IMPAIRED GLUCOSE TRANSPORT AS A CAUSE OF DECREASED INSULIN-STIMULATED MUSCLE GLYCOGEN SYNTHESIS IN TYPE 2 DIABETES

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ABSTRACT

Background Insulin resistance, a major factor in the pathogenesis of type 2 diabetes mellitus, is due mostly to decreased stimulation of glycogen synthesis in muscle by insulin. The primary rate-controlling step responsible for the decrease in muscle glycogen synthesis is not known, although hexokinase activity and glucose transport have been implicated.

Methods We used a novel nuclear magnetic resonance approach with carbon-13 and phosphorus-31 to measure intramuscular glucose, glucose-6-phosphate, and glycogen concentrations under hyperglycemic conditions (plasma glucose concentration, approximately 180 mg per deciliter [10 mmol per liter]) and hyperinsulinemic conditions in six patients with type 2 diabetes and seven normal subjects. In vivo microdialysis of muscle tissue was used to determine the gradient between plasma and interstitial-fluid glucose concentrations, and open-flow microperfusion was used to determine the concentrations of insulin in interstitial fluid.

Results The time course and concentration of insulin in interstitial fluid were similar in the patients with diabetes and the normal subjects. The rates of whole-body glucose metabolism and muscle glycogen synthesis and the glucose-6-phosphate concentrations in muscle were approximately 80 percent lower in the patients with diabetes than in the normal subjects under conditions of matched plasma insulin concentrations. The mean (±SD) intracellular glucose concentration was 2.0±8.2 mg per deciliter (0.11±0.46 mmol per liter) in the normal subjects. In the patients with diabetes, the intracellular glucose concentration was 4.3±4.9 mg per deciliter (0.24±0.27 mmol per liter), a value that was 1/25 of what it would be if hexokinase were the rate-controlling enzyme in glucose metabolism.

Conclusions Impaired insulin-stimulated glucose transport is responsible for the reduced rate of insulin-stimulated muscle glycogen synthesis in patients with type 2 diabetes mellitus. (N Engl J Med 1999;341: 240-6.)

DIABETES mellitus is the most common metabolic disease in the world.[1] Over 90 percent of patients with diabetes have type 2, and although the primary factors that cause this disease are unknown, it is clear that insulin resistance has an important role in its development. Evidence of this role comes from longitudinal studies showing that insulin resistance precedes the onset of the disease by 10 to 20 years,[2-4] from cross-sectional studies in which insulin resistance is a consistent finding in patients with type 2 diabetes,[5-7] and from prospective studies showing that insulin resistance is the best predictor of the development of diabetes.[2-4]

Insulin resistance in patients with type 2 diabetes can be attributed mostly to decreased stimulation of muscle glycogen synthesis by insulin,[8,9] and defects in glycogen synthase,[10-12] hexokinase,[13-17] and glucose transport[16-19] have all been implicated in the reduced rate of glycogen synthesis (Fig. 1). To determine the relative importance of these factors as determinants of the uptake and metabolism of muscle glucose, we used nuclear magnetic resonance (NMR) spectroscopy with carbon-13 and phosphorus-31 ($^{13}C-^{31}P$ NMR) to measure intracellular concentrations of glucose, glucose-6-phosphate, and glycogen in muscle in patients with type 2 diabetes and normal subjects. Because it has been proposed that decreased delivery of insulin to the muscle underlies the insulin resistance in patients with type 2 diabetes,[20] we also measured insulin concentrations in interstitial fluid.

METHODS

Subjects

We performed NMR measurements and hyperglycemic–hyperinsulinemic clamp studies in seven normal subjects (four men and three women; age range, 24 to 77 years) and in six men with type 2 diabetes (age range, 39 to 67 years). The mean (±SD) body-mass index (calculated as the weight in kilograms divided by the square of the height in meters) was 22.3±1.9 in the normal subjects and 30.7±3.7 in the patients with diabetes. The hyperglycemic–hyperinsulinemic clamp studies were performed after the subjects had eaten an isocaloric diet (55 percent carbohydrate, 20 percent protein, and 25 percent fat) for three days and then fasted overnight. In the group of patients with diabetes, the mean duration of the disease was 9±7 years, and the mean value for glycosylated hemoglobin was 11.8±3.1 percent (normal range, 6 to 8 percent); none of the patients had taken any oral hypoglycemic drugs for at least eight days before the study.

We measured insulin concentrations in interstitial fluid by open-flow microperfusion techniques, and we used microdialysis techniques to determine the gradient between plasma and interstitial glucose concentrations during a similar clamp study in five normal subjects (two men and three women; age range, 34 to 67

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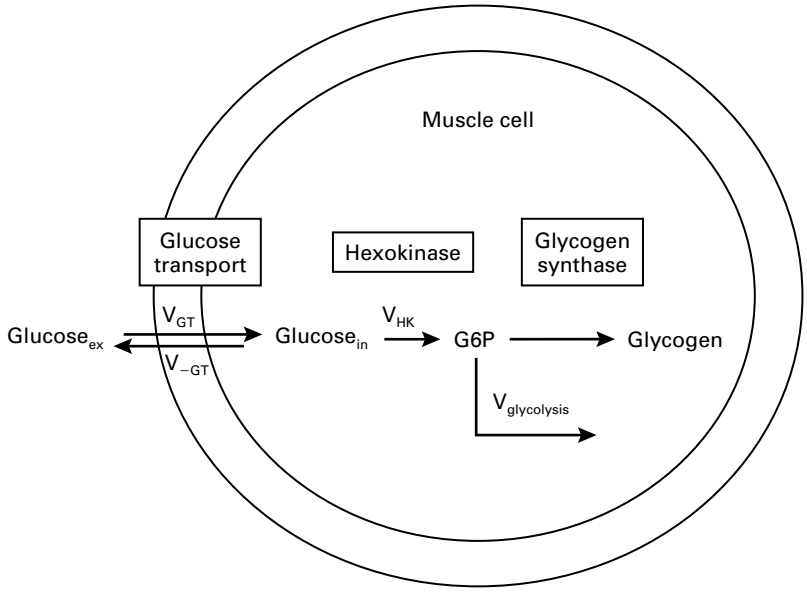

Figure 1. Potential Rate-Controlling Steps in Insulin Stimulation of Glycogen Synthesis in a Muscle Cell. $Glucose_{ex}$ and $glucose_{in}$ denote the extracellular and intracellular glucose concentrations, respectively; V_{GT} and V_{-GT} the velocity of glucose transport into and out of the muscle cell, respectively; V_{HK} the velocity of glucose phosphorylation by hexokinase; G6P glucose-6-phosphate; and $V_{glycolysis}$ the net velocity of the glycolytic flux of glucose-6-phosphate.

years; body-mass index, 28.8±7.8) and six patients with type 2 diabetes (five men and one woman; age range, 33 to 69 years; body-mass index, 30.9±5.6). Two of the patients with type 2 diabetes participated in both the NMR studies and the interstitial-fluid studies. The six patients participating in the interstitial-fluid studies had had diabetes for a mean of 11±7 years and had a mean glycosylated hemoglobin value of 11.4±2.8 percent; all six were receiving their usual treatment while these studies were done. None of the patients in any of the studies were being treated with insulin.

The study protocols were reviewed and approved by the Human Investigation Committee of Yale University School of Medicine. All the subjects gave written informed consent.

NMR and Hyperglycemic–Hyperinsulinemic Clamp Studies

Protocol 1

After the subjects had fasted overnight, Teflon catheters were inserted into an antecubital vein in each arm for blood drawing and infusions. The subjects were placed in a 2.1-T NMR spectrometer, and glucose-6-phosphate in the gastrocnemius muscle was measured by ^{31}P NMR spectroscopy. An infusion of somatostatin (0.1 μg per kilogram of body weight per minute) was then started and was continued throughout the study to inhibit endogenous insulin secretion. Five minutes later, insulin was given as a primed continuous infusion (40 mU per square meter of body-surface area per minute) along with a primed continuous infusion of glucose (20 to 40 percent enriched with [1-^{13}C]glucose) for 220 minutes. The latter infusion was periodically adjusted to maintain the plasma glucose concentration at about 180 mg per deciliter (10 mmol per liter) for the duration of the experiment. Blood samples were obtained every 15 minutes from a warmed arm vein for measurements of plasma insulin, glucose, and mannitol and ^{13}C enrichment of glucose and mannitol. The mean rate of glucose infusion, minus the rate of urinary glucose excretion, served as a measure of the rate of whole-body glucose metabolism.

Protocol 2

To determine the relative roles of glucose transport and hexokinase activity in glycogen synthesis in the patients with diabetes, four of the patients were studied again under identical hyperglycemic conditions but with the insulin-infusion rate increased by a factor of 10 (400 mU per square meter per minute).

Protocols 1 and 2

The change in glucose-6-phosphate in response to insulin stimulation was measured between 45 and 60 minutes after the start of the insulin infusion. Thereafter, serial ^{13}C NMR spectra of the muscle were recorded to determine the rate of muscle glycogen synthesis between 90 and 120 minutes after the start of the infusion.[9] At 120 minutes, an infusion of mannitol (99 percent enriched with [1-^{13}C]mannitol) was begun, and intracellular glucose was measured between 180 and 220 minutes.[21]

Glucose and Insulin Concentrations in Interstitial Fluid

After the subjects had fasted overnight, two probes were inserted into the gastrocnemius muscle to obtain samples of interstitial fluid: a microdialysis probe (CMA60, CMA/Microdialysis, Solna, Sweden) for the measurement of interstitial glucose, and an open-flow microperfusion probe for the measurement of interstitial insulin.[22] After a three-hour equilibration period, a hyperglycemic–hyperinsulinemic clamp study was performed as described above.

The microdialysis probe was perfused with artificial extracellular fluid (sodium chloride, 135 mmol per liter; potassium chloride, 3 mmol per liter; magnesium chloride, 1 mmol per liter; calcium chloride, 1.2 mmol per liter; ascorbate, 200 μmol per liter; and sodium phosphate buffer, 2 mmol per liter, adjusted to pH 7.4) at a flow rate of 0.3 μl per minute with the use of a portable pump (CMA106, CMA/Microdialysis). At this flow rate, the rate of recovery with the microdialysis probe is almost 100 percent.[23] The open-flow microperfusion probe was perfused at a flow rate of 1 μl per minute. The inlet of the double-lumen probe was connected to a bag containing artificial extracellular fluid of

TABLE 1. MEAN (±SD) PLASMA GLUCOSE AND INSULIN CONCENTRATIONS AND RATE OF GLUCOSE INFUSION IN NORMAL SUBJECTS AND PATIENTS WITH TYPE 2 DIABETES DURING NMR MEASUREMENTS.*

GROUP	NO. OF SUBJECTS	PLASMA GLUCOSE		PLASMA INSULIN DURING CLAMP STUDY	RATE OF GLUCOSE INFUSION DURING CLAMP STUDY
		BASAL	DURING CLAMP STUDY		
		mg/dl		μU/ml	mg/kg/min
Normal subjects (protocol 1)	7	85±5	176±5	52±16	15.8±3.4
Patients with diabetes (protocol 1)	6	178±32	187±12	62±17	3.2±1.7
Patients with diabetes (protocol 2)	4	218±28	184±4	670±156	11.2±0.8

*The mean rate of glucose infusion, minus the rate of urinary glucose excretion, served as a measure of the rate of whole-body glucose metabolism. To convert the values for plasma glucose to millimoles per liter, multiply by 0.056. To convert the values for plasma insulin to picomoles per liter, multiply by 6. To convert the values for the glucose-infusion rate to micromoles per kilogram of body weight per minute, multiply by 5.56. NMR denotes nuclear magnetic resonance.

the same composition as that used for microdialysis, and the outlet was connected to a peristaltic pump (Minipuls 3, Gilson, Middleton, Wis.). Effluent samples for the measurement of insulin were collected at 10-minute intervals. Although the equilibration between the perfusate and the interstitial fluid is not complete at this flow rate (the effluent concentration is lower than the interstitial concentration), the perfusion flow rates were the same in all studies. Therefore, the dilution of insulin in the effluent was the same for all subjects, and differences in the effluent insulin concentrations reflected differences in the interstitial insulin concentrations.

Analytic Procedures

Glucose in plasma and muscle-tissue effluent was measured by the glucose oxidase method (Glucose Autoanalyzer II, Beckman Instruments, Fullerton, Calif.). Plasma insulin was measured with the use of a double-antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, Tex.), and interstitial fluid insulin was measured with the use of an ultrasensitive human insulin radioimmunoassay (Linco Research, St. Charles, Mo.). The relative concentrations of plasma [1-^{13}C]glucose and [1-^{13}C]mannitol were determined by ^{13}C NMR spectroscopy, and ^{13}C enrichment of plasma glucose and mannitol was measured by gas chromatography–mass spectrometry.[24]

Calculations

The rate of muscle glycogen synthesis was determined according to the increase in the amplitude of the signal for carbon 1 in muscle glycogen, with the use of ^{13}C NMR spectroscopy.[9] The rate of glycogen synthesis was calculated from the slope of the least-squares linear fit of the glycogen-concentration curve.

Intracellular glucose concentrations and the ratio of the intracellular volume to the extracellular volume were determined by comparing ^{13}C NMR spectra for muscle and plasma concentrations of glucose and mannitol, as described previously.[24] The concentration of intracellular glucose was calculated as the difference between total tissue glucose, measured with the use of [1-^{13}C]mannitol as an in vivo internal concentration standard, and the extracellular glucose concentration corrected for the ratio of the volume of the intracellular space to the volume of the extracellular space.[24] The extracellular glucose concentration was calculated on the basis of the plasma glucose concentration and the gradient between the plasma and interstitial-fluid glucose concentrations, as determined by microdialysis. The ratio of the volume of the intracellular space to the volume of the extracellular space was determined on the basis of the relative ^{13}C NMR signal intensities for extracellular mannitol and intracellular creatine (creatine plus creatine phosphate).[24]

The hypothetical intracellular glucose concentration resulting from reduced hexokinase flux with normal glucose-transport kinetics was calculated by simplifying the metabolic steps regulating glycogen-synthesis flux in muscle, as shown in Figure 1. The steady state is represented by the following equation:

$$\Delta[\text{glucose}_{in}] \div \Delta t = V_{GT} - V_{-GT} - V_{HK} = 0,$$

where glucose_{in} is the intracellular glucose concentration, t is time, V_{GT} is the velocity of glucose transport into the cell, V_{-GT} is the velocity of glucose transport out of the cell, and V_{HK} is the velocity of glucose phosphorylation by hexokinase.

Assuming Michaelis–Menten kinetics yields the following equations:

$$V_{GT} = V_{GTmax}[\text{glucose}_{ex}] \div (K_{m1} + [\text{glucose}_{ex}])$$

and

$$V_{-GT} = V_{-GTmax}[\text{glucose}_{in}] \div (K_{m-1} + [\text{glucose}_{in}]),$$

where V_{GTmax} is the maximal velocity of glucose transport into the cell, glucose_{ex} is the extracellular glucose concentration, K_{m1} is the Michaelis–Menten constant for glucose transport into the cell, V_{-GTmax} is the maximal velocity of glucose transport out of the cell, and K_{m-1} is the Michaelis–Menten constant for glucose transport out of the cell.

We then made the additional assumptions that the transport of glucose by GLUT-4 is the same in both directions ($V_{GTmax} = V_{-GTmax}$, $K_{m1} = K_{m-1}$) and that the velocity of glucose phosphorylation by hexokinase is approximately equal to the glycogen-synthesis rate in the control subjects (35 mg per liter of muscle per minute [0.20 mmol per liter of muscle per minute]). The use of a Michaelis–Menten constant of 90 mg per deciliter (5 mmol per liter) for GLUT-4,[25] with an extracellular glucose concentration of 180 mg per deciliter and an intracellular glucose concentration of 1.8 mg per deciliter (0.1 mmol per liter), resulted in the following calculation of the velocity of glycogen synthesis:

$$V_{GTmax}[glucose_{ex}] \div (K_{m1} + [glucose_{ex}]) - V_{-GTmax}[glucose_{in}] \div (K_{m-1} + [glucose_{in}]),$$

$$V_{GTmax}[180 \div (90 + 180)] - V_{-GTmax}[1.8 \div (90 + 1.8)] = 35 \text{ mg per liter of muscle per minute},$$

and

$$V_{GTmax} = 54 \text{ mg per liter of muscle per minute (0.30 mmol per liter of muscle per minute)}.$$

Assuming that the velocity of glucose phosphorylation by hexokinase is decreased in patients with diabetes but not in normal persons and that it is approximately equal to the glycogen-synthesis rate of 4.9 mg per liter of muscle per minute (0.027 mmol per liter of muscle per minute), this calculation can be repeated with a value of 54 mg per liter of muscle per minute for the maximal rate of glucose transport into the cell:

$$54[180 \div (90 + 180)] - 54[[glucose_{in}] \div ([glucose_{in}] + 90)] = 4.9 \text{ mg per liter of muscle per minute},$$

and

$$glucose_{in} = 122 \text{ mg per deciliter (6.8 mmol per liter)}.$$

Statistical Analysis

Unpaired two-tailed t-tests were used for comparisons between groups. Paired two-tailed t-tests were used to compare the results of the low-dose and high-dose insulin studies in the patients with type 2 diabetes.

RESULTS

Under conditions of a steady-state plasma insulin concentration (approximately 57 μU per milliliter [340 pmol per liter]) and a steady-state plasma glucose concentration (approximately 180 mg per deciliter), which are similar to the postprandial concentrations, the mean (±SD) rate of glucose infusion in the patients with diabetes was approximately 80 percent lower than that in the normal subjects during the NMR measurements (Table 1). The mean rate of muscle glycogen synthesis and the increment in glucose-6-phosphate concentrations in muscle were also approximately 80 percent lower in the patients with diabetes than in the normal subjects (Fig. 2), results that are consistent with our previous findings.[9,16] The reduction in glucose metabolism and glycogen synthesis could not be attributed to a reduced rate of insulin delivery to the muscle, because the interstitial-fluid insulin concentrations during the hyperglycemic–hyperinsulinemic clamp studies were similar in the patients with diabetes and the normal subjects (Fig. 3).

On the basis of a qualitative comparison of the ^{13}C NMR spectra of muscle and plasma during the infusion period (Fig. 4), the relative concentrations of glucose and mannitol in the total tissue space were similar to those in the plasma space. Since the mannitol signal in muscle arises only from the extracellular space, any intracellular glucose will contribute to an increase in the ratio of the glucose signal to the mannitol signal as compared with this ratio in plasma. The in vivo glucose-to-mannitol ratio is proportional to the ratio of intracellular to extracellular glucose concentrations weighted by the distribution volumes. On the basis of the ^{13}C NMR–measured ratio of mannitol to creatine, the ratio of the volume of intracellular space to the volume of extracellular space was the same in the normal subjects and the patients with diabetes (8±1). Using microdialysis, we found no gradient between the arterialized plasma glucose concentration and the interstitial-fluid glucose concentration (ratio of plasma glucose to interstitial-fluid glucose in the normal subjects, 1.02±0.02; ratio in the patients with diabetes, 1.01±0.07). Thus, in muscle, glucose is confined almost exclusively to the extracellular space. The intracellular glucose

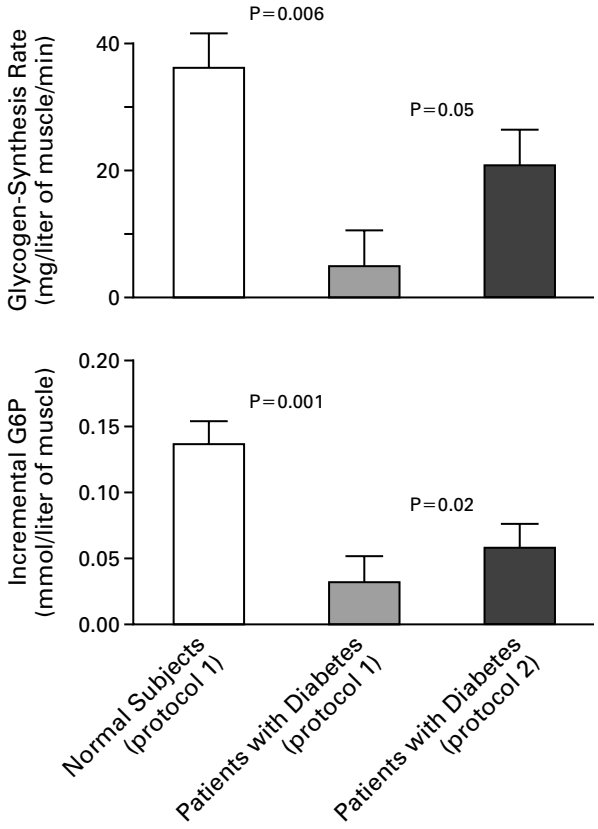

Figure 2. Mean (+SD) Rates of Glycogen Synthesis (Upper Panel) and Incremental Glucose-6-Phosphate (G6P) Concentrations (Lower Panel) in Normal Subjects and Patients with Type 2 Diabetes during Hyperglycemic–Hyperinsulinemic Clamp Studies.

The rate of glycogen synthesis was determined between 90 and 120 minutes after the start of the insulin infusion, and the glucose-6-phosphate concentrations were measured from 5 to 20 minutes before and from 45 to 60 minutes after the start of the insulin infusion. In the normal subjects and the patients with diabetes, the mean plasma insulin concentration was approximately 57 μU per milliliter (340 pmol per liter) in protocol 1 and approximately 670 μU per milliliter (4000 pmol per liter) in protocol 2. To convert the values for the glycogen-synthesis rate to micromoles per liter per minute, multiply by 5.56.

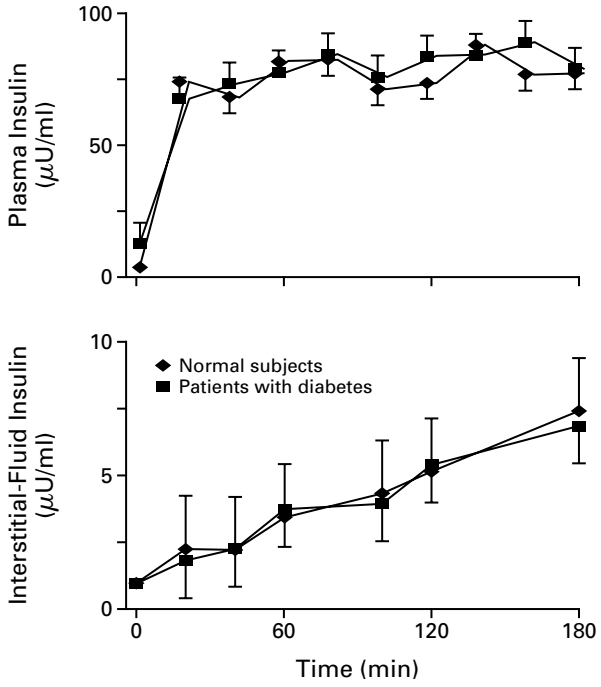

Figure 3. Mean (±SD) Insulin Concentrations in Plasma and Interstitial Fluid (Open-Flow Microperfusion Effluent) in Muscle over Time in Normal Subjects and Patients with Type 2 Diabetes during Hyperglycemic–Hyperinsulinemic Clamp Studies.

The plasma glucose concentration was approximately 180 mg per deciliter (10 mmol per liter), and the plasma insulin concentration was approximately 80 μU per milliliter (500 pmol per liter). The interstitial-fluid concentrations of insulin were diluted by the open-flow microperfusion technique. Since the perfusion flow rates were similar in all studies, the dilution of insulin in the effluent was the same for all subjects. To convert the values for insulin to picomoles per liter, multiply by 6.

concentration was calculated to be 2.0±8.2 mg per deciliter (0.11±0.46 mmol per liter) in the normal subjects. In the patients with diabetes, the concentration was 4.3±4.9 mg per deciliter (0.24±0.27 mmol per liter), a value that was 1/25 what it would be if hexokinase were the primary rate-controlling enzyme for glycogen synthesis.

When the insulin-infusion rate was increased by a factor of 10 in the patients with diabetes, the rates of whole-body glucose metabolism and glycogen synthesis increased by a factor of approximately 4, over the results with a low-dose insulin infusion (Table 1 and Fig. 2). These changes were accompanied by an increase in glucose-6-phosphate by a factor of approximately 2 (Fig. 2) and a slight decrease in the intracellular glucose concentration, to a measured value of −2.2±5.8 mg per deciliter (−0.12±0.32 mmol per liter, P=0.05). The negative value reflects the imprecision of the measurement of the intracellular glucose concentration when it is less than 5.0 mg per deciliter (0.3 mmol per liter).

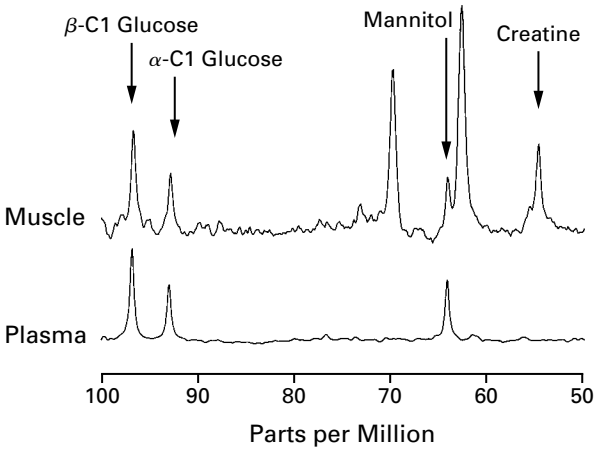

Figure 4. Representative ^{13}C NMR Spectra of Muscle and Plasma during an Infusion of [1-^{13}C]Glucose and [1-^{13}C]Mannitol under Hyperglycemic–Hyperinsulinemic Conditions in a Patient with Type 2 Diabetes.

The plasma glucose concentration was approximately 180 mg per deciliter (10 mmol per liter), and the plasma insulin concentration was approximately 57 μU per milliliter (340 pmol per liter). α-C1 and β-C1 denote carbon 1 of the alpha and beta anomers, respectively, of glucose.

DISCUSSION

Intracellular glucose-6-phosphate is an intermediary between glucose transport and glycogen synthesis, and its intracellular concentration is responsive to the relative activities of these two processes. If the activity of glycogen synthase is decreased in patients with diabetes, glucose-6-phosphate will be higher in such patients than in normal subjects. The blunted incremental changes in glucose-6-phosphate in patients with type 2 diabetes in response to insulin stimulation can therefore be ascribed to either decreased glucose-transport activity or decreased hexokinase activity and are consistent with our previous results.[16]

In the same manner, intracellular glucose is an intermediary between glucose transport and hexokinase activity, and its concentration is responsive to the relative activities of these two processes. To distinguish between impaired glucose transport and impaired hexokinase activity in the patients with diabetes, we measured intracellular glucose using a ^{13}C NMR technique. Unlike the biopsy method, this approach is noninvasive and is not subject to the errors caused by contamination of biopsy tissue with plasma glucose or incomplete removal of nonmuscle constituents. If hexokinase activity were reduced relative to glucose transport in patients with diabetes, one would predict a substantial increase in intracellular glucose. In keeping with this prediction, we recently observed that intracellular glucose is increased in transgenic mice that overexpress the GLUT-1 glucose transporter in skeletal muscle.[26] In contrast, if glucose transport is

primarily responsible for maintaining intracellular glucose metabolism, intracellular glucose and glucose-6-phosphate should change proportionately. In a recent study in which we used the same NMR approach with ^{13}C and ^{31}P as in this study, insulin resistance in skeletal muscle induced by increasing the plasma free-fatty-acid concentration was associated with a concomitant fall in both glucose-6-phosphate and intracellular glucose, a finding indicative of control at the level of glucose transport.[27] The patients with diabetes in our current study did not have a marked accumulation of intracellular glucose in association with a substantially lower rate of glycogen synthesis.

When the rates of muscle glycogen synthesis in four of the patients with diabetes were increased by increasing the insulin-infusion rate by a factor of 10, the changes in the concentrations of intracellular glucose and glucose-6-phosphate (Fig. 2) indicated that the rates of glucose transport were matched by increases in the rates of glucose phosphorylation and glycogen synthesis. These data support the hypothesis that glucose-transport activity has a predominant role in insulin-stimulated muscle glycogen synthesis in patients with diabetes,[28] but they do not rule out the possibility of additional abnormalities in the glycogen-synthesis pathway, which under these conditions would not have a strong rate-controlling effect. In our study in which free-fatty-acid–induced insulin resistance resulted in decreased glucose-transport activity,[27] insulin-stimulated phosphatidylinositol-3-kinase activity was also decreased, and this decrease, in turn, could lead to other defects in the action of insulin. This mechanism would also be consistent with the data in the present study.

A triple-tracer, forearm-infusion technique, combined with a multicompartmental model, has been used in an attempt to distinguish the rate of muscle glucose transport from that of hexokinase activity.[17,29] Using an alternative approach with dynamic imaging of [^{18}F]2-deoxyglucose uptake and positron-emission tomography, Kelley et al. found that in patients with type 2 diabetes, unlike normal subjects, there was no stimulation of glucose transport by insulin, and at the same time, there was a blunted increase in glucose phosphorylation.[13] These other studies have suggested that the patients had a resistance to the actions of insulin in stimulating both glucose transport and glucose phosphorylation. The inherent assumptions and particular model chosen to interpret the kinetic data in these other studies leave in question the relative importance of glucose transport and glucose phosphorylation in causing insulin resistance in patients with type 2 diabetes.

It has also been hypothesized that decreased delivery of substrate or insulin to the tissue bed might be responsible for insulin resistance in type 2 diabetes.[20] In regard to substrate delivery, we found no difference in the ^{13}C NMR–measured ratio of the volume of intracellular space to the volume of extracellular space in the normal subjects and the patients with diabetes, suggesting that there was no substantial difference in insulin-mediated vasodilatation between the two groups. We also found no difference in the interstitial-fluid insulin concentrations during the hyperinsulinemic clamp studies in the two groups, suggesting that the delivery of insulin is not responsible for insulin resistance in patients with type 2 diabetes. This result is consistent with previous studies that found similar time courses for insulin-receptor autophosphorylation in normal subjects and patients with type 2 diabetes.[30]

Overall, the results of our study are consistent with the hypothesis that glucose transport is the rate-controlling step in insulin-stimulated muscle glycogen synthesis in patients with type 2 diabetes.

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