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Research Article

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Regulation of Glycogen Synthase and Phosphorylase Activities by Glucose and Insulin in Human Skeletal Muscle

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Abstract

We examined the insulin dose-response characteristics of human muscle glycogen synthase and phosphorylase activation. We also determined whether increasing the rate of glucose disposal by hyperglycemia at a fixed insulin concentration activates glycogen synthase.

Physiological increments in plasma insulin but not glucose increased the fractional activity of glycogen synthase. The ED₅₀:s for insulin stimulation of whole body and forearm glucose disposal were similar and unaffected by glycemia. Glycogen synthase activation was exponentially related to the insulin-mediated component of whole body and forearm glucose disposal at each glucose concentration. Neither insulin nor glucose changed glycogen phosphorylase activity.

These results suggest that insulin but not the rate of glucose disposal per se regulates glycogen synthesis by a mechanism that involves dephosphorylation of glycogen synthase but not phosphorylase. This implies that the low glycogen synthase activities found in insulin-resistant states are a consequence of impaired insulin action rather than reduced glucose disposal.

Introduction

The stimulation of glucose disposal is one of the most characteristic actions of insulin. Muscle tissue plays a quantitatively dominating role in the disposal of glucose by insulin (1, 2). Insulin stimulates both glucose oxidation and nonoxidative glucose disposal, which presumably largely consists of storage as muscle glycogen (1, 3–5). Net glycogen synthesis is determined by the balance of glycogen synthase and phosphorylase activities and by the rate of the glucose flux not entering glycolysis (6). In insulin-resistant subjects, the fractional activity of glycogen synthase is low and correlates with the rates of glucose disposal (3). In rat muscle, the fractional activity of glycogen synthase can be increased by increasing the glucose-6-phosphate (G-6-P)¹ content, which dephosphorylates the G-6-P dependent (D) form of glycogen synthase by increasing the activity of glycogen phosphatase (7, 8). It has also been demonstrated in isolated rat hemidiaphragms that an increase in the extracellular glucose concentration leads to elevated G-6-P concentrations in muscle (9). Therefore, it is possible that the fractional activity of glycogen

synthase in human muscle could be determined by the flux of glucose into the cell or by insulin's ability to stimulate the fractional activity of the enzyme (10). However, whether the fractional activity of glycogen synthase responds to changes in the glucose flux in vivo in human skeletal muscle is not known.

Breakdown of glycogen is limited by the activity of glycogen phosphorylase. The enzyme exists in two forms, designated as phosphorylase *a* and phosphorylase *b*, which both can stimulate glycogenolysis. In resting muscle under aerobic conditions, phosphorylase *a* is more active than *b* and thus the form mainly responsible for glycogenolysis under most circumstances (11). In isolated rat diaphragm, phosphorylase *a* activity, like that of glycogen synthase, can be modulated by G-6-P and by glucose (12). In contrast, insulin either alone (13–15) or after elevation of basal phosphorylase activity by epinephrine (13–15) has not been found to change the activity of the enzyme in rat diaphragm (15) or heart muscle (13, 14). The role of glucose and insulin in the regulation of human skeletal muscle phosphorylase activities has not been studied.

In the present study we determined the insulin dose-response characteristics of glycogen synthase activation at different levels of glycemia and its relationship to whole body glucose disposal and glucose disposal across forearm muscle in normal volunteers. We also studied whether glycogen phosphorylase activity is influenced by short-term exposure to varying combinations of glycemia and insulinemia in vivo.

Methods

Subjects and study protocol

22 male Caucasian volunteers were admitted to the metabolic ward of the Clinical Diabetes and Nutrition Section for 15 d. After written informed consent, all subjects were physically examined and a 12 lead electrocardiogram recorded. After an overnight fast, blood was drawn for complete blood count, liver function tests, blood-urea nitrogen, creatinine, electrolytes, calcium, total protein, and albumin. None of the subjects was taking any medications and all had a normal physical examination, electrocardiogram, and blood tests. After 3 d on a weight-maintaining diet containing at least 200 g of carbohydrate per day, a 3-h oral glucose tolerance test (16) was performed. The percent body fat of each volunteer was determined by underwater weighing with correction for the simultaneously measured residual lung volume by helium dilution (17).

A total of 88 glucose disposal measurements were performed at four different glucose concentrations in the 22 subjects. In each subject, glucose disposal was measured on four separate days (days 5, 8, 11, and 14 from admission) at the same insulin infusion rate at plasma glucose concentrations of 90, 160, 250, and 400 mg/dl. The order of studies at the different glucose levels was randomized. Insulin was infused at rates of 0 ($n = 6$), 20 ($n = 5$), 60 ($n = 6$), and 400 ($n = 5$) mU/m²·min for 120 min. The subgroups differing with respect to the insulin infusion rate were matched for age, body weight, and composition, and for glucose and insulin concentrations (Table I). Glucose tolerance was normal in each subject according to National Diabetes Data Group criteria (16).

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1. Abbreviations used in this paper: G-6-P, glucose-6-phosphate; R_a , appearance rate; R_d , disappearance rate.

Table I. Characteristics of the Subjects

	Insulin infusion rate (mU/m ² · min)			
	0	20	60	400
n	6	5	6	5
Age (yr)	27±2	28±2	29±2	28±2
BMI (kg/m ²)	23.1±1.4	23.8±1.1	21.7±1.1	21.2±1.0
% fat (%)	15±4	17±3	17±3	14±3
Fasting glucose (mg/dl)	91±2	86±4	90±2	92±2
Fasting insulin (μU/ml)	16±2	14±1	15±2	18±2

Results are expressed as means±SEM.

Glucose disposal measurements

After an overnight fast three catheters were inserted. Catheter 1 was placed in an antecubital vein for infusion of glucose, somatostatin, [³H]glucose and insulin. Catheter 2 was inserted in an ipsilateral heated dorsal hand vein for sampling of arterialized venous blood (18). The use of a heated superficial hand vein as a replacement for an artery has been previously validated for measurement of glucose kinetics in man (18, 19). Catheter 3 was retrogradely inserted into the contralateral arm in the deep branch of the median cubital vein for sampling of blood draining the forearm muscle (20). The insulin infusions (porcine monocomponent insulin, Nordisk-USA, Bethesda, MD) were given in a primed continuous manner as previously described (3, 21). Somatostatin (Sigma Chemical Co., St. Louis, MO) was infused in all studies for 120 min at a rate of 500 μg/h to suppress endogenous insulin secretion. Plasma glucose was adjusted to the desired concentration within 0 to 30 min from start of the insulin infusion using a variable rate 20% infusion of glucose based on plasma glucose determinations (22) every 2.5 to 5 min (21). Plasma insulin concentrations were measured by radioimmunoassay using Herbert's modification (23) in arterialized venous blood samples taken at 0, 30, 60, 90, and 120 min. The [³-H]glucose infusion was given as a bolus (30 μCi) followed by 0.3 μCi/min. The mean plasma glucose and insulin concentrations attained in the different studies are shown in Table II.

Whole body glucose disposal. The appearance rate of glucose (R_a) in the plasma was calculated from the blood [³-H]glucose specific activities (24) using Steele's equations (25, 26). Steady state values over the last 30 min of each study were used for statistical analyses. In the group, which received no insulin but only somatostatin and glucose, endogenous R_a was suppressed by 22±9, 40±11, 47±5, and 83±6% at glucose concentrations of 90, 160, 250, and 400 mg/dl, respectively. Complete suppression of endogenous R_a occurred at the insulin infusion rate of 20 mU/m² · min at all glucose levels. At this insulin infusion rate, the rates of glucose disappearance (R_d 's) for glucose were comparable as determined either from the infusion rate of exogenous glucose, or from glucose specific activities using Steele's steady-state (25) or nonsteady state (26, pool fraction 0.65) equations (3.1±0.3 vs. 2.8±0.3 vs. 2.7±0.6 mg/kg body weight (BW)/min at 90 mg/dl, 5.3±0.5 vs. 4.7±0.5 vs. 4.9±0.5 at 160 mg/dl, 5.8±0.7 vs. 5.0±0.4 vs. 5.1±0.7 mg/kg BW per min at 250 mg/dl and 8.2±2.0 vs. 8.8±1.1 vs. 7.8±1.0 at 400 mg/dl, for R_d 's calculated from glucose infusion rates, steady vs. nonsteady state equations, respectively). At higher rates of glucose disposal, the isotopically determined R_d 's became negative, as reported by others (27, 28). The actual glucose infusion rate was used as the measure of total R_d in all groups that received an insulin infusion. Total R_d was then corrected for urinary glucose loss to reflect the actual R_d by tissues.

Forearm glucose disposal. Total forearm glucose disposal (milligrams per deciliter forearm tissue per minute) was determined by multiplying total forearm blood flow (milliliters per deciliter forearm per minute) times the [arterialized venous blood - deep venous blood] - difference (mg/ml) for glucose. Forearm blood flow was measured using capacitance

Table II. Plasma Glucose and Insulin Levels during the Studies

Glucose (goal)	Insulin infusion rate (mU/m ² · min)			
	0	20	60	400
90 mg/dl SSPG (mg/dl)	91±2	90±1	89±1	90±1
SSPG-CV* (%)	3±1	2±1	3±1	2±1
SSPI (μU/ml)	7±2	45±1	170±14	1,710±251
SSPI-CV (%)†	15±6	4±1	9±3	3±1
160 mg/dl SSPG (mg/dl)	160±2	159±2	157±3	161±1
SSPG-CV (%)	3±1	3±1	4±1	2±1
SSPI (μU/ml)	7±1	47±2	165±9	2,018±184
SSPI-CV (%)	20±5	7±3	10±3	2±2
250 mg/dl SSPG (mg/dl)	259±3	253±4	247±4	255±3
SSPG-CV (%)	2±1	2±1	2±1	3±1
SSPI (μU/ml)	11±2	48±4	162±10	1,800±241
SSPI-CV (%)	7±5	10±4	15±4	4±1
400 mg/dl SSPG (mg/dl)	402±5	431±6	405±11	424±10
SSPG-CV (%)	2±1	2±1	2±1	4±1
SSPI (μU/ml)	10±2	48±4	169±10	1,731±285
SSPI-CV (%)	28±6	5±2	10±2	8±1

* Coefficient of variation of plasma glucose.

† Coefficient of variation of serum insulin, (mean±SEM of 30, 60, 90 and 120 min values).

plethysmography (model 2560, UFI, Morro Bay, CA). The change in voltage induced by venous occlusion was compared to the voltage induced by injection of a standard volume into the forearm. Forearm volume was determined by water displacement. Arterialized venous and deep venous blood samples for measurement of plasma glucose and glucose specific activities were obtained simultaneously and at 90, 100, 110, and 120 min in each study. Plasma values were converted to whole blood values by multiplying the plasma value by (1 - 0.30·hematocrit) (29). Before and during blood sampling, blood flow to the hand was interrupted for 2 min by a pediatric blood pressure cuff inflated to 250 mmHg. Blood flow was measured immediately after blood withdrawal.

The amount of glucose taken up by forearm muscle [muscle blood flow (ml/kg muscle · min)·(arterialized venous glucose - deep venous glucose) (mg/dl)] was calculated based on the following experiments and assumptions.

Muscle mass. In six separate subjects, the percent muscle by volume in forearm was determined by computerized planimetry (Hipad Digitalizer, Austin, TX) from serial nuclear magnetic resonance (NMR) scans covering the section of the forearm between the blood pressure cuffs. The percent muscle in forearm was related to the percent fat free mass (ffm) [100 - % fat (underwater weighing)] as follows:

$$\% \text{ muscle in forearm} = -4.090 + 0.745 \cdot (\% \text{ fat free mass})$$

($r = 0.79$, $P < 0.05$). In the 22 subjects with a mean % fat of 14.5% (range 5.0 to 29.0%) the mean of the predicted % muscle in forearm was 59.6% (range 48.7 to 66.7%).

Flow. The blood flow in forearm muscle follows the function: muscle flow = 0.47 × total forearm flow + 0.83 (30).

Muscle biopsies

Before and 2 h after the start of the infusions, a percutaneous muscle biopsy (30-60 mg) was performed using a Bergstrom needle under local anesthesia (2% lidocaine) (31). The samples were obtained from the vastus lateralis muscle from opposite sites in each study. The biopsy site was progressed distally in the vastus lateralis during the 2-wk study period. The specimen was frozen in liquid nitrogen in less than 10 s, stored at

-70°C and assayed for glycogen synthase and phosphorylase activities within 2 mo.

Enzyme analyses

Glycogen synthase activity was assayed using a modification (32, 33) of the method of Nuttall et al. (34). Enzyme activity was measured at a low (0.17 mM) glucose-6-phosphate concentration, which is an estimate of in vivo enzyme activity (33), and at a maximally stimulating (7.2 mM) glucose-6-phosphate concentration (total activity). The UDP-glucose concentration was 0.14 mM in both assays (33). The activity was measured using UDP-[¹⁴C]glucose and is expressed as nanomoles of glucose incorporated into glycogen per minute per milligram of protein (35). The fractional activity of glycogen synthase is calculated from the activity at the low glucose-6-phosphate concentration divided by total activity.

Glycogen phosphorylase activity was measured according to Tan et al. (36). The assay is based on the incorporation of U-[¹⁴C]glucose-1-phosphate into glycogen in the absence (phosphorylase *a*) and presence (total activity) of 3 mM AMP. Phosphorylase *b* was defined as the activity remaining after subtraction of activity in the absence of AMP from total activity. The fractional activity of glycogen phosphorylase, which is subsequently referred to as "glycogen phosphorylase activity," is calculated from the ratio phosphorylase *a* activity to total phosphorylase activity.

Data analysis

The dose-response curves for R_d vs. insulin were fitted to a four parameter logistic equation using a least mean square iterative routine (37):

$$R_d = (a - V_{\max})/[1 + (I/ED_{50})^b] + V_{\max};$$

where a = response at 0 insulin, V_{\max} = response at the highest insulin level (~1700 μ U/ml), ED_{50} = the 50% maximally efficient dose. b = slope factor and I the plasma insulin concentration. The a 's (rates of noninsulin-dependent glucose disposal) were defined by linear extrapolation of the three lowest plasma insulin concentrations to zero insulin as described by Gottesman et al. (38). The rate of insulin-dependent glucose disposal was then calculated by subtracting the rate of noninsulin-dependent glucose disposal from the rates of total glucose disposal. Before searching for the best fit for the observed means, nonuniformity of the variance of the response was estimated by a weighing function (37). Comparison of means was done by the unpaired Student's *t* test after one-way analysis of variance.

Results

Dose-response characteristics of glycogen synthase activation by insulin at different plasma glucose concentrations. At all glucose concentrations, the fractional activity of glycogen synthase increased as a function of increasing insulin concentrations (Fig. 1). The ED_{50} 's of insulin for stimulation of glycogen synthase activity were comparable at all glucose levels (47 ± 8 , 74 ± 36 , 60 ± 14 , 47 ± 9 μ U/ml at glucose concentrations of 90, 160, 250, and 400 mg/dl, respectively, Fig. 1). At the insulin level of ~160 μ U/ml glycogen synthase activity was not significantly lower than at the highest insulin level (~1,700 μ U/ml) at any glucose level (Fig. 1). Thus, maximal fractional activity of glycogen synthase was obtained with insulin concentrations in the high physiological range. Total enzyme activity did not change in response to a change in glycemia or insulinemia (data not shown).

Whole body and forearm glucose disposal and their relationship to glycogen synthase activation. The ED_{50} 's for stimulation of whole body and forearm glucose disposal were comparable and independent of glycemia (Figs. 2 and 3). At each glucose concentration, glycogen synthase activity was exponentially related to the rate of glucose disposal both in whole body ($r = 0.81$, $r = 0.89$, $r = 0.80$, and $r = 0.82$ at glucose levels of

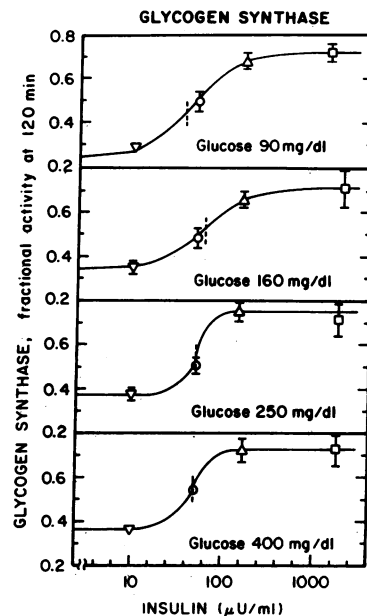


Figure 1. Glycogen synthase activity (mean \pm SE) at the end of each experiment as a function of the serum insulin concentration at four glucose levels. The vertical hatched line represent the half-maximally effective insulin concentration.

90, 160, 250, and 400 mg/dl, $P < 0.001$ for all, Fig. 4), and across forearm muscle ($r = 0.81$, $r = 0.79$, $r = 0.67$, and $r = 0.73$, respectively, $P < 0.001$ for all).

Glycogen synthase activation in relation to noninsulin- vs. insulin-dependent glucose disposal. The estimated rates of noninsulin-dependent glucose disposal averaged 2.3 ± 0.8 , 3.8 ± 1.3 , 3.4 ± 1.2 , and 5.4 ± 2.5 mg/kg ffm \cdot min at glucose levels of 90, 160, 250, and 400 mg/dl in whole body. The proportion of glucose disposal in whole body and across the forearm attributable to insulin-dependent glucose uptake at each glucose level as a function of the serum insulin level is depicted in Figs. 2 and 3.

The relationship between glycogen synthase activation and the noninsulin- vs. insulin-dependent components of whole body glucose disposal is shown in Fig. 4. In the range of rates of total glucose disposal where the insulin-dependent component constituted $< 50\%$ of total disposal, the relationship between glycogen synthase activation and total disposal was weak or

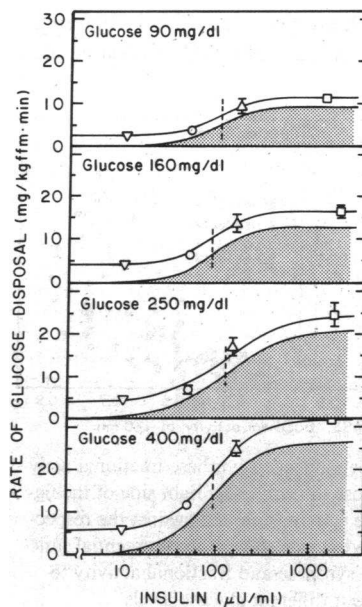


Figure 2. Rates of whole body glucose disposal (mean \pm SE) as a function of the serum insulin concentration at four glucose levels. The vertical hatched line represent the half-maximally effective insulin concentration. \square , noninsulin dependent; \circ , insulin dependent rate of disappearance.

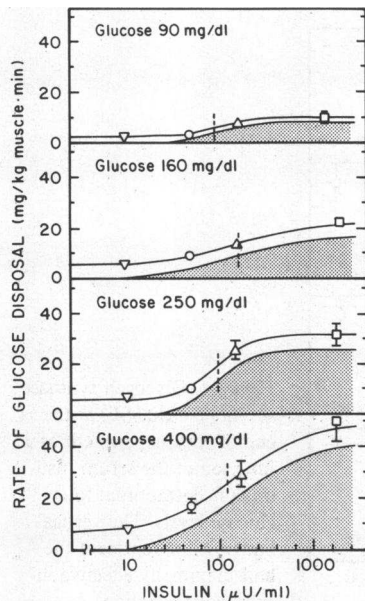


Figure 3. Rates of forearm glucose disposal (mean \pm SE) as a function of the serum insulin concentration at four glucose levels. The vertical hatched line represent the half-maximally effective insulin concentration. \square , noninsulin dependent R_d ; \blacksquare , insulin dependent R_d .

nonexistent (Fig. 4). In contrast, when insulin-dependent glucose disposal predominated (> 50%), the slope of the relationship between glycogen synthase activity and total glucose disposal was much steeper (Fig. 4).

Role of glucose, insulin and basal enzyme activity in the regulation of glycogen phosphorylase activity. Neither insulin nor glucose changed glycogen phosphorylase activity. However, a significant inverse relationship was found between basal (0 h) glycogen phosphorylase activity and its change during the 2-h study period when all the basal values were plotted against all changes in glycogen phosphorylase activity ($r = -0.77$, $n = 85$, $P < 0.0001$), or when basal activity was plotted against the change at each insulin (Fig. 5) or glucose (data not shown) level. In multiple linear regression analysis, basal enzyme activity but not the glucose or the insulin concentration was a significant ($P < 0.0001$) determinant of the change in glycogen phosphorylase activity when all studies were analyzed together.

Discussion

In subjects with normal as well as abnormal glucose tolerance, glucose storage is a major determinant of insulin-mediated glu-

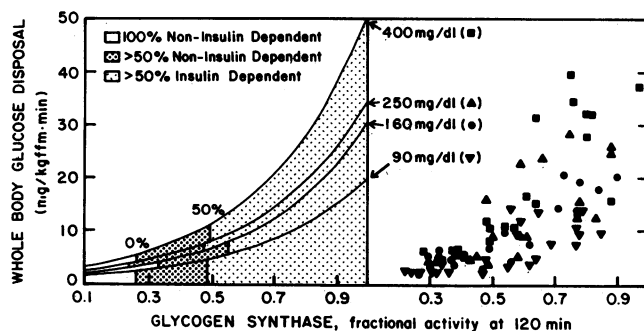


Figure 4. The relationship between glycogen synthase fractional activity and rates of whole body glucose disposal. The right side of the figure shows the individual enzyme activities plotted against the respective rates of glucose disposal, the left side shows the exponential functions (best fits) relating glycogen synthase and fractional activity to rates of glucose disposal at the four different glucose levels.

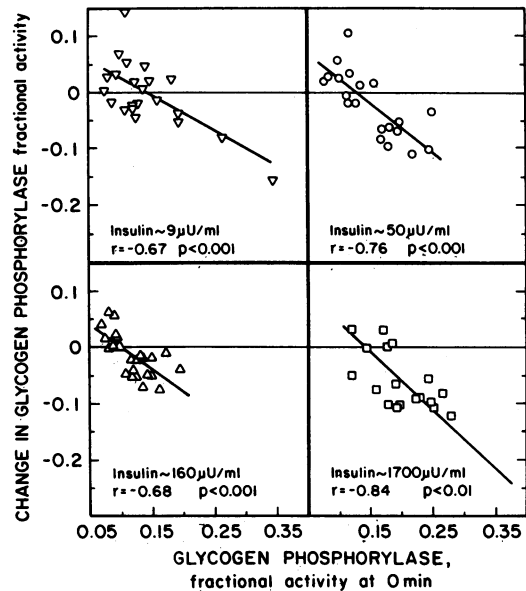


Figure 5. The relationship between glycogen phosphorylase fractional activity at 0 min and the change in fractional activity during the 2-h study period at different insulin levels.

cose disposal (39, 40). Both after oral (2) and intravenous (1) glucose administration, muscle tissue plays an important role in the disposal of glucose. Thus, knowledge of factors regulating the activity of enzymes involved in cellular glucose metabolism could be of considerable physiological importance. However, in vitro determinations of enzyme activity can only be regarded as approximations of in vivo enzyme activity. Glycogen synthase activity is subject to multiple regulatory factors (6). After in vivo exposure to various substances, only factors that change the phosphorylation state (e.g., insulin, glucose-6-phosphate, epinephrine) of the enzyme are measurable in vitro, whereas rapid modulation of glycogen synthase activity by noncovalent regulators (e.g., glucose-6-phosphate, UDP-glucose, Mg^{2+} , ATP, ADP, AMP) cannot be assayed. Despite these limitations, we found a close correlation between the fractional activity of glycogen synthase and insulin-mediated glucose disposal both in whole body and across forearm muscle (Fig. 4). This relationship does not permit us to conclude that insulin's ability to activate glycogen synthase determines the rate of insulin-dependent glucose disposal. However, recent kinetic studies both in vivo in man (27) and in the perfused rat hindlimb (41) have indicated that the rate of glucose disposal in vivo is not determined by the activity of the glucose transport system but rather by some step beyond transport. The intracellular location of the posttransport step limiting glucose disposal has so far not been determined. In search of this process, it would seem appropriate, based on these kinetic studies and the above-mentioned relationship to consider glycogen synthase activation as one of the possible contributors to the rate-determining steps of insulin-mediated glucose disposal.

Exposure of mouse diaphragm (8) or rat thigh muscle (7) to increasing concentrations of glucose-6-phosphate or 2-deoxyglucose increases the fractional activity of glycogen synthase. This effect seems to be mediated via stimulation of glycogen synthase phosphatase activity (7, 8, 42). Thus, under in vitro conditions in the rat, a noninsulin-dependent mechanism capable of dephosphorylating glycogen synthase exists in skeletal

muscle. However, glucose in the presence (43–45) or absence (43–45) of insulin causes only a small (43) or no increase (43–45) in glycogen synthase activity in rat muscle. Our present findings in human muscle tissue, where increasing the glucose level or its disposal had no effect on the fractional or total activity of glycogen synthase indicates that glucose-transport dependent dephosphorylation of the enzyme does not occur or, is not significant under in vivo conditions.

The rate of glucose disposal increased approximately three-fold when the glucose level was increased from 90 to 400 mg/dl at a constant insulin level. Most of this increase could be attributed to an increase in insulin-dependent glucose disposal by muscle (Fig. 2). Since the rate of glucose oxidation does not exceed 4 to 6 mg/kg ffm · min when measured in the presence of high glucose and insulin concentrations (46), it is conceivable that the glucose-induced increase in insulin-dependent glucose disposal was mostly due to an increase in nonoxidative glucose disposal. In the rat, increasing glucose disposal by the mass-action effect of glucose leads to increased glycogen formation despite unaltered fractional activity of glycogen synthase (47, 48). The increase in glycogen formation by glucose mass-action could be mediated via an increase in UDP-glucose concentration (47) or an increase in the affinity of the glycogen synthase I- or D-form for UDP-glucose by glucose-6-phosphate (48). In man, the expected increase in total muscle glycogen content even during maximal glucose and insulin stimulation is within the coefficient of variation for measurement of muscle glycogen content from biopsy samples (~ 10–20%). Therefore it is not possible to prove by direct measurement of muscle glycogen content that the glucose-induced increase in nonoxidative glucose disposal consisted of glycogen deposition.

In the isolated rat diaphragm muscle, both glucose and glucose-6-phosphate can decrease phosphorylation of phosphorylase *a* (12). In contrast to rat muscle, glucose did not decrease phosphorylase activity in the present study, but in keeping with findings in rat muscle (13–15), insulin had no effect on phosphorylase activity. Thus, exposure of human muscle to insulin results in dephosphorylation of glycogen synthase but not phosphorylase. Specific dephosphorylation of synthase but not phosphorylase seems possible in light of the multiple kinases and phosphatases that control phosphorylation of the enzyme at specific sites. In rabbit muscle, insulin administration leads to specific dephosphorylation of sites 3*a*, *b*, and *c* with no change in the phosphorylation of other sites including site 2, which is dephosphorylated by phosphorylase kinase (6).

In conclusion, insulin-dependent glucose disposal in whole body and across forearm muscle closely parallels activation of muscle glycogen synthase activity. In contrast, insulin does not change glycogen phosphorylase activity, suggesting that insulin causes dephosphorylation of glycogen synthase via regulators that are not common for these two enzymes. The finding that the rate of glucose disposal per se has no effect on the phosphorylation state of glycogen synthase suggests that the low fractional activities found in insulin-resistant subjects (3) are a consequence of impaired insulin action rather than secondary to the reduced rates of glucose disposal.

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