Direct assessment of muscle glycogen storage after mixed meals in normal and type 2 diabetic subjects

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To understand the day-to-day pathophysiology of impaired muscle glycogen storage in type 2 diabetes, glycogen concentrations were measured before and after the consumption of sequential mixed meals (breakfast: 190.5 g carbohydrate, 41.0 g fat, 28.8 g protein, 1,253 kcal; lunch: 203.3 g carbohydrate, 48.1 g fat, 44.0 g protein, 1,497.5 kcal) by use of natural abundance 13C magnetic resonance spectroscopy. Subjects with diet-controlled type 2 diabetes (n = 9) and age- and body mass index-matched nondiabetic controls (n = 9) were studied. Mean fasting gastrocnemius glycogen concentration was significantly lower in the diabetic group (57.1 ± 3.6 vs. 68.9 ± 4.1 mmol/l; P < 0.05). After the first meal, mean glycogen concentration in the control group rose significantly from basal (97.1 ± 7.0 mmol/l at 240 min; P = 0.005). After the second meal, the high level of muscle glycogen concentration in the control group was maintained, with a further rise to 108.0 ± 11.6 mmol/l by 480 min. In the diabetic group, the postprandial rise was markedly lower than that of the control group (65.9 ± 5.2 mmol/l at 240 min, P < 0.005, and 70.8 ± 6.7 mmol/l at 480 min, P = 0.01) despite considerably greater serum insulin levels (752.0 ± 109.0 vs. 372.3 ± 78.2 pmol/l at 300 min, P = 0.013). This was associated with a significantly greater postprandial hyperglycemia (10.8 ± 1.3 vs. 5.3 ± 0.2 mmol/l at 240 min, P < 0.005). Basal muscle glycogen concentration correlated inversely with fasting blood glucose (r = −0.55, P < 0.02) and fasting serum insulin (r = −0.57, P < 0.02). The increment in muscle glycogen correlated with initial increment in serum insulin only in the control group (r = 0.87, P < 0.002). This study quantitates for the first time the subnormal basal muscle glycogen concentration and the inadequate glycogen storage after meals in type 2 diabetes.

METHODS

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ketones at the time of diagnosis and a Hb A1C of ≤8.5% were recruited. Matched nondiabetic subjects with no family history of type 2 diabetes were also recruited as controls. Subjects were excluded if they were medicated with thiazide diuretics, β-blockers, oral hypoglycemic agents or steroids, or if they had evidence of hepatic or renal impairment. Weight, height, and waist-to-hip ratio were documented after an overnight fast. Triceps skin-fold thickness and mid-arm circumference were measured as previously described (13) and used to estimate total muscle mass. Bio-impedence was performed using a Holtain BC Analyser (Holtain, Dyfed, UK), and percentage of body fat was derived. Nine subjects with diet-controlled type 2 diabetes (6 male, 3 female) were recruited, along with 9 age- and weight-matched control subjects (5 male, 4 female). The clinical and metabolic characteristics of the two groups are shown in Table 1. Both groups reported similar calorie intake (1,952 ± 10 kcal for diabetic and control subjects, respectively) and similar food composition (carbohydrate, 211 ± 14 g; fat, 92 ± 7 g; protein, 83 ± 8 g; vs. 241 ± 14 g; fat, 92 ± 5 vs. 100 ± 11 g; protein, 83 ± 7 vs. 87 ± 6 g).

**Study design.** Subjects abstained from alcohol or vigorous exercise for 3 days before the study. Before this period, they kept a food diary to record all intake. They fasted from 1800 on the evening before the study, although water was permitted. On the morning of the study, subjects were transported to the Magnetic Resonance Centre by taxi. Baseline-arterialized blood samples were taken for estimation of blood glucose, free fatty acids (FFA), triglycerides, lactate, insulin, and C-peptide. The subject was then positioned in the NMR scanner and a baseline muscle glycogen measurement recorded. The baseline blood samples were repeated, and time was set at 0 h. The subject was then given a standard carbohydrate-rich breakfast (190.5 g carbohydrate, 41.0 g fat, 28.8 g protein, 1,253 kcal). Repeat glycogen measurements and blood samples were taken at 60, 120, and 240 min. At 240 min, the subjects were given a further standard meal (203.3 g carbohydrate, 48.1 g fat, 44.0 g protein, 1,497.5 kcal). Muscle glycogen was measured at 300, 360, and 480 min, and arterialized blood samples were taken.

**13C Magnetic resonance spectroscopy.** Muscle glycogen measurements were taken from the subject’s gastrocnemius muscle with a 3.0-Tesla whole body magnetic resonance scanner. A 13C surface coil was used for transmission and reception, and quadrature 1H coils were used for 1H decoupling. The subject was placed in a comfortable supine position, with the calf muscle placed directly over the coil. To ensure reproducibility of positioning of the subject’s leg for repeated measurements, a vacuum pillow was used to make a cast of the leg.

Manual shimming was performed on the water resonance peak. The shimmmed signal was localized to the volume of interest by using the 13C surface coil at the proton frequency. Both the FID and the spectral linewidth were monitored throughout shimming to give optimal signal. Water linewidths of 25–45 Hz were achieved, and the broadband decoupling frequency was centered on the glycogen resonance.

All three coils were tuned and matched before each glycogen measurement with a Network Analyser (HP model 8751A, 5–500 KHz).

13C NMR spectra were acquired according to the following pulse sequence. Two cycles of the WALTZ-8 (31) pulse sequence were applied with a peak power of 68 ± 2 W during acquisition to decouple proton signals. A 100-μs rectangular pulse was used for 13C excitation, with a peak power of 390 ± 10 W at a resonant frequency of 32.154973 MHz. CYCLOPS phase cycling was used to cancel any phase-coherent noise. A repetition rate of 360 ms was used, and spectra were collected in blocks of 1,000 scans, giving a temporal resolution of 6 min. The sampling time was set to 142 ms, and 512 data points were collected during the acquisition period. RF power values were monitored throughout the acquisition so that any drift in these values could be corrected for and, more importantly, to ensure that they did not exceed the maximal values allowed according to specific absorption ratio guidelines recommended by the National Radiological Protection Board (26).

All spectra were analyzed using the Matlab version of the mru software package (version 96.3) (40). All data were converted to a mru-compatible format using sun2mru, a conversion routine written in-house, and the FID math function was used to sum together the three spectra acquired at each time point to improve the signal-to-noise ratio. All peaks in the spectra were selected using the peakpick function, and the AMARES algorithm was used to fit them to Lorentzian line shapes. During the fitting, the noise level was estimated from the last 50 data points. The integral of the glycogen peak was then expressed as a fraction of that from the formate peak, derived from a phantom containing [13C]formate and placed at the center of the 13C coil. These data were then converted into absolute glycogen concentration with the following method.

A calf-shaped phantom was constructed containing a glycogen solution of 139 ± 7 mmol/l with 150 mmol/l KCl. These spectra were analyzed as we have described. Again, the integral from the glycogen peak was expressed as a fraction of that from the formate peak. Glycogen concentrations were then calculated using the general formula

\[
\frac{R_{Gly}(s)}{R_{Gly}(ph)} \times \frac{[Glyc]}{R_{Gly}(ph)}
\]

where \(R_{Gly}(s)\) is the ratio of the glycogen to formate peaks in the subject, \([Glyc]\) is the concentration of the glycogen in the phantom in mmol/l, and \(R_{Gly}(ph)\) is the ratio of the glycogen to formate peaks in the phantom.

**Short insulin sensitivity test.** Insulin sensitivity was determined by the short insulin sensitivity test (KITT), as previously described (2). Arterialized plasma glucose samples were collected from a wrist vein before and after injection of 0.1 U/kg Actrapid insulin in a total volume of 2 ml. Insulin sensitivity (KITT) was determined by the slope of the regression line of the logarithm of plasma glucose against time during the first 3–15 min after the administration of insulin. Indirect calorimetry data were derived by use of a constant-

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**Table 1. Clinical characteristics of the study subjects**

<table>
<thead>
<tr>
<th></th>
<th>Diabetic (6M, 3F)</th>
<th>Control (5M, 4F)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>52.2 ± 3.1</td>
<td>51.3 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.2 ± 1.2</td>
<td>29.0 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>86.7 ± 5.1</td>
<td>82.4 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>WHR</td>
<td>1.01 ± 0.02</td>
<td>0.87 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%Body fat</td>
<td>38.8 ± 3.5</td>
<td>39.8 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Muscle mass, kg</td>
<td>29 ± 2.8</td>
<td>26.7 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>FBG, mmol/l</td>
<td>7.1 ± 0.3</td>
<td>4.6 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb A1C, %</td>
<td>6.9 ± 0.2</td>
<td>5.4 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chol, mmol/l</td>
<td>5.3 ± 0.2</td>
<td>3.9 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>2.9 ± 0.7</td>
<td>1.4 ± 0.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. M and F, male and female subjects, BMI, body mass index; WHR, waist-to-hip ratio; FBG, fasting blood glucose; Hb A1C, hemoglobin type A1C; Chol, cholesterol; TG, triglycerides; NS, not significant.
flow hood calorimeter (Deltatrac, Datex Ohmeda, Hatfield, Hertfordshire, UK) over an initial 20-min period before the insulin sensitivity test and were repeated for a further 20-min period between 20 and 40 min after insulin injection. Data for analysis were collected after an equilibrium period of 10 min for each time point.

**Metabolite and hormone assays.** Plasma glucose was measured on a Yellow Springs glucose analyzer (YSI, Yellow Springs, OH). Blood glucose was measured with a HemoCue photometer analyzer (HemoCue, Angelholm, Sweden). Plasma FFA were measured on a Roche Cobas centrifugal analyzer with a Wako kit (Wako Chemicals, Neuss, Germany). Plasma triglycerides were also measured on a Roche Cobas centrifugal analyzer, with a colorimetric assay (ABX Diagnostics, Montpellier, France). Serum insulin and C-peptide assays were both measured using Dako ELISA kits (Dako, Ely, Cambridgeshire, UK).

**Statistical methods.** Data are presented as means ± SE unless otherwise stated. Comparisons between groups were performed with Student’s paired *t*-test or the Mann-Whitney test as appropriate. Relationships were tested by linear correlation analysis.

**RESULTS**

**Blood glucose.** Mean fasting blood glucose was higher in the diabetic group than in the control group (7.1 ± 0.3 vs. 4.6 ± 0.1 mmol/l; *P* < 0.001). One hour after breakfast, mean blood glucose increased to 14.1 ± 0.1 mmol/l in the diabetic group, and this level was sustained over the next hour. In the control group, the rise after breakfast was limited, blood glucose being 6.6 ± 0.3 mmol/l at 60 min. Before the second meal at 240 min, mean blood glucose had fallen to 10.8 ± 1.3 mmol/l in the diabetic group vs. 5.3 ± 0.2 mmol/l in the control group (*P* < 0.005). After the second meal, the rise in blood glucose was considerably less than after breakfast in both groups (0.8 vs. 0.7 mmol/l, respectively; *P* = NS), although the mean glucose levels remained >10.0 mmol/l in the diabetic group compared with <6.0 mmol/l in the control subjects (Fig. 1).

**Serum insulin and C-peptide.** Mean fasting serum insulin was significantly higher in the diabetic group (68.1 ± 9.0 vs. 30.8 ± 2.5 pmol/l; *P* < 0.001). After breakfast, the rise in serum insulin in the two groups was similar (464.0 ± 60.4 vs. 450.9 ± 71.7 pmol/l at 60 min; *P* = 0.89). Mean serum insulin continued to rise in the diabetic group (584.1 ± 76.9 pmol/l at 120 min), but it fell in the control group (307.3 ± 65.2 pmol/l at 120 min). There was a further increase in both groups after lunch, peaking at 60 min postprandially, when mean serum insulin was over twofold elevated compared with the control group (752.0 ± 109.0 vs. 372.3 ± 78.2 pmol/l; *P* = 0.013).

Fasting C-peptide levels were also significantly higher in the diabetic group (0.98 ± 0.06 vs. 0.57 ± 0.04 nmol/l; *P* < 0.001). After breakfast, levels in the two groups rose similarly (3.04 ± 0.22 vs. 3.60 ± 0.38 nmol/l; *P* = 0.2). However, C-peptide levels subsequently fell in the control group but rose further in the diabetic group (4.37 ± 0.76 vs. 2.36 ± 0.26 nmol/l at 240 min; *P* < 0.05). C-peptide levels in both groups peaked after the second meal (5.19 ± 0.31 vs. 3.71 ± 0.40 nmol/l; *P* < 0.02).

**Plasma FFA and triglycerides.** Mean fasting FFA were significantly higher in the diabetic group (0.56 ± 0.06 vs. 0.39 ± 0.04 mmol/l; *P* = 0.039). In both groups, FFA fell to a nadir after 120 min (0.09 ± 0.02 vs. 0.05 ± 0.02 mmol/l; *P* = 0.08) and rose similarly toward the end of the study (0.17 ± 0.02 vs. 0.14 ± 0.02 mmol/l at 480 min).

Mean fasting triglyceride levels were over twofold higher in the diabetic group (2.75 ± 0.72 vs. 1.27 ± 0.16 mmol/l; *P* = 0.03) and remained approximately twofold higher to 480 min (4.25 ± 1.1 vs. 2.09 ± 0.22 mmol/l; *P* = 0.052). Triglyceride levels increased steadily in both groups during the course of the study.

![Fig. 1. Change in blood glucose (A), insulin (B), and C-peptide (C) concentrations during the study period in the type 2 diabetic group (●) and the matched normal control group (○). Data are shown as means ± SE.](http://ajpendo.physiology.org/Downloadedfrom)
Muscle glycogen. Mean fasting glycogen concentration was significantly lower in the diabetic group (57.1 ± 3.6 vs. 68.9 ± 4.1 mmol/l; P < 0.05). After the first meal, mean glycogen concentration remained unchanged during the first 2 h in both groups (Fig. 3). Thereafter, in the control group, it rose significantly from basal (97.1 ± 7.0 mmol/l at 240 min; P = 0.005). There was only a small rise in muscle glycogen in the diabetic group (65.9 ± 5.2 mmol/l), and levels were significantly lower than in the control group at 240 min (P < 0.005). After the second meal, the high level of muscle glycogen concentration in the control group was maintained, with a further rise to 108.0 ± 11.6 mmol/l that was reached at 480 min. Although there was a 26% increase from fasting levels of muscle glycogen in the diabetic group, this was not significant (70.8 ± 6.7 mmol/l at 480 min, P = 0.08).

Basal muscle glycogen concentration correlated inversely with fasting blood glucose (r = -0.55, P < 0.02) and fasting serum insulin (r = -0.57, P < 0.02). There was a direct relationship between basal muscle glycogen concentration and $K_{	ext{ITT}}$ (r = 0.49, P = 0.04) in the whole group. However, there was no relationship between increment in muscle glycogen and $K_{	ext{ITT}}$ in either the control or the diabetic group (Fig. 4). The increment in muscle glycogen correlated with the initial increment in serum insulin only in the control group (r = 0.87, P < 0.002). Given that the response to any increment in serum insulin would be modified by insulin resistance, the relationships of the product of $K_{	ext{ITT}}$ and the increment in plasma insulin (0–60 min) were examined. This index correlated with the increment in muscle glycogen both in the control subjects (r = 0.86, P < 0.003) and in the whole group (r = 0.63, P < 0.05).

Plasma lactate. Fasting plasma lactate levels in the two groups were similar (728 ± 60 vs. 685 ± 60 μmol/l).

Fig. 2. Change in free fatty acids (FFA, A) and triglyceride (B) concentrations during the study period in type 2 diabetic (●) and matched normal control (○) groups. Data are shown as means ± SE.

Fig. 3. Change in muscle glycogen concentration during the study period in type 2 diabetic (●) and matched normal control (○) groups. Data are shown as means ± SE.

Fig. 4. Relationships between change in muscle glycogen during the study period and insulin sensitivity as measured by the short insulin tolerance test ($K_{	ext{ITT}}$, A) and product of increment in serum insulin and $K_{	ext{ITT}}$ (B) in type 2 diabetic (●) and matched normal control (○) groups.
There was a significant increase from basal after the first meal (1,818 ± 259 vs. 1,844 ± 147 μmol/l at 60 min, \( P < 0.01 \) both groups). Plasma lactate levels subsequently fell, but there was a further significant increase after the second meal (1,374 ± 60 vs. 1,335 ± 81 μmol/l at 300 min, \( P < 0.02 \), both groups compared with 240 min). There was no significant difference between the two groups at any time point during the study.

Short insulin sensitivity test. Diabetic subjects had significantly lower insulin sensitivity than the control subjects (\( K_{ITT} \) 1.1 ± 0.2 vs. 1.9 ± 0.2 mmol\( \cdot \)\( \text{min}^{-1} \)\( \cdot \)kg\(^{-1} \)), \( P < 0.005 \). Mean blood glucose fell from 7.7 ± 0.4 to 5.7 ± 0.4 mmol/l in the diabetic group and from 5.2 ± 0.1 to 3.0 ± 0.2 mmol/l in the control group. FFA fell from 0.59 ± 0.04 to 0.34 ± 0.05 mmol/l in the diabetic group and from 0.48 ± 0.04 to 0.17 ± 0.03 mmol/l in the control group.

Fasting glucose oxidation rates were 1.15 ± 0.17 mg/kg\( \cdot \)\( \text{min}^{-1} \) in the diabetic group and 1.10 ± 0.17 mg/kg\( \cdot \)\( \text{min}^{-1} \) in the control group, and fasting lipid oxidation rates were 0.79 ± 0.10 and 0.62 ± 0.08 mg/kg\( \cdot \)\( \text{min}^{-1} \), respectively (\( P = \text{NS} \)). Between 30 and 40 min after insulin injection, glucose oxidation increased to 1.89 ± 0.22 and 2.45 ± 0.19 mg/kg\( \cdot \)\( \text{min}^{-1} \) (\( P = 0.08 \)), and lipid oxidation decreased to 0.52 ± 0.12 and 0.23 ± 0.09 mg/kg\( \cdot \)\( \text{min}^{-1} \) (\( P = 0.08 \)) in the diabetic and control groups, respectively.

DISCUSSION

In this study, natural abundance \(^{13}\)C MRS has been used to make direct observation of muscle glycogen concentration after breakfast and lunch in subjects with type 2 diabetes and normal control subjects. The diabetic subjects were selected on the basis of being in the early phase of type 2 diabetes with reasonable blood glucose control on diet alone, but despite this, fasting muscle glycogen concentration was observed to be significantly lower than normal. After breakfast, mean muscle glycogen concentration increased by only 15% in the diabetic group compared with 40% in the control group, the difference being maintained after lunch. Serum insulin concentrations were higher in the diabetic group, especially after the second meal, indicating the day-to-day relevance of insulin resistance at the level of muscle.

Full understanding of the pathophysiology of type 2 diabetes requires accurate information on glucose stores in muscle and liver in the fasting state and knowledge of rate of change after eating. Although much information is available on fasting hepatic glycogen stores in type 2 diabetes (14, 23, 35), it is remarkable that no previous MRS studies of fasting muscle glycogen concentration in type 2 diabetes have been published. Many studies of glycogen concentration measured on the basis of muscle biopsy samples have been reported, but only a general, nonsignificant trend to lower concentrations in diabetic subjects has been observed (17, 18, 42, 43). The present study establishes that fasting muscle glycogen concentrations are almost 20% lower than normal in subjects with diet-controlled type 2 diabetes despite elevated plasma glucose and insulin concentrations.

Muscle glycogen concentration did not start to rise immediately after eating, and this slow rise would not have been anticipated in view of the more rapid time course of arteriovenous difference in plasma glucose measured across forearm or leg after oral glucose (16, 20, 25, 29) or during insulin infusion (39). This phenomenon has previously been observed in young healthy subjects (36). It may be inferred that the predominant fate of glucose taken up by muscle in the immediate postprandial period is oxidation, whereafter a gradual change to net glycogen synthesis takes place. This is consistent with our observation of rapid increase in glucose oxidation within 40 min of the intravenous injection of insulin during the insulin sensitivity test. Interestingly, close inspection of data on leg glucose balance in normal subjects after a mixed meal shows a biphasic pattern, with the second peak of uptake starting at 120 min, the time at which we observe commencement of net glycogen synthesis (7, 21). Both the increase in muscle glycogen concentration (57% in control subjects and 24% in diabetic subjects) and the increase in whole body glucose oxidation (120% in control subjects and 47% in diabetic subjects) were subnormal in the diabetic subjects. Overall, this is consistent with the previous MRS observation that the initial step of glucose uptake into the muscle is rate limiting (8).

The rate of muscle glucose uptake is subnormal in subjects with type 2 diabetes under euglycemic clamp conditions (4). However, early limb balance studies of oral glucose administration indicated that the higher blood glucose concentration in diabetic subjects (fasting blood glucose ~12 mmol/l) compensates for this, leading to similar overall rates of glucose uptake by muscle (11, 19, 25). More recently, limb balance studies on type 2 diabetic subjects with similarly raised fasting blood glucose levels showed 30% lower leg glucose uptake, the difference being most pronounced from 120 min after ingestion of a mixed meal (21). The present data demonstrate that, although the rate of glucose delivery to muscle may be moderately impaired, under everyday conditions postprandial storage of glucose as glycogen in muscle is markedly impaired in type 2 diabetes. This observation is consistent with the demonstration of subnormal insulin-stimulated rate of incorporation of \(^{13}\)C-glucose into muscle glycogen in type 2 diabetic subjects (32). Although both the subnormal rise in muscle glycogen concentration after the meals and the insulin sensitivity (\( K_{ITT} \)) were subnormal in the diabetic group, there was no simple correlation between the two parameters. This would certainly have been expected if the experimental design had fixed the extent of the insulin stimulus, but under the normal diurnal conditions studied, the insulin-secretory response varied markedly between individuals. In the control group, the initial insulin response (0–60 min) correlated with the rapid phase of glycogen synthesis (\( r = 0.87, P < 0.002 \)). No
such relationship existed in the diabetic group, possibly because of the small increment in muscle glycogen concentration.

The rise in blood glucose was considerably less after the second of the two similar meals, a phenomenon first described over 80 years ago (34, 38). More recent work has confirmed this effect (1, 15, 41), although a single study using intravenous glucose is quoted to suggest that it does not occur in type 2 diabetes (30). The blood glucose profiles shown in Fig. 1 clearly demonstrate the second-meal phenomenon. The rise in blood glucose after lunch in the normal control subject was significantly less than after breakfast (0.63 ± 0.29 vs. 2.06 ± 0.28 mmol/l, P < 0.004) despite the similar meal composition. The effect was considerably more pronounced in the diabetic subjects, the postlunch increment being only 10% of the postbreakfast rise. However, it is clear from Fig. 3 that the phenomenon was not a consequence of insulin priming of skeletal muscle and, hence, a more immediate increase in glycogen storage, as there was minimal change in muscle glycogen concentration over the 2-h period after either meal in either group. It may be postulated that the second-meal effect is likely to relate to continued suppression of hepatic glucose release, as we have previously demonstrated that meal-induced suppression continued for ~4 h in both nondiabetic and type 2 diabetic subjects, even after a much smaller meal (33, 35). It is important to recognize the physiological consequences of this effect, because most studies of postprandial metabolism are conducted after an overnight fast, with extrapolation of results to the second and third meals of the day.

The role of fatty acids in impairing insulin-stimulated glucose storage has received much attention, although discussion has focused upon plasma FFA. In the present study, subjects were selected who had type 2 diabetes but could control it by diet alone. The extent of muscle insulin resistance in these subjects was reflected both in the KTG data and in the failure to increase glycogen storage despite marked hyperinsulinemia. Plasma FFA were only 19% higher than in the control subjects in the fasting state, and they were almost normal between 2 and 8 h. However, plasma triglyceride concentrations were twofold higher throughout the test day, and it must be considered that delivery of fatty acid to skeletal muscle by lipoprotein lipase activity would ensure that the fatty acid effect in muscle tissue was considerably greater than would be suggested by plasma FFA levels (10). This effect would be in addition to that of the increased intramyocellular triglyceride stores in type 2 diabetic subjects (3, 5, 22, 24, 28).

Further work is required to establish whether muscle glycogen dynamics can be restored in type 2 diabetes by specific therapy. Given the extent of acute hyperinsulinemia observed in this study, insulin treatment itself would not be predicted to restore metabolic flexibility. However, the effect of direct insulin-sensitizing agents upon diurnal muscle glycogen storage needs to be assessed.

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REFERENCES
