Effects of raising muscle glycogen synthesis rate on skeletal muscle ATP turnover rate in type 2 diabetes

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Mitochondrial dysfunction has been implicated in the pathogenesis of type 2 diabetes. We hypothesized that any impairment in insulin-stimulated muscle ATP production could merely reflect the lower rates of muscle glucose uptake and glycogen synthesis, rather than cause it. If this is correct, muscle ATP turnover rates in type 2 diabetes could be increased if glycogen synthesis rates were normalized by the mass-action effect of hyperglycemia. Isoglycemic- and hyperglycemic-hyperinsulinemic clamps were performed on type 2 diabetic subjects and matched controls, with muscle ATP turnover and glycogen synthesis rates measured using 31P- and 13C-magnetic resonance spectroscopy, respectively. In diabetic subjects, hyperglycemia increased muscle glycogen synthesis rates to the level observed in controls at isoglycemia [from 19 ± 9 to 41 ± 12 μmol·l⁻¹·min⁻¹ (P = 0.012) vs. 40 ± 7 μmol·l⁻¹·min⁻¹ in controls]. This was accompanied by a modest increase in muscle ATP turnover rates (7.1 ± 0.5 vs. 8.6 ± 0.7 μmol·l⁻¹·min⁻¹, P = 0.04). In controls, hyperglycemia brought about a 2.5-fold increase in glycogen synthesis rates (100 ± 24 vs. 40 ± 7 μmol·l⁻¹·min⁻¹, P = 0.028) and a 23% increase in ATP turnover rates (8.1 ± 0.9 vs. 10.0 ± 0.9 μmol·l⁻¹·min⁻¹, P = 0.025) from basal state. Muscle ATP turnover rates correlated positively with glycogen synthesis rates (r = 0.46, P = 0.005). Changing the rate of muscle glucose metabolism in type 2 diabetic subjects alters demand for ATP synthesis at rest. In type 2 diabetes, skeletal muscle ATP turnover rates reflect the rate of glucose uptake and glycogen synthesis, rather than any primary mitochondrial defect.

Skeletal muscle mitochondrial dysfunction has been hypothesized to contribute to the development of insulin resistance and type 2 diabetes. Muscle biopsy studies of type 2 diabetic subjects have demonstrated smaller mitochondria and reduced activities of oxidative enzymes compared with glucose-tolerant subjects (13, 18). Magnetic resonance (MR) spectroscopy allows noninvasive measurement of a critical aspect of mitochondrial function by measuring rates of ATP synthesis (20). With use of this methodology, decreased ATP turnover rates have been reported in skeletal muscle of type 2 diabetic subjects and healthy subjects at risk of developing diabetes (i.e., insulin-resistant offspring of type 2 diabetic subjects) (31, 45). In young healthy individuals, insulin stimulated muscle ATP turnover rate, whereas no response was seen in the insulin-resistant offspring of type 2 diabetic subjects (32). Decreased expression of nuclear-encoded genes that regulate mitochondrial biogenesis, such as peroxisome proliferation-activated receptor-γ coactivator 1α, has also been reported in nondiabetic individuals with impaired glucose tolerance and in healthy first-degree relatives of type 2 diabetic subjects (27, 30). Short-term exercise training has been shown to increase insulin sensitivity and ATP synthesis in healthy humans, but not in first-degree relatives of type 2 diabetic subjects (17). These studies support the concept that abnormalities in oxidative metabolism contribute to the development of insulin resistance and, hence, type 2 diabetes (5, 29).

However, a substantial number of recent studies in humans and rodents directly challenge the view that a preexisting defect in mitochondrial function is responsible for the development of insulin resistance. In obese humans, muscle biopsy studies have shown improvement in mitochondrial function after programs of weight loss and physical exercise (24). Similarly, improvement in insulin sensitivity through calorie restriction has been demonstrated in overweight and obese subjects in the absence of any measurable change in mitochondria DNA content and NADH-oxidase activity (46). In addition, several studies of genetically modified mice have failed to demonstrate a clear effect of alteration of mitochondrial function on insulin action (4, 12). In light of all the evidence presented, the reduced capacity of the mitochondria to produce ATP in muscle of type 2 diabetes could be secondary to the metabolic state itself and a feature of insulin resistance, rather than its cause (33, 44).

Application of 13C-MR spectroscopy demonstrated that insulin-stimulated muscle glycogen synthesis was the major metabolic pathway of glucose disposal under postprandial conditions and that a defect in muscle glycogen synthesis was responsible for the decrease in insulin sensitivity in type 2 diabetes (42). Further study showed that a defect in muscle glucose transport was responsible for the decreased rate of insulin-stimulated glycogen synthesis in muscle of type 2 diabetic subjects (7). We therefore hypothesized that impairment in insulin-stimulated ATP turnover rates in muscle of type 2 diabetic subjects merely reflects the lower rates of muscle glucose uptake and glycogen synthesis, rather than causes it. Consequently, muscle ATP turnover rates would be expected to normalize if glycogen synthesis rates were normalized by an increase in plasma glucose concentration. To test this hypothesis, MR spectroscopy was used to quantify muscle ATP turnover rates and muscle glycogen synthesis rates during isoglycemic-hyperglycemic hyperinsulinemia in type 2 diabetic and nondiabetic control subjects.

METHODS

Subjects

Ten well-controlled type 2 diabetic subjects (7 men and 3 women) and eight normoglycemic controls (6 men and 2 women) matched for sex, age, body mass index, and physical activity were studied. Their
anthropometric and metabolic characteristics are summarized in Table 1. All subjects were recruited by means of advertisement. After a complete medical history was obtained from all subjects, clinical examination and laboratory tests were carried out to exclude hepatic and renal diseases. Diabetic subjects treated with insulin or any oral hypoglycemic medications, except metformin, were excluded. Subjects in the control group had no family history of diabetes, nor were they taking any medication (e.g., steroids, β-blockers, or diuretics) known to affect glucose tolerance or insulin sensitivity. Normal glucose metabolism was confirmed by a standard 75-g oral glucose tolerance test. None of the subjects performed moderate or intense exercise on a regular basis. Physical activity was assessed over 3 days using the Body Monitoring System and SenseWear Armband (BodyMedia, Pittsburgh, PA), which provides a measure of total daily energy expenditure and number of steps taken per day (25). The study protocol was approved by Newcastle upon Tyne Ethics Committee No. 2, and informed consent was obtained from all subjects.

Experimental Protocol

All subjects refrained from physical exertion during the 3 days preceding the studies and fasted overnight for 12 h before the experiments. Metformin was withdrawn 3 days before each experiment. Each subject underwent two experimental protocols, an isoglycemic-hyperinsulinemic clamp test and a hyperglycemic-hyperinsulinemic clamp test, completed 4–8 wk apart (Fig. 1). Isoglycemic, rather than euglycemic, clamps were used to allow observation of the true fasting state in each group. The subjects’ body weights and lifestyles remained unchanged throughout the study. Data on the isoglycemic clamps have previously been reported as part of a study of effects of plasma free fatty acid (FFA) suppression (21). For all experiments, subjects travelled to the MR facility by taxi and were transported within the center by wheelchair. At 0830 (–270 min), one cannula was inserted into an antecubital vein for administration of glucose and insulin. A second cannula was inserted into the contralateral wrist vein for blood sampling. Use of a hand-warming device ensured arterIALIZATION of venous blood. To permit the frequent 31P- and 13C-MR spectroscopy measurements, subjects were studied lying in the MR scanner for the duration of each study.

Protocol A: isoglycemic-hyperinsulinemic clamp. Isoglycemia was maintained to ensure that the true basal condition of each subject could be observed. Isoglycemic hyperinsulinemia was induced with the insulin-glucose clamp technique (9). Insulin (Actrapid, NovoNordisk, Bagsvaerd, Denmark) was administered as a primed-continuous infusion (40 μU·m–2·min–1) for 150 min. To inhibit pancreatic hormone secretion, somatostatin was infused at 0.06 μg·kg–1·min–1 (Somatostatin-UCB, UCB Pharma) from 5 min before the start of the insulin infusion and continued for the duration of the clamp. Fasting isoglycemia was maintained by a variable glucose infusion based on plasma glucose measurements performed at 5-min intervals. To increase sensitivity of measurement of the muscle glycogen synthesis by 13C-MR spectroscopy, the variable glucose infusion contained 20% [1-13C]glucose (Cambridge Isotope Laboratories, Andover, MA). Whole body insulin sensitivity was determined from calculated whole body glucose disposal during the last 30 min of the hyperinsulinemic glucose clamp (9). Whole body glucose disposal was calculated from glucose infusion rate (39). To assess rate of oxidation of infused glucose, breath samples for 13CO2 were obtained. As this measure would be affected by differences in plasma [13C]glucose enrichment, the index of whole body glucose oxidation was calculated as the ratio of breath to plasma 13C atom percent excess (APE): (breath APE/plasma APE) × 100.

Protocol B: hyperglycemic-hyperinsulinemic clamp. A hyperglycemic-hyperinsulinemic clamp was performed on a separate day to examine the combined effect of insulin and glucose. The experimental protocol was the same as that described for protocol A, except plasma glucose was clamped at a stable level 5 mmol/l above fasting concentrations.

MR Spectroscopy

MR data were acquired using a 3-T Achieva scanner (Philips, Best, The Netherlands) with a built-in body coil used for imaging. A 14-cm-diameter surface coil was used for phosphorus spectroscopy, and a 6-cm-diameter 13C coil with an integral quad 1H decoupling coil (PulseTeq, Wotton under Edge, UK) was used for 13C spectroscopy. Subjects remained supine inside the MR spectrometer, with each coil positioned beneath the left calf during each investigation. The coil position was marked on the leg with indelible ink. Scout images were acquired to ensure identical coil positioning on repeat scans. To prevent movement during each study, the coil was secured in place using webbing straps around the calf. All spectra were analyzed with jMRUI (version 3.0) (28) using the AMARES fitting (advanced method for accurate, robust, and efficient spectral fitting) algorithm (48).

31P-MR spectroscopy was carried out as previously described (22). Briefly, a saturation transfer sequence was used to measure the transfer magnetization between γ-ATP and P(1,20). The steady-state magnetization of P(1,20) was measured during selective irradiation of γ-ATP (P(1,20)) and compared with the equilibrium P(1,20) magnetization with the irradiation placed symmetrically downfield from the P(1,20) frequency (M(0,20)). The fractional reduction of P(1,20) magnetization upon saturation of γ-ATP, (M(0,20) – M(0,20))/M(0,20), was used to calculate the pseudo-first-order rate constant using the Forsen-Hoffman equation: k = [(M(0,20) – M(0,20))/M(0,20)](1/Tγ), where Tγ is the spin-lattice relaxation time for the phosphorus nucleus of P(1,20) when ATP is saturated (10). Unidirectional turnover rate of ATP synthesis was then calculated by multiplying the constant k by the P(1,20) concentration. 31P-MR spectroscopy measurements were acquired at baseline from −240 to −210 min and twice further during the clamp, from 15 to 45 min and from 90 to 120 min. For 13C-MR spectroscopy, spectra were acquired as previously described (21) at baseline from −205 to −185 min and twice further during the clamp, from 50 to 70 min and from 130 to 150 min. The concentration of muscle glycogen at baseline, [Glyc]muscle, was calculated using the following formula: [Glyc]muscle = (Sphantom × [Glyc]phantom)/Sphantom , where Sphantom and Smuscle are the signal intensities arising from glycogen in the phantom and muscle, respectively, and [Glyc]phantom is the concentration of glycogen in the phantom (100 mmol/l). The increments in muscle glycogen concentration at 70 and 150 min of the clamp, Δ[Glyc]70 and Δ[Glyc]150, respectively, were calculated from the equation previously reported (16): [ΔGlyc]70 = [(S70 – S0) × [Glyc]70 × f70/(S0 × f70)] and [ΔGlyc]150 = [(S150 – S70) × [Glyc]150 × f150/(S0 × f150)], where S0, S70, and S150 represent the signal intensity of [1-13C]glycogen at 0, 70, and 150 min, respectively, [Glyc]0 is the basal glycogen concentration (in mmol/l), f70 is the natural abundance enrichment of [1-13C]glycogen at baseline (1.1%), and f70 and f150 represent the mean percent 13C enrichment of plasma glucose at 70 and 150 min, respectively. Each increment was then

Table 1.  Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th>T2DM</th>
<th>Control</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>57 ± 2</td>
<td>53 ± 3</td>
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<tr>
<td>BMI, kg/m²</td>
<td>28.7 ± 1.2</td>
<td>28.1 ± 1.1</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>26.1 ± 2.0</td>
<td>26.4 ± 1.7</td>
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<tr>
<td>Fat-free mass, kg</td>
<td>54.7 ± 3.8</td>
<td>58.5 ± 3.6</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>7.7 ± 0.3</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>93 ± 14</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>HbA1C, %</td>
<td>6.6 ± 0.2</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Fasting triglyceride, mmol/l</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Mean daily energy expenditure, kcal</td>
<td>2,455 ± 198</td>
<td>2,248 ± 76</td>
</tr>
<tr>
<td>Mean daily steps taken</td>
<td>6,160 ± 385</td>
<td>5,701 ± 288</td>
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Values are means ± SE of 10 (7 men and 3 women) subjects with type 2 diabetes mellitus (T2DM) and 8 (6 men and 2 women) controls. BMI, body mass index.
added to the previous concentration, and the slope was calculated by linear regression analysis to yield the rate of glycogen synthesis (42).

**Breath ^13^C Enrichments**

Breath samples for ^13^C enrichments were collected at five time points. The subjects were asked to exhale fully through a short straw into a glass tube (Exetainer, Laboco, Buckinghamshire, UK), which was immediately sealed with a stopper. ^13^C enrichments of breath samples were determined by continuous-flow isotope ratio mass spectrometry (ABCA system; PDZ Europa). The coefficient of variation for the analysis was 0.07%, and the coefficient of variation for the collection was 0.3%. All results of the ^13^C enrichment of expired air are expressed as APE.

**Analytic Techniques**

Plasma glucose concentration was measured by the glucose oxidase method with a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). The ^13^C enrichment in plasma glucose was determined by gas chromatography-mass spectrometry of the pentaacetate derivatives of plasma glucose after deproteinization and deionization, as previously described (49). Plasma insulin concentration was determined using ELISA kits (Dako, Ely, UK). HbA1c was measured using high-performance liquid chromatography (TOSOH, Tokyo, Japan).

**Statistical Analysis**

Statistical analyses were performed using SPSS 15.0 software (SPSS, Chicago, IL). Values are means ± SE. Statistical comparisons between diabetic and control groups were performed using Student’s t-test; within-group differences were determined using paired t-test where appropriate. Changes of sequential data within experiments were evaluated by repeated-measures ANOVA with Tukey’s post hoc testing. Nonparametric correlations were tested by Spearman’s rank test (r_s). Statistical significance was accepted at P < 0.05.

**RESULTS**

**Plasma Glucose and Insulin**

The steady-state plasma glucose concentrations necessary to test the hypothesis were achieved. During the basal period, plasma glucose concentration decreased steadily in the diabetic group (from 7.7 ± 0.3 to 6.5 ± 0.3 mmol/l, P = 0.002) and remained steady in the control subjects (5.1 ± 0.1 vs. 5.0 ± 0.1 mmol/l, P = 0.186). After observation of the basal state, (−240 to 0 min), the clamp period was characterized by stable plasma glucose and plasma insulin concentrations in all studies (Fig. 2). During isoglycemia, plasma glucose concentration was clamped at 10.6 ± 0.2 and 13.0 ± 0.3 mmol/l for control and diabetic subjects, respectively (P < 0.01).

**Glucose Disposal Rates**

At isoglycemia, the glucose disposal rate was lower in the diabetic than the control group [4.8 ± 0.6 vs. 6.6 ± 0.5 mg kg\(^{-1}\) min\(^{-1}\) (where kg\(_{ffm}\) is kilograms of fat-free mass), P = 0.04; Fig. 3A]. During the hyperglycemic clamp in the diabetic group, the glucose disposal rate increased 1.6-fold compared with the isoglycemic clamp (7.7 ± 0.9 vs. 4.8 ± 0.6 mg kg\(_{ffm}\) \(^{-1}\) min\(^{-1}\), P = 0.005). Hence, the glucose disposal rate became similar to that of the control subjects at isoglycemia (7.7 ± 0.9 vs. 6.6 ± 0.5 mg kg\(_{ffm}\) \(^{-1}\) min\(^{-1}\), P = 0.573). During hyperglycemia in the control group, glucose disposal
rate also increased 1.6-fold (10.5 ± 1.1 vs. 6.6 ± 0.5 mg·kg⁻¹·min⁻¹, P = 0.01).

Muscle Glycogen

Fasting muscle glycogen concentrations were similar on the isoglycemia and hyperglycemia clamp days (67.5 ± 4.5 and 67.0 ± 3.8 mmol/l, respectively, for diabetic subjects and 71.1 ± 2.6 and 72.3 ± 6.2 mmol/l, respectively, for controls). Glycogen synthesis rates were examined between 70 and 150 min of the clamp. At isoglycemia, rates were lower in the diabetic than the control group (19 ± 9 vs. 40 ± 7 μmol·l⁻¹·min⁻¹, P = 0.012; Fig. 3B). The subnormal rate in the diabetic subjects at isoglycemia was increased 2.2-fold by hyperglycemia (from 19 ± 9 to 41 ± 12 μmol·l⁻¹·min⁻¹, P = 0.013), making the glycogen synthesis rate almost identical to that of the controls at isoglycemia (40 ± 7 μmol·l⁻¹·min⁻¹, P = 0.460; Fig. 3B).

In control subjects, the mean rate of muscle glycogen synthesis between 70 and 150 min was increased 2.5-fold by hyperglycemia compared with isoglycemia (100 ± 24 vs. 40 ± 7 μmol·l⁻¹·min⁻¹, P = 0.028).

The absolute increment in glycogen concentration in the diabetic subjects during hyperglycemia was similar to that during isoglycemic clamp conditions in the control group (3.8 ± 1.3 vs. 3.8 ± 0.8 mmol/l, P = 0.515). In control subjects during hyperglycemia, the increment in glycogen concentration was threefold higher than during isoglycemia (11.1 ± 2.7 vs. 3.8 ± 0.8 mmol/l, P = 0.012).

Muscle ATP Turnover Rates

Muscle ATP turnover rates are shown in Table 2 and Fig. 3C. Basal ATP turnover rates were similar in control and diabetic subjects. Muscle ATP turnover rates in control subjects remained unchanged during the isoglycemic-hyperinsulinemic clamps (8.6 ± 0.7 vs. 8.6 ± 1.3 μmol·g⁻¹·min⁻¹, P = 0.40). In diabetic subjects, during isoglycemia, muscle ATP turnover tended to decline (8.6 ± 0.8 vs. 7.1 ± 0.5 μmol·g⁻¹·min⁻¹, P = 0.09), whereas hyperglycemia prevented this decline in insulin-stimulated muscle ATP turnover rates (7.1 ± 0.5 and 8.6 ± 0.7 μmol·g⁻¹·min⁻¹ for isoglycemia and hyperglycemia, respectively, P = 0.04). In control subjects, during hyperglycemic-hyperinsulinemic clamps, insulin increased muscle ATP turnover rate by 23% (from 8.1 ± 0.9 to 10.0 ± 0.9 μmol·g⁻¹·min⁻¹, P = 0.025). Overall, muscle ATP turnover rates correlated positively with muscle glycogen synthesis rates (r = 0.46; P = 0.005; Fig. 4).

Plasma and Breath ¹³C Enrichments

Plasma enrichment of [¹³C]glucose increased steadily during the [¹⁻¹³C]glucose infusion in all the clamps. In diabetic and control groups, the ¹³C APE in expired breath increased steadily during isoglycemia: from 0.14 ± 0.01 to 0.36 ± 0.07 (P < 0.01) and from 0.23 ± 0.04 to 0.44 ± 0.05 from 90 to 150 min in diabetic and control subjects, respectively. The same pattern was observed during hyperglycemia: from 0.28 ±

### Table 2. Muscle ATP turnover rates

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<th>Muscle ATP Turnover Rates, μmol·g⁻¹·min⁻¹</th>
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<tr>
<td></td>
<td>Baseline</td>
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<tr>
<td>Isoglycemia clamps</td>
<td></td>
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<tr>
<td>Control</td>
<td>8.6 ± 0.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8.6 ± 0.8</td>
</tr>
<tr>
<td>Hyperglycemia clamps</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.1 ± 0.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8.7 ± 0.7</td>
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Values are means ± SE, *P < 0.05 vs. baseline.
Prolonged fasting for 60 h in humans lowered insulin-stimulated muscle ATP production (3). Other recent studies support this interpretation. A 0.21-fold decrease in insulin-stimulated ATP turnover rate in vivo during 3 h of elevated FFA levels, despite a marked reduction in whole body glucose disposal. They only observed a decrease in insulin-stimulated ATP turnover rate when FFA levels were elevated for >4 h (3). In the present study, the time resolution of the technique allowed acquisition of data over 30-min periods. This revealed a gradual fall in ATP turnover rate.
ATP turnover rate in muscle of type 2 diabetic subjects during the isoglycemia study. Although the reasons for this cannot be determined from the present study, we postulate that the downward trend occurred as a consequence of prolonged fasting (~15 h) and a decrease in blood glucose concentration during the basal period of the study protocol (6.7 ± 0.3 to 5.8 ± 0.3 mmol/l) and that increased intracellular mobilization of fatty acid from intramyocellular lipid could possibly have suppressed muscle ATP turnover rates. During hyperglycemia, this fall in muscle ATP turnover rates in the diabetic subjects was prevented. In a related study on the same subjects, we previously showed that suppression of FFA levels over ~6 h brought about an increase in ATP turnover rate in muscle of type 2 diabetic subjects (21). This further emphasizes that muscle ATP turnover rate is dependent on intracellular substrate supply and that ATP turnover rates are not limited by the presence of type 2 diabetes but respond to metabolic conditions.

Previous work suggested that acute elevation of insulin to postprandial levels would increase muscle ATP turnover rate in healthy normoglycemic controls, but not diabetic subjects (31, 32, 45). As such, the present study protocol was designed with this expectation. However, when no effect of physiological hyperinsulinemia on muscle ATP turnover rate was observed in the initial control subjects in this experiment, we conducted a substudy on a group of young healthy subjects to investigate the relationship between insulin’s activation of glucose metabolism and muscle ATP turnover rate over a 150-min time frame (22). As we were able to measure ATP turnover rate over 30-min periods, we were able to track the time course of muscle ATP turnover rate during the onset of insulin action. We observed the greatest increase in glucose metabolism during the first 45 min of the euglycemic hyperinsulinemia, whereas there was no concurrent change in muscle ATP turnover rate. In the cohort of young, insulin-sensitive subjects, muscle ATP turnover rate increased 8% above basal values after 2 h of insulin stimulation. This is in contrast to findings of an 11–90% increase in muscle ATP turnover rate with insulin in the literature (3, 32, 45). The latter studies used measurements of muscle ATP turnover rate averaged over 120–350 min of insulin stimulation and made the assumption that an insulin-stimulated increase in muscle ATP turnover rate was responsible for the changes in glucose metabolism. The acute metabolic effect of insulin does not appear to depend on any measurable increase in muscle ATP turnover rate. It is likely that processes other than insulin’s effect on glucose metabolism, such as on mitochondrial fusion and proliferation (26, 50) or mitochondrial protein synthesis (43), may affect muscle ATP turnover rate on a time scale of several hours of insulin stimulation. These processes are not directly related to the early effects of insulin on glucose metabolism. There is considerable variation among ATP turnover rates at different glycogen synthesis rates during iso- and hyperglycemia (Fig. 4). The reason for this is unclear, as is the proportion of ATP utilization related to glycogen synthesis. Baijepi et al. (1) also reported a broad range of maximal ATP synthetic rates, determined from the rate of phosphocreatine recovery, within type 2 diabetes subjects: 52% of the diabetic subjects had maximum ATP synthesis rates that were within the range observed in healthy sedentary controls and 24% had maximum ATP synthesis rates that overlapped with those of the active control group.

Glucose infusion rate is a useful measure of overall glucose disposal but does not take into account the possibility of incomplete suppression of hepatic glucose production during the clamp, especially among the diabetic subjects. An estimate of endogenous glucose production can be obtained from the measured isotopic enrichments of infusate and plasma together with glucose infusion rate (37). Hepatic glucose production was 0.01 ± 0.28 and 1.13 ± 0.18 mg·kg⁻¹·min⁻¹ in control and diabetic subjects, respectively, during the isoglycemic-hyperinsulinemic clamps and 0.04 ± 0.30 and −0.17 ± 0.32 mg·kg⁻¹·min⁻¹ in control and diabetic subjects, respectively, during the hyperglycemic-hyperinsulinemic clamps. By addition of this value to the glucose infusion rate, total glucose disposal rates can be estimated to be 6.58 ± 0.28 (control/isoglycemia), 5.94 ± 0.62 (diabetes/isoglycemia), 10.57 ± 1.34 (control/hyperglycemia), and 7.54 ± 0.67 (diabetes/hyperglycemia) mg·kg⁻¹·min⁻¹. Hence, consideration of glucose endogenous production does not change the interpretation of the primary results.

The present data demonstrate that normalization of the rate of glycogen synthesis by hyperglycemia is associated with an increase in muscle ATP turnover rate in type 2 diabetes. Therefore, muscle ATP turnover rates reflect prevailing substrate availability, and defects in mitochondrial function are unlikely to underlie and initiate the metabolic abnormalities of type 2 diabetes.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.L.L. and R.T. are responsible for conception and design of the research; E.L.L., K.G.H., and F.E.S. analyzed the data; E.L.L., K.G.H., and R.T. interpreted the results of the experiments; E.L.L. prepared the figures; E.L.L. drafted the manuscript; E.L.L., K.G.H., and F.E.S. read and revised the manuscript; E.L.L. and R.T. approved the final version of the manuscript.

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