

Hyperglycemia Normalizes Insulin-stimulated Skeletal Muscle Glucose Oxidation and Storage in Noninsulin-dependent Diabetes Mellitus

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Abstract

The diminished ability of insulin to promote glucose disposal and storage in muscle has been ascribed to impaired activation of glycogen synthase (GS). It is possible that decreased glucose storage could occur as a consequence of decreased glucose uptake, and that GS is impaired secondarily. Muscle glucose uptake in 15 diabetic subjects was matched to 15 nondiabetic subjects by maintaining fasting hyperglycemia during infusion of insulin. Leg muscle glucose uptake, glucose oxidation (local indirect calorimetry), release of glycolytic products, and muscle glucose storage, as well as muscle GS and pyruvate dehydrogenase (PDH) were determined before and during insulin infusion. Basal leg glucose oxidation and PDH were increased in the diabetics. Insulin-stimulated leg glucose uptake in the diabetics ($8.05 \pm 1.41 \mu\text{mol}/[\text{min} \cdot 100 \text{ ml leg tissue}]$) did not differ from controls (5.64 ± 0.37). Insulin-stimulated leg glucose oxidation, nonoxidized glycolysis, and glucose storage (2.48 ± 0.27 , 0.68 ± 0.15 , and $5.04 \pm 1.34 \mu\text{mol}/[\text{min} \cdot 100 \text{ ml}]$, respectively) were not different from controls (2.18 ± 0.12 , 0.62 ± 0.16 , and 2.83 ± 0.31). PDH and GS in noninsulin-dependent diabetes mellitus (NIDDM) were also normal during insulin infusion. When diabetics were restudied after being rendered euglycemic by overnight insulin infusion, GS and PDH were reduced compared with hyperglycemia. Thus, fasting hyperglycemia is sufficient to normalize insulin-stimulated muscle glucose uptake in NIDDM, and glucose is distributed normally to glycogenesis and glucose oxidation, possibly by normalization of GS and PDH. (*J. Clin. Invest.* 1990. 86:1999–2007.) Key words: noninsulin-dependent diabetes mellitus • muscle glucose metabolism • glycogen synthase • pyruvate dehydrogenase

Introduction

Skeletal muscle is thought to be a major site of the insulin resistance characteristic of noninsulin-dependent diabetes mellitus (NIDDM)¹ (1–4). When subjects with NIDDM are

rendered euglycemic by means of an insulin infusion, the deficit in insulin-stimulated systemic glucose disposal can largely be accounted for by decreased glucose storage in muscle (5). Because impaired insulin activation of muscle glycogen synthase is correlated with impaired glucose storage, it has been suggested that an intra-cellular defect is responsible for decreased glucose disposal (5). However, it is possible that decreased glucose storage could occur solely as a consequence of decreased glucose uptake, and that impaired activation of glycogen synthase is a secondary effect.

Distinguishing between these possibilities is critical to understanding the biochemical mechanism(s) responsible for skeletal muscle insulin resistance. One way of distinguishing between the two would be to match rates of muscle glucose uptake in NIDDM and nondiabetic subjects at equivalent hyperinsulinemia and assess oxidative and nonoxidative muscle glucose metabolism. Revers et al. (6) found that maintaining fasting hyperglycemia in NIDDM subjects during infusion of insulin was sufficient to match rates of systemic glucose disposal to those obtained in euglycemic nondiabetics. This experimental approach would be useful to probe the regulation of intracellular muscle glucose metabolism. Thorburn et al. (7) adjusted hyperglycemia in NIDDM subjects to obtain equivalent systemic glucose disposal to euglycemic controls (at matched insulin infusions), and found reduced systemic glucose oxidation and increased plasma lactate concentrations. These investigators inferred that there was a higher rate of muscle Cori cycle activity in NIDDM. However, under those experimental conditions these abnormalities cannot be attributed solely to skeletal muscle, because the extreme hyperglycemia induced in that study ($\sim 20 \text{ mmol/liter}$) would increase lactate production by other tissues such as liver (8–17). In another study, Shulman et al. (18), using nuclear magnetic resonance techniques, found that during hyperglycemia in NIDDM the effects of insulin resistance in muscle are accounted for, nearly entirely, by impaired glycogen formation. However, the activity of muscle glycogen synthase, muscle glucose uptake, and lactate release were not determined in that study, leaving the biochemical mechanism of insulin resistance unknown.

These studies were undertaken to resolve the controversy of which metabolic pathways within skeletal muscle are affected by insulin resistance in NIDDM and, more specifically, to determine whether or not defects in intracellular glucose metabolism occur independently of glucose uptake. In order to more directly address these questions in skeletal muscle, limb balance rather than systemic techniques were used to estimate muscle glucose uptake and metabolism.

The limb balance technique is a direct in vivo method for assessment of glucose metabolism across a large bed of muscle, and has been used to specifically measure glucose uptake (8, 11, 19), nonoxidized glycolysis (release of lactate) (20), and glucose oxidation (21, 22) (net indirect calorimetry based on

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Received for publication 20 October 1990 and in revised form 8 August 1990.

1. Abbreviations used in this paper: FV, femoral venous; GS, glycogen synthase; G6P, glucose 6 phosphate; NIDDM, noninsulin-dependent diabetes mellitus; PDH, pyruvate dehydrogenase; PDH_a , PDH_{FV} , PDH_t , active, fractional velocity, total PDH.

J. Clin. Invest.

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0021-9738/90/12/1999/09 \$2.00

Volume 86, December 1990, 1999–2007

gas exchange across the limb). The subtraction of the latter two pathways from glucose uptake provides an estimate of muscle glucose storage (22). The limb balance method provides direct data on muscle metabolism which cannot be obtained with studies of systemic glucose metabolism. Additionally, enzyme activities in biopsies of this muscle bed can be directly related to flux through the various pathways of glucose metabolism.

Our findings demonstrate that, in NIDDM, the fasting level of hyperglycemia is sufficient to normalize muscle glucose uptake during an insulin infusion. These normal rates of insulin-stimulated glucose uptake allow glucose storage, oxidation, and nonoxidized glycolysis to proceed at normal rates, which suggests that the site of muscle insulin resistance in NIDDM may be proximal to all these pathways. However, at hyperglycemia, insulin-stimulated muscle glycogen synthase (GS) and pyruvate dehydrogenase (PDH) activities were normalized in NIDDM, indicating that regulation of intracellular pathways of glucose metabolism may also be partially responsible for normalization of muscle glucose storage and oxidation. Finally, the finding of increased basal muscle glucose oxidation and PDH activity provides evidence that defects in basal muscle metabolism may contribute to systemic basal metabolic abnormalities in NIDDM.

Methods

Subjects (Table I). The studies were approved by the Institutional Review Board of the University of Pittsburgh School of Medicine and The Eye and Ear Institute of Pittsburgh, and informed written consent was obtained from each subject. The clinical characteristics of each subject are shown in Table I. All of the nondiabetic volunteers had normal glucose tolerance, as determined with a 1 g/kg (maximum 75 g) 2 h oral glucose tolerance test (23). 14 of the 15 subjects with NIDDM were being treated with oral hypoglycemic agents that were discontinued at least 2 wk before the study. One of the diabetics had been treated by diet alone, and none of these subjects had been treated with insulin. Subjects were instructed to consume a diet containing at least 200 g of carbohydrate per day for the 3 d preceding admission and to not exercise on the day before the study. Body fat percentages were determined using bio-impedance (24).

Study design. Subjects were admitted to the University of Pittsburgh General Clinical Research Center on the evening before the study. They were fed a 10 kcal/kg evening meal (50% carbohydrate, 35% protein, and 15% fat) at 6 p.m., and fasted overnight. In the morning, catheters were placed in an antecubital vein for infusion of glucose and insulin, and a radial artery and a femoral vein for blood sampling. After an additional 90 min, simultaneous arterial and

venous blood samples were obtained for 30 min at 10-min intervals for glucose, lactate, and alanine, and at 5-min intervals for O₂ and CO₂. Blood flow to the thigh was measured using impedance plethysmography (BIA II; BioMed Instruments Inc., La Jolla, CA). After the period of basal determinations, a percutaneous needle muscle biopsy was obtained from the vastus lateralis muscle under local superficial anesthesia (1% xylocaine without epinephrine). The biopsy was performed in the opposite thigh to that with the femoral vein catheter. The muscle sample was frozen in liquid nitrogen within 15 s for later determination of GS and PDH activity.

Immediately after the biopsy, a primed, continuous infusion of insulin (U-100 Humulin R; Eli Lilly and Co., Indianapolis, IN) was administered at 40 mU/(m²·min) for 240 min. Whole blood arterial glucose was determined every 5 min throughout the insulin infusion. In the diabetic subjects, arterial glucose was maintained at the individual's level of fasting hyperglycemia, and in nondiabetic subjects euglycemia was maintained with a variable infusion of 50% dextrose. During the insulin infusion, arterial and/or venous blood was obtained every 30 min for glucose, lactate, alanine, free fatty acid (FFA), and insulin. During the final 30 min of the insulin infusion, (210–240 min) simultaneous arterial and venous samples were obtained at 10-min intervals for glucose, lactate, alanine, FFA, and insulin, and every 5 min for O₂ and CO₂. After blood sampling was concluded but before stopping infusion of insulin, a second muscle biopsy was obtained from the vastus lateralis at a site ~ 3 cm distal to the first biopsy. Patients remained supine throughout the study.

In four of the subjects with NIDDM, another study was conducted on a separate day. The subjects were admitted to the General Clinical Research Center in the afternoon on the day before the study and fed a standard dinner. A slow, intravenous infusion of insulin was then begun at a rate of 4–12 mU/(m²·min) and continued overnight. The plasma glucose concentration was measured hourly overnight, and the insulin infusion was adjusted so as to achieve euglycemia. The next morning, while the overnight insulin infusion was still running, a biopsy of the vastus lateralis muscle was performed as described above. The insulin infusion was then increased to 40 mU/(m²·min) and the plasma glucose was maintained at euglycemia (5.3±0.2 mmol/liter) by infusion of exogenous glucose. After 4 h, a second muscle biopsy was taken and the insulin infusion was stopped. Biopsies were assayed for glycogen synthase and PDH activities.

Leg indirect calorimetry. During the 30-min basal period and during the final 30 min of the insulin infusion, simultaneous arterial and femoral venous blood samples were collected at 5-min intervals for measurement of O₂ and CO₂ content. These repetitive blood gas samples were drawn into heparinized syringes, immersed in ice, and analyzed within 5 min. Plasma CO₂ content was calculated from measured CO₂ tension and pH (System 1304 pH/Blood Gas Analyzer; Allied Instrumentation Laboratory, Lexington, MA) using established equations and a solubility constant of 0.0307. The blood gas analyzer was calibrated with a quantitative gasometric chemical technique. The accuracy of the equipment is 0.1 mmol/liter for CO₂. Plasma CO₂ content was adjusted to whole blood CO₂ content based on hemoglobin content, saturation, and pH using an empirically derived regression equation (25). This equation gives values of whole blood CO₂ content that are in close agreement with determination of blood CO₂ content by the Van Slyke method. A comparative advantage of the automated equipment used in this study over a Van Slyke apparatus is that six samples can be analyzed in approximately the time required to perform a single Van Slyke determination. A co-oximeter (IL282 Co-Oximometer System; Allied Instrument Laboratory) was used to measure hemoglobin content and oxygen saturation. O₂ content was calculated from the hemoglobin content and percent saturation, using a constant of 1.34. The accuracy of this equipment is 0.3 mmol/liter for O₂ content.

The basal and clamp arterial and venous blood CO₂ and O₂ contents were estimated as the mean of six respective determinations in each period. The coefficient of variation was calculated for each individual, and the mean intrasubject coefficient of variation was ~ 1% for

Table I. Clinical Characteristics of the Subjects

	Age	BMI	Body fat (percent of weight)	Waist to hip ratio	Blood glucose	Plasma IRI
	yr	kg/m ²			mmol/liter	mU/liter
NIDDM						
Mean	58	28.5	24	0.98*	11.0 [‡]	12.8
SEM	2	2.0	2	0.01	0.6	1.5
Controls						
Mean	54	27.1	19	0.93	4.88	9.6
SEM	2	1.0	2	0.01	0.10	0.8

* $P < 0.05$ vs. controls; [‡] $P < 0.001$ vs. controls.

arterial CO₂, femoral venous CO₂, and arterial O₂, and 4% for femoral venous O₂ content.

Leg O₂ consumption (VO₂) and CO₂ production (VCO₂) were calculated as the product of the arteriovenous difference and leg blood flow. Rates of leg carbohydrate and lipid oxidation were calculated using the equations of Frayn (26), employing a constant for limb protein oxidation of 85 nmol/(min · 100 ml tissue), as kindly suggested by E. Ferrannini (personal communication), based on previous studies of muscle protein oxidation (27).

Analysis. Whole blood glucose was measured with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Lactate and alanine determinations were made using microfluorometric assays (28, 29) performed on extracts of whole blood samples which had been immediately deproteinized with an equal volume of chilled 7% perchloric acid at the bedside. The supernatant was separated and stored at -70°C. Arterial plasma insulin was assayed by the method of Herbert et al. (30). Plasma FFA was measured enzymatically on arterial plasma samples using a commercial kit (NEFA C; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

PDH and GS activities. Assays of skeletal muscle PDH activity and GS activity were performed as previously described (31). PDH was assayed in the presence of fluoride with 50 μM magnesium to measure active (PDA_a), and in the absence of fluoride with 10 mM magnesium to measure total PDH activity (PDH_t). The pyruvate concentration in both cases was 1 mmol/liter. The fractional velocity of PDH (PDH_{FV}) is defined as PDA_a/PDH_t. GS was assayed in the presence of 0.1 or 10 mmol/liter glucose 6-phosphate (G6P) and 0.3 mmol/liter UDPG. GS_{FV} is defined as the activity of GS assayed at 0.1 mmol/liter G6P divided by the activity of the enzyme assayed at 10 mmol/liter G6P.

Calculations. Glucose, lactate, alanine, O₂, and CO₂ balance across the leg were calculated as the product of the arterio-venous concentration difference and blood flow and are expressed in units of μmol/(min · 100 ml leg tissue). Leg glucose clearance was calculated by dividing leg glucose uptake by arterial glucose concentration.

Net leg glucose storage was calculated as the difference between glucose uptake and the sum of glucose oxidation and release of glycolytic products. This estimate of net storage can be positive or negative. If positive, it was interpreted as net glycogen synthesis, while if negative, as net glycogenolysis. When combined as a measure of release of glycolytic products, net leg lactate and alanine release was expressed in terms of glucose equivalents.

Statistics. Data are expressed as mean ± SEM. Statistical comparisons were made using paired or unpaired *t* tests, where appropriate, using a 0.05 significance level. All correlation analyses were performed by parametric methods (Pearson's *r*). Statistical analyses were performed using RS/1 (BBN Software, Cambridge, MA).

Results

Clinical characteristics (Table I). The diabetic and nondiabetic subjects were matched for age, body mass index (BMI), and

percentage body fat, although the diabetics had a higher waist to hip ration. All subjects were male. The groups were moderately obese, as indicated by the mean BMIs, which is characteristic of the population of patients with NIDDM. Fasting plasma glucose ranged from 7.1 to 14.9 mmol/liter in the NIDDM subjects. Each control subject had normal glucose tolerance as determined with a 75 g oral glucose tolerance test; the mean plasma glucose values were 5.15 ± 0.12 at 0 min, 9.37 ± 0.39 at 60 min and 6.56 ± 0.36 mmol/liter at 120 min.

Glucose clamp. In the postabsorptive state, blood glucose was 11.01 ± 0.59 mmol/liter in NIDDM subjects and was kept constant during insulin infusion (10.96 ± 0.54 mmol/liter) by exogenous glucose infusion. In controls, blood glucose was 4.88 ± 0.10 basally and 4.94 ± 0.10 mmol/liter during insulin infusion. The coefficient of variation of arterial blood glucose concentration was < 4% throughout the insulin infusion. Basal plasma insulin tended to be higher in NIDDM (12.8 ± 1.5 mU/liter) than in the controls (9.6 ± 0.8), but this was not significant (*P* = 0.07). Insulin concentrations were equivalent during the last 30 min of insulin infusion (78 ± 3 vs. 77 ± 3 mU/liter, NS) in NIDDM and control subjects. Basal leg blood flow was lower in the NIDDM subjects (5.34 ± 0.18 ml/[min · 100 ml tissue]) than in the controls (6.04 ± 0.28, *P* < 0.05), and did not change significantly in either group during the insulin infusion. The rate of glucose infusion required to maintain euglycemia in the nondiabetics during insulin infusion was 234 ± 16 mg/(m² · min), which was not significantly different from that required to maintain hyperglycemia in the subjects with NIDDM (330 ± 48 mg/[m² · min]).

Leg glucose uptake (Table II). Basal leg glucose uptake was similar in controls and NIDDM (0.57 ± 0.06 vs. 0.73 ± 0.16 μmol/[min · 100 ml tissue], respectively). During infusion of insulin, the time course of stimulation of leg glucose uptake was similar in both groups and steady-state conditions were attained by 180 min. During the final 30 min of insulin infusion, leg glucose uptake was 5.64 ± 0.37 μmol/(min · 100 ml tissue) in controls and 8.06 ± 1.41 μmol/(min · 100 ml tissue) in NIDDM. Due to significantly greater variability among subjects with NIDDM (*F*-test on ratio of variances, *P* < 0.01), the difference in leg glucose uptake between NIDDM and controls was not significant (*P* = 0.06). Leg glucose clearance was reduced in NIDDM during the final 30 min of insulin infusion (0.74 ± 0.13 vs. 1.14 ± 0.07 ml/[min · 100 ml tissue], *P* < 0.01). Neither basal nor insulin-stimulated leg glucose uptake was correlated with fasting blood glucose in the diabetic subjects. Rates of exogenous glucose infusion were correlated with rates of leg glucose uptake during insulin infusion in NIDDM (*r* = 0.83, *P* = 0.001) and in controls (*r* = 0.68, *P* = 0.005).

Table II. Leg Glucose Metabolism

	Basal		Clamp	
	Controls	NIDDM	Controls	NIDDM
Glucose uptake	0.57 ± 0.06	0.73 ± 0.16	5.64 ± 0.37	8.06 ± 1.41
Nonoxidized glycolysis (glucose equivalents)	0.52 ± 0.12	0.62 ± 0.16	0.62 ± 0.16	0.68 ± 0.15
Glucose oxidation	1.27 ± 0.18	1.85 ± 0.26*	2.18 ± 0.31 [‡]	2.48 ± 0.27 [‡]
Glucose storage	-1.22 ± 0.27	-1.71 ± 0.30	2.83 ± 0.31	5.04 ± 1.34

All rates are expressed in terms of μmol glucose/min per 100 ml leg tissue. Nonoxidized glycolysis is the sum of net alanine plus lactate balance expressed in glucose equivalents. * *P* < 0.05 Diabetics vs. Controls; † *P* < 0.05 and †† *P* < 0.01, Basal vs. insulin infusion.

Table III. Leg Balance of Lactate and Alanine

	Basal		Clamp	
	Controls	NIDDM	Controls	NIDDM
Lactate				
Arterial (mmol · liter ⁻¹)	0.58±0.06	0.91±0.10*	0.74±0.06†	1.22±0.10‡
A-FV (mmol · liter ⁻¹)	-0.10±0.02	-0.12±0.04	-0.15±0.03	-0.17±0.04
Leg balance (μmol · min ⁻¹ · 100 ml tissue ⁻¹)	-0.60±0.15	-0.67±0.21	-0.94±0.22	-1.00±0.23‡
Alanine				
Arterial (mmol · liter ⁻¹)	0.27±0.02	0.25±0.02	0.27±0.02	0.29±0.02‡
A-FV (mmol · liter ⁻¹)	-0.07±0.01	-0.09±0.01	-0.05±0.01	-0.06±0.01
Leg balance (μmol · min ⁻¹ · 100 ml tissue ⁻¹)	-0.43±0.09	-0.51±0.08	-0.30±0.09	-0.35±0.06‡

Data are given as means±SEM. * $P < 0.01$ vs. control values; † $P < 0.05$ vs. basal values.

Leg nonoxidized glycolysis (lactate and alanine balance, Tables II and III). Basally, arterial blood lactate was significantly higher in NIDDM subjects than euglycemic controls (Table III). During insulin infusion, arterial lactate increased in controls (0.58±0.06 to 0.74±0.06 mmol/liter, $P < 0.01$) and in NIDDM (0.91±0.10 to 1.22±0.10 mmol/liter, $P < 0.01$). Despite increased arterial lactate concentrations in NIDDM, release of lactate by the leg was equivalent in the two groups basally (0.60±0.15 vs. 0.67±0.21 μmol/[min · 100 ml tissue], controls vs. NIDDM), and during the final 30 min of insulin infusion (0.94±0.22 vs. 1.00±0.23 μmol/[min · 100 ml tissue]).

Arterial blood alanine concentrations (Table III) were equivalent in controls and diabetics and did not change during insulin infusion in controls (0.27±0.02 vs. 0.27±0.02 mmol/liter), whereas in NIDDM arterial alanine increased modestly (0.25±0.02 vs. 0.29±0.02 mmol/liter, $P < 0.05$). Basally, there was a net release of alanine by leg tissue, which was similar in controls and in NIDDM. Infusion of insulin decreased net alanine release by leg in controls (0.43±0.09 to 0.30±0.09 μmol/[min · 100 ml tissue], $P < 0.05$) and in NIDDM (0.51±0.08 to 0.35±0.06 μmol/[min · 100 ml tissue], $P < 0.05$).

In controls and diabetics, infusion of insulin produced similar increases in net release of lactate and decreases in net release of alanine by the leg (Table III). The increment in arterial blood lactate between basal and steady-state levels during the insulin infusion was correlated with the increment in arterial alanine ($r = 0.86$, $P < 0.01$), as was the increment in arterial lactate and the rate of exogenous glucose infusion ($r = 0.64$, $P < 0.05$). However, there was no correlation between the changes in net release of lactate by leg tissues during insulin infusion and increments in arterial lactate ($r = 0.025$, NS).

The sum of leg net lactate and alanine release can be considered to be an estimate of the rate of nonoxidized glycolysis (22). For this purpose, lactate and alanine release were expressed in terms of glucose equivalents (1 mol glucose = 2 mol lactate or alanine). As seen in Table II, nonoxidized glycolysis increased slightly but not significantly in controls (0.52±0.12 vs. 0.62±0.16 μmol/[min · 100 ml tissue], basal vs. insulin infusion) and NIDDM (0.62±0.16 vs. 0.68±0.15 μmol/[min · 100 ml tissue]). There were no differences between the groups.

Leg glucose and lipid oxidation (local indirect calorimetry, Figs. 1–3, Tables II and IV. Data concerning leg O₂ consumption and CO₂ release are given in Table IV. Arterial blood O₂

and CO₂ content were similar in controls and NIDDM. Femoral venous (FV) oxygen content was lower in NIDDM than in controls due to reduced oxyhemoglobin saturation level in NIDDM basally (61.5±1.8% vs. 67.5±1.4%, $P < 0.05$) and during insulin infusion (58.6±2.0% vs. 66.4±2.4%). The resulting increased basal A-FV difference for O₂ in NIDDM was offset by reduced leg blood flow such that basal leg oxygen consumption was equivalent to that in controls (15.36±0.80 vs. 15.31±0.85 μmol/[min · 100 ml tissue], controls vs. diabetics, respectively). Basal CO₂ production by the leg was greater in diabetics than in controls (14.03±0.87 vs. 13.0±0.82 μmol/[min · 100 ml tissue]), which resulted in a significantly higher value for the basal RQ across the leg in NIDDM (0.92±0.03 vs. 0.84±0.02, $P < 0.01$), as can be seen in Fig. 1.

Oxygen consumption by leg tissue during the final 30 min of insulin infusion did not change compared with the basal rate in controls (15.36±0.80 vs. 15.24±1.00 μmol/[min · 100 ml tissue]) or diabetics (15.31±0.85 vs. 16.71±0.89). However, production of CO₂ by leg tissues increased during infusion of insulin and glucose in controls (13.0±0.82 vs. 14.58±0.80 μmol/[min · 100 ml tissue]), and in NIDDM (14.03±0.87 vs. 16.14±0.99 μmol/[min · 100 ml tissue]). Because of the increased CO₂ production during insulin infusion, the RQ across the leg tissue increased significantly in controls (from 0.84±0.02 to 0.97±0.02, $P < 0.01$), but the increment in RQ in NIDDM (0.92±0.03 vs. 0.96±0.02) was not significant (see Fig. 1).

Rates of gas exchange across the leg were used to calculate rates of glucose and lipid oxidation by the leg (Table II, Fig. 2). As expected from the elevated basal RQ in patients with NIDDM, basal glucose oxidation by leg tissues was increased in NIDDM compared with controls (1.85±0.26 vs. 1.27±0.18 μmol/[min · 100 ml tissue], $P < 0.05$) and rates of lipid oxida-

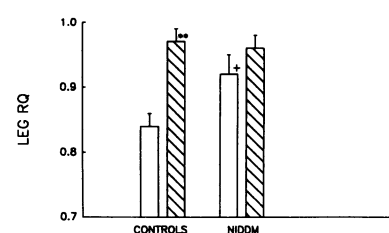


Figure 1. Basal (open bars) and insulin-stimulated (hatched bars) leg RQ in control and NIDDM subjects. RQ is calculated as VCO₂/VO₂, where VCO₂ and VO₂ are determined as the A-V differences in blood CO₂ and O₂ content multiplied by blood flow. ** $P < 0.01$ insulin vs. basal; + $P < 0.05$ controls vs. diabetics.

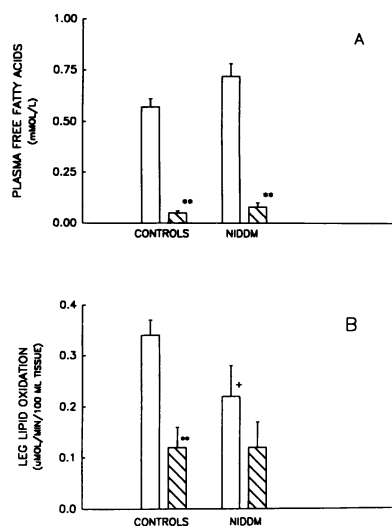


Figure 2. Plasma free fatty acid concentrations (A) and leg lipid oxidation (B) in control and NIDDM subjects. Basal data are given as open bars; insulin-suppressed data as hatched bars. ** $P < 0.01$ insulin vs. basal; + $P < 0.05$ controls vs. diabetics.

tion were lower in NIDDM (0.22 ± 0.06 vs. 0.34 ± 0.03 $\mu\text{mol}/[\text{min} \cdot 100 \text{ ml tissue}]$, $P < 0.05$). Insulin infusion increased the rates of glucose oxidation by leg tissue significantly above basal rates in both groups, and insulin-stimulated leg glucose oxidation did not differ significantly between NIDDM and controls (2.48 ± 0.27 vs. 2.18 ± 0.12 $\mu\text{mol}/[\text{min} \cdot 100 \text{ ml tissue}]$, respectively). In controls, there was a significant decrease in leg lipid oxidation during the clamp (from 0.34 ± 0.03 to 0.12 ± 0.04 $\mu\text{mol}/[\text{min} \cdot 100 \text{ ml tissue}]$, $P < 0.05$), but the decrease in leg lipid oxidation in NIDDM was not significant. Despite lower lipid oxidation rates, plasma FFA levels (Fig. 2) were higher in NIDDM during the basal period (0.72 ± 0.06 vs. 0.57 ± 0.04 mmol/liter, $P < 0.05$) and were suppressed by insulin infusion to equivalent levels (0.08 ± 0.02 vs. 0.05 ± 0.01 mmol/liter, NIDDM vs. controls, respectively). In control subjects, insulin stimulation of glucose oxidation was correlated with that of insulin-stimulation of release of glycolytic products (Fig. 3; $r = 0.79$, $P < 0.01$), but there was no such correlation in the diabetics.

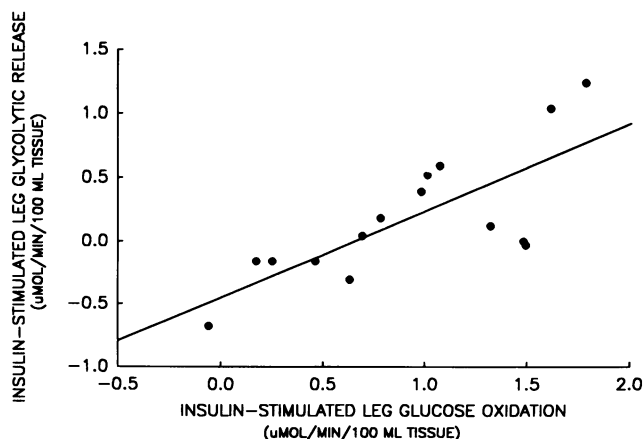


Figure 3. Relationship between insulin-stimulated leg glucose oxidation and release of glycolytic products in nondiabetic subjects. Data are expressed as insulin-stimulated differences from basal rates of leg glucose oxidation or nonoxidized glycolysis. $r = 0.79$; $P < 0.01$.

Leg glucose storage (Table II). Rates of leg glucose uptake, leg nonoxidized glycolysis, and leg glucose oxidation were used to calculate net leg glucose storage. In diabetics and controls during postabsorptive conditions, leg glucose oxidation was more than double the rate of leg glucose uptake, and net release of lactate plus alanine was equivalent to leg glucose uptake. As a consequence, there was a negative rate of net glucose storage (interpreted as glycogenolysis) during post absorptive conditions in controls and NIDDM (-1.22 ± 0.27 vs. -1.71 ± 0.30 $\mu\text{mol}/[\text{min} \cdot 100 \text{ ml tissue}]$, NS). During infusion of insulin, the rate of leg glucose storage increased in both groups (5.04 ± 1.34 vs. 2.83 ± 0.31 $\mu\text{mol}/[\text{min} \cdot 100 \text{ ml tissue}]$, diabetics vs. controls), although the values were not significantly different due to variability in leg glucose storage rates in NIDDM.

Relationship between activities of muscle enzymes and leg metabolic pathways (Tables V and VI, and Fig. 4). Under basal conditions, glycogen synthase activity at 0.1 and 10 mmol/liter G6P in diabetics did not differ from control values. In both groups, infusion of insulin resulted in significant stimulation of GS activity assayed at 0.1 mmol/liter G6P, and this stimulation did not differ from that observed in controls (2.82 ± 0.43 vs. 3.05 ± 0.39 nmol/[min · mg], controls vs. NIDDM). Insulin infusion did not increase GS activity assayed with 10 mmol/liter. As a result, infusion of insulin-stimulated GS_{FV} (Table IV) in controls (from 0.272 ± 0.047 to 0.391 ± 0.046 , $P < 0.001$) and subjects with NIDDM (from 0.322 ± 0.041 to 0.484 ± 0.054 , $P < 0.001$). GS_{FV} during insulin infusion in subjects with NIDDM was not statistically greater than control values, nor was the increment between basal and insulin-stimulated GS_{FV} (0.117 ± 0.018 vs. 0.161 ± 0.022 , controls vs. NIDDM, NS).

The relationships between insulin activation of GS_{FV} and insulin stimulation of leg glucose uptake and storage in subjects with NIDDM are shown in Fig. 4, *a* and *b*, respectively. Insulin stimulation of GS_{FV} was correlated with insulin stimulation of leg glucose uptake ($r = 0.651$, $P < 0.01$) and leg glucose storage ($r = 0.592$, $P < 0.02$). There were no significant correlations in the control subjects between insulin stimulation of glycogen synthase and leg glucose uptake ($r = 0.05$) or leg glucose storage ($r = -0.133$).

Basally, PDH_a (Table VI) was similar in diabetics and controls (1.06 ± 0.15 vs. 0.93 ± 0.147 nmol/[min · mg protein]), and although PDH_i was higher in controls, this difference was not significant (2.86 ± 0.38 vs. 2.44 ± 0.37 nmol/[min · mg]). Basal PDH_{FV} in diabetics was significantly greater than controls (0.467 ± 0.068 vs. 0.330 ± 0.022 , $P < 0.05$). Insulin infusion did not significantly increase PDH_a or PDH_i in either the diabetics or the controls. When diabetics and controls were considered together, basal muscle PDH_{FV} and basal leg glucose oxidation were significantly, although weakly, correlated ($r = 0.505$, $P < 0.01$; see Fig. 10), as were PDH_a and basal leg glucose oxidation ($r = 0.393$, $P < 0.05$).

Glycogen synthase and PDH activity in NIDDM subjects during euglycemia (Table VII). In four subjects with NIDDM, GS and PDH were assayed in biopsies taken under euglycemic conditions after slow overnight insulin infusion ($4\text{--}12$ mU/[m² · min]) and then after four hours of infusion of insulin at a rate of 40 mU/(m² · min) while euglycemia was maintained. After overnight insulin, the plasma insulin concentration was 17 ± 4 mU/ml in these subjects; during the 40 mU/(m² · min) insulin infusion, plasma insulin reached a

Table IV. Leg Indirect Calorimetry

	Basal		Clamp	
	Controls	NIDDM	Controls	NIDDM
Oxygen				
Arterial (mmol/liter)	8.63±0.15	8.56±0.14	8.30±0.16	8.16±0.18
A-FV (mmol/liter)	2.56±0.11	2.89±0.16	2.58±0.20	3.11±0.17
VO ₂	15.36±0.80	15.31±0.85	15.24±1.00	16.71±0.89
Arterial saturation (%)	94.4±0.62	93.4±0.34	94.9±0.52	94.0±0.27
FV saturation (%)	67.5±1.36	61.48±1.76*	66.4±2.44	58.6±1.99*
Carbon dioxide				
Arterial (mmol/liter)	21.99±0.28	22.06±0.37	21.46±0.32	21.61±0.42
A-FV (mmol/liter)	-2.17±0.12	-2.65±0.17*	-2.47±0.17†	-3.01±0.19†
VCO ₂	13.00±0.82	14.03±0.87	14.58±0.80†	16.14±0.99

Data are given as means±SEM. * $P < 0.05$ Control vs. NIDDM; † $P < 0.01$ Clamp vs. basal.

steady-state plateau of 67±4 mU/ml, which was not different from those insulin concentrations achieved during insulin infusion at hyperglycemia.

Basal GS activity and GS_{FV} were significantly decreased by 75% during euglycemia as compared with hyperglycemia (Table VII). Muscle GS activity and GS_{FV} were similarly decreased at euglycemia during insulin infusion. In like fashion, overnight euglycemia resulted in a 50–60% decrease in muscle PDH_a basally and during insulin infusion. Unlike the case for GS, though, total PDH activity (PDH_t) was also decreased during euglycemia to a comparable extent as was PDH_a. Therefore, PDH_{FV} during euglycemia was not different to that during hyperglycemia (Table VII).

Discussion

Critical to understanding the mechanism responsible for skeletal muscle insulin resistance in NIDDM is the question of whether impaired muscle glucose storage and oxidation occur independently of defects in glucose uptake. To address this issue, we used the limb balance technique to compare skeletal muscle glucose oxidation and storage, as well as muscle GS and PDH activities under conditions in which rates of muscle glucose uptake were matched in NIDDM and nondiabetic subjects at equivalent plasma insulin concentrations.

As an initial attempt to achieve comparable rates of muscle glucose uptake in NIDDM and nondiabetic subjects, we studied the NIDDM subjects at their prevailing fasting plasma glucose levels. This was done because Revers et al. had reported that fasting hyperglycemia was able to fully compensate for insulin resistance in NIDDM (6). This approach was successful since, in the present study, we found that basal and insulin-stimulated rates of leg glucose uptake were equivalent in NIDDM subjects studied at their fasting plasma glucose levels and nondiabetic volunteers studied at euglycemia. These results suggest that fasting hyperglycemia in a patient with NIDDM is the equilibrium point at which glucose production by the liver normalizes the rate of muscle glucose uptake. Although rates of systemic and leg glucose uptake were equivalent, glucose clearance systemically and across the leg was reduced in the NIDDM subjects. Thus, hyperglycemia in

NIDDM subjects did not completely eliminate insulin resistance. In both groups of subjects, leg glucose uptake was strongly correlated with systemic glucose disposal, accounting for nearly three-quarters of the variability in insulin-stimulated glucose disposal. Therefore, muscle is a key tissue in which compensation for insulin resistance by hyperglycemia takes place, and the prevailing fasting hyperglycemia of an individual with NIDDM is sufficient for this phenomenon to occur.

Despite normal basal leg glucose uptake, there were significant abnormalities in basal muscle glucose and lipid metabolism in the subjects with NIDDM. The basal rate of leg glucose oxidation was significantly greater in the diabetics, and this was associated with significantly increased muscle PDH activity. This increase in basal leg glucose oxidation in NIDDM occurred in spite of significantly greater plasma FFA concentrations, which suggests that there may be an abnormality in FFA clearance by muscle in NIDDM. Decreased systemic clearance of FFA in subjects with NIDDM has been reported (32). Although the extent to which muscle contributes to this defect is unknown, it may be significant, since muscle in normal subjects predominantly uses FFA as its oxidative substrate. Parenthetically, decreased use of FFA by muscle could lead to increased plasma FFA levels and increased hepatic lipid oxidation, which could in turn lead to increased hepatic glucose production (33) and be a mechanism that links defects in skeletal muscle with those in liver in NIDDM. Such an increase in hepatic lipid oxidation could offset the decrease in

Table V. Muscle Glycogen Synthase Activities

Glycogen synthase activity	Basal		Clamp	
	Controls	NIDDM	Controls	NIDDM
(0.1 mM G6P)	1.59±0.29	2.15±0.35	2.82±0.43*	3.05±0.39*
(10 mM G6P)	6.14±0.62	8.03±1.49	7.21±0.67	7.85±1.15
(GS _{FV})	0.272±0.047	0.322±0.041	0.391±0.046*	0.484±0.054*

Data are given as means±SEM in units of nmol ¹⁴C-UDPG converted to [¹⁴C]glycogen/min per mg extract protein. * $P < 0.001$ vs. basal values.

Table VI. Muscle PDH Activities

	Basal		Clamp	
	Controls	NIDDM	Controls	NIDDM
PDH _a	0.93±0.15	1.06±0.15	1.00±0.13	1.19±0.16
PDH _t	2.86±0.38	2.44±0.37	2.90±0.35	2.95±0.33
PDH _{FV}	0.330±0.022	0.467±0.068*	0.365±0.043	0.417±0.055

Data are given as means±SEM in units of nmol 1-[¹⁴C]pyruvate converted to ¹⁴CO₂/min per mg extract protein. * *P* < 0.05 vs. control values.

muscle lipid oxidation, resulting in unchanged or even increased systemic lipid oxidation in NIDDM. Finally, even though muscle glucose oxidation was increased, which suggests increased glycolysis, under basal conditions in NIDDM release of glycolytic products from the leg was normal. This is consistent with the observed increase in PDH_{FV} in that group. In spite of this, arterial lactate levels were greater than in controls, implying that increased blood lactate in NIDDM does not come from muscle.

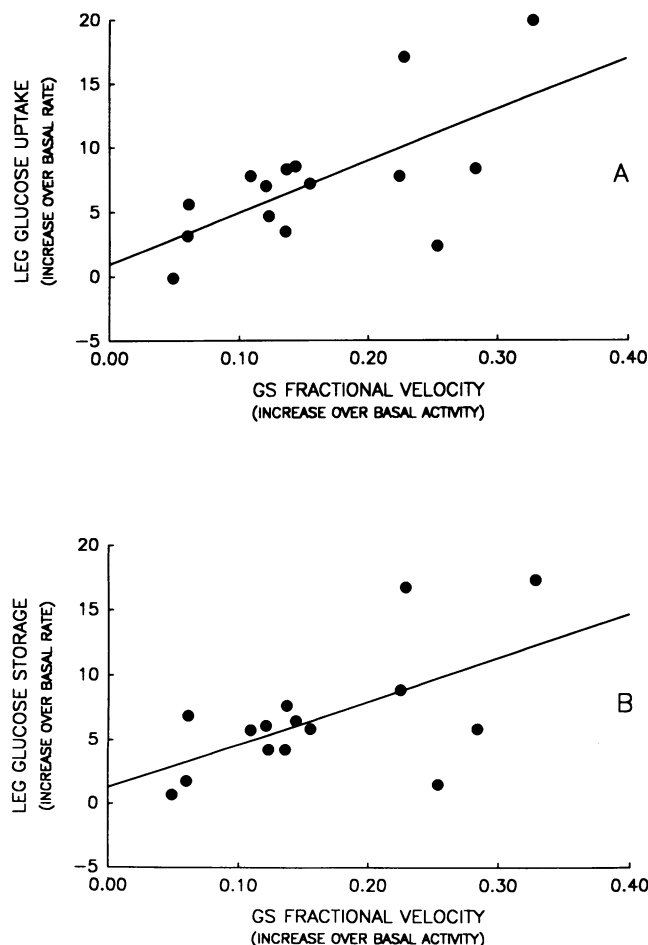


Figure 4. Relationships between insulin stimulation of glycogen synthase (GS_{FV}) and leg glucose uptake (A) (*r* = 0.651, *P* < 0.01), and storage (B) (*r* = 0.592, *P* < 0.02) in fifteen subjects with NIDDM.

Having successfully attained equivalent rates of insulin-stimulated muscle glucose uptake in NIDDM and nondiabetic subjects, it was possible to assess whether normalization of glucose uptake compensated for intracellular pathways of glucose metabolism which are decreased when glucose uptake is reduced. If this were the case, it would provide evidence that impaired muscle glucose oxidation and storage in NIDDM are secondary to reduced muscle glucose uptake.

In contrast to the abnormalities found in leg glucose metabolism in NIDDM subjects studied under basal conditions, leg glucose, storage, oxidation, and release of glycolytic products proceeded at normal rates in NIDDM subjects studied when their fasting hyperglycemia was maintained during an insulin infusion. It could be concluded from this that the defect in insulin-stimulated glucose metabolism in muscle occurs at a point that is proximal to all the pathways studied. A likely candidate for the step responsible for this defect is glucose transport. The simplest explanation for the phenomena observed in the present study is that, in NIDDM, hyperglycemia normalizes insulin-stimulated glucose uptake by mass action. Normalization of flux of glucose into the cell would then result in normal rates of intracellular glucose metabolism. A similar explanation for muscle insulin resistance in patients with insulin dependent diabetes has recently been put forth (34).

However, these findings do not exclude the possibility that hyperglycemia in NIDDM normalizes leg glucose metabolism by means of direct or indirect effects on muscle GS and PDH. This study provides some evidence that is consistent with this idea. We found that hyperglycemia normalized muscle GS and PDH activities in NIDDM subjects who had a profound defect in the activity of this enzyme when they were studied after being rendered euglycemic by an overnight insulin infusion. One interpretation of these data is that hyperglycemia increased glucose transport by mass action, resulting in increased intracellular concentrations of effectors of GS phosphatase (35, such as G6P) or PDH phosphatase (such as pyruvate), which could result in increased activities of GS and PDH through covalent modification. The normalized GS and PDH activities could then have participated at least in part in normalization of insulin-stimulated muscle glucose storage and oxidation. This effect may be limited to NIDDM, since hyperglycemia only minimally stimulates muscle glycogen synthase in nondiabetic controls (36).

These findings in subjects with NIDDM who were studied while hyperglycemic, differ significantly from those recently reported by Thorburn et al. (7), who reported decreased insulin-stimulated systemic glucose oxidation and muscle glycogen synthase activity, but paradoxically increased systemic glucose storage and elevated plasma lactate concentrations. They concluded from this that, despite normal rates of systemic glucose disposal, muscle glucose metabolism was not normalized, and attributed the increased plasma lactate concentration in NIDDM to increased muscle lactate release. These findings indicate that matching leg glucose uptake to control values by maintaining fasting hyperglycemia normalizes muscle glucose oxidation and storage in NIDDM, and that increased blood lactate concentrations in the diabetics do not come from muscle. The differences between these results and those of Thorburn et al. (7) most likely result from differences in study design and subject selection. In that study, in order to normalize glucose disposal, plasma glucose had to be increased to a mean of 20 mmol/liter, with some subjects being studied at a

Table VII. Muscle Glycogen Synthase and PDH Activities During Euglycemia Compared with Hyperglycemia in NIDDM

	Euglycemia		Hyperglycemia	
	Basal	Clamp	Basal	Clamp
GS activity at 0.1 mM G6P	0.562±0.311*	0.489±0.100*	3.13±0.77	2.78±0.51
GS activity at 10 mM G6P	5.17±0.60	3.38±0.37	7.96±2.04	5.19±1.44
GS _{FV}	0.099±0.047*	0.146±0.029*	0.396±0.062	0.605±0.090 [‡]
PDH _a	0.300±0.094*	0.583±0.215	0.990±0.310	1.02±0.17
PDH _t	0.902±0.301*	1.58±0.59	3.04±1.22	2.48±0.53
PDH _{FV}	0.341±0.014	0.365±0.065	0.372±0.049	0.457±0.12

Data are given as means±SEM; units are nmol/min · mg, as described in legends to Tables IV and V. * $P < 0.05$ less than values during hyperglycemia; [‡] $P < 0.05$ greater than basal values.

plasma glucose concentration as high as 30 mmol/liter. The increase in hyperglycemia necessary to match glucose disposal rates to controls was probably due to the fact that the nondiabetic control subjects in the Thorburn study appear to be extremely insulin sensitive compared with those in this study and those used by Revers et al. The reasons for the disparity in insulin sensitivity between the control subjects of Thorburn et al. and those in this study are not readily apparent. Nevertheless, at such high glucose concentrations in the NIDDM subjects, significant uptake and metabolism of glucose (including glycogen deposition and glycolysis) must have occurred in the liver and splanchnic bed (9–17). These tissues are likely to be the sources of the increased plasma lactate concentrations as well as much of the increase in systemic nonoxidative glucose metabolism they observed in the diabetics. Under such conditions, it is not surprising that muscle glycogen synthase activity did not reflect systemic nonoxidative glucose metabolism. Therefore, under conditions of hyperinsulinemia and hyperglycemia, inferences made about muscle from knowledge of systemic glucose metabolism must be made judiciously.

These findings should not be interpreted to mean that in daily life hyperglycemia is somehow beneficial for the patient with NIDDM. The conclusions apply only to conditions of steady-state plasma insulin concentrations matched to controls. After a meal, the pattern of plasma insulin levels in the diabetic will be different than the nondiabetic, so there may not be complete normalization of the pathways of skeletal muscle glucose metabolism, to the extent this depends on insulin concentration. Nevertheless, this study provides data that is useful in determining the biochemical site of insulin resistance.

In conclusion, our results show that basal muscle glucose oxidation is increased in NIDDM in spite of increased plasma FFA levels, suggesting that there is a defect in utilization of FFA by muscle in NIDDM. Further studies will be required to determine whether such a defect in FFA clearance exists as well as what effects it might have on systemic metabolism. Our results also indicate that, during insulin stimulation, maintenance of prevailing hyperglycemia in NIDDM subjects is sufficient to normalize muscle glucose uptake, the oxidative and nonoxidative pathways of muscle glucose metabolism, and muscle glycogen synthase and PDH activities. These findings are consistent with a mechanism whereby fasting hyperglycemia increases until uptake of glucose by muscle is normalized. Once inside muscle cells, glucose is then distributed normally to glycogen formation and glucose oxidation, possibly via nor-

malization of glycogen synthase and PDH activities by hyperglycemia.

Acknowledgments

The expert technical assistance of L. Weiss, J. Finlayson, and R. Thorne, and the nursing staff of the GCRC is gratefully acknowledged. J. Smith and G. Gutkowski provided excellent editorial assistance. We also wish to thank Dr. John Gerich for helpful suggestions.

This work was supported by funds from the American Diabetes Association, Medical Research Service, Department of Veterans Affairs, National Institutes of Health (grant R01-DK-41075), General Clinical Research Center (grant 5 M01-RR-00056), The Pennsylvania Lions, Research To Prevent Blindness, Inc., and The Eye and Ear Institute of Pittsburgh. We also thank the volunteers who participated in this study.

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