Intracellular Skeletal Muscle Glucose Metabolism Is Differentially Altered by Dexamethasone Treatment of Normoglycemic Relatives of Type 2 Diabetic Patients

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Young first-degree relatives of type 2 diabetic patients are insulin-resistant, with the insulin resistance mainly located in skeletal muscle due to decreased insulin-induced nonoxidative glucose metabolism and muscle glycogen synthase activation. We investigated whether the mechanism differs for dexamethasone (dex)-induced insulin resistance in first-degree relatives of type 2 diabetics versus healthy control subjects by quantifying intracellular glucose processing in muscle biopsies taken before and after 5 days of dex treatment (4 mg/d) in 20 normal glucose-tolerant relatives of type 2 diabetic patients and 20 matched controls (age, 29.4 ± 1.7 *v* 29.4 ± 1.6 years; body mass index, 25.1 ± 1.0 *v* 25.1 ± 0.9 kg/m^2). In addition, an intravenous glucose tolerance test (IVGTT) combined with continuous indirect calorimetry was performed. Following 5 days of dex treatment, glucose tolerance deteriorated in both the relatives and the control subjects. Fasting dry-weight muscle glucose and fasting intracellular muscle glucose concentrations increased in response to dex only in the relatives (2.43 ± 0.21 *v* 2.97 ± 0.26 mmol/kg dry weight, $P < .05$; 0.28 ± 0.07 *v* 0.45 ± 0.08 mmol/L intracellular water, $P < .05$); no increases were observed in the control subjects. Fasting dry-weight muscle lactate also increased post-dex only in the relatives (7.37 ± 0.40 *v* 10.77 ± 1.22 mmol/kg dry weight, $P < .001$). Both basal muscle glucose and lactate concentrations from the IVGTT study correlated with the 2-hour post-dex glucose value obtained during the OGTT study in the relatives ($R = .76$ and $R = .74$, respectively, both $P < .0001$) but not in the control subjects. Basal intramuscular glycogen synthase activity decreased approximately 25% in both the relatives and control subjects post-dex; the decrement was significant ($P < .01$) only in control subjects. Indirect calorimetry during the post-dex IVGTT demonstrated increased glucose oxidation ($P < .03$) and reduced lipid oxidation ($P < .03$) in the relatives only. We postulate that the insulin resistance induced by dex in first-degree relatives of type 2 diabetic patients is associated with a preferential channeling of glucose into the glycolytic pathway (increased glucose oxidation and lactate production), probably associated with a preexisting downregulation of the glycosen synthase pathway.
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NORMAL GLUCOSE-TOLERANT first-degree relatives of non–insulin-dependent diabetic (type 2 diabetes) patients carry a lifetime risk of diabetes of approximately 40%[1] and are characterized by a decreased insulin sensitivity (due to diminished glycogen synthase activity[2]), an altered insulin secretion, and a compensatory increase in glucose-mediated glucose disposal (glucose effectiveness), the latter accounting for the maintenance of normal glucose tolerance.[3] Glucocorticoids induce insulin resistance by promoting hepatic gluconeogenesis,[4] thereby reducing the suppression of hepatic glucose production by insulin, and decrease peripheral muscle glucose uptake,[5] with a reduction in glycogen synthesis and glucose storage.[6] In addition, glucocorticoids attenuate the expected hyperinsulinemic response to the induced insulin-resistant state.[7]

Recently, we demonstrated that exposure of normal glucose-tolerant relatives of type 2 diabetics to short-term stress with dexamethasone (dex) induced insulin resistance in all subjects and compromised β-cell function in one third of the subjects.[8] These latter insulinopenic subjects developed transient dex-induced glucose intolerance. Importantly, the degree of insulin resistance induced by dex was similar in the matched controls and the relatives,[8] regardless of the pre-dex insulin resistance status[8] and the known defect of muscle glucose storage present in the normoglycemic relatives.[2,9-11] However, glucocorticoids are known to inhibit insulin-stimulated glycogen synthase activity and to reduce glycogen synthase protein content.[6] We postulated that despite the similarity in whole-body insulin resistance in controls and relatives following exposure to dex, the dex-induced mechanisms responsible for the changes in muscle glucose metabolism and insulin action were different in the relatives.

Thus, the aim of the present study was to determine basal intracellular glucose metabolism in skeletal muscle before and after exposure to dex in control subjects and relatives of type 2 diabetics. In particular, we measured the muscle intracellular glucose, glucose-6-phosphate (G-6-P), lactate, and glycogen concentration and glycogen synthase activity, as well as whole-body glucose and lipid oxidation rates, in the matched controls and normoglycemic relatives to establish the mechanism(s) for glucocorticoid-induced skeletal muscle insulin resistance in these subjects. Whole-body insulin action, glucose metabolism, and insulin secretion dynamics in these subjects have been previously reported.[3,8]

SUBJECTS AND METHODS

Subjects

Twenty subjects with one first-degree and at least one second-degree relative or two (or more) first-degree relatives with type 2 diabetes were identified by questioning verified type 2 diabetic patients from the

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Department of Endocrinology, Odense University Hospital. All relatives had normal oral glucose tolerance and were not prescribed any medication known to influence glucose homeostasis. The relatives were matched according to sex (female to male ratio, 8:12 per group), age (29.4 ± 1.7 *v* 29.4 ± 1.6 years), and body mass index (25.1 ± 1.0 *v* 25.1 ± 0.9 kg/m^2) to a group of normoglycemic control subjects (n = 20) without any family history of type 2 diabetes.[3,8]

Protocol

All subjects were studied in the fasting state with an oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT) during two periods over 3 weeks, ie, before and after 5 days' treatment with oral dex 4 mg/d (2 mg morning and evening) as previously described.[3,8] On the IVGTT days, a muscle biopsy was taken from the vastus lateralis using a Bergström needle (including suction) under local anesthesia[2] and frozen at −70°C, always within 10 seconds, for later analysis.[2] All muscle biopsies were taken after an overnight fast without the subjects' having performed any form of physical activity, since they were admitted to the ward on the evening before each study. Fasting blood samples (the mean of three for the OGTT and four for the IVGTT, respectively) were analyzed for plasma glucose, insulin, and lactate concentrations, and post–glucose load samples for plasma insulin and glucose.[3,8] During both of the 3-hour IVGTTs, continuous indirect calorimetry was performed (apart from a short break from 110 to 120 minutes) using a computerized flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland) as formerly described.[12] Samples of expired and inspired air were analyzed for oxygen and carbon dioxide concentrations at 1-minute intervals, and oxygen consumption and carbon dioxide production were calculated by the computer and recorded at 1-minute intervals from the nonprotein respiratory quotient tables of Lusk.[13] At the commencement of each study and after an initial equilibration period of 10 minutes, the mean gas-exchange rates were recorded for 30 minutes prior to the intravenous glucose load, and were used to calculate the rates of basal glucose and lipid oxidation.[14,15] After the intravenous glucose load, glucose and lipid oxidation rates were calculated as the mean integrated oxidation rate for 20-minute intervals from the 1-minute estimates over the 180-minute test periods. The protein oxidation rate was estimated from urinary nitrogen excretion (1 g nitrogen = 6.25 g protein) from urine collected over the test period.

The protocol was approved by the local Ethics Committee, and informed written consent was obtained from all participants before testing.

Assays

The frozen muscle biopsies were freeze-dried, dissected free of blood, fat, and connective tissue, and analyzed for intramuscular substrates, glycogen concentration, and glycogen synthase activity as previously described by our laboratory.[2,12] Glucose, G-6-P, and lactate levels were measured fluorometrically on neutralized perchloric acid extracts,[16] and the values are expressed as millimoles per kilogram of dry muscle weight The assays have been previously reported to have an intraassay and interassay precision of less than 10% and less than 20%, respectively.[17] Skeletal muscle glucose and lactate levels are also expressed as millimoles per liter of intracellular water, assuming a muscle biopsy extracellular water content of 0.3 L/kg dry weight and intracellular water content of 2.8 L/kg dry weight[18,19] to correct for plasma concentrations of glucose and lactate. The glycogen content in muscle biopsies was measured as glucose residues after hydrolysis with 1 mol/L HCl at 100°C for 2 hours.[16] Glycogen synthase activity was measured as previously described[20] as activity without the addition of G-6-P and in the presence of 0 05, 0.1, and 10 mmol/L G-6-P, the latter taken as the maximal activity of the enzyme. The total concentration of uridine diphosphate glucose (UDPG) ([^{14}C]UDPG + cold UDPG) in the reaction mixture was 0.31 mmol/L Activity levels are expressed as nanomoles of UDPG, incorporated into glycogen per minute per milligram of extract protein. The protein content of the extract was determined by the method of Lowry et al.[21] The fractional velocity (FV) of glycogen synthase is calculated as the ratio between the activity at 0.0 and 10 mmol/L G-6-P (FV 0.01), 0.05 and 10 mmol/L G-6-P (FV 0.05), and 0.1 and 10 mmol/L G-6-P (FV 0.1). The GLUT-4 level was measured in the muscle powder of the frozen muscle biopsies as previously described[12] by sodium dodecyl sulfate–polyacrylamide gel by electrophoresis and Western blot analysis[22] followed by quantification of GLUT-4 by densitometric scanning of immunoreactivity. The antibody used for detection of GLUT-4 was affinity-purified polyclonal rabbit antibody directed against an artificial peptide identical to the 13 COOH-terminal amino acids of GLUT-4. All assays were completed within 6 months of obtaining the muscle samples.

The plasma glucose concentration was measured bedside by the glucose oxidase method on a Glucose Analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin was determined by a double-antibody radioimmunoassay in duplicate (Kabi Pharmacia Diagnostics, Uppsala, Sweden). Plasma lactate was determined by a fluorometric method. Urinary nitrogen content was calculated as reported by Tappy et al.[23]

Statistical Analysis

The results are presented as the mean ± SE. Differences between groups were compared by a Mann-Whitney rank-sum test. Paired comparisons were performed using the Wilcoxon matched-pairs signed-rank test, and an F test was used to test the equality of variance. Correlation analyses were performed using Spearman correlation analysis. *P* values of .05 or less were considered significant.

RESULTS

Glucose Tolerance and Plasma Glucose, Insulin, and Lipid Concentrations

Before dex treatment, the fasting plasma glucose concentration was statistically increased approximately 5% in the relatives compared with the control subjects (5.41 ± 0.08 *v* 5.16 ± 0.08 mmol/L, $P < .05$). In response to dex, a significant ($P < .05$) increase was observed in each group, with a significant difference of about 6% remaining between groups (5.70 ± 0.12 *v* 5.38 ± 0.06 mmol/L, $P < .05$). Glucose tolerance was similar in relatives and control subjects before dex (2-hour plasma glucose, 5.5 ± 0.2 (range, 3.2 to 7.0) *v* 5.5 ± 0.2 (3.7 to 7.4) mmol/L, nonsignificant [NS]), but after dex, the relatives showed a more heterogenous change in the 2-hour OGTT plasma glucose versus the control subjects (Fig 1), with a significant difference for the variation in 2-hour plasma glucose between relatives and controls (8.5 ± 0.7 (3.9 to 17.0) *v* 7.5 ± 0.3 (5.7 to 9.8) mmol/L; F test, $P < .05$) as previously described.[8] Fasting plasma insulin prior to treatment with dex was similar in relatives and controls (45.6 ± 3.0 *v* 41.4 ± 3.0 pmol/L, NS) and increased significantly ($P < .005$) but equally in both groups after dex (97.2 ± 9.0 *v* 81.6 ± 5.4 pmol/L, NS). The fasting plasma lactate concentration was not significantly different between relatives and controls either before (0.97 ± 0.07 *v* 0.94 ± 0.05 mmol/L, NS) or after exposure to dex (1.31 ± 0.10 *v* 1.17 ± 0.10 mmol/L, NS), although fasting plasma lactate increased significantly after dex in both groups ($P < .05$).

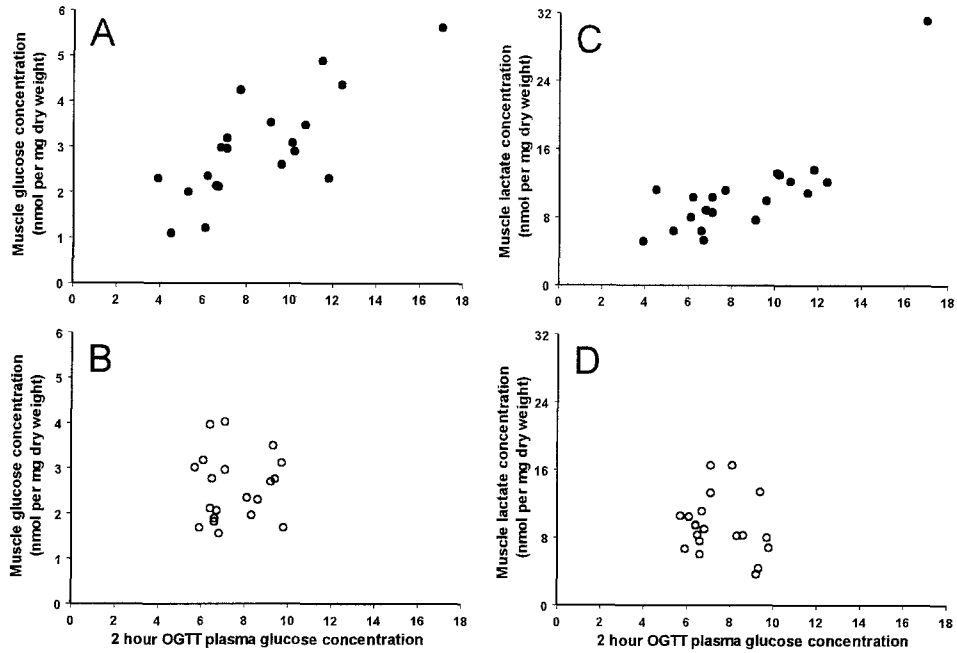

Fig 1. Skeletal muscle glucose (A + B) and lactate (C + D) concentrations versus glucose tolerance, expressed as the 2-hour OGTT, plasma glucose concentration, during dex treatment in relatives of type 2 diabetics (●, $R = .77$ and .74, respectively, both $P < .0001$) and control subjects (○, $r = .01$ and $-.18$, respectively, both NS).

Intramuscular Substrate Concentrations

Fasting concentrations of glucose, G-6-P, and lactate in the freeze-dried muscle biopsies taken before treatment with dex were similar in each group. In response to dex, a significant increase in the muscle concentration of glucose and lactate was observed in the relatives only, although basal plasma concentrations of the latter substrates were comparable in both groups. No increase occurred for the control group. No change was observed in either of the groups for the muscle G-6-P concentration after dex (Table 1).

Table 1. Skeletal Muscle Concentrations of Glucose, G-6-P, and Lactate in Freeze-Dried Muscle Biopsies (and after recalculation to intracellular water) in Relatives of Type 2 Diabetic Patients and Control Subjects Before and After Treatment With Dex

	Relatives		Controls	
Parameter	Pre-dex	Post-dex	Pre-dex	Post-dex
Glucose				
mmol/kg	2.43 ± 0.21	2.97 ± 0.26*	2.45 ± 0.18	2.57 ± 0.17
mmol/L intracellular water	0.28 ± 0.07	0.45 ± 0.08*	0.32 ± 0.06	0.34 ± 0.06
G-6-P (mmol/kg)	0.66 ± 0.07	0.61 ± 0.07	0.85 ± 0.09	0.79 ± 0.09
Lactate				
mmol/kg	7.37 ± 0.40	10.77 ± 1.22†	8.31 ± 0.52	9.40 ± 0.78
mmol/L intracellular water	2.53 ± 0.14	3.71 ± 0.43†	2.87 ± 0.19	3.23 ± 0.27

NOTE. Results are the mean ± SE. Values are shown as the absolute concentration in freeze-dried muscle biopsies (mmol/kg dry · muscle weight) and the calculated intracellular skeletal muscle substrate concentration, the latter calculated as the dry-weight concentration (mmol/kg dry weight) minus 0.3 (L/kg dry weight) times the plasma concentration (mmol/L), divided by 2.8 (L/kg dry weight).

*$P < .05$, †$P < .001$ *v* pre-dex.

Because of the heterogeneity of the glucose tolerance in the relatives after treatment with dex, we examined whether there was a relation between basal post-dex intramuscular substrates from the day of the IVGTT study and 2-hour OGTT plasma glucose concentrations obtained on another day. A significant positive association ($R = .77$, $P < .001$) was documented between the post-dex IVGTT (day 5) fasting dry-weight glucose concentration and the 2-hour OGTT plasma glucose concentration (day 4) for the relatives, but not for the control group ($R = .00$, NS). In the relatives, a significant association ($R = .74$, $P < .001$) between the dry-weight lactate concentration in the post-dex fasting muscle biopsy and the post-dex 2-hour plasma glucose concentration also occurred, but, again, not in the control subjects ($R = -.18$, NS) (Fig 1). These relationships did not change when the data were expressed as millimoles per liter of intracellular water in either the relatives (glucose, $R = .76$, $P < .001$; lactate, $R = .74$, $P < .001$) or the controls (glucose, $R = .00$, NS; lactate, $R = -.22$, NS).

Indirect Calorimetry

In response to dex, no changes occurred in the basal oxidation rates for glucose and lipid in either the relatives (pre-*v* post-dex: glucose, 1.94 ± 0.16 *v* fat-free mass 2.14 ± 0.15 mg/kg [FFM]/min, NS; lipid, 1.30 ± 0.08 *v* 1.19 ± 0.07 mg/kg FFM/min, NS) or the controls (glucose, 1.88 ± 0.19 *v* 1.92 ± 0.16 mg/kg FFM/min, NS; lipid, 1.21 ± 0.08 *v* 1.22 ± 0.08 mg/kg FFM/min, NS). In contrast, the post-dex integrated 3-hour mean glucose and lipid oxidation rates during the IVGTT were significantly increased and decreased, respectively, compared with the pre-dex state in the relatives (glucose, 2.87 ± 0.16 *v* 2.37 ± 0.15 mg/kg FFM/min, $P < .03$; lipid, 1.05 ± 0.08 *v* 1.16 ± 0.07, $P < .03$), but not in the controls

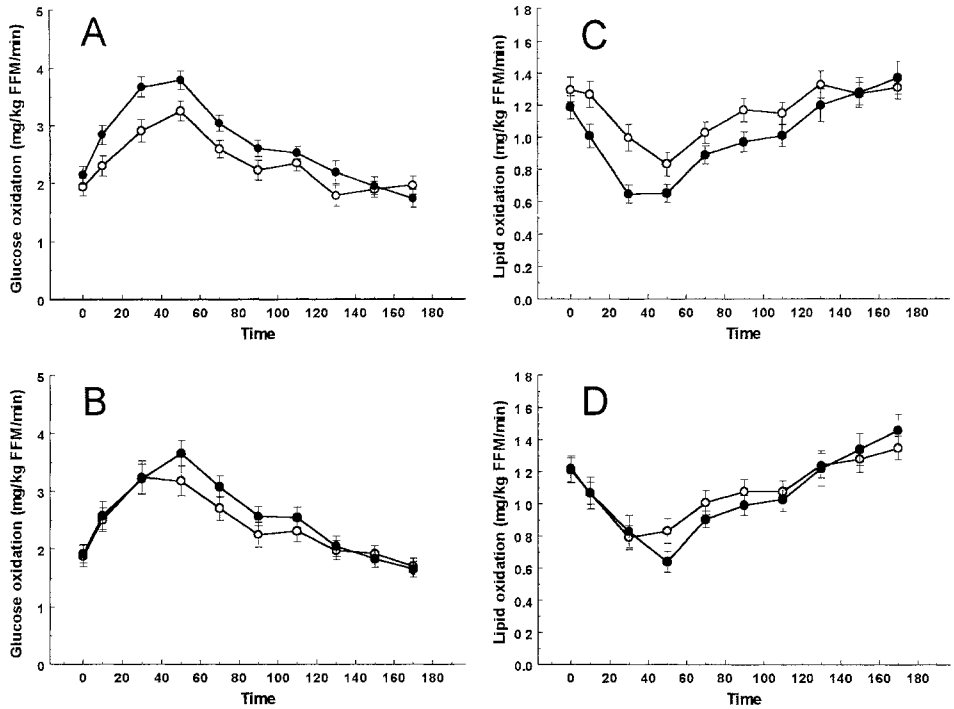

Fig 2. Time profile of glucose (A + B) and lipid (C + D) oxidation rates during the 3-hour IVGTT before (○) and after (●) dex treatment in relatives of type 2 diabetic patients (A + C) and control subjects (B + D). Mean post–intravenous glucose load glucose and lipid oxidation rates were significantly different only for the relatives pre- *v* post-dex ($P < .05$).

(glucose, 2.65 ± 0.16 *v* 2.44 ± 0.18 mg/kg FFM/min, NS; lipid, 1.04 ± 0.06 *v* 1.06 ± 0.06 mg/kg FFM/min, NS). This is also evident from the time profiles for glucose and lipid oxidation rates shown in Fig 2. No associations were noted between either the basal or integrated IVGTT glucose and lipid oxidation rates and the 2-hour OGTT glucose concentration.

Skeletal Muscle Glycogen Synthase Activity, Muscle Glycogen Content, and GLUT-4

Basal glycogen synthase activity, expressed either as the content of UDPG incorporated into glycogen in the presence of 0, 0.05, 0.1, and 10 mmol/L G-6-P or as the FV, was similar for both groups before and after treatment with dex. Although the absolute decrements in glycogen synthase activity in response to dex were similar in both groups (~25%), only for control subjects was this decrement significant ($P < .001$). In the relatives, an inverse correlation between the post-dex FV glycogen synthase and the 2-hour OGTT plasma glucose concentration (FV 0.1/10, $R = -.46$, $P < .05$) was noted, but not in the control subjects (FV 0.1/10, $R = -.15$, NS). Basal concentrations of intramuscular glycogen were similar in both groups both before and after dex, with no changes occurring in either group in response to dex. Similarly, basal levels of the insulin-dependent glucose transporter GLUT-4 before treatment with dex were comparable between relatives and control subjects, and no changes occurred in either group in response to dex (Table 2).

As previously reported, seven relatives had a transient frank impairment of glucose tolerance and were labeled hyperglycemic, as opposed to the normoglycemic relatives.[8] When the same above-mentioned statistical analysis is performed (ie, pre-*v* post-dex comparisons), most of the differences found in the combined group of relatives were observed in the hyperglycemic relatives subgroup. However, due to the limited number of subjects (n = 7), not all comparisons reached statistical significance. Thus, in the subgroup of hyperglycemic relatives, there was an increased skeletal muscle glucose and lactate concentration as compared with the pre-dex situation (glucose, 3.81 ± 0.45 *v* 2.79 ± 0.35 mmol/kg dry weight, $P = .09$; lactate,

Table 2. Skeletal Muscle Glycogen Synthase Activity, Glycogen Level, and GLUT-4 Level in Relatives of Type 2 Diabetic Patients and Control Subjects Before and After Treatment With Dex

	Relatives		Controls	
Parameter	Pre-dex	Post-dex	Pre-dex	Post-dex
G-6-P activity				
0.00	0.23 ± 0.04	0.16 ± 0.02	0.21 ± 0.03	0.15 ± 0.02*
0.05	0.92 ± 0.11	0.71 ± 0.08	0.92 ± 0.10	0.69 ± 0.07*
0.10	1.52 ± 0.17	1.19 ± 0.12	1.54 ± 0.15	1.21 ± 0.11*
10.0	4 63 ± 0.48	4.38 ± 0.45	4.69 ± 0.37	4.70 ± 0.31
G-6-P FV (%)				
0.00/10	4.7 ± 0.5	3.9 ± 0.4	4.7 ± 0.5	3.3 ± 0.4†
0.05/10	19.9 ± 1.2	16.9 ± 1.4	19.3 ± 1.1	14.9 ± 1.2†
0.10/10	33.1 ± 1.6	28.5 ± 1.9	32.2 ± 1.4	26.1 ± 1.9†
Glycogen (mmol/kg dry weight)	419 ± 22	440 ± 25	423 ± 15	426 ± 15
GLUT-4 (arbitrary units)	0.51 ± 0.04	0.53 ± 0.03	0.58 ± 0.03	0.54 ± 0.03

NOTE. Values are the mean ± SE Glycogen synthase activity is expressed as nmol UDPG incorporation into glycogen/mg extract protein/min in the absence (0.00) or presence of 0.05, 0.1, and 10 mmol/L allosteric activator G-6-P.

*$P < .05$, †$P < .001$ *v* pre-dex.

15.19 ± 2.69 *v* 7.43 ± 0.78, $P = .018$), an increased mean integrated IVGTT glucose (2.93 ± 0.20 *v* 2.15 ± 0.25 mg/kg FFM/min, $P = .063$), and a decreased lipid oxidation rate (0.91 ± 0.09 *v* 1.23 ± 0.10 mg/kg FFM/min, $P = .018$) and basal glycogen synthase activity ($FV_{0.1}$, 22.6% ± 3.09% *v* 32.8% ± 2.36%, $P = .06$). When the normoglycemic relatives (n = 13) were examined,[8] they behaved metabolically like the control group (data not shown).

DISCUSSION

Glucocorticoids are known to enhance hepatic gluconeogenesis,[4] to increase hepatic glucose production and reduce hepatic insulin sensitivity,[24] and to induce peripheral insulin resistance, especially in skeletal muscle, by decreasing both insulin-stimulated oxidative and nonoxidative glucose disposal.[5] In rats, glucocorticoids also reduce glycogen storage and glycogen synthase activity. Similarly, patients exposed to long-term high-dose glucocorticoids following renal transplantation are characterized by reduced nonoxidative glucose disposal, glycogen synthase activity, and glycogen synthase enzyme concentration.[6] Together, these data suggest significant shifts in glucose processing as a consequence of glucocorticoid exposure. However, the precise changes in intramuscular glucose metabolism following exposure to more modest doses of glucocorticoids such as dex in healthy subjects and normoglycemic relatives of type 2 diabetics at increased risk to develop diabetes are unknown.

In the present study, heterogeneous changes in fasting skeletal muscle substrate concentrations occurred in the dex-treated relatives, in contrast to the control subjects, in whom no dex-induced changes in skeletal muscle substrate concentrations were observed. Thus, only the relatives, particularly the hyperglycemic subgroup, developed significantly elevated basal intramuscular glucose and lactate concentrations post-dex exposure that correlated significantly with their glucose tolerance, expressed by the post-dex 2-hour OGTT plasma glucose concentration on a separate day. In addition, accompanying this apparent delay of intracellular skeletal muscle glucose metabolism, glucose oxidation during the post-dex IVGTT was increased with a corresponding reduction in lipid oxidation in the relatives compared with the matched controls, despite the similar or slight decrease of insulin secretion in the relatives during the IVGTT.[8] Together, these intramuscular and indirect calorimetry data suggest that in the relatives, an apparent preferential shift of post-dex glucose metabolism to the glucose oxidative-glycolytic pathway occurs as a consequence of the known downregulation of the insulin-activated glycogen-storage pathway.[2,9] Interestingly, these changes in intramuscular insulin action and glucose processing were accompanied by a dex-induced reduction of whole-body insulin sensitivity and first-phase insulin secretion in the relatives who developed significant glucose intolerance during treatment with dex.[8]

Previous studies in "non-stressed" normoglycemic relatives of type 2 diabetics on the intracellular muscle concentration of glucose and G-6-P under basal and euglycemic-hyperinsulinemic clamp conditions have found these substrate levels to be comparable to the concentrations observed in control subjects.[2,9] In contrast, Rothman et al,[10] Perseghin et al,[11] and Price et al,[25] using the nuclear magnetic resonance (NMR) technique, studied such relatives under hyperglycemic-hyperinsulinemic conditions and proposed that decreased glucose transport/phosphorylation was an early defect, as well as reduced nonoxidative glucose uptake, glycogen synthesis, and insulin-stimulated glucose oxidation, in the relatives compared with matched controls. They also noted reduced concentrations of G-6-P in muscle in the nonstressed relatives. Perseghin et al[11] examined whether an exercise program to enhance insulin sensitivity in a highly selected small subset of insulin-resistant normoglycemic relatives of type 2 diabetic patients would alter skeletal muscle glucose metabolism and insulin action. They found that exercise did improve insulin sensitivity, due to improved insulin-stimulated glycogen synthesis in the relatives during the hyperglycemic-hyperinsulinemic clamps. Although both before and after exercise, normoglycemic relatives still had ongoing decreased insulin-stimulated glycogen synthesis compared with the exercized control subjects, the preexisting low G-6-P concentrations were normalized in the exercised muscles of the relatives, whereas no change in G-6-P levels occurred in control subjects.[11]

In the present study, we examined in the relatives of type 2 diabetic patients the opposite scenario of the above-mentioned insulin sensitivity enhancing exercise studies.[11,25] Specifically, we examined the dex-induced insulin-resistant state in normoglycemic relatives of type 2 diabetic patients under basal conditions.[8] Despite similar basal skeletal muscle intracellular glucose, G-6-P, lactate, and glycogen concentrations and glycogen synthase activity in our relatives and control subjects before treatment with dex, post-dex differences emerged both in absolute terms and in the interrelationships between intracellular muscle glucose metabolism and 2-hour OGTT plasma glucose levels obtained on a separate day. Thus, although there was an apparent similar decrease of basal muscle glycogen synthase activity in both groups, post-dex, especially the hyperglycemic subgroup, intramuscular glucose concentrations were elevated post-dex in the relatives only, and this correlated with the degree of dex-induced glucose intolerance. These data suggest some delay in basal skeletal muscle glucose transport/phosphorylation during dex treatment in the relatives. However, in the presence of the known reduced insulin-activated glycogen synthesis[2,9-11,25,26] the relatives had normal basal intramuscular G-6-P levels, consistent with a compensatory enhancement in glycolysis under basal or stimulated conditions. This is supported by our observation of increased glycolysis (ie, indirect calorimetry showed enhanced whole-body glucose oxidation but suppressed lipid oxidation during the IVGTT, and increased basal muscle lactate concentrations) in the relatives following dex exposure. It should also be noted that during the IVGTT, we have previously reported no increase in the activity of intramuscular glycogen synthase at 30, 60, or 120 minutes despite the brief surge of insulin after the glucose load.[12] Similar findings were noted in the present study for biopsies taken at 60 minutes during the IVGTT (data not shown). Most recently, a study in rats using the IVGTT reported only a small (~30%) enhancement of glycogen synthase activity 15 minutes after the glucose load, which was completely dissipated by 30 minutes. The investigators suggested that this small brief increase in glycogen synthase activity is important to the overall clearance of the intravenous glucose load.[27] Thus, in our group of relatives

with a known decrease in the insulin sensitivity of glycogen synthase,[2,9-11,25,26] it is possible that a blunted glycogen synthase response contributed to the apparent delay in intracellular glucose metabolism and increased intracellular glucose in the relatives post-dex. The aims of the present study were several,[3,8] including the evaluation of insulin secretion and glucose effectiveness in relatives of type 2 diabetic patients both pre- and post-dex, and we therefore chose the IVGTT technique. Future studies using the euglycemic-hyperinsulinemic clamp technique are needed to determine if the postulated changes in glycogen synthase activity with dex in the relatives are correct. However, from the present data, we postulate that during the dex-induced decompensation of glucose tolerance (characterized by the elevated 2-hour OGTT plasma glucose concentration), a further impairment of the ongoing inherited defect in insulin-stimulated glycogen synthase activity occurs. This leads to the increase in intracellular glucose, which is channeled into the oxidative glycolytic pathway (as determined from indirect calorimetry) and the nonoxidative glycolytic pathway (as indicated by increased intramuscular basal lactate). This ability to enhance the intramuscular glycolytic pathway in the presence of a compromised glycogen-storage pathway permits ongoing metabolism of G-6-P and maintenance of normal basal muscle G-6-P concentrations with dex treatment.

However, there are two important issues of potential concern. First, there is a possibility that the elevated intramuscular lactate concentration in the relative is due to the technical failure to rapidly freeze the initial muscle biopsy specimens or the muscle preparations that were thawed at the time of the muscle lactate assays, either of which would encourage in vitro nonoxidative glycolysis.[28] This is unlikely, because the muscle samples were dealt with identically for both the healthy controls and the relatives and yet an elevated intramuscular lactate was noted only in the latter group. Further, if uncontrolled in vitro glycolysis did occur in the muscle specimens due to warming, G-6-P should also be elevated preferentially in these samples,[28] which was clearly not the case. The G-6-P levels in the present study are comparable to the findings from other studies using the needle-biopsy technique, which average approximately 0.61 mmol/kg dry-weight muscle.[29-33] However, with the NMR technique in man[34] or certain rapid in situ freeze-clamping techniques in rats,[28,35] lower concentrations of muscle G-6-P have been reported. In a study of rhesus monkeys using the freeze-clamp technique,[36] G-6-P levels were comparable to our data. In addition, a recent direct comparison of G-6-P concentrations between the freeze-clamp and biopsy techniques (frozen within 8 seconds) in normal rats showed a small decrease in G-6-P with the former method ($n = 8$, 0.44 ± 0.06 *v* 0.69 ± 0.14 mmol/kg dry weight, $P = .07$; E. Richter and A. Vaag, unpublished data, September 1997). Importantly, intracellular glucose and glycogen concentrations were identical with the two techniques. These data collectively may suggest that the needle-biopsy technique in man may result in slightly higher G-6-P intramuscular levels due to postbiopsy activation of glycogenolysis. However, the finding of a very low fractional glycogen phosphorylase in human compared with rat muscle biopsies argues against this possibility.[37,38]

The second potential concern is that there may be a direct association between the freeze-dried muscle absolute glucose concentration and the prevailing glycemia because of "contamination" of the muscle biopsy with extracellular glucose. We believe this error is likely minimal. First, the biopsies were taken in the fasting state, in which glucose concentrations were comparable in all groups, being only slightly increased by about 5% in the relatives, in contrast to the muscle biopsy glucose concentrations, where the increase was greater than 20%. Second, the interstitial glucose concentration at steady state, ie, fasting, is almost identical to the plasma glucose concentration.[39,40] Third, the absolute freeze-dried muscle glucose concentration from the IVGTT study was compared with the 2-hour OGTT plasma glucose concentration obtained on the separate OGTT study day, but the relationship between these two variables differed between the two groups of subjects (Fig 1). Fourth, when the intramuscular glucose concentrations were recalculated assuming an extracellular water content for the muscle biopsy of 0.3 L/kg dry weight and an intracellular water content of 2.8 L/kg dry weight,[18,19] a clear increase in muscle intracellular glucose was still observed in the relatives post-dex, but not in the control subjects. Finally, it has been questioned as to whether intracellular free glucose is measurable in skeletal muscle. With the NMR technique at normoinsulinemia (~30 pmol/L) and plasma hyperglycemia (~22 mmol/L), the intracellular glucose concentration in skeletal muscle is about 1.2 mmol/L intracellular water.[41] Most recently, Shulman's group,[42] using a modified NMR technique,[43] have reported intracellular glucose concentrations of 0 to 0.33 mmol/L wet muscle at basal euglycemia and normoinsulinemia. These data are comparable to the intracellular glucose concentration that we estimated at euglycemia and normoinsulinemia of about 0.35 mmol/L intracellular water, and to the other published muscle biopsy estimates of intracellular glucose.[29,33,44] Interestingly, Bonadonna et al,[45] using a multicompartmental model of glucose kinetics, have calculated that glucose exists in a free form in skeletal muscle at a concentration of 1.27 ± 0.18 mmol/L in control subjects. This latter glucose concentration is reported as glucose per liter of glucose distribution volume, which was higher than the estimated intracellular water volume,[45] thereby yielding an apparently higher concentration compared with the other noted studies.

One other aspect of the present study must be considered. Previously, in the same study population of relatives, we demonstrated that glucose-mediated glucose uptake (GE) at basal insulin was increased in the relatives compared with control subjects.[3] No change occurred in this glucose pathway following dex and GE remained significantly increased post-dex in the relatives compared with the controls.[8] Thus, the increase in glycolysis following dex could occur via increased GE into muscle, which leads to elevated muscle glucose concentrations and enhanced in vivo glucose oxidation. However, no association could be demonstrated between post-dex GE and muscle substrates or glycogen synthase activity or in vivo glucose oxidation (data not shown).

Finally, GLUT-4 in the skeletal muscle of relatives of type 2 diabetics is not decreased in the basal[3,46] or insulin-stimulated[46] state, and no changes were observed with dex treatment in the skeletal muscle GLUT-4 concentration in either of the groups. Similar conclusions have been noted previously for GLUT-4 in steroid-treated rats.[47] However, these observations do not

exclude the possibility that the intrinsic activity or translocation of the glucose transporter protein may be altered by steroid treatment.

In conclusion, during dex-induced insulin resistance, the response of intracellular glucose processing is different in control subjects versus relatives of type 2 diabetic patients. In particular, intramuscular concentrations of glucose and lactate were increased and glucose oxidation was enhanced in the relatives, whereas no such changes occurred in the control subjects. These alterations in muscle glucose metabolism during steroid treatment are postulated to be secondary to the enhanced glucose-mediated glucose uptake, which results from a further reduction by dex of the preexisting compromised glycogen synthase–glucose-storage pathway[2] in these relatives.

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