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NMR studies of muscle glycogen synthesis in insulin-resistant offspring of parents with non-insulin-dependent diabetes mellitus immediately after glycogen-depleting exercise

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(diabetes/glucose 6-phosphate)

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ABSTRACT **To examine the impact of insulin resistance on the insulin-dependent and insulin-independent portions of muscle glycogen synthesis during recovery from exercise, we studied eight young, lean, normoglycemic insulin-resistant (IR) offspring of individuals with non-insulin-dependent diabetes mellitus and eight age-weight matched control (CON) subjects after plantar flexion exercise that lowered muscle glycogen to ≈25% of resting concentration. After ≈20 min of exercise, intramuscular glucose 6-phosphate and glycogen were simultaneously monitored with ^{31}P and ^{13}C NMR spectroscopies. The postexercise rate of glycogen resynthesis was nonlinear. Glycogen synthesis rates during the initial insulin independent portion (0–1 hr of recovery) were similar in the two groups (IR, 15.5 ± 1.3 mM/hr and CON, 15.8 ± 1.7 mM/hr); however, over the next 4 hr, insulin-dependent glycogen synthesis was significantly reduced in the IR group [IR, 0.1 ± 0.5 mM/hr and CON, 2.9 ± 0.2 mM/hr; ($P \leq 0.001$)]. After exercise there was an initial rise in glucose 6-phosphate concentrations that returned to baseline after the first hour of recovery in both groups. In summary, we found that following muscle glycogen-depleting exercise, IR offspring of parents with non-insulin-dependent diabetes mellitus had (*i*) normal rates of muscle glycogen synthesis during the insulin-independent phase of recovery from exercise and (*ii*) severely diminished rates of muscle glycogen synthesis during the subsequent recovery period (2–5 hr), which has previously been shown to be insulin-dependent in normal CON subjects. These data provide evidence that exercise and insulin stimulate muscle glycogen synthesis in humans by different mechanisms and that in the IR subjects the early response to stimulation by exercise is normal.**

After intense exercise that depletes muscle glycogen concentrations to <35 mM glycogen, resynthesis proceeds in an approximately biphasic manner in both animal and human skeletal muscles (1–4). In normal healthy humans, there is an initial phase of rapid glycogen resynthesis (12–30 mM/hr) lasting ≈45 min that is insulin independent (1). The subsequent period of glycogen resynthesis (beyond ≈35 mM glycogen) is much slower (≈3 mM/hr) and insulin dependent (5). Exercise and insulin are both known to stimulate muscle glucose uptake and subsequent glycogen synthesis in an independent and additive manner (6–10). Under resting conditions, the effect of insulin stimulation on glycogen synthesis has been compared in healthy control (CON) subjects and in subjects with non-insulin-dependent diabetes mellitus (NIDDM) by ^{13}C NMR (11). In both CON subjects and NIDDM subjects placed under hyperglycemic-hyperinsulinemic conditions, the major pathway of insulin-dependent glucose metabolism was muscle glycogen synthesis (11). However, in the NIDDM subjects the rate of muscle glycogen synthesis was significantly impaired (11).

^{31}P NMR has been used to measure concentrations of glucose 6-phosphate (G6P), an intermediate of glycogen synthesis, under hyperglycemic-hyperinsulinemic conditions (12). Under these conditions, the G6P concentration was reduced in subjects with NIDDM compared with healthy CON subjects, suggesting that impairment of an earlier step (e.g., glucose transport or phosphorylation) is responsible for the reduction in the rate of muscle glycogen synthesis (12). Further evidence that glucose transport/phosphorylation is impaired in these subjects has been obtained with forearm balance studies of nonobese NIDDM patients using D-mannitol and 3-*O*-methyl-D-glucose (13). Recent studies have reported a reduction of both muscle glucose transport and muscle glycogen synthesis rates in the insulin-resistant (IR) offspring of parents with NIDDM under the same conditions of hyperglycemia-hyperinsulinemia, showing that reduced glucose transport/phosphorylation is present before the onset of NIDDM (14, 15).

While insulin-stimulated muscle glycogen synthesis has been studied in the IR offspring of subjects with NIDDM, exercise-stimulated glycogen synthesis has not. This study examines the impact of insulin resistance on the insulin-independent and insulin-dependent portions of muscle glycogen synthesis during recovery from exercise by comparing young normoglycemic IR offspring of individuals with NIDDM with age- and weight-matched CON subjects. Simultaneous ^{31}P NMR and ^{13}C NMR were used to measure the time courses of intramuscular G6P and glycogen concentrations following a standardized exercise protocol (1).

METHODS

Subjects. Eight (three males and five females) lean IR offspring of individuals with NIDDM (age 31 ± 3 years, weight 70 ± 4 kg, height 170 ± 3 cm) were compared with eight (five males and three females) age- and weight-matched CON subjects (age 29 ± 4 years, weight 67 ± 4 kg, height 170 ± 3 cm). In both the IR and the CON groups, fasting plasma glucose and 2-hr postglucose tolerance test glucose levels were <6 mM. Glycosylated hemoglobin, measured using an ion exchange chromatography method (Isolab), was within the normal range (IR, 5.81% ± 0.66% and CON, 5.75% ± 0.35%; normal range, 4–8%). Subjects in the IR group had either one

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; G6P, glucose 6-phosphate; IR, insulin resistant; CON, control; PME, phosphomonoester; GLUT4, glucose transporter.

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($n = 4$) or both parents ($n = 4$) with NIDDM, while subjects in the CON group had no family history of NIDDM traced through their grandparents. Subjects were screened by determining rates of whole body glucose metabolism (M values) during a 1 milliunit euglycemic-hyperinsulinemic clamp according to a standardized protocol (16).

Screening Procedure. Subjects arrived at the Yale–New Haven Hospital General Clinical Research Center at 8 a.m. after an overnight fast. A Teflon catheter was inserted into an antecubital vein for glucose and insulin infusions and an additional catheter was inserted retrogradely into a wrist vein for blood sampling. During the study, the hand was kept in a heated box (70°C) to allow sampling of arterialized venous blood. After obtaining three baseline samples for plasma insulin and glucose concentrations, a euglycemic-hyperinsulinemic clamp was begun at an insulin infusion rate of 1 milliunit·kg^{-1}·min^{-1} to achieve a plasma insulin concentration of ≈400 pmol per liter. During the clamp, plasma glucose concentration was determined every 5 min and a variable infusion of 20 g per liter of glucose was adjusted to maintain the plasma glucose concentration constant at the basal level. Blood samples were obtained every 10 min for insulin concentration. IR subjects were selected if their M value was in the lowest quartile (<3.5 mg/kg per min). The M values were significantly different ($P \leq 0.01$) in the two groups (IR, 3.0 ± 0.4 mg/kg per min and CON, 6.2 ± 0.3 mg/kg per min). The protocol was reviewed and approved by the Human Investigation Committee of Yale University School of Medicine and all subjects gave informed written consent.

Experimental Protocol. On a separate day, each subject's maximum voluntary contraction (MVC) for the gastrocnemius muscle was assessed (1, 17). MVC values for the two groups were not significantly different (CON, 143 ± 4 kg and IR, 137 ± 4 kg). The exercise protocol was single-leg toe-raises from an erect standing position (knee fully extended) to isolate the gastrocnemius muscle (1). The exercise intensity was determined by each subject's body mass and workloads for the two groups were not significantly different (CON, 49 ± 4% MVC and IR, 55 ± 4% MVC). To minimize intramuscular lactate accumulation, subjects exercised by alternating 1 min of toe raises (≈35 raises per min) with 1 min of rest throughout the period of exercise (1). The exercise durations were determined so as to deplete gastrocnemius glycogen to ≈25% of resting levels and were not significantly different (CON, 20 ± 2 min and IR, 21 ± 1 min). Mean exercise-induced glycogen depletion rates determined by ^{13}C NMR were not significantly different in the two groups (CON, 143 ± 17 mM/hr and IR,123 ± 17 mM/hr) nor were the total amounts of work performed (CON, 25.1 ± 1.4 kJ and IR, 27.3 ± 2.6 kJ).

All studies were begun at 8 a.m. after an overnight fast of ≥12 hr. At least 30 min before any samples were drawn, a Teflon catheter was inserted into an antecubital vein for blood assays. Two interleaved natural abundance ^{13}C-^{31}P NMR spectra were obtained to establish baseline levels of the ^{13}C and ^{31}P metabolites as well as baseline pH. During this same period, baseline blood samples were obtained. Subjects were then asked to perform the exercise protocol. Interleaved ^{13}C-^{31}P NMR spectra were obtained at 15 min of exercise as well as at the end of exercise; blood samples were obtained at the end of exercise. When the exercise protocol was completed, recovery was monitored over 5 hr with blood sampling and interleaved ^{13}C-^{31}P NMR spectroscopy. NMR spectra were obtained continuously over the first hour of recovery (5.4 min per spectrum) and on 30-min time intervals from hours 2 to 5 (1). From hours 2 to 5 of recovery, subjects rested quietly outside the spectrometer between scans. During the exercise and recovery periods, dietary intake was restricted to water only.

NMR Spectroscopy. Interleaved natural abundance ^{13}C-^{31}P NMR spectroscopy was performed at 4.7T on a Bruker (Billerica, MA) model Biospec spectrometer with a 30-cm diameter magnet bore. During the measurements, subjects remained supine with one leg positioned within the homogeneous volume of the magnet and with the lower portion of that leg resting on the stage of a surface coil radiofrequency (RF) probe. The spectrometer was equipped with a modified RF relay switch that allowed the hardware to switch the RF power between ^{13}C (50.4 MHz) and ^{31}P (81.1 MHz) channels with a 10 μsec switching time (18). A modified pulse sequence allowed switching of the acquisition parameters and preamplifiers between the two channels during the 10 μsec switching time. A 5.1-cm diameter circular ^{13}C-^{31}P double-tuned surface coil RF probe was used for interleaved acquisitions (18). The double-tuned circuit was optimized for the ^{31}P channel so that the NMR sensitivity would be enhanced to detect G6P. Shimming, imaging, and ^{1}H decoupling at 200.4 MHz were performed with a 9 cm × 9 cm series butterfly coil. Proton linewidths were shimmed to <50 Hz. A microsphere containing ^{13}C and ^{31}P reference standards was fixed at the center of the double-tuned RF coil for calibration of RF pulse widths. Subjects were positioned by an image-guided localization routine that used a T_1-weighted gradient-echo image (repetition time = 82 msec, echo time = 21 msec). The subject's lower legs were typically positioned so that the isocenter of the magnetic field was ≈1 cm into the medial head of the gastrocnemius muscle. By determining the 180° flip angles at the center of the observation coil from the microsphere standard, RF pulse widths were set so that the 90° pulse was sent to the center of the muscle. This maximized suppression of the lipid signal that arises from the subcutaneous fat layer and optimized signal from the muscle.

The interleaved ^{1}H decoupled ^{13}C-^{31}P RF pulse sequence was designed so that 72 ^{31}P transients were acquired during the same period that 2736 ^{13}C transients were obtained (38 ^{13}C scans per ^{31}P relaxation period), and free induction decays were saved separately in two blocks (18). The repetition time for ^{31}P acquisition was 4.6 sec to allow for the long T_1 of ^{31}P resonances. Because the acquisition times of both channels had to be identical due to a spectrometer limitation, the optimized acquisition time was 87 msec. ^{1}H continuous wave decoupling could not be turned on during the entire acquisition time because RF power deposition would have been excessive. Therefore, the decoupling time was truncated to 25 msec at the beginning of each ^{13}C acquisition. Power deposition, assessed by magnetic vector potential specific absorption rate calculation (19), was <4 W/kg. The total scan time for each interleaved spectrum was 5.4 min.

Intramuscular glycogen concentrations were determined by comparison with an external standard solution (150 mM glycogen + 50 mM KCl) in a cast of the subject's leg that loaded the RF coil in the same way the as subject's legs (1, 13). ^{13}C spectra were processed by methods that have been described in detail (1, 13, 14, 17). Briefly, gaussian-broadened spectra (30 Hz) were baseline corrected ± 1000 Hz on either side of the 1-^{13}C glycogen resonance of both subject spectra and sample spectra. Areas were then assessed ± 200 Hz about the resonance. The ^{13}C NMR technique for assessing intramuscular glycogen concentrations has been validated *in situ* in frozen rabbit muscle (20) and by comparison with biopsied human gastrocnemius muscle tissue samples (21).

Concentrations of inorganic phosphate (P_i) and creatine phosphate (PCr) were also calculated by comparison with β-ATP (13, 14). Values of pH were calculated according to the chemical shift difference between the P_i peak and the PCr peak using the equation:

$$pH = 6.77 + \log[(\Delta\delta - 3.29)/(5.68 - \Delta\delta)]$$

where $\Delta\delta$ is the chemical shift difference between P_i and PCr.

Corrections were made when exercise resulted in swelling of the muscle that could alter the NMR sensitive volume and make the NMR peaks appear smaller. In the processing mode of the spectrometer, the postexercise ^{31}P total spectral intensity was corrected to equal the resting spectrum. MRIs that were performed on a subset of subjects before and after the exercise protocol revealed an increase in cross-sectional area of the medial and lateral heads of the gastrocnemius (+16% ± 1%) that returned to resting values 15 min into the recovery period. This is in agreement with the normal efflux of water from recovering muscle, which is rapid and results in minimal muscle swelling within 10–15 min following exercise (22, 23). When the total spectrum area correction factor was applied, there were no significant differences in the β-ATP resonances after exercise. Time domain data were apodized and zero-filled using a 6-Hz exponential function. Intramuscular G6P was quantified by comparison with the β-ATP resonance as an internal reference standard (13, 14). A constant concentration of 5.5 mM was assumed for resting muscle ATP (24). The quantification of muscle G6P differed from the method described by Rothman *et al.* (12) in that the chemical shift of G6P was determined relative to P_i rather than PCr. Pan *et al.* (25) have shown that over the pH range of 6.60–7.05 exercise-induced changes in the chemical shift of P_i paralleled those of G6P. We measured the chemical shifts of the major constituents of the phosphomonoester (PME) region at pH 6.60–7.05 relative to P_i at 0.00 ppm and found them to remain constant [G6P, 2.29 ppm; α-glycerol phosphate, 2.04 ppm; inosine 5′-monophosphate (IMP), 1.61 ppm]. The basal G6P concentration was determined by integrating over the chemical shift range of 2.61–2.29 ppm and multiplying the area by two in order to minimize any contribution from upfield (lower ppm) PME resonances (12). When difference spectra were obtained by subtracting resting spectra from spectra obtained after exercise, the increase in G6P after exercise was cleanly resolved at 2.29 ppm. Measurement of G6P by ^{31}P NMR has been validated in an animal model by comparison with chemical assay of G6P done on rat muscle frozen *in situ* (26).

Blood Samples. Venous blood samples were assayed for glucose, lactate, insulin, epinephrine, and glucagon. Glucose and lactate were assayed by enzymatic methods (27, 28). Glucagon, insulin, and epinephrine were determined by radioimmunoassay methods (29). Glycosylated hemoglobin was assayed with an ion exchange chromatography method (Isolab).

Statistical Analysis. Blood data and NMR-determined metabolite concentrations are presented as means ± SE of all exercise/recovery sessions unless otherwise noted. Overall NMR precision was calculated by pooled variance analysis (17, 24). Paired two-tailed *t* tests were used for comparison of data within individual subjects. Comparisons of data between groups were performed using ANOVA. Glycogen repletion rates for each subject were determined by least-squares linear regression analysis of the increase in glycogen, either over a specified time period or within a specified range of glycogen concentrations.

RESULTS

Before exercise, plasma glucose concentrations in the IR offspring were similar to those of the CON group (IR, 5.1 ± 0.2 mM and CON, 5.3 ± 0.1 mM), whereas the plasma insulin concentrations were significantly greater ($P \leq 0.01$) in the IR group (IR, 56.4 ± 7.8 pM and CON, 28.2 ± 4.2 pM). Resting levels of glucagon, epinephrine, and lactate were not significantly different in the two groups (Table 1). After exercise, plasma levels of glucose and insulin did not change significantly in either group; however, plasma insulin concentrations in the IR group remained significantly greater at the end of exercise ($P \leq 0.05$) and 30 min into the recovery period ($P \leq 0.05$). Plasma epinephrine and glucagon did not change significantly as a result of exercise in either group. Basal plasma lactate concentrations were not significantly different in the IR group when compared with the CON group; however, the initial rise in plasma lactate concentration was significant in the IR group ($P \leq 0.05$) but not significantly different from the CON group at this time (Table 1).

The intramuscular levels of creatine phosphate and inorganic phosphate before and immediately after exercise were not significantly different, although there was a trend toward an increase in both PCr and P_i for both groups (Table 2). Resting levels of both PCr and P_i were slightly higher ($P \leq 0.05$) in the IR group (PCr, 21.7 ± 1.0 mM and P_i, 2.0 ± 0.1 mM) when compared with the CON group (PCr, 18.7 ± 0.8 mM and P_i, 1.6 ± 0.1 mM). In both groups, the resting pH (IR, 7.03 ± 0.02 and CON, 7.03 ± 0.02) dropped ($P \leq 0.01$) immediately after exercise (IR, 6.86 ± 0.04 and CON, 6.89 ± 0.04) and returned to resting pH within the first 25 min of recovery. The time of recovery of pH back to ≥ 7.00 was similar in the two groups (IR, 24.6 ± 3.6 min and CON, 19.6 ± 5.9 min). After exercise, in addition to increases in G6P, there were increases in the two other major portions of the PME region in both groups. While intensity increases in the α-glycerol phosphate region were significant in both groups (IR, $P \leq 0.05$ and CON, $P \leq 0.0001$), increases in the IMP region were not consistently observed. The changes observed in the α-glycerol phosphate and IMP resonances of the PME region were not quantified because they would have required deconvolution of the resonances. The G6P resonance did not have to be deconvoluted because of its downfield position in the PME region.

G6P was quantified before exercise and during the 5-hr timecourse after exercise. Resting levels of G6P were not significantly different in the two groups (IR, 0.16 ± 0.02 mM

Table 1. Concentrations of plasma constituents in the CON and IR groups at rest, at the end of exercise, and 30 min into the recovery period

	Glucose, mM	Insulin, pM	Epinephrine, pg/ml	Glucagon, pg/ml	Lactate, mM
CON					
Rest	5.3 ± 0.1	28.2 ± 4.2	30 ± 6	54 ± 3	1.0 ± 0.1
End of exercise	5.6 ± 0.1	24.6 ± 6.6	45 ± 7	60 ± 7	1.4 ± 0.2
30 min into recovery	5.5 ± 0.2	20.4 ± 5.4	33 ± 6	56 ± 7	1.1 ± 0.2
IR					
Rest	5.1 ± 0.2	56.4 ± 7.8*	23 ± 5	55 ± 4	1.0 ± 0.2
End of exercise	5.4 ± 0.2	61.2 ± 13.8*	38 ± 14	59 ± 5	1.8 ± 0.2
30 min into recovery	5.3 ± 0.2	44.4 ± 5.4*	18 ± 5	55 ± 4	1.1 ± 0.2

Rest, $t = -30$ min; end of exercise, $t = 0$.
*$P \leq 0.05$ compared with CON group.

Table 2. Intramuscular concentrations of PCr, P_i, and muscle pH in the CON and IR groups

	PCr, mM	P_i, mM	pH
CON (n = 8)			
Rest	18.70 ± 0.75	1.62 ± 0.13	7.03 ± 0.01
End of exercise	20.70 ± 1.96	3.13 ± 0.89	6.89 ± 0.04*
IR (n = 8)			
Rest	21.69 ± 1.03	2.01 ± 0.07	7.03 ± 0.02
End of exercise	24.77 ± 2.25	5.43 ± 1.78	6.86 ± 0.04*

Concentrations measured before and 7.5 min after cessation of glycogen-depleting exercise. Values are mean ± SE.
*pH after exercise compared with pH at rest ($P \leq 0.01$).

and CON, 0.13 ± 0.01 mM) (Table 3). Immediately after exercise, G6P levels increased (IR, 0.94 ± 0.18 mM and CON, 0.56 ± 0.08 mM) (Table 3). A typical difference ^{31}P spectrum for an individual exercise session is shown in Fig. 1, where the top spectrum was obtained 7.5 min after cessation of exercise, the center spectrum was obtained at rest immediately before exercise, and the bottom spectrum represents the difference between the top two spectra. The timecourse of G6P after exercise is shown in Fig. 2*B*. Mean G6P levels were consistently greater in the IR group than in the CON group; however, there was no significant difference between G6P concentrations in the two groups. In both groups, after an initial high G6P concentration immediately after exercise (7.5 min of recovery), G6P declined rapidly to resting concentrations over the first hour of recovery. Over the next 4 hr of recovery, G6P concentrations were not significantly greater than the resting G6P concentration. The G6P concentrations exhibited higher variability between subjects in the IR group than in the CON group. When spectra collected between hours 1 and 5 of recovery were added, processed G6P concentrations were not significantly different in the two groups (IR, 0.24 ± 0.04 mM and CON, 0.15 ± 0.02 mM) (Table 3). In both groups, the period of rapid change in G6P concentration that occurred

Table 3. Individual muscle G6P concentrations for eight CON and eight IR subjects calculated at rest, immediately following glycogen-depleting exercise (t = 7.5 min), and during 1 hr–5 hr of recovery

	G6P, mM		
		Post exercise	
Subject	Rest	t = 7.5 min	t = 1 hr–5 hr
Control			
1	0.158	0.328	0.119
2	0.100	0.348	0.137
3	0.138	0.791	0.137
4	0.128	0.438	0.160
5	0.168	0.728	0.210
6	0.058	0.957	0.092
7	0.134	0.375	0.140
8	0.177	0.503	0.268
Mean ± SE	0.133 ± 0.014	0.559 ± 0.083*	0.158 ± 0.020
IR			
1	0.099	0.873	0.131
2	0.204	0.996	0.273
3	0.136	0.672	0.112
4	0.200	0.426	0.240
5	0.102	1.859	0.148
6	0.266	0.505	0.420
7	0.192	1.547	0.312
8	0.104	0.628	0.254
Mean ± SE	0.163 ± 0.022	0.938 ± 0.181†	0.236 ± 0.037

*$P \leq 0.001$ when G6P at rest is compared with G6P at t = 7.5 min after exercise.
†$P \leq 0.05$ when G6P at rest is compared with G6P at t = 7.5 min after exercise.

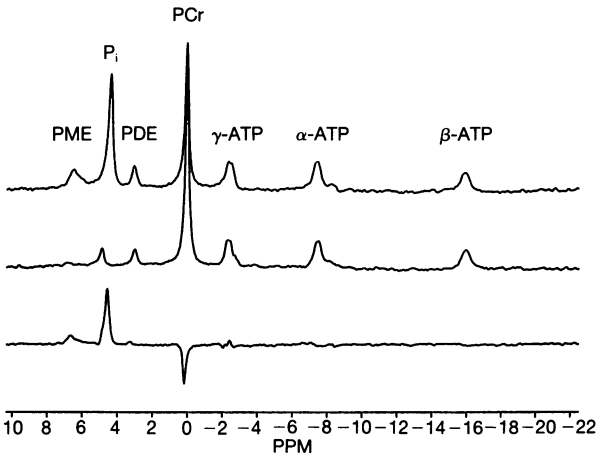

FIG. 1. ^{31}P NMR spectra from an individual subject in the IR group. The top spectrum was obtained 7.5 min after the cessation of 18 min of glycogen-depleting exercise (t = 7.5 min). The middle spectrum was obtained at rest before the exercise protocol was begun (t = −30 min). The bottom spectrum is a difference spectrum between the top two spectra. This spectrum shows the change that resulted from exercise in the PME region, P_i, the phosphodiester region (PDE), and PCr; there was no change in the adenosine triphosphate region (γ-ATP, α-ATP, and β-ATP).

during the first hour following exercise corresponded with rapid, exercise-induced muscle glycogen synthesis (Fig. 2).

Preexercise muscle glycogen concentrations were not significantly different (IR, 58.9 ± 5.8 mM and CON, 66.5 ± 6.1

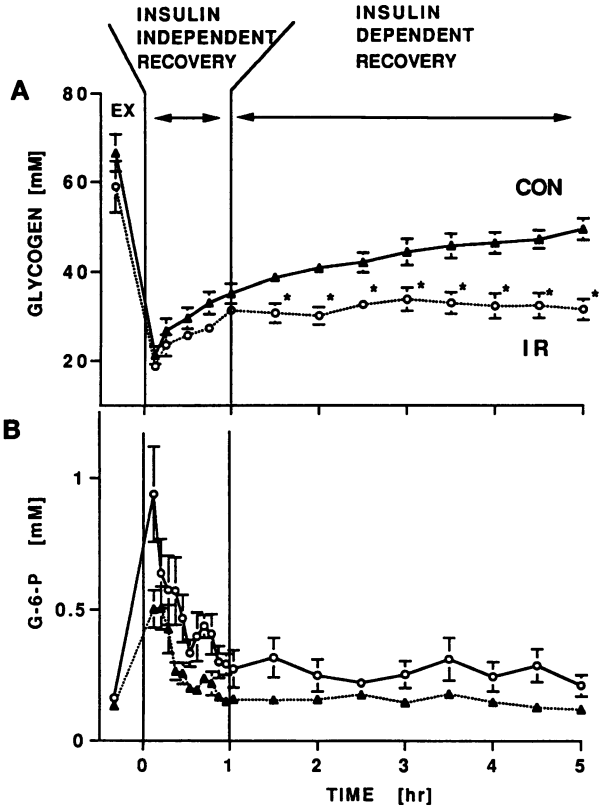

FIG. 2. Time courses of glycogen (*A*) and G6P (*B*) concentrations during 5-hr recovery of glycogen-depleted gastrocnemius muscle. Glycogen-depleting exercise was performed by a group of CON (▲) subjects (n = 8) and a group of IR offspring of NIDDM parents (○) (n = 8). Recovery was followed during the initial insulin-independent phase and during the subsequent insulin-dependent phase. *$P \leq 0.05$ IR versus CON. Concentrations are presented as means ± SE.

Table 4. Individual muscle glycogen synthesis rates for eight CON and eight IR subjects calculated during the initial hour of recovery (insulin-independent phase) and during the subsequent 1–5 hr of recovery (insulin-dependent phase)

	Glycogen synthesis rate, mM/hr	
Subject	0 hr–1 hr	1 hr–5 hr
CON		
1	23.0	2.7
2	14.7	3.3
3	17.3	3.7
4	22.2	2.5
5	14.3	3.3
6	10.5	2.5
7	9.8	2.3
8	14.7	2.9
Mean ± SE	15.8 ± 1.7	2.9 ± 0.2
IR		
1	16.4	0.6
2	17.2	−2.1
3	14.8	1.8
4	21.0	−0.1
5	17.2	1.7
6	12.8	−0.4
7	16.3	−0.1
8	8.3	−1.0
Mean ± SE	15.5 ± 1.3	0.1 ± 0.5*

*$P \leq 0.001$ compared with the CON group.

mM). The precision of the natural abundance ^{13}C NMR measurements, determined as the coefficient of variation (17), was not significantly different in the two groups (IR, CV = 8.4% ± 1.7% and CON, CV = 5.2% ± 1.3%). The glycogen concentration in the two groups following the glycogen-depleting exercise was not significantly different (IR, 17.3 ± 1.8 mM and CON, 20.0 ± 1.7 mM). After 1 hr of recovery, muscle glycogen concentrations were not significantly different in the two groups, although there was a trend toward lower concentrations in the IR group (IR, 32.2 ± 0.6 mM and CON, 36.8 ± 2.6 mM). During the initial 45 min to 1 hr period after exercise, the glycogen resynthesis rates were calculated as the rate of glycogen synthesis when glycogen concentrations were ≤ 35 mM (insulin-independent period). These calculated rates were not significantly different between the two groups (IR, 15.5 ± 1.3 mM/hr and CON, 15.8 ± 1.7 mM/hr) (Table 4, Fig. 3). As reported (1), insulin-independent glycogen resynthesis rates were greater at lower glycogen concentrations; therefore, we calculated resynthesis rates at glycogen concentrations ≤ 25 mM and found them to be similar to those previously reported (IR, 31.0 ± 9.7 mM/hr and CON, 35.7 ± 10.0 mM/hr) (1). Over the next 4 hr (2–5 hr recovery), insulin-dependent glycogen resynthesis was significantly reduced ($P \leq 0.001$) in the IR group (0.1 ± 0.5 mM/hr) when compared with the CON group (2.9 ± 0.2 mM/hr) (Table 4, Fig. 3). After 5-hr recovery, muscle glycogen concentrations were significantly lower ($p \leq 0.001$) in the IR group when compared with the CON group (IR, 33.7 ± 2.0 mM and CON, 49.7 ± 1.9 mM).

DISCUSSION

Previous studies have shown that glucose transport/phosphorylation is impaired under hyperglycemic-hyperinsulinemic conditions both in subjects with NIDDM (12) and in IR offspring of subjects with NIDDM (14). The presence of this defect in IR offspring shows that the reduction of muscle glucose transport/phosphorylation is fully expressed before the development of diabetes (14). This study examines the effect of exercise stimulation on glucose transport/phosphorylation and muscle glycogen synthesis in these same subjects. After glycogen-depleting exercise, the insulin-independent portion of muscle glycogen resynthesis is not impaired in the IR group. In contrast, the insulin-dependent portion of muscle glycogen synthesis is reduced in the IR group.

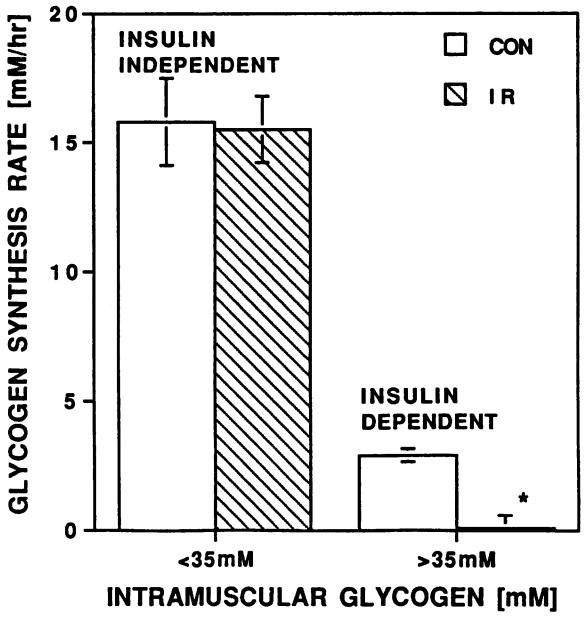

FIG. 3. Glycogen synthesis rates (mM/hr) in the gastrocnemius muscle in CON (□) subjects (n = 8) and IR (▨) subjects (n = 8) at glycogen concentrations below ≈35 mM and at concentrations above ≈35 mM. *$P \leq 0.001$ IR versus CON. Rates are given as means ± SE.

Animal studies have shown increases in both the number and activity of glucose transporters (GLUT4) on the muscle plasma membrane following exercise (6, 9). Goodyear *et al.* (9) reported a 4-fold increase in glucose transport by rat gastrocnemius muscle immediately after exercise (1.8-fold increase in transporter number, 1.9-fold increase in intrinsic activity) that declined to a 1.8-fold increase in transport after 30 min of recovery (1.6-fold increase in transporter number, 1.1-fold increase in intrinsic activity). Both transporter number and intrinsic activity had returned to basal levels by 2 hr into the recovery period (9). The reported time course of increase and subsequent decline in GLUT4 transporter number and activity (immediately after exercise, 4-fold; 30 min after exercise, 1.8-fold; 2 hr after exercise, 1.0-fold) (6, 9) is similar to the time course of the increase in G6P seen in the two groups of the current study (CON, immediately after exercise, 4.2-fold; 30 min after exercise, 1.9-fold; 2 hr after exercise, 1.2-fold; and IR, immediately after exercise, 5.8-fold; 30 min after exercise, 2.3-fold; 2 hr after exercise, 1.4-fold). The increases in G6P currently seen in both the CON and IR groups, which are consistent with the increases in transporter number and activity reported in the earlier animal studies (6, 9), suggest that an exercise-induced increase in GLUT4 transporter number and activity is responsible for the rise in G6P immediately after exercise and that this is not impaired in the IR offspring of subjects with NIDDM.

The intracellular source of GLUT4 transport proteins has been examined in animals to determine whether the insulin- and exercise-stimulated transporters originate from a single pool or two separate pools (6, 8). Wallberg-Henriksson *et al.* (8) examined the permeability of rat epitrochlearis muscle to 3-*O*-methylglucose exposed to a range of insulin concentrations at different timepoints after exercise. This study concluded that the actions of insulin and exercise result in activation/translocation of glucose transporters from two separate pools (8). This finding is consistent with the results of

a recent study by Lund *et al.* (30) in which wortmannin, an inhibitor of phosphatidylinositol 3-kinase, was found to inhibit insulin-stimulated GLUT4 translocation in isolated rat soleus muscle, whereas it had no effect on exercise-induced GLUT4 translocation. Douen *et al.* (6) have used an immunoblotting technique with an anti-GLUT4 polyclonal antibody to show that insulin stimulation increased the GLUT4 transporters in the cell membrane while decreasing the transporters in the intracellular membrane fraction. In contrast, exercise stimulation increased the number of GLUT4 transporters in the cell membrane but did not significantly decrease them in the intracellular membrane fraction, further supporting the existence of two separate intracellular pools of glucose transporters (6, 8). The results of the present study support the existence of distinct mechanisms for exercise and insulin action on glucose transport/phosphorylation because normal glucose transport was present in the IR subjects after exercise despite their previously demonstrated impairment of insulin-stimulated glucose transport/phosphorylation (14).

During the insulin-dependent portion of muscle glycogen resynthesis, the CON subjects synthesized glycogen at a rate of 2.9 ± 0.2 mM/hr while the IR subjects showed a reduced rate of muscle glycogen synthesis (0.1 ± 0.5 mM/hr) despite similar plasma concentrations of glucose and higher concentrations of insulin (Table 2). The reduced rate of muscle glycogen synthesis during this period may be due to an insulin-dependent impairment of either glucose transport/phosphorylation or glycogen synthase. The mean of the G6P data was not significantly different from the CON subjects and was similar to preexercise values during the insulin-dependent phase. The finding of similar G6P concentration with decreased glycogen synthesis suggests that both glucose transport/phosphorylation and glycogen synthase activity are reduced in a coordinated manner (12).

The finding of reduced glucose transport/phosphorylation and glycogen synthase activity differs from previous observations under conditions of high-infused insulin ($\approx$480 pM) and glucose ($\approx$10 mM) in which a similar group of IR subjects showed reduced activity primarily in the glucose transport/phosphorylation step (14). These differences may reflect alterations in the insulin regulation of glucose transport/phosphorylation and glycogen synthase activity after exercise as opposed to the resting state. In the CON group, insulin-stimulated glycogen synthesis occurs in the second phase despite plasma glucose and insulin levels that do not promote glycogen synthesis in the resting leg (1). Further studies will be necessary to elucidate the differences in insulin regulated glycogen synthesis between the late postexercise period and the resting state.

In summary, we found that following muscle glycogen depleting exercise, IR offspring of parents with NIDDM had (*i*) normal rates of muscle glycogen synthesis and intracellular G6P concentrations during the insulin-independent phase of recovery from exercise and (*ii*) severely diminished rates of muscle glycogen synthesis during the subsequent recovery period (2–5 hr), which has previously been shown to be insulin dependent in normal CON subjects. These data provide evidence that exercise and insulin stimulate muscle glycogen synthesis in humans by different mechanisms and that in the IR subjects the early response to stimulation by exercise is normal.

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