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Research Article

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Relative Contribution of Glycogen Synthesis and Glycolysis to Insulin-mediated Glucose Uptake

A Dose–Response Euglycemic Clamp Study in Normal and Diabetic Rats

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Abstract

To examine the relationship between plasma insulin concentration and intracellular glucose metabolism in control and diabetic rats, we measured endogenous glucose production, glucose uptake, whole body glycolysis, muscle and liver glycogen synthesis, and rectus muscle glucose-6-phosphate (G-6-P) concentration basally and during the infusion of 2, 3, 4, 12, and 18 mU/kg · min of insulin. The contribution of glycolysis decreased and that of muscle glycogen synthesis increased as the insulin levels rose. Insulin-mediated glucose disposal was decreased by 20–30% throughout the insulin dose–response curve in diabetics compared with controls. While at low insulin infusions (2 and 3 mU/kg · min) reductions in both the glycolytic and glycogenic fluxes contributed to the defective tissue glucose uptake in diabetic rats, at the three higher insulin doses the impairment in muscle glycogen repletion accounted for all of the difference between diabetic and control rats. The muscle G-6-P concentration was decreased (208±11 vs. 267±18 nmol/g wet wt; $P < 0.01$) compared with saline at the lower insulin infusion, but was gradually increased twofold (530±16; $P < 0.01$ vs. basal) as the insulin concentration rose. The G-6-P concentration in diabetic rats was similar to control despite the reduction in glucose uptake.

These data suggest that (*a*) glucose transport is the major determinant of glucose disposal at low insulin concentration, while the rate-limiting step shifts to an intracellular site at high physiological insulin concentration; and (*b*) prolonged moderate hyperglycemia and hypoinsulinemia determine two distinct cellular defects in skeletal muscle at the levels of glucose transport/phosphorylation and glycogen synthesis. (*J. Clin. Invest.* 1990. 85:1785–1792.) glycolysis • glycogen synthesis • glucose-6-phosphate • rate-limiting step • euglycemic clamp • diabetic rats

Introduction

Insulin resistance is a major determinant of glucose intolerance in non-insulin-dependent (1–3) and insulin-dependent diabetes mellitus (4, 5) in humans. Insulin contributes to the maintenance of glucose homeostasis by inhibiting endogenous glucose production and stimulating hepatic and peripheral glucose uptake. Once glucose is transported into the cell, it is phosphorylated to glucose-6-phosphate (G-6-P)[1] and enters one of two major pathways, glycogen synthesis or glycolysis. In turn, glycolysis leads to either lactate formation or pyruvate oxidation in the Krebs cycle. In non–insulin-dependent diabetes mellitus defects have been demonstrated at the level of the glucose transport system (6, 7), glycogen synthase (8, 9), and pyruvate dehydrogenase (9). However, the precise contribution of each of these pathways to overall insulin-induced glucose disposal is controversial, mainly due to uncertainty about the rate-limiting step(s) for glucose metabolism (10–15). Recently, techniques have been developed to measure in vivo the rates of insulin-mediated glucose metabolism in awake, chronically catheterized rats (16–18). However, although this technique has been modified to measure glucose uptake in individual tissues (19), it does not presently discriminate between the two major metabolic fates of the intracellular glucose, i.e., glycolysis and glycogen synthesis.

Skeletal muscle is the major site of insulin-mediated glucose disposal during a euglycemic clamp study (20). A severe impairment in insulin-mediated glucose storage is characteristic of the majority of the insulin-resistant states (21), and the defective stimulation of glycogen synthesis in skeletal muscle has been implicated as its major cellular mechanism (8). The net flux through this pathway is mostly determined by the balance between the enzymes glycogen synthase and glycogen phosphorylase (22), but may also be regulated by the G-6-P concentration through stimulation of glycogen synthase phosphatase (23, 24). Since changes in G-6-P concentration in the physiological range may also modulate the flux through hexokinase and glucose transport systems (25), it appears important to evaluate the total glucose flux and its intracellular distribution in relation to the G-6-P levels.

Therefore, in this study we propose a methodology that, by quantitating endogenous glucose production, liver and skeletal muscle glycogen synthesis, and whole body glycolysis during insulin clamp studies, allows us to quantitate the contribution of each of these pathways to the overall glucose metabolism in the conscious rat. The comparison of the glucose fluxes with the intracellular G-6-P concentration allows us to speculate on the rate-limiting step(s) for insulin action and on the site(s) of insulin resistance in diabetic rats.

Methods

Animals. Two groups of male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were studied: group I, sham-operated controls ($n = 48$); group II, partially pancreatectomized rats ($n = 48$). At 3–4 wk of age all rats (80–100 g) were anesthetized with phenobarbital (50 mg/kg body wt i.p.), and in group II 90% of

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1. *Abbreviations used in this paper:* G-6-P, glucose-6-phosphate; HGP, hepatic glucose production; NEFA, nonesterified fatty acid.

their pancreas was removed according to the technique of Foglia (26), as modified by Bonner-Weir et al. (27). Group I underwent a sham pancreatectomy in which the pancreas was disengaged from the mesentery and gently rubbed between the fingers.

Immediately after surgery (i.e., pancreatectomy or sham pancreatectomy) rats were housed in individual cages and subjected to a standard light (6 a.m. to 6 p.m.)–dark (6 p.m. to 6 a.m.) cycle. Based on prior experience, rats received the identical daily allotment of rat chow (Ralston-Purina Co., St. Louis, MO) in an amount (0.1 g/g body wt per d) that sustained normal growth and was completely consumed by all of the animals.

After surgery rats were weighed twice weekly and tail vein blood was collected for the determination of nonfasting plasma glucose and insulin concentrations at the same time (8 a.m.). The fasting plasma glucose and insulin concentrations also were determined weekly on tail vein blood.

Euglycemic clamp study. Insulin-mediated whole body glucose uptake was measured in awake, unstressed, chronically catheterized rats using the euglycemic clamp in combination with [3-^{3}H]glucose infusion as previously described (17, 18). Briefly, 5 wk after pancreatectomy or sham-pancreatectomy rats were anesthetized with an intraperitoneal injection of phenobarbital (50 mg/kg body wt), and indwelling catheters were inserted in the right internal jugular vein and 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 (17, 18). 1 wk after catheter placement (6 wk after pancreatectomy or sham-operation) rats received an infusion of saline (n = 6) or insulin at either 2 (n = 6), 3 (n = 6), 4 (n = 8), 12 (n = 5), or 18 (n = 7) mU/kg · min for 2 h. A variable infusion of 25% glucose solution was started at time 0 and adjusted to clamp the plasma glucose concentration at ~ 100 mg/dl. A prime-continuous (0.4 μCi/min) infusion of [3-^{3}H]glucose (New England Nuclear, Boston, MA) was initiated at time 0 and continued throughout the study (28, 29). Plasma samples for determination of [3-^{3}H]glucose and tritiated water specific activities were obtained at 5–10-min intervals throughout the insulin clamp study. Plasma samples for determination of plasma insulin, lactate, and nonesterified fatty acid (NEFA) concentrations were obtained at −30, 0, 60, 90, and 120 min during the study. The total volume of blood withdrawn was < 3 ml/study. To prevent volume depletion and anemia, insulin was diluted in a solution (1:1, vol/vol) of ~ 4 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 U/ml). At the end of the 120-min study rats were injected with phenobarbital (60 mg/kg body wt), the abdomen was quickly opened, the rectus abdominal muscle was clamped in situ, and the liver and hindlimb muscle were freeze-clamped with aluminum tongs precooled in liquid nitrogen. All tissue samples were kept frozen at −80°C for subsequent analysis. To determine the effect of the sampling procedure on the G-6-P levels, we compared the metabolite concentrations in abdominal rectus muscle freeze-clamped in situ with those of the same muscle excised and immediately freeze-clamped. The concentration of G-6-P was significantly increased in the excised muscle sample (622±59 vs. 257±19 nmol/g; $P < 0.01$). This discrepancy may suggest some caution in the interpretation of data obtained with different sampling procedures and from muscle biopsy in humans.

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Glycogen formation in vivo. Muscle glycogen synthesis was quantitated by two independent means: first, by determining the increment in cold glycogen concentration above fasting levels, and second, by measuring the incorporation of [3-^{3}H]glucose counts into glycogen. Liver and muscle glycogen concentrations were determined after digestion with amyloglucosidase as previously described (30, 31). The intra- and interassay coefficients of variation were < 10% (at 0.250 g% tissue weight) when a liver or muscle homogenate was assayed as multiple aliquots. Aliquots of the tissue homogenate (200 μl) were used to determine the amount of tritium label in glycogen. Glycogen was precipitated by washing in 10 vol of absolute ethanol and 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 [3-^{3}H]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 [3-^{3}H]glucose radioactivity in glycogen (disintegrations per minute per gram tissue) by the mean specific activity of [3-^{3}H]glucose in plasma during the insulin clamp (disintegrations per minute per microgram plasma glucose). The rate of net glycogen synthesis is expressed as micrograms of glucose in glycogen per gram of tissue. During the initial 30 min after starting insulin, the plasma-tritiated glucose specific activity is not constant and blood was drawn every 5 min to accurately define the mean specific activity during this time period. This number was time-averaged with the mean tritiated glucose during the last 90 min of the insulin clamp to calculate the glycogen synthetic rate (Fig. 1).

Whole body glycolytic flux in vivo. Aliquots of plasma were precipitated with $Ba(OH)_2$ and $ZnSO_4$ and centrifuged. Plasma-tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present either in tritiated water or [3-^{3}H]glucose (32). Although tritium may also be released during fructose-6-phosphate cycling and/or pentose phosphate cycling, these pathways account for only a small percentage of glucose turnover (33–35). Additionally, some of the glucose carbons that enter the pentose phosphate pathway will re-enter the glycolytic pathway through glyceraldhyde and will be correctly interpreted as glycolytic flux with the present methodology.

Rates of whole body glycolysis were estimated from the increment per unit time in tritiated water (disintegration per minute per milliliter per minute) × body water mass (milliliters)/[3-^{3}H]glucose specific activity (disintegrations per minute per milligram). Plasma water was assumed to be 93% of the total plasma volume and total body water mass was assumed to be 65% of the body mass.

Tritiated water infusion. The calculation of glycolytic flux using the above approach assumes that the appearance of ^3H_2O in plasma is representative of that of whole body and that the loss of ^3H_2O during the 2 h of the experiment is negligible. Both of these assumptions appear to be reasonable since the turnover of body water is extremely slow and there are no barriers (i.e., active transport) to the diffusion of water between various intra- and extracellular compartments. To provide experimental validation of these assumptions, we performed either saline infusions or euglycemic insulin clamp studies in normal (n = 10) and diabetic (n = 10) rats in combination with a constant infusion of ^3H_2O (0.2 μCi/min). The tracer infusion rate was chosen to approximate the rate of appearance of counts in plasma water during the [3-^{3}H]glucose studies previously described. Plasma samples for determination of tritiated water specific activity were obtained at 5–10-min intervals throughout the insulin clamp study. If the above

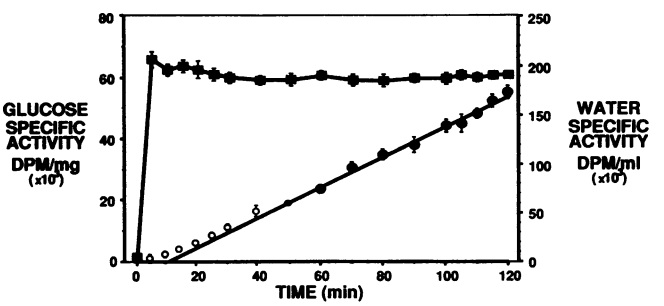

Figure 1. Plasma [3-^{3}H]glucose (*solid squares*) and tritiated water (*circles*) specific activity time courses during the 18 mU/kg · min insulin clamp study in control rats.

Table I. General Characteristics of the Control (Group I) and Diabetic (Group II) Rats

Group	n	Body weight	Fasting plasma		Nonfasting plasma		Fasting plasma	
			Glucose	Insulin	Glucose	Insulin	Lactate	NEFA
		g	mg/dl	μU/ml	mg/dl	μU/ml	μM	μM
I Controls	48	314±4	101±2	29±3	134±3	63±7	563±24	469±24
II Diabetics	48	305±7	126±6*	26±4	322±12*	33±4*	596±37	496±31

* $P < 0.01$ vs. controls (I).

outlined assumptions are correct, the rate of appearance of tritiated water in the plasma during the experiment, multiplied by the whole body water space, should closely predict the ^3H_2O infusion rate. In all rats the increment in plasma ^3H_2O was linear between 60 and 120 min. The whole body ^3H_2O rate of appearance as extrapolated from the plasma-tritiated water was 100±1% in control and diabetic rats.

Tissue-tritiated water specific activity was estimated by liquid scintillation counting of deproteinized (6% wt/vol perchloric acid) urine or tissue samples before and after evaporation to dryness. Tissue water was determined by weighing before and after lyophilization.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (glucose analyzer; Beckman Instruments, Inc., Palo Alto, CA) and plasma insulin by RIA using rat and porcine insulin standards. Plasma lactate and muscle G-6-P were measured spectrophotometrically as described by Michal (36). Plasma NEFA concentrations were determined according to the microfluorometric method of Miles et al. (37). Plasma [3-^{3}H]glucose radioactivity was measured in duplicate in the supernatants of barium hydroxide–zinc sulphate precipitates (Somogyi procedure) of plasma samples after evaporation to dryness to eliminate tritiated water.

Calculation. Data for total body glucose uptake and suppression of hepatic glucose production (HGP) represent the mean values during the last 30 min. The HGP was calculated as the difference between the tracer-derived rate of appearance and the infusion rate of glucose. Total body glucose disposal was calculated by adding the rate of residual HGP during the last 30 min of each insulin clamp to the glucose infusion rate during the same 30-min time period. The rate of net glycogen synthesis was calculated as the number of [3-^{3}H]glucose disintegrations per minute in glycogen per gram of muscle tissue divided by the time-weighed mean plasma [3-^{3}H]glucose specific activity (disintegrations per minute per microgram glucose). For each rat the mean of four determinations on rectus abdominal muscle and four on hindlimb muscle were used to approximate the mean whole body muscle glycogen concentration. The whole body glycolytic rate was calculated from the increment per minute in plasma ^3H_2O radioactivity from 60 to 120 min multiplied by the body water space and divided by the [3-^{3}H]glucose specific activity. This time period was selected since the appearance in ^3H_2O counts in the plasma became linear after 40–50 min following the [3-^{3}H]glucose infusion (Fig. 1). All values are presented as mean±SEM. Differences between groups were determined using the one-way analysis of variance in conjunction with the student Newman-Kuels test.

Results

General characteristics of the animals. There were no differences in the mean body weights between control and diabetic rats (Table I). Both the fasting ($P < 0.05$) and postmeal ($P < 0.01$) plasma glucose concentrations during the 2-wk period before the insulin clamp study were significantly higher in the diabetic group (II) compared with the control group (I). The fasting plasma insulin, lactate, and NEFA concentrations were similar in the two groups, while the postmeal plasma insulin concentration was significantly diminished in diabetic rats (group II) compared with controls (group I) ($P < 0.01$).

Insulin clamp study. During the saline infusion studies (Table II, Figs. 2 and 3 *a*) the plasma glucose concentration was higher in diabetic compared with control rats (121±3 vs. 101±1 mg/dl). Steady-state plasma glucose and insulin concentrations during the insulin clamp studies were similar in the two groups (Table II). The coefficients of variation in plasma glucose and insulin levels were < 5 and < 10%, respectively, in all studies. After hyperinsulinemia the absolute plasma NEFA as well as the decrement in plasma NEFA concentration was

Table II. Plasma Glucose, Insulin, Lactate, and NEFA Concentrations during the Euglycemic Clamp Studies

Groups	Insulin infusion rate (mU/kg · min)					
	0	2	3	4	12	18
I Controls						
SSPG (*mg/dl*)	101±1	100±1	102±1	101±1	100±1	100±1
SSPI (*μU/ml*)	31±4	63±5	87±6	107±8	286±16	403±8
Lactate (*μM*)	554±18	637±29	719±43	969±38	1,347±69	1,482±84
NEFA (*μM*)	481±32	317±27	263±34	193±42	179±31	159±24
II Diabetics						
SSPG (*mg/dl*)	121±3	102±1	101±1	102±1	101±1	100±1
SSPI (*μU/ml*)	28±3	59±6	83±11	103±8	281±14	399±9
Lactate (*μM*)	573±34	709±41	835±37	1,095±58	1,458±96	1,593±93
NEFA (*μM*)	501±41	361±42	298±31	203±36	149±43	162±37

SSPG, steady-state plasma glucose; SSPI, steady-state plasma insulin.

similar in control and diabetic rats (Table II). The plasma lactate concentration was similar in the two groups in the basal state and during the insulin infusions (Table II). Fig. 2 shows the relationships between plasma insulin concentration and HGP in control and diabetic rats. After a 24-h fast there was no significant difference in HGP between the two groups; the suppression of HGP (Fig. 2) during the insulin infusions was similar in the diabetic and control groups, either expressed as percentage suppression from basal or as absolute values. The tissue glucose uptake (Fig. 3 *a*) was significantly decreased in diabetic compared with control rats at all the insulin concentrations examined. From similar basal levels the tissue glucose uptake rose to 16.3±1.2, 21.8±3, 27.3±0.9, 36.0±0.7, and 35.9±9 mg/kg·min in controls, and to 13.1±1.1, 15.9±1.2, 19.4±0.6, 24.8±0.7, and 25.3±0.8 mg/kg·min in diabetics at steady-state plasma insulin concentrations of ~ 50, 75, 100, 300, and 450 μU/ml, respectively.

Muscle glycogen synthesis. The fasting muscle glycogen concentration was similar in groups I and II: 0.54±0.01 (n = 11) in group I and 0.53±0.01 in group II (n = 11) (Table III, Fig. 3 *b*).

The muscle glycogen concentration was significantly reduced in diabetic rats (group II) compared with controls (group I) at the end of each of the insulin clamp studies (Table III). As previously shown (28, 29), the two methods for the assessment of the rate of net muscle glycogen synthesis gave similar results. In response to insulin, net muscle glycogen synthesis, estimated from the increment in cold glycogen concentration (Table III), was significantly stimulated during all the insulin infusions in control rats; the insulin-stimulated muscle glycogen synthesis was severely impaired in diabetic animals compared with controls. During the two lowest insulin doses the increment in muscle glycogen concentration from fasting level did not reach statistical significance, and at the higher insulin doses it was < 40% of control values (Table III). In Fig. 3 *b* the glycogenic rates are extrapolated to whole body muscle mass to allow comparison with the tissue glucose uptake and the glycolytic flux. Liver glycogen concentration (not shown) was significantly higher in diabetic compared with control rats; however, no significant increment was detected during the insulin compared with the saline infusion studies in either controls or diabetics.

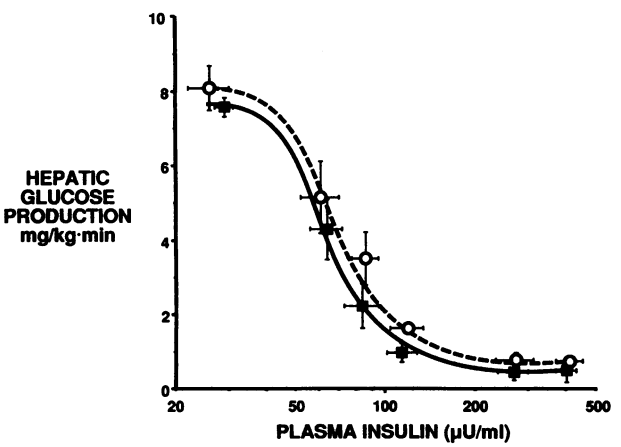

Figure 2. Effect of euglycemic insulin infusions on HGP in control (*solid squares*) and diabetic (*open circles*) rats.

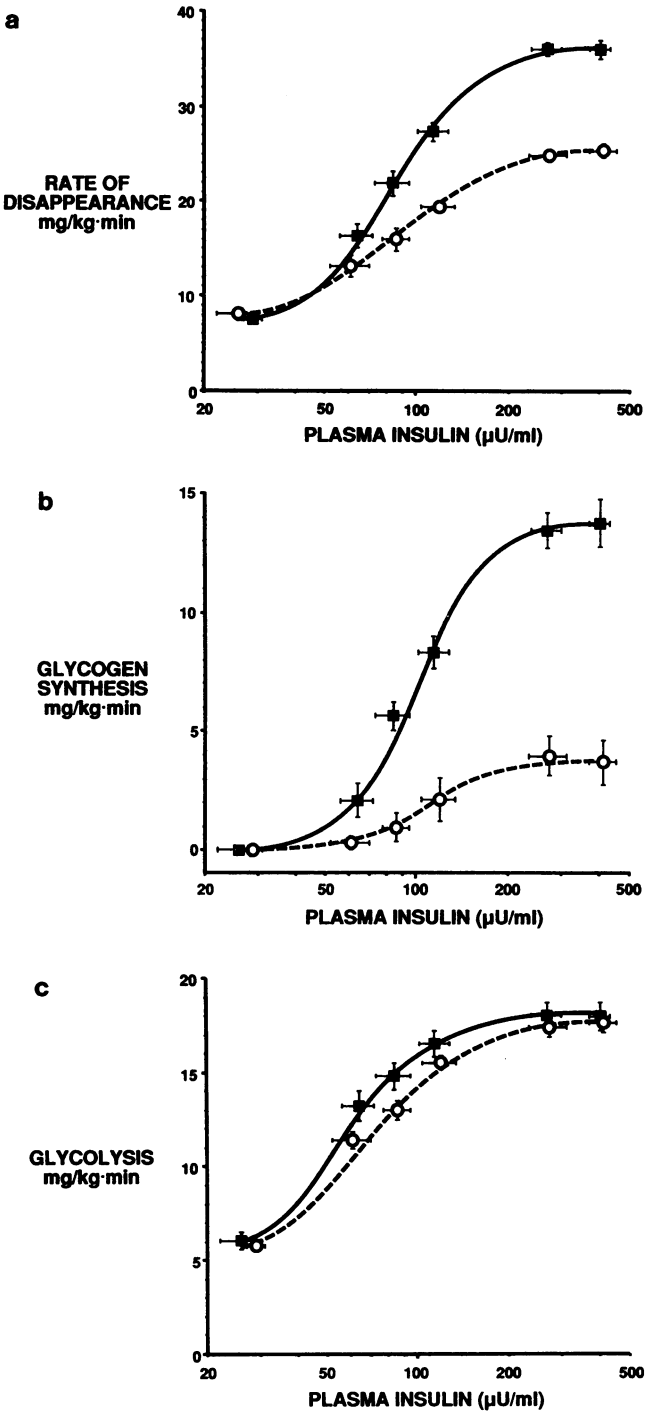

Figure 3. Effect of euglycemic insulin infusions on rates of glucose disposal (*a*), muscle glycogen synthesis (*b*), and glycolysis (*c*) in control (*solid squares*) and diabetic (*open circles*) rats. Glucose disposal and glycolytic rates were increased significantly ($P < 0.01$) over basal values at all insulin infusion rates in both controls and diabetics. The rate of net muscle glycogen synthesis was increased significantly ($P < 0.01$) over basal values at all insulin infusion rates in controls, but in diabetics was significantly increased ($P < 0.01$) during the 4, 12, and 18 mU/kg·min insulin infusions only. Glucose disposal and muscle glycogen synthetic rates were significantly decreased ($P < 0.01$) in diabetics compared with controls at all insulin infusion rates. The rate of glycolysis was ($P < 0.01$) significantly decreased in diabetics compared with controls during the 2 and 3 mU/kg·min insulin infusions only.

Table III. Skeletal Muscle Glycogen Concentrations and Rates of Net Glycogen Synthesis during the Euglycemic Clamp Studies

Groups	Insulin infusion rate (mU/kg · min)					
	0	2	3	4	12	18
I Controls						
MG conc (*g%*)	0.54±.01	0.60±.02	0.71±.02	0.79±.02	0.94±.03	0.95±.04
MG rate (*μg/g · min*)	0	5.2±1.7	14.1±1.5	20.8±1.6	33.6±1.9	34.4±2.5
II Diabetics						
MG conc (*g%*)	0.53±.01	0.54±.01	0.56±.03	0.61±.03	0.68±.02	0.67±.03
MG rate (*μg/g · min*)	0	0.8±.7	2.4±1.6	7.0±2.3	12.4±2.0	11.7±2.3

MG conc, skeletal muscle glycogen concentration; MG rate, rate of net glycogen synthesis.

Whole body glycolytic flux. Glycolytic rate was estimated from the appearance of tritiated water in plasma water after the infusion of [3-^{3}H]glucose during the last 60 min of the insulin clamp study (Fig. 3 *c*). Fig. 1 shows the time course of the tritiated glucose and water specific activities. The increment in ^{3}H appearance in plasma water was linear in all experimental conditions between 60 and 120 min. In the basal state, whole body glycolytic flux was 5.8±0.3 mg/kg · min in control rats, and rose to 13.2±0.8, 14.8±0.7, 16.5±0.7, 18.0±0.7, and 17.9±0.8 mg/kg · min, respectively, during the 2, 3, 4, 12, and 18 mU/kg · min euglycemic clamp studies. In diabetic rats compared with controls the glycolytic flux was similar during the basal state (6.0±0.4 mg/kg · min) and during the 4, 12, and 18 mU/kg · min insulin clamp studies (15.5±0.4, 17.4±0.5, and 17.6±0.6 mg/kg · min), but was significantly decreased during the two low-dose insulin clamp studies (11.3±0.4 vs. 13.2±0.8 and 12.9±0.5 vs. 14.8±0.7 mg/kg · min; $P < 0.01$).

Tritiated water specific activity was measured in atrial and portal vein plasma and in liver, kidney, skeletal muscle, and urine at the end of the study. The highest specific activity was detected in skeletal muscle and the lowest in urine and kidney in all studies. This suggests that skeletal muscle is the major site of glycolysis under the present experimental conditions and is consistent with the notion that muscle uptake is responsible for 75–85% of the whole body glucose uptake in hyperinsulinemic euglycemic conditions (18).

Muscle G-6-P concentration. G-6-P, measured in abdominal skeletal muscle, was decreased during the low dose and increased during the highest insulin clamp study compared with saline infusions in both controls and diabetic rats (Fig. 4). Since the basal G-6-P concentration was assessed at a higher plasma glucose concentration in diabetic compared with control rats (Table II), this value may have been slightly overestimated in the diabetic group. When the relationship between glucose uptake and G-6-P concentration (Fig. 4 *b*) was examined at a rate of glucose disposal higher than ~ 18 mg/kg · min, the G-6-P concentration was increased in diabetics compared with controls. Fig. 4 *c* depicts the relationship between glycogen synthesis and G-6-P: the glycogenic pathway appears to be severely impaired in diabetic rats despite a relative increase in G-6-P levels. Finally, the glycolytic flux was identical in diabetic and control rats when evaluated at equal concentrations of G-6-P (Fig. 4 *d*).

Discussion

Insulin's effect on glucose homeostasis involves the balance of endogenous glucose production and glucose disposal (38). The major pathways by which glucose is disposed of are glycogen synthesis and glycolysis. The present study describes a technique for examining, in the conscious intact animal, the effect of plasma insulin concentration on endogenous glucose production, glucose uptake, glycogen synthesis, and glycolytic flux. We have also examined the contribution of these ongoing processes to insulin resistance in diabetic rats. Our results provide a physiological validation of the technique and suggest that the degree of hyperinsulinemia has considerable influence on the intracellular fate of the infused glucose and on the contribution of various metabolic alterations to the insulin resistance of the diabetic state.

The relationship between circulating plasma insulin concentration (and rate of glucose disposal) and the rate-limiting step for insulin-mediated glucose uptake has been examined by several methodological approaches (10–15). Kubo and Foley (10) in perfused hindlimb and Yki-Jarvinen et al. (11) in human forearm observed an increase in K_m for glucose utilization as insulin concentrations rose, suggesting a shift in the rate-limiting step from glucose transport to a posttransport event. Similar conclusions were also reached by Ferrannini et al. (15) by using a compartmental model of the glucose kinetics in man. It has therefore been hypothesized that at physiological glucose concentrations, in the presence of maximally stimulating insulin levels, the rate-limiting step for insulin-mediated glucose uptake and metabolism in skeletal muscle shifts from glucose transport to some intracellular step (10, 11). In contrast, the absence of detectable accumulation of free intracellular glucose under similar experimental conditions (12, 14) has been advocated as a definitive proof for the rate-limiting role of glucose transport in insulin-mediated glucose metabolism (12). However, the accuracy of these measurements has been questioned, particularly at high rates of glucose metabolism, since it may be misleading to equate the extracellular glucose level with the plasma glucose concentration in those experimental conditions. Additionally, in the study of Ziel et al. (12) the maximal rate of glucose disposal attained during the euglycemic clamp in anesthesized rats was only 37% (14 vs. 38 mg/kg · min) of that in awake, unstressed rats (16–18).

In this study the muscle glycogen synthesis accounted for 13% of glucose uptake in control rats, while whole body glycolysis represented 81% of the total glucose disposal during the low-dose insulin clamp study. When maximally insulin-stimulated glucose uptake was examined, the contribution of the muscle glycogenic rate rose to 38%, while the glycolysis was 51% of the rate of disappearance. Therefore, with increasing

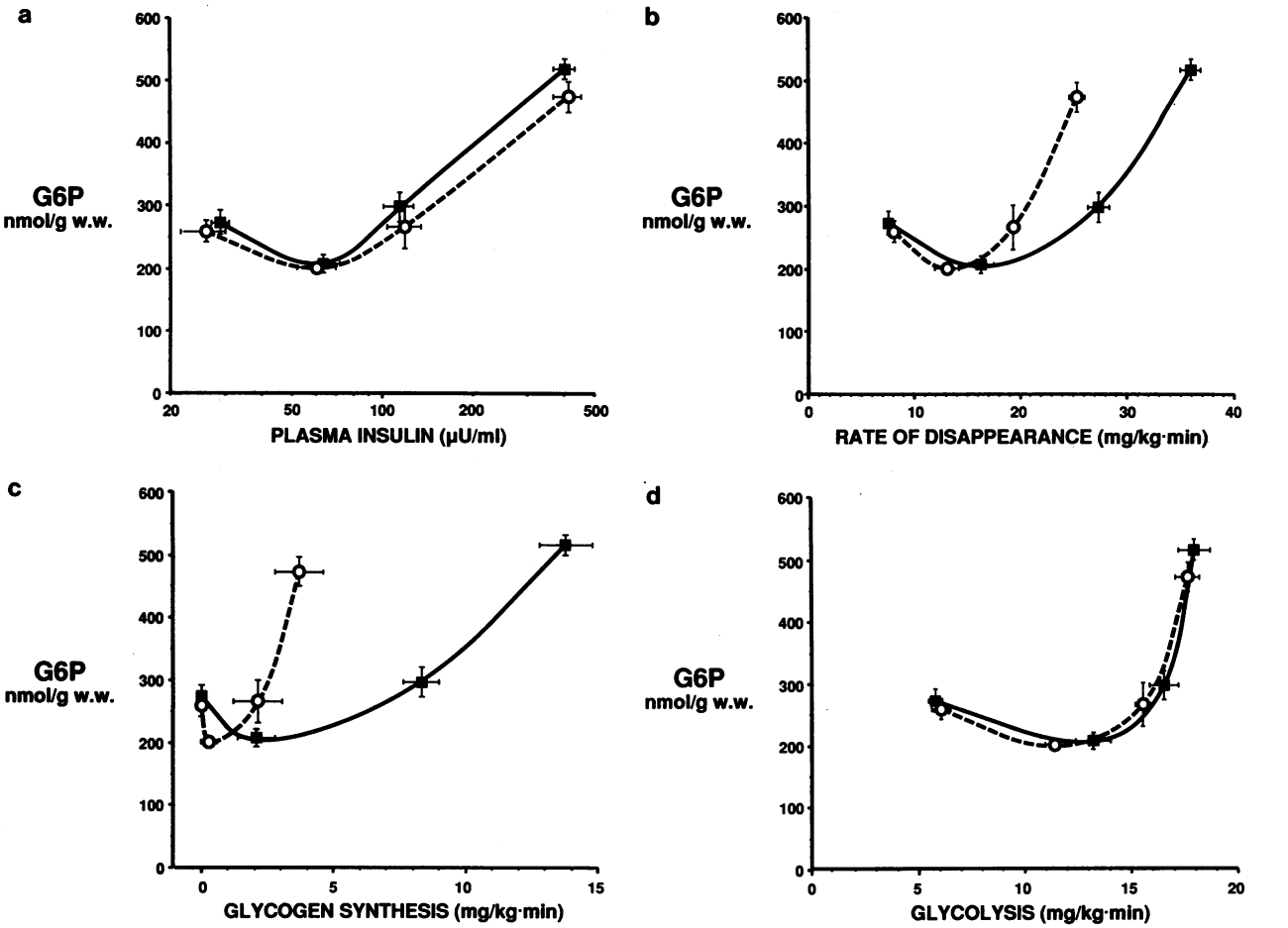

Figure 4. Skeletal muscle G-6-P concentrations vs. plasma insulin levels (*a*), glucose disposal rates (*b*), rates of net muscle glycogen synthesis (*c*), and glycolytic rates (*d*). In both control and diabetic rats, the muscle G-6-P concentration was decreased ($P < 0.01$ vs. basal) during the 2 mU/kg · min and increased significantly ($P < 0.01$ vs. basal) during the 18 mU/kg · min insulin infusions.

plasma insulin concentrations, the contribution of muscle glycogen synthesis to overall glucose metabolism is enhanced, while that of glycolysis is decreased. These results support findings, obtained with indirect calorimetry in man, that oxidative glucose metabolism is more sensitive to insulin than is nonoxidative metabolism (39, 40). However, it must be noted that in this study we directly measured glycogen synthesis, while the tracer-derived glycolytic rate includes both oxidative and nonoxidative glycolysis.

In diabetic rats a reduction in insulin-mediated glucose metabolism (between 20 and 30% of control values) was detected at all the insulin levels examined; however, the contributions of the glycogenic and glycolytic pathways to insulin resistance were influenced by the circulating insulin concentration (Fig. 3). During the lower-dose insulin clamp studies both the muscle glycogen synthesis and the whole body glycolysis were significantly reduced compared with controls, accounting for ~ 56 and 35%, respectively, of the defect in glucose metabolism. On the contrary, when glucose metabolism was maximally stimulated by insulin, the glycolytic flux was completely normalized, while the muscle glycogen synthesis was severely impaired compared with controls and accounted for 86% of the decrease in glucose disposal. However, the percent contribution to overall glucose disposal of glycogen repletion (2 to 19% vs. 13 to 38%) was decreased, while the contribution of glycolysis (92 to 71% vs. 81 to 51%) was increased in diabetic rats compared with controls throughout the insulin dose–response curve. These results suggest that the major intracellular defect in diabetic rats resides in the glycogenic pathway. However, the observation that a significant impairment in glycolytic flux is detectable at the two lowest insulin doses introduces several possible interpretations. Since the glycolytic pathway represents 80–90% of the total glucose metabolism during the low dose insulin clamp, while it accounts for only 35% of the difference between diabetic and control groups, the present data may suggest that the slight decrease in the glycolytic rate is a consequence of the reduced transport of glucose into the cell. This would be consistent with the hypothesis that glucose transport is the rate-limiting step for glucose disposal at the lower insulin infusion rate, while glycogen synthase becomes more relevant at high insulin levels (8, 10, 11). However, these results may also suggest that the glycolytic flux, impaired at lower insulin concentrations, is normalized in the presence of maximal insulin stimulation by a relative increase in G-6-P, which in turn decreases the rates of phosphorylation and transport (25) and increases the flux through phosphofructokinase.

The concentration of muscle G-6-P (Fig. 4, *a–d*) may help to elucidate further the flux data in Fig. 3. In both control and diabetic rats the response of skeletal muscle G-6-P to insulin

was biphasic, with a slight but significant decrement at the lower insulin concentration, 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 the combined rate of the glycolytic and glycogenic fluxes exceeded the reactions proximal to the substrate (i.e., phosphorylation and/or transport) at the lower insulin dose, while at the highest concentration of the hormone the combined fluxes through phosphofructokinase and glycogen synthase are less stimulated by insulin than the proximal step (i.e., hexokinase and/or glucose transporters). Whether the elevation in G-6-P concentration during the highest insulin infusion may inhibit the rates of glucose transport/phosphorylation cannot be determined by our data. However, a recent report by Foley and Hueckstead (25) suggests that similar levels of this metabolite can decrease glucose transport in adipose cells. This results are also consistent with the kinetic studies of Kubo and Foley (10) and Yki-Jarvinen et al. (11), which suggested a shift in the rate-limiting step from glucose transport to some intracellular step at high insulin levels. Furthermore, when the G-6-P concentrations are examined at similar values of glucose flux (Fig. 4 *b*), they are significantly increased in diabetic compared with control rats for values of tissue glucose uptake > 18 mg/kg · min, thus emphasizing the important role of the intracellular defect, particularly at high rates of glucose metabolism. The equal G-6-P concentration in diabetic compared with control rats, despite severely impaired glycogen synthesis, suggests that this in vivo alteration is not the consequence of a decrease in G-6-P level, but rather of the impaired activation of the rate-limiting enzyme, glycogen synthase. This observation may further validate the correlation between decreased in vitro glycogen synthase activation and in vivo glycogen repletion observed in this rat model (29) as well as in humans (8, 9, 21, 40). On the contrary, when the in vivo glycolytic flux is examined at comparable G-6-P concentrations (Fig. 4 *d*), no impairment can be demonstrated in diabetic compared with control rats, again suggesting that the defect in this pathway, detected at low insulin levels, is solely the consequence of the reduced glucose uptake/phosphorylation. Consistent with this was the observation that in diabetic rats at low insulin concentration, when glucose uptake was restored to control levels by means of an increase in plasma glucose concentration, G-6-P level rose slightly and the glycolytic flux was completely normalized (data not shown).

Finally, to reconcile the glycolytic and glycogenic flux data with the G-6-P concentration, it is necessary to hypothesize the presence of two major and independent defects in the diabetic skeletal muscle: a proximal defect (i.e., glucose transport or phosphorylation) primarily responsible for the impaired total glucose uptake at low plasma insulin concentration; and a distal defect (i.e., glycogen synthesis) primarily responsible for the altered intracellular distribution of glucose. Moreover, the rise in G-6-P concentration at high rates of glucose metabolism supports a role for the impaired activation of glycogen synthase in the defective glucose uptake. The metabolic characteristics of this diabetic model are consistent with a major role of hyperglycemia and hypoinsulinemia in the impairment of these two metabolic processes. In fact, the 90% partially pancreatectomized rat is characterized by the absence of significant dehydration or weight change, hyperglucagonemia, and elevation in plasma free fatty acid concentration, all features that often accompany more severe diabetic states.

In conclusion, our results provide a quantitative approach to the in vivo measurement of the major intracellular pathways of glucose metabolism in the intact and conscious rat. Our data suggest that glucose transport is the major determinant of glucose disposal at low insulin concentrations, while the rate-limiting step shifts to an intracellular site at high physiological and pharmacological insulin levels. The presence of prolonged moderate hyperglycemia and hypoinsulinemia determines two distinct cellular defects in skeletal muscle at the levels of glucose transport/phosphorylation and glycogen synthase.

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