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Decreased muscle glucose transport/phosphorylation is an early defect in the pathogenesis of non-insulin-dependent diabetes mellitus

(¹³C NMR spectroscopy/glycogen metabolism/nonoxidative glucose metabolism)

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ABSTRACT **Recent studies have demonstrated that reduced insulin-stimulated muscle glycogen synthesis is the major cause of insulin resistance in patients with non-insulin-dependent diabetes mellitus (NIDDM). This reduced rate has been assigned to a defect in either glucose transport or hexokinase activity. However it is unknown whether this is a primary or acquired defect in the pathogenesis of NIDDM. To examine this question, we measured the rate of muscle glycogen synthesis and the muscle glucose 6-phosphate (G6P) concentration using ^{13}C and ^{31}P NMR spectroscopy as well as oxidative and nonoxidative glucose metabolism in six lean, normoglycemic offspring of parents with NIDDM and seven age/weight-matched control subjects under hyperglycemic (≈11 mM)–hyperinsulinemic (≈480 pM) clamp conditions. The offspring of parents with NIDDM had a 50% reduction in total glucose metabolism, primarily due to a decrease in the nonoxidative component. The rate of muscle glycogen synthesis was reduced by 70% ($P < 0.005$) and muscle G6P concentration was reduced by 40% ($P < 0.003$), which suggests impaired muscle glucose transport/hexokinase activity. These changes were similar to those previously observed in subjects with fully developed NIDDM. When the control subjects were studied at similar insulin levels (≈440 pM) but euglycemic plasma glucose concentration (≈5 mM), both the rate of glycogen synthesis and the G6P concentration were reduced to values similar to the offspring of parents with NIDDM. We conclude that insulin-resistant offspring of parents with NIDDM have reduced nonoxidative glucose metabolism and muscle glycogen synthesis secondary to a defect in muscle glucose transport/hexokinase activity prior to the onset of overt hyperglycemia. The presence of this defect in these subjects suggests that it may be the primary factor in the pathogenesis of NIDDM.**

Insulin resistance is a primary factor responsible for glucose intolerance in type II, or non-insulin-dependent diabetes mellitus (NIDDM). Studies on both families and populations with a high incidence of NIDDM have found that reduced insulin-dependent glucose metabolism is frequently found in nondiabetic relatives (1–4) and that it is the best predictor for the later development of the disease (1, 4). Using ^{13}C NMR, we have shown that muscle glycogen synthesis accounts for the majority of insulin-dependent glucose metabolism under hyperglycemic–hyperinsulinemic conditions in healthy control subjects and that reduced muscle glycogen synthesis is the major factor responsible for impaired glucose metabolism in subjects with NIDDM (5). Glucose 6-phosphate (G6P) is an intermediate in the muscle glycogen synthesis pathway, and its concentration depends on the relative activities of the muscle glycogen synthase enzyme and glucose transport into muscle (6–9). In a recent study using ^{31}P NMR, we (6) found that muscle G6P concentration was reduced in subjects with NIDDM, suggesting that reduced activity of muscle glucose transporters or of hexokinase is responsible for the impaired muscle glycogen synthesis.

The presence of altered muscle glycogen metabolism prior to the development of diabetes is supported by studies that have found a high percentage of nondiabetic offspring of patients with NIDDM to be insulin resistant (1–4). However, the impaired muscle glycogen metabolism and glucose transport/hexokinase activity in NIDDM may be a consequence of the disease, since studies in rats (10, 11) and patients with type I diabetes (12) have demonstrated that chronic hyperglycemia can reduce insulin-stimulated glucose uptake. The present study was designed to determine whether reduced muscle glycogen synthesis and glucose transport/hexokinase activity is present in nondiabetic offspring of patients with NIDDM. ^{31}P and ^{13}C NMR were used in conjunction with the glucose–insulin clamp technique (13).

METHODS

Subjects. Six (two males, four females) lean (body mass index, 24 ± 1 kg/m²), nondiabetic subjects (age 37 ± 4 years) with fasting plasma glucose <6 mM and normal glycosylated hemoglobin (5.7% ± 0.1%, normal range 4–8%) who had one ($n = 1$) or both parents ($n = 5$) with NIDDM were studied (offspring of NIDDM parents). Seven control subjects (two males, five females) with fasting plasma glucose concentration <6 mM, normal glycosylated hemoglobin (5.7% ± 0.1%), and no family history of NIDDM traced through their grandparents were studied as controls. The age (32 ± 3 years) and body mass index (24 ± 1 kg/m²) of the control subjects were matched to the offspring of NIDDM parents.

Experimental Protocol. Written consent was obtained from all subjects after explanation of the purpose, nature, and potential risks of the study. The protocol was reviewed and approved by the Human Investigation Committee of Yale University School of Medicine. The subjects were admitted to the Yale–New Haven Hospital General Clinical Research Center the evening before the study. All studies were begun at 8 a.m. after an overnight fast of 12–14 hr. A teflon catheter was inserted into an antecubital vein in each arm for blood drawing and glucose/hormone infusion. A baseline ^{31}P NMR spectrum

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; G6P, glucose 6-phosphate; Pi, intracellular phosphate; PCr, phosphocreatine.

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($t = -40$ to 0 min) was obtained. An infusion of somatostatin (0.1 μg/kg of body weight per min) was initiated at time $t = -5$ min to inhibit endogenous insulin secretion and was continued throughout the study. At $t = 0$ min, insulin (Novolin; Novo-Nordisk, Copenhagen) was administered in a priming dose of 240 pmol/m^2 of body surface area per min followed by a continuous infusion (6 pmol/kg per min) to raise the plasma insulin concentration rapidly and to maintain it at ≈480 pM. Simultaneously, a variable priming infusion of [1-^{13}C]glucose (1.11 M, 20% ^{13}C enriched) was begun to maintain plasma glucose levels at ≈11 mM for 120 min. At $t = 120$ min, the glucose infusion rate was reduced in the control subjects to allow plasma glucose concentrations to fall to euglycemic levels (≈5 mM), where it was clamped until $t = 240$ min while plasma insulin concentration was maintained constant. The mean glucose infusion rate minus urinary glucose excretion (which was negligible) was used to measure the total rate of glucose metabolism. Blood samples were taken for measurement of plasma glucose concentration every 5 min, plasma glucose ^{13}C enrichment every 15 min, and plasma insulin concentration every 30 min.

Measurements. Continuous indirect calorimetry was performed to determine total-body glucose oxidation at 40–60 min and 100–120 min as described (5, 14). Nonoxidative glucose metabolism was calculated by subtracting the amount of glucose oxidized from the total amount of glucose infused.

Plasma glucose ^{13}C atom percent excess was measured using GC-MS (15). Plasma glucose concentration was measured by the glucose oxidase method (Beckman). Plasma immunoreactive insulin concentration was measured with a double antibody RIA (Ventrex Laboratories, Portland, ME).

NMR Spectroscopy. During the measurements, the subjects remained supine within an NMR spectrometer (Bruker, Billerica, MA; 1-m bore, 2.1 T). The gastrocnemius muscle of the right leg was positioned within the homogeneous volume of the magnet on top of a concentric surface coil probe. The probe consisted of a 9-cm-diameter inner coil for ^{31}P and ^{13}C acquisition and a 13-cm outer coil for ^{1}H acquisition, decoupling, and shimming.

^{31}P NMR spectra were obtained using a 70° pulse at coil center (100 μsec) with a repetition time of 2 sec and a 256-msec acquisition time (6). During acquisition, ^{1}H power (1 W) was applied at the position of the C6 protons of G6P to decouple the $J_{H\text{-}P}$ interaction. Spectra were acquired in 288 scans with 4096 data points. Time domain data were zero filled and apodized using either a 2-Hz or 10-Hz exponential function and a mild 500-Hz profile correction. Concentrations of metabolites were calculated from the data as described (6). The area of the β resonance of ATP was used as an internal concentration standard and assumed to represent a concentration of 5.5 mmol/kg of muscle (16). The ^{31}P NMR G6P measurement has been validated in an animal model by comparison with chemical assay of G6P done on muscle frozen *in situ* (17).

^{13}C NMR spectra were obtained with a pulse-acquire sequence in 10-min blocks consisting of 4800 scans using a 90° pulse at coil center and a repetition time of 120 msec. Decoupling at the ^{1}H frequency at a power of 15 W was applied at the glycogen Cl proton resonance frequency during the 25.6-msec acquisition period. Quantitation of the [1-^{13}C]glycogen concentration in glucosyl units was relative to a solution of oyster glycogen in a mold of the right calf of each subject (1). A 2-cm sphere containing [^{13}C]formic acid was used as a pulse power and loading calibration. The ^{13}C NMR muscle glycogen measurement has been validated by comparison with biochemical assay of glycogen measured in biopsied human muscle tissue (18) and rabbit muscle frozen *in situ* (19).

Calculations. Increments in muscle glycogen concentration were calculated from the change in [1-^{13}C]glycogen concentration and the plasma [1-^{13}C]glucose atom percent excess as described (5). The rate of glycogen synthesis was calculated from the slope of the least-squares linear fit to the glycogen concentration curve from $t = 60$ to 120 min during hyperglycemia and $t = 180$ to 240 min during euglycemia. In five of the most insulin-resistant subjects, the hyperglycemic clamp was extended to 180 min to obtain a more accurate rate of muscle glycogen synthesis.

Statistics. Measurements are given as the group mean ± SE. Statistical differences between groups were performed using the Student's *t* test.

RESULTS

After the overnight fast, the offspring of parents with NIDDM had a mean plasma glucose concentration similar to the control subjects (5.3 ± 0.2 versus 5.2 ± 0.1 mM), whereas basal plasma insulin concentration was slightly but not significantly higher (97 ± 18 versus 60 ± 6 pM). During the hyperglycemic–hyperinsulinemic clamp, the offspring of parents with NIDDM and the control subjects had similar plasma glucose (9.8 ± 0.2 versus 9.4 ± 0.1 mM) and plasma insulin (474 ± 36 versus 492 ± 12 pM) concentrations. To assess whether decreased glycogen synthase activity contributes to impaired glycogen synthesis in the offspring of parents with NIDDM, the rate of glycogen synthesis in the control subjects was matched to that of the offspring of parents with NIDDM by lowering the plasma glucose level to euglycemia. During the euglycemic–hyperinsulinemic clamp, the average plasma glucose concentration was 4.9 ± 0.1 mM, and the plasma insulin concentration (444 ± 48 pM) was similar to the level achieved during the hyperglycemic–hyperinsulinemic clamp.

Fig. 1 shows the total and nonoxidative glucose metabolism for both groups as well as for nonobese older subjects with NIDDM studied previously under the same hyperglycemic–hyperinsulinemic clamp conditions (5). The mean rate of whole-body glucose metabolism of the offspring of parents with NIDDM was half the rate of the control subjects (32 ± 4 versus 67 ± 6 μmol/kg of body weight per min, $P < 0.002$). The majority of decrease in whole-body glucose metabolism was due to a reduced rate of nonoxidative glucose metabolism [25 ± 1 ($n = 5$)¶ versus 49 ± 6 μmol/kg of body weight per min, $P < 0.005$]. Oxidative glucose metabolism was also significantly reduced in the diabetic offspring [11 ± 2 ($n = 5$) versus 17 ± 1 μmol/kg of body weight per min, $P < 0.05$]. The reduced rates of insulin-dependent whole-body glucose metabolism and nonoxidative and oxidative glucose metabolism measured in the offspring of parents with NIDDM were similar to those previously measured in subjects with NIDDM (30 ± 4 μmol/kg of body weight per min, 22 ± 3 μmol/kg of body weight per min, and 8 ± 4 μmol/kg of body weight per min, respectively) (5). When the plasma glucose concentration in the control subjects was reduced to euglycemic levels, the rates of total and nonoxidative glucose metabolism decreased relative to the hyperglycemic portion of the clamp (42 ± 4 μmol/kg of body weight per min, $P < 0.0005$ and 26 ± 3 μmol/kg of body weight per min, $P < 0.005$) to values similar to the offspring of parents with NIDDM during the hyperglycemic–hyperinsulinemic clamp.

To determine the factors that might account for the reduced nonoxidative glucose metabolism, the rate of muscle glycogen synthesis and the concentration of G6P were measured by NMR and are shown in Fig. 2. In the offspring of parents with NIDDM, both the rate of muscle glycogen synthesis and muscle G6P concentration were significantly lower than in the control subjects (65 ± 15 versus 194 ± 32 μmol/liter of muscle per min, $P < 0.005$; 0.16 ± 0.02 versus 0.25 ± 0.01 mmol/kg

¶One subject was not included in this result due to technical problems with the indirect calorimetry measurement.

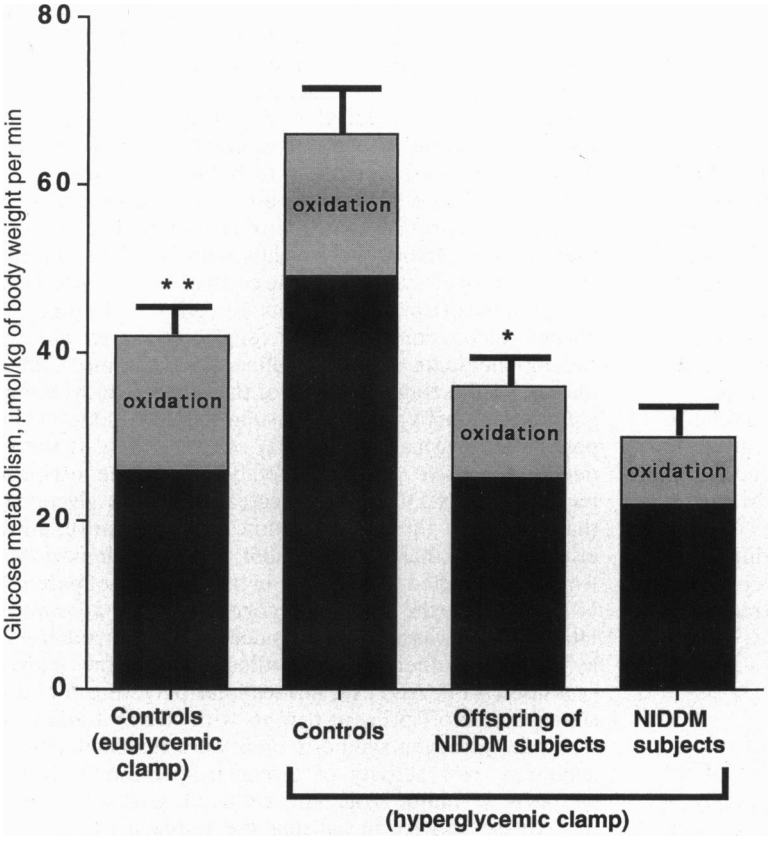

FIG. 1. Mean rates of nonoxidative and oxidative whole-body glucose metabolism in the offspring of parents with NIDDM, control subjects, and subjects with NIDDM during the same conditions of hyperglycemia–hyperinsulinemia and in the same control subjects under conditions of euglycemia–hyperinsulinemia. The reduced rate of whole-body glucose metabolism in the offspring of NIDDM subjects was mostly attributed to a reduced rate of nonoxidative glucose metabolism. The reduced rate of whole-body glucose metabolism in the offspring of parents with NIDDM was similar to subjects with NIDDM. The data for NIDDM subjects was obtained from ref. 5. *, $P < 0.005$; **, $P < 0.0005$ (compared to control subjects at hyperglycemic–hyperinsulinemia).

of muscle, $P < 0.003$) and were similar to subjects with clinically manifest NIDDM (78 ± 28 μmol/liter of muscle per min and 0.17 ± 0.02 mmol/kg of muscle) (5, 6) (Fig. 2). These values were also similar to the rate of muscle glycogen synthesis

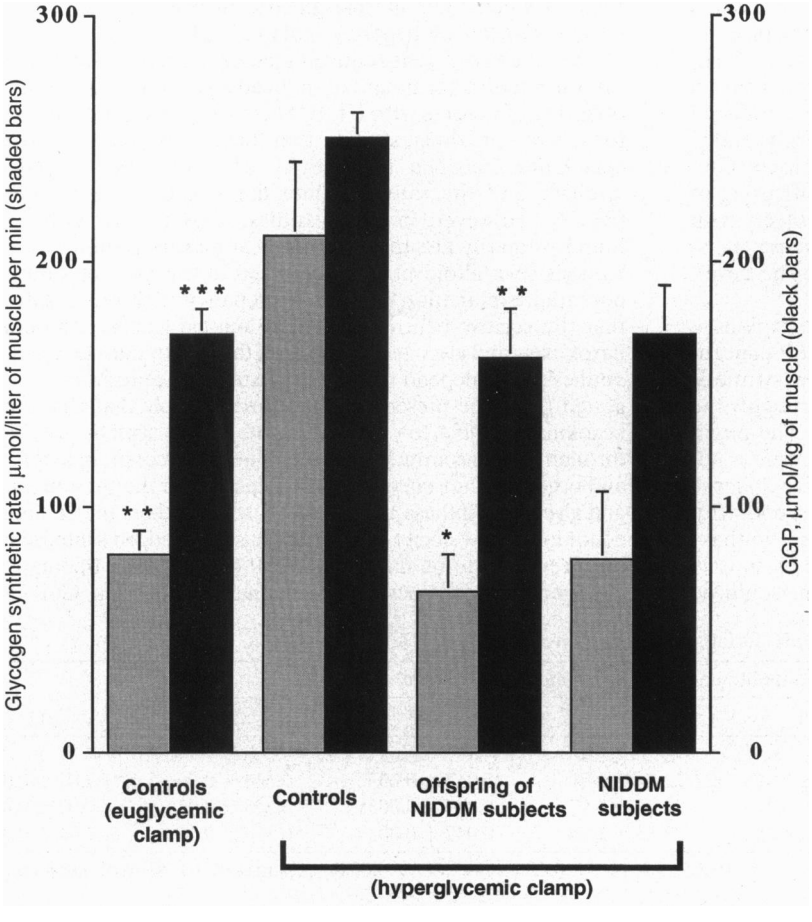

FIG. 2. Mean rates of muscle glycogen synthesis and muscle G6P concentration in the offspring of parents with NIDDM, control subjects, and subjects with NIDDM during the same conditions of hyperglycemia–hyperinsulinemia and in the same control subjects under conditions of euglycemia–hyperinsulinemia. Both the mean rate of glycogen synthesis and G6P concentration were reduced in the offspring of parents with NIDDM to values similar to that previously observed in subjects with NIDDM. For the NIDDM subjects, glycogen synthetic data were obtained from ref. 5 and the G6P data were obtained from ref. 6. *, $P < 0.005$; **, $P < 0.003$; ***, $P < 0.0004$ (compared to the control subjects at hyperglycemic–hyperinsulinemia).

and the concentration of G6P during the euglycemic–hyperinsulinemic clamp in the control subjects (76 ± 4 μmol/liter of muscle per min, $P < 0.002$; 0.17 ± 0.01 mmol/kg of muscle, $P < 0.0004$ relative to the same subjects at hyperglycemic–hyperinsulinemia).

No significant differences between the groups were observed for the mean basal muscle concentrations of G6P, ADP, phosphocreatine (PCr), intracellular phosphate (Pi), and intracellular pH, which are summarized in Table 1. During the hyperglycemic–hyperinsulinemic clamp, only the G6P concentration was significantly different in the offspring of parents with NIDDM (Table 1) compared with controls. An increment in Pi over basal values was measured in both the offspring of parents with NIDDM and control subjects. In the euglycemic–hyperinsulinemic clamp, the muscle G6P, ADP, PCr, and Pi concentrations and pH in the control subjects were similar to offspring of parents with NIDDM during the hyperglycemic clamp. The increment of Pi concentration during the euglycemic clamp in the control subjects was ≈2-fold lower than during the hyperglycemic clamp (0.293 ± 0.07 versus 0.644 ± 0.06 mmol/kg of muscle, $P < 0.005$). There was no difference between the groups under basal or clamp conditions in the β-ATP chemical shift, which reflects the free intracellular Mg^{2+} concentration (20).

DISCUSSION

In previous studies, we have found that the rate of muscle glycogen synthesis (5) and the concentration of muscle G6P (6) were reduced in subjects with NIDDM during a hyperglycemic–hyperinsulinemic clamp. From these results, we concluded that subjects with NIDDM have a defect in muscle glucose transport or hexokinase activity (6). However, it was unclear whether this reduction in glucose transport/hexokinase activity was involved in the pathogenesis of diabetes or was induced by the chronic alterations in plasma glucose and insulin concentrations associated with the disease. The present study shows that healthy, normoglycemic, lean, insulin-resistant offspring of parents with NIDDM also have a reduced rate of muscle glycogen synthesis and a reduced concentration of muscle G6P during a hyperglycemic–hyperinsulinemic clamp. The similarity of the muscle G6P concentration and glycogen synthesis rate in the offspring of parents with NIDDM compared to the older subjects with NIDDM indicates that the reduction in muscle glucose transport/hexokinase activity is fully expressed prior to the development of diabetes.

The conclusion of reduced glucose transport/hexokinase activity is based on the finding of lower muscle G6P concentration in conjunction with reduced muscle glycogen synthesis and reduced glucose outflow from the G6P pool (roughly the sum of muscle glycogen synthesis and glycolysis). The possibility of enhanced muscle glycolysis explaining this result is not tenable since rates of oxidative and nonoxidative glucose metabolism were both lower in the offspring of parents with NIDDM (6). If reduced activity of muscle glycogen synthase was solely responsible for the observed decrease in muscle glycogen synthesis, the muscle G6P concentration would be higher than in the control subjects (6). Since the concentrations of the other major effectors of hexokinase (Pi, ADP, and pH; ref. 21) were similar in the offspring of NIDDM parents and the controls, the finding of lower G6P concentrations in conjunction with reduced glycogen synthesis suggests that insulin-stimulated muscle glucose transport/hexokinase activity is reduced in the offspring of NIDDM parents.

To assess whether decreased muscle glycogen synthase activity also contributes to the reduced muscle glycogen synthesis in the offspring of parents with NIDDM, the rate of muscle glycogen synthesis in the control subjects was matched to that of the offspring of parents with NIDDM by reducing the plasma glucose concentration to euglycemic levels while maintaining the same plasma insulin concentration. Under the plasma insulin concentrations of the present study, the glycogen synthase activity in non-insulin-resistant subjects is independent of plasma glucose (22) concentration. If the major defect in muscle glycogen metabolism in the offspring of parents with NIDDM were decreased muscle glycogen synthase activity, the concentration of the positive allosteric effectors of synthase, of which G6P is the most important (23), would be expected to be higher in the offspring of parents with NIDDM when the rates of glycogen synthesis were matched. However, this was not the case since the concentrations of G6P as well as the other important allosteric effectors of glycogen synthase (ATP, ADP, Pi, intracellular pH) were found to be similar in the offspring of parents with NIDDM and controls when the glycogen synthesis rates were matched, suggesting similar *in vivo* activity of glycogen synthase. This finding supports a major role for reduced glucose transport/hexokinase activity in causing the reduced rate of muscle glycogen synthesis observed in the offspring of parents with NIDDM. Our finding is supported by the finding of similar muscle glycogen synthesis and *in vitro* glycogen synthase activity in NIDDM and insulin-dependent diabetes mellitus subjects when their muscle glucose uptake was matched to normal subjects by hyperglycemia (22, 24).

The conclusion that reduced glucose transport/hexokinase was the major determinant of reduced glycogen synthesis in the offspring of parents with NIDDM is in apparent contradiction to several previous studies that have reported a positive correlation between the activity of semipurified glycogen synthase and the rate of nonoxidative glucose metabolism (24–26). However, in these studies, a good correlation was found primarily at supraphysiological plasma insulin concentrations severalfold higher than used in the present study. A potential explanation for the discrepancy with our results is that the relative control exerted by muscle glucose transport/hexokinase and glycogen synthase on the rate of muscle glycogen synthesis may depend on plasma insulin concentration as suggested (27). The present findings further imply that transport/hexokinase is able to control the rate of the synthase reaction through the concentration of G6P. Under the conditions of ATP and other effector concentration measured in the present study and glycogen synthase activity measured by others under similar conditions, the velocity of purified muscle glycogen synthase has been reported to be linear with G6P (23, 28). The finding that glycogen synthesis increases fractionally more than G6P may

Table 1. Intramuscular metabolite concentrations and pH during glucose–insulin clamps

Subject	Metabolite concentration, nmol/kg of muscle					
	G6P	Pi	PCr	ADP	ΔPi	pH
Control						
Hyperglycemia	0.25 ± 0.01	3.35 ± 0.16	22.44 ± 0.91	0.047 ± 0.003	0.644 ± 0.06	7.02 ± 0.01
Euglycemia	0.17 ± 0.01***	3.01 ± 0.37	22.88 ± 0.97	0.047 ± 0.003	0.293 ± 0.07*	7.02 ± 0.01
Offspring of NIDDM	0.16 ± 0.02**	2.91 ± 0.25	23.33 ± 1.09	0.047 ± 0.003	0.454 ± 0.16	7.04 ± 0.01

ΔPi is the increment in Pi above the baseline. *, $P < 0.005$; **, $P < 0.003$; ***, $P < 0.0004$ (compared to control subjects at hyperglycemic–hyperinsulinemic conditions).

suggest that the *in vivo* kinetics of synthase differ from the purified enzyme. Alternatively the concentration of UDP-glucose, which was kept constant in the *in vitro* studies (23, 28), may also increase. Caution must be used in determining the *in vivo* dependence of synthase on G6P from the present data since the resting concentration of G6P may have been overestimated due to overlap with other compounds in the ^{31}P NMR spectrum. This could explain the apparently supralinear response.

The present data cannot be used to distinguish whether the reduced rate of muscle glycogen synthesis in the offspring of parents with NIDDM was due to a reduction in the activity of either glucose transport or hexokinase. Insulin-stimulated muscle glucose transport occurs by facilitated diffusion (29) using the Glut 4 transporter (30). Measurements of Glut 4 protein and mRNA in the muscle of subjects with NIDDM have generally shown no difference compared to control subjects (31). However, total glucose transporter number may not reflect glucose transport activity, since insulin-stimulated glucose transport requires the translocation of Glut 4 to the plasma membrane (32). Reduced glucose transport activity (33, 34) and number (35) has been found in studies of adipocytes from patients with NIDDM, which also express the insulin-sensitive Glut 4 transporter, and in *in vitro* muscle preparations (36) from patients with NIDDM. *In vivo* evidence for reduced muscle glucose transport activity associated with insulin resistance has recently been provided by Bonadonna *et al.* (37), who found reduced unidirectional muscle glucose transport and net muscle glucose uptake in subjects with NIDDM as well as a correlation between transport and uptake in nondiabetic subjects. Hexokinase has previously not been believed to be insulin stimulated and therefore not considered as a potential rate-limiting step. However, a recent study has suggested that *in vivo* hexokinase activity is insulin dependent and may in some conditions be rate limiting (38). In addition a reduction in glucose transport may be secondary to impaired glucose and insulin delivery due to reduced muscle capillary density or blood flow (39, 40).

In summary, we found a significant reduction in muscle glycogen synthesis and G6P concentration in lean, normoglycemic, insulin-resistant offspring of parents with NIDDM. Decreased muscle glucose transport/hexokinase activity was found to be the primary factor responsible for the reduced rate of muscle glycogen synthesis. The presence of reduced muscle glucose transport/hexokinase activity in this group of subjects, who have been shown to have a high risk of developing diabetes, suggests that it may be a primary factor in the pathogenesis of NIDDM.

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