesis. This observation indicates that the major role of the insulin's activation of the skeletal muscle glycogen synthase at low physiological plasma insulin levels is to limit net glycogenolysis and that this is an exquisitely sensitive insulin action.

The relationship between circulating plasma insulin concentration and skeletal muscle glycogenolysis has not been previously examined. However, the effect of euglycemic insulin infusions on skeletal muscle glycogen synthase has been extensively examined in both humans (2, 4, 8, 12, 13, 40, 41) and animals (31, 34, 35, 42-46). Although the great majority of the

Table IV. Activity of AMP-independent and AMP-dependent Forms of the Skeletal Muscle Glycogen Phosphorylase at the End of Saline Infusions (Basal) and at the End of Euglycemic Clamp Studies Performed in the Presence of Increasing Plasma Insulin Concentrations in 24-h (Upper Panel) and 6-h (Lower Panel) Fasted Conscious Rats

	Insulin infusion rate						
		pmol/kg per min					
AMP	Basal	1.5	3	6	12	24	108
Independent form, μmol/g wet wt per min	14.6±3.3	13.1±2.4	16.9±3.4	11.5±3.3	13.6±3.2	15.6±2.0	16.7±1.8
Dependent form, μmol/g wet wt per min	65.4±6.9	70.5±5.5	77.8±7.3	61.8±7.1	64.5±7.3	66.3±3.1	69.3±7.7
AMP	Basal	3	6	9	18	36	108
Independent form, μmol/g wet wt per min	14.6±4.2	16.2±3.5	17.2±4.4	17.0±3.3	12.9±3.2	17.6±3.1	15.5±1.8
Dependent form, μmol/g wet wt per min	90.2±12.7	81.8±13.9	89.3±7.3	98.7±10.1	81.5±9.6	86.3±5.5	89.3±10.7

Values are mean±SEM.

previous studies focused on the effect of high physiological or pharmacological increments in the plasma insulin concentrations, Mandarino et al. (41) demonstrated that the skeletal muscle glycogen synthase was significantly activated at plasma insulin concentrations as low as 132 pM (+90 pM from basal insulin levels) in normal subjects. Perhaps more importantly, the same authors recognized the apparent discrepancy between the stimulation (~ 60%) of the muscle glycogen synthase by insulin and the lack of insulin's stimulation of nonoxidative glucose metabolism in response to this modest increase in the circulating insulin concentrations. However, the rate of nonoxidative glucose metabolism, as measured by indirect calorimetry, includes other components beside glycogen synthesis, and a direct assessment of muscle glycogen synthesis was not performed in that study. Thus, the insulin's stimulation of net muscle glycogen synthesis may have not been detected due to a concomitant reduction in other components of nonoxidative glucose metabolism, e.g., lactate formation.

In the present study, an increase in the plasma insulin concentration of ~ 150 pM was unable to significantly stimulate the net rate of muscle glycogen synthesis in both 6-h and 24-h fasted rats. This was demonstrated by the tracer incorporation in isolated muscle glycogen (shown in Figs. 3 A and 4 A) and confirmed by the lack of increase in the muscle glycogen concentrations. This observation is consistent with the finding in humans of a lack of insulin-mediated stimulation of the non-oxidative pathways of glucose disposal up to ~ 200 pM (41, 47). However, in response to these modest increments in the plasma insulin concentrations the skeletal muscle glycogen synthase activity was significantly stimulated and the intracellular specific activity of the exose-phosphates was promptly and progressively equilibrating with the extracellular glucose specific activity, thus suggesting the inhibition of net muscle glycogenolysis by insulin.

Muscle glucose-6-phosphate levels were decreased in response to an increment in the plasma insulin concentration of ~ 100 pM. This decline appears to be the consequence of the unbalance between the insulin's effects on muscle glycogenolysis and perhaps glycolysis, and the lack of significant activation

of glucose transport/phosphorylation. Furthermore, the decrease in both glucose-6-phosphate and UDP-glucose concentrations may contribute to the lack of net glycogen deposition despite significant activation of glycogen synthase at these low insulin concentrations. Together our results suggest that insulin's stimulation of glucose uptake and subsequent rise in the skeletal muscle glucose-6-phosphate concentration toward basal or suprabasal levels are necessary for the onset of net glycogen synthesis in skeletal muscle.

The concentration of muscle glucose-6-phosphate may also help to elucidate an additional discrepancy between the effect of insulin on muscle glycogen synthase and net glycogen synthesis. The insulin's activation of muscle glycogen synthase was almost maximal at ~ 500 pM plasma concentration in both 6- and 24-h fasted animals. However, the net rate of muscle glycogen synthesis was progressively and markedly increased by further elevations in the plasma insulin concentration from ~ 500 to ~ 2500 pM. The muscle glucose-6-phosphate concentration returned to approximately the basal levels at ~ 500 pM and progressively increased at higher plasma insulin concentrations. It is tempting to speculate that, in the presence of a fully activated glycogen synthase, the increase in the muscle glucose-6-phosphate concentration modulates the in vivo rate of glycogenesis. Consistent with this hypothesis, a > 40% decrease in the K_m of muscle glycogen synthase was demonstrated in vitro by increasing the G-6-P concentration from 0.11 to 0.25 mM. In both experimental groups, the response of skeletal muscle glucose-6-phosphate to insulin was biphasic, with a decrement at the lower insulin concentrations followed by a return to basal level at the intermediate insulin dose and a significant increment above basal at the maximal insulin level. This pattern suggests that, at the lower insulin concentrations, glucose uptake, i.e., transport and phosphorylation, is not significantly stimulated by insulin. This, in turn, contributes to the drop in the glucose-6-phosphate concentration and prevents the onset of net muscle glycogen synthesis. Once glucose uptake is stimulated, the glucose-6-phosphate concentration begins to rise and muscle glycogen synthesis can occur. Although the dose-dependent insulin's suppression of net skeletal muscle glycogenol-

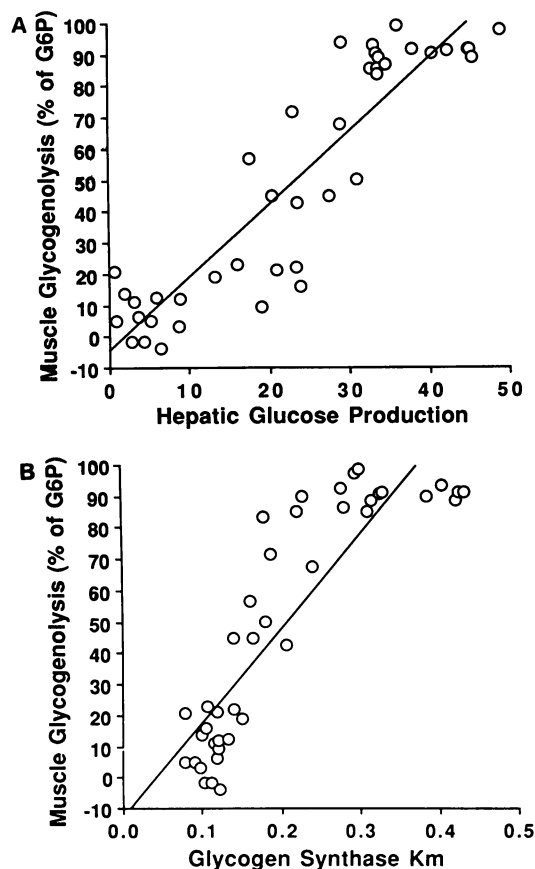


Figure 7. Correlation between the percent contribution of net skeletal muscle glycogenolysis to the G6P pool and the hepatic glucose production (*upper panel*) and the K_m for UDPglucose of the muscle glycogen synthase (*lower panel*) in 24-h fasted rats. The percent contribution of net skeletal muscle glycogenolysis to the G6P pool was positively correlated with the rate of hepatic glucose production ($r = 0.827$) and with the K_m of the muscle glycogen synthase (in the presence of 0.11 mM G6P) ($r = 0.767$).

ysis was highly correlated with the hormone's activation of glycogen synthase, there was no detectable effect of insulin on glycogen phosphorylase. This finding is consistent with previous *in vivo* studies (35, 48) and appears to indicate that the activation of glycogen synthase plays the major role in insulin's inhibition of net muscle glycogenolysis. However, these results should be cautiously interpreted, since the *in vitro* assay of glycogen phosphorylase may not reflect its *in vivo* activity (49) and an additional effect of insulin on the *in vivo* activity of glycogen phosphorylase cannot be ruled out.

In the present study the insulin-dependent inhibition of skeletal muscle glycogenolysis was highly correlated with the insulin's inhibition of hepatic glucose production. Furthermore, the EC_{50} for the effects of insulin on hepatic glucose production and skeletal muscle glycogenolysis were similar. The reproducibility of these findings under different metabolic and nutritional conditions strengthens this conclusion. Thus, some of the *in vivo* peripheral and hepatic effects of the hormone follow similar dose-response characteristics. Recent evidence has indicated that the ability of insulin to inhibit hepatic glucose production *in vivo* is partially due to its extrahepatic effects (20–24). Particularly, several studies have shown that

the intraportal and the peripheral delivery of insulin were similarly effective in suppressing hepatic glucose production (21, 23, 50–52). Consistent with this hypothesis, Bradley et al. (24) have demonstrated that the dynamics of the hepatic and peripheral effects of insulin are identical and suggest a common rate-limiting step for hepatic and peripheral *in vivo* insulin action. The present study indicates that the skeletal muscle sensitivity to insulin may be higher than previously reported and that the most sensitive action of insulin in skeletal muscle, the inhibition of net glycogenolysis, is as sensitive to insulin as is the hepatic glucose production. Several factors may contribute to the postulated "indirect" effects of insulin in regard to the suppression of hepatic glucose production. Modest increments in the peripheral insulin concentrations have been shown to inhibit the plasma glucagon concentration, which is a major determinant of the rate of hepatic glucose output in the presence of low insulin concentrations (53, 54). An effect of insulin on the CNS may also affect the hepatic glucose output through changes in the firing of hepatic sympathetic and parasympathetic innervation (55). Finally, the hormone may regulate the supply of energy and key substrates to the liver (25, 28). Such

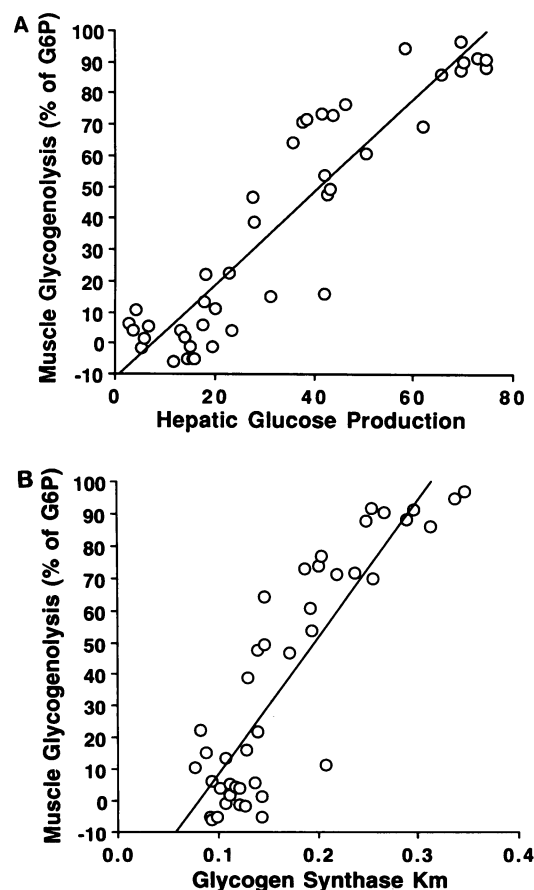


Figure 8. Correlation between the percent contribution of net skeletal muscle glycogenolysis to the G6P pool and the hepatic glucose production (*upper panel*) and the K_m for UDPglucose of the muscle glycogen synthase (*lower panel*) in 6-h fasted rats. The percent contribution of net skeletal muscle glycogenolysis to the G6P pool was positively correlated with the rates of hepatic glucose production ($r = 0.847$) and with the K_m of the muscle glycogen synthase (in the presence of 0.11 mM G6P) ($r = 0.774$).

an effect may be particularly important, in combination with direct hormonal signals, for the inhibition of hepatic gluconeogenesis. Under postabsorptive conditions a large portion of lactate and alanine carbons are derived from skeletal muscle glycogenolysis (25), thus, the exquisite sensitivity of this process to insulin may suggest its role in decreasing the supply of gluconeogenic precursors to the liver. However, it is important to point out that the correlation between the effects of insulin on skeletal muscle glycogenolysis and hepatic glucose production was observed in both 24-h fasted rats, which are depleted of hepatic glycogen and largely depend on gluconeogenesis for sustaining their hepatic glucose output, and in 6-h fasted animals, which equally rely on hepatic gluconeogenesis and glycogenolysis. Therefore, the proposed peripheral signal to the liver has to involve a concomitant effect on hepatic gluconeogenesis and glycogenolysis. It may be tempting to speculate that one or more of the substrates that are affected by the peripheral effects of insulin may also contribute to the modulation of hepatic glycogen metabolism (56).

This study may also be relevant to the interpretation of commonly used whole body tracer methodology. In fact, several tracer approaches to the study of glucose and lactate turnover and oxidation assume a complete equilibrium between intracellular and extracellular specific activities (32, 57, 58). For skeletal muscle, the source of intracellular dilution of specific activity at the level of the exose-phosphate pool is the preexisting unlabeled glycogen. With an elegant experimental approach in humans, Butler and co-workers (58) suggested that high physiological to pharmacological insulin concentrations were required for the complete equilibration of the intracellular specific activity of glucose-6-phosphate with the plasma glucose specific activity. The present observation provides experimental support for the notion that under basal insulin conditions a large portion of the intracellular exose-phosphates is derived from glycogen rather than plasma glucose (58). However, it indicates that, at least in conscious rats, the equilibration of the extracellular and intracellular specific activity is very sensitive to insulin, since it occurs in response to a modest physiological increment in the plasma insulin concentrations.

In summary, the present data demonstrate that insulin suppresses in vivo hepatic glucose production and skeletal muscle glycogenolysis with virtually identical sensitivity. The insulin-mediated suppression of skeletal muscle glycogenolysis is highly correlated to its activation of glycogen synthase and contributes to the drop in the skeletal muscle glucose-6-phosphate concentration. The sensitivity to insulin of both Rd and muscle glycogen synthesis is much lower than that of muscle glycogenolysis. Since net muscle glycogen synthesis does not initiate despite submaximal insulin's activation of glycogen synthase, it is suggested that at low plasma insulin concentration glycogen synthesis in skeletal muscle is limited by glucose transport or phosphorylation.

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