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

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ABSTRACT

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 causing 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-hyperinsulinemic and hyperglycemic-hyperinsulinemic clamps were performed on type 2 diabetic subjects and matched controls with measurements of muscle ATP turnover and glycogen synthesis rates using ^{31}P and ^{13}C MR spectroscopy respectively. In diabetic subjects hyperglycemia increased muscle glycogen synthesis rates to the level observed in controls at isoglycemia (19 ± 9 to 41 ± 12 μmol·l^{-1}·min^{-1}, p=0.012; controls 40 ± 7 μmol·l^{-1}·min^{-1}). 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 from basal state (8.1 ± 0.9 vs. 10.0 ± 0.9 μmol·l^{-1}·min^{-1}; p = 0.025). Muscle ATP turnover rates correlated positively with glycogen synthesis rates (r_s = 0.46, p = 0.005). Changing the rate of muscle glucose metabolism in type 2 diabetes 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.

Keywords: ATP turnover rate, mitochondrial function, muscle glycogen synthesis, type 2 diabetes

INTRODUCTION

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 diabetes subjects have demonstrated smaller mitochondria and reduced activities of oxidative enzymes compared with glucose-tolerant subjects (13, 18). Magnetic resonance (MR) spectroscopy allows non-invasive measurement of a critical aspect of mitochondrial function by measuring rates of ATP synthesis (20). Using this methodology, decreased ATP turnover rates have been reported in skeletal muscle in type 2 diabetes and in healthy subjects at risk of developing diabetes (i.e. insulin-resistant offspring of type 2 diabetic patients) (31, 45). In young healthy individuals, insulin was observed to stimulate muscle ATP turnover rate whereas no response was seen in the insulin-resistant offspring of type 2 diabetic patients (32). Decreased expression of nuclear-encoded genes that regulate mitochondrial biogenesis such as peroxisome-proliferation-activated receptor γ co-activator 1α (PGC-1α) has also been reported in non-diabetic individuals with impaired glucose tolerance and in healthy first-degree relatives to type 2 diabetes patients (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 diabetes patients (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 both in humans and rodents directly challenge the view that a pre-existing defect in mitochondrial function is responsible for the development of insulin resistance. In obese humans, muscle biopsy studies have observed

74 improvement in mitochondrial function after programs of weight loss and physical exercise
75 (24). Similarly, improvement in insulin sensitivity through calorie restriction has been
76 demonstrated in overweight and obese subjects in absence of any measurable change in
77 mitochondria DNA content and NADH-oxidase activity (46). In addition, several studies of
78 genetically modified mice have failed to demonstrate a clear effect of altering mitochondrial
79 function on insulin action (4, 12). In light of all the evidence presented, the reduced capacity
80 of the mitochondria to produce ATP in muscle of type 2 diabetes could be secondary to the
81 metabolic state itself, and a feature of insulin resistance rather than its cause (33, 44).
82
83 Application of ^{13}C MR spectroscopy demonstrated that insulin-stimulated muscle
84 glycogen synthesis was the major metabolic pathway of glucose disposal under post-prandial
85 conditions, and that a defect in muscle glycogen synthesis was responsible for the decrease in
86 insulin sensitivity in type 2 diabetes (42). Further study showed that defect in muscle glucose
87 transport was responsible for the decreased rate of insulin-stimulated glycogen synthesis in
88 muscle of type 2 diabetes (7). We therefore hypothesized that impairment in insulin-
89 stimulated ATP turnover rates in muscle of type 2 diabetes merely reflects the lower rates of
90 muscle glucose uptake and glycogen synthesis, rather than causing it. Consequently, muscle
91 ATP turnover rates would be expected to normalize if glycogen synthesis rates were
92 normalized by raising plasma glucose concentration. To test this hypothesis, MR
93 spectroscopy was used to quantify muscle ATP turnover rates and muscle glycogen synthesis
94 rates during isoglycemic- and hyperglycemic hyperinsulinemia in type 2 diabetic and non-
95 diabetic control subjects.
96
97

METHODS

Subjects

Ten well-controlled type 2 diabetic patients (seven males and three females) and eight normoglycemic controls (six males and two females) matched for sex-, age-, body mass index (BMI)- and physical activity were studied. Their anthropometrical and metabolic characteristics are summarised in Table 1. All subjects were recruited by means of advertisement and underwent a complete medical history, clinical examination, and lab tests to exclude hepatic and renal diseases. Patients with diabetes on insulin treatment or any oral hypoglycemic medications apart from metformin were excluded. The individuals in the control group had neither a family history of diabetes nor were taking any medication (for example steroids, beta-blockers or diuretics) known to affect glucose tolerance or insulin sensitivity. Normal glucose metabolism was confirmed by a standard 75g 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, USA) which provides a measure of total daily energy expenditure and number of steps taken per day (25). The study protocol was approved by the Newcastle upon Tyne Ethics Committee No. 2, and informed consent was obtained from all subjects.

Experimental Protocol

All subjects refrained from any 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: (A) isoglycemic-hyperinsulinemic clamp test and (B) hyperglycemic-hyperinsulinemic clamp test, completed

4-8 weeks apart (Figure 1). Isoglycemic rather than euglycemic clamps were used in order to allow observation of the true fasting state in each group. The subjects' body weights and lifestyle remained unchanged during the period of the study. Data on the isoglycemic clamps has previously been reported as part of a study of the effects of plasma free fatty acid (FFA) suppression (21). For all experiments, subjects travelled to the magnetic resonance facility by taxi and were transported within the centre by wheelchair. At 0830 hours (-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 arterialisation of venous blood. To permit the frequent ^{31}P and ^{13}C MR spectroscopy measurements, subjects were studied lying in the MR scanner for the entire duration of each study.

Protocol A: Isoglycemic-Hyperinsulinemic Clamp

Isoglycemia was maintained in order 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 $mU \cdot m^{-2} \cdot min^{-1}$) for 150 min. To inhibit pancreatic hormone secretion, somatostatin was infused at 0.06 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (Somatostatin-UCB; UCB-Pharma, Netherlands) 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. In order to increase sensitivity of measurement of the muscle glycogen synthesis by ^{13}C NMR spectroscopy, the variable glucose infusion contained 20% [1-^{13}C] glucose (Cambridge Isotope Laboratories, Andover, MA, USA). Whole-body insulin sensitivity was determined from calculated whole-body

146 glucose disposal during the last 30 min of the hyperinsulinemic glucose clamp (9). Whole-
147 body glucose disposal was calculated from glucose infusion rate (39). To assess rate of
148 oxidation of infused glucose, breath samples for $^{13}CO_2$ were obtained. As this measure would
149 be affected by differences in plasma ^{13}C glucose enrichment, the index of whole-body glucose
150 oxidation was calculated as the ratio of breath to plasma ^{13}C atom percentage excess (APE)
151 ([breath APE/plasma APE] x 100).

152

153 ***Protocol B: Hyperglycemic-Hyperinsulinemic Clamp***

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

158

159 ***MR Spectroscopy***

160 Magnetic resonance data were acquired using a 3 Tesla Achieva scanner (Philips, Best, The
161 Netherlands) with an in-built body coil used for imaging. A 14-cm diameter surface coil was
162 used for phosphorus spectroscopy and a 6-cm diameter ^{13}C coil with an integral quad ^{1}H
163 decoupling coil (PulseTeq, Wotton under Edge, UK) was used for ^{13}C spectroscopy. Subjects
164 remained supine inside the magnetic resonance spectrometer with each coil positioned
165 beneath the left calf during each investigation. The coil position was marked on the leg with
166 indelible ink. Scout images were acquired to ensure identical coil positioning on repeat scans.
167 To prevent movement during each study, the coil was secured in place using webbing straps
168 around the calf. Analysis of all spectra was performed with jMRUI (version 3.0) (28) using
169 the AMARES fitting algorithm (48).

^{31}P MR spectroscopy was carried out as previously described (22). In brief, a saturation transfer sequence was used to measure the transfer magnetization between γ-ATP and inorganic phosphate (P_i) (20). The steady-state magnetization of P_i was measured during selective irradiation of γ-ATP (M_z) and compared with the equilibrium P_i magnetization with the irradiation placed symmetrically down-field from the P_i frequency (M_o). The fractional reduction of P_i magnetization upon saturation of γ-ATP, $(M_o - M_z)/M_o$, was used to calculate the pseudo-first order rate constant using the Forsen-Hoffman equation: $k_1 = [(M_o - M_z)/M_o](1/T_1^*)$, where T_1^* is the spin-lattice relaxation time for the phosphorus nucleus of P_i when ATP is saturated (10). Unidirectional turnover rate of ATP synthesis was then calculated by multiplying the constant k_1 by the P_i concentration. ^{31}P MR spectroscopy measurements were acquired at baseline from -240 to -210 min and twice further during the clamp from 15-45 min and from 90-120 min. For ^{13}C MR spectroscopy, spectra were acquired as previously described (21) at baseline from -205 to -185 min and twice further during the clamp from 50-70 min and from 130-150 min. The concentration of muscle glycogen at baseline, $[\text{Glyc}]_{\text{muscle}}$, was calculated using the formula:

$$[\text{Glyc}]_{\text{muscle}} = \frac{S_{\text{muscle}} \times [\text{Glyc}]_{\text{phantom}}}{S_{\text{phantom}}}$$

where S_{phantom} and S_{muscle} are the signal intensities arising from glycogen in the phantom and muscle, respectively, and $[\text{Glyc}]_{\text{phantom}}$ is the concentration of glycogen in the phantom (100 mmol/l). The increments in muscle glycogen concentration at 70 min and 150 min of the clamp, $[\Delta\text{Glyc}_{70}]$ and $[\Delta\text{Glyc}_{150}]$ respectively, were calculated from the equation previously reported (16):

$$[\Delta\text{Glyc}_{70}] = \frac{(S_{70} - S_0) \times [\text{Glyc}_0] \times f_0}{S_0 \times f_{70}}$$

193 $$[\Delta \mathrm{Glyc}_{150}] = \frac{(S_{150} - S_{70}) \times [\mathrm{Glyc}_0] \times f_0}{S_0 \times f_{150}}$$

194 where S_0, S_{70} and S_{150} represent the signal intensity of ^{13}C-glycogen at 0, 70 and 150 min,
195 [$Glyc_0$] is the basal glycogen concentration in mmol/l. f_0 corresponds to the natural abundance
196 enrichment of ^{13}C-glycogen at baseline (1.1%), and f_{70} and f_{150} correspond to the mean
197 percentage ^{13}C enrichment of plasma glucose at 70 min and 150 min respectively. Each
198 increment was then added to the previous concentration, and the slope calculated by linear
199 regression analysis to yield the rate of glycogen synthesis (42).
200
201 ***Breath ^{13}C Enrichments***
202 Breath samples for ^{13}C enrichments were collected at 5 time points during the study. The
203 subjects were asked to exhale fully through a short straw into a glass tube (Labco Exetainer;
204 Laboco Ltd., Buckinghamshire, UK). The tubes were immediately stoppered. ^{13}C
205 enrichments of breath samples were determined by continuous flow isotope ratio mass
206 spectrometry (ABCA system; PDZ Europa). The coefficient of variation (CV) for the
207 analysis was 0.07% and CV for the collection was 0.3%. All results of the ^{13}C enrichment of
208 expired air are expressed as APE.
209
210 ***Analytical Techniques***
211 Plasma glucose concentration was measured by the glucose oxidase method (YSI glucose
212 analyser; Yellow Springs, OH, USA). The ^{13}C enrichment in plasma glucose was determined
213 by gas chromatography-mass spectrometry of the pentaacetate derivatives of plasma glucose
214 after deproteinization and deionization as previously described (49). Plasma insulin
215 concentration was determined using ELISA kits (Dako, Ely, UK). HbA_{1c} was measured using
216 high performance liquid chromatography (TOSOH Corporation, Tokyo, Japan).

Statistical Analysis

Statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as means ± SE. Statistical comparisons between diabetes and control groups were performed Student's *t* test while within group differences were determined using paired *t* test where appropriate. Changes of sequential data within experiments were evaluated by repeated measures ANOVA with post hoc Tukey testing. Non-parametric correlations were tested by Spearman 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 (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, $p = 0.186$). Following observation of the basal state, (-240 min to 0 min), the clamp period was characterised by stable plasma glucose and plasma insulin concentrations in all studies (Figure 2). During isoglycemia, plasma glucose concentration was clamped at the level observed by the end of the baseline period (diabetic subjects: 6.6 ± 0.2 mmol/l and control subjects: 5.0 ± 0.1; $p < 0.01$). For the hyperglycemia protocol, plasma glucose concentration was clamped at 10.6 ± 0.2 and 13.0 ± 0.3 mmol/l for control and diabetic subjects respectively ($p < 0.01$).

241 ***Glucose Disposal Rates***

242 At isoglycemia, the glucose disposal rate in the diabetic group was lower compared with the
243 control group (4.8 ± 0.6 vs. 6.6 ± 0.5 $mg \cdot kg_{ffm}^{-1} \cdot min^{-1}$; $p = 0.04$, Figure 3A). During the
244 hyperglycemic clamp in the diabetic group, the glucose disposal rate increased 1.6-fold
245 compared with isoglycemic clamp (7.7 ± 0.9 vs. 4.8 ± 0.6 $mg \cdot kg_{ffm}^{-1} \cdot min^{-1}$, $p = 0.005$).
246 Hence the glucose disposal rate became similar to that of the control subjects at isoglycemia
247 (7.7 ± 0.9 vs. 6.6 ± 0.5 $mg \cdot kg_{ffm}^{-1} \cdot min^{-1}$, $p = 0.573$). During hyperglycemia in the control
248 group, glucose disposal rate also increased 1.6-fold (10.5 ± 1.1 vs. 6.6 ± 0.5 $mg \cdot kg_{ffm}^{-1} \cdot min^{-1}$,
249 $p = 0.01$).

250

251 ***Muscle Glycogen***

252 Fasting muscle glycogen concentrations were similar on the isoglycemia and hyperglycemia
253 clamp days (diabetic subjects: 67.5 ± 4.5 vs. 67.0 ± 3.8 mmol/l; control subjects: 71.1 ± 2.6
254 and. 72.3 ± 6.2 mmol/l respectively). Glycogen synthesis rates were examined between 70
255 and 150 min of the clamp. At isoglycemia, rates were lower in the diabetic group compared
256 to controls (19 ± 9 vs. 40 ± 7 $\mu mol \cdot l^{-1} \cdot min^{-1}$, $p = 0.012$; Figure 3B). The subnormal rate in
257 the diabetic subjects at isoglycemia was increased 2.2-fold by hyperglycemia (19 ± 9 to 41 ±
258 12 $\mu mol \cdot l^{-1} \cdot min^{-1}$ ($p = 0.013$), making the glycogen synthesis rate almost identical to that of
259 the controls at isoglycemia (40 ± 7 $\mu mol \cdot l^{-1} \cdot min^{-1}$, $p = 0.460$; Figure 3B). In control subjects,
260 the mean rate of muscle glycogen synthesis between 70 and 150 min was increased 2.5-fold
261 by hyperglycemia compared with isoglycemia (100 ± 24 vs. 40 ± 7 $\mu mol \cdot l^{-1} \cdot min^{-1}$, $p =$
262 0.028).

263

The absolute increment in glycogen concentration in the diabetic subjects during hyperglycemia was similar to that seen 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 three-fold higher compared to 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 Figure 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 $\mu mol \cdot g^{-1} \cdot min^{-1}$, $p = 0.40$). In diabetic subjects, during isoglycemia a tendency to decline was observed (8.6 ± 0.8 vs. 7.1 ± 0.5 $\mu mol \cdot g^{-1} \cdot min^{-1}$, $p = 0.09$), whereas hyperglycemia prevented this decline in insulin-stimulated muscle ATP turnover rates (isoglycemia: 7.1 ± 0.5 vs. hyperglycemia: 8.6 ± 0.7 $\mu mol \cdot g^{-1} \cdot min^{-1}$, $p = 0.04$). In control subjects, during hyperglycemic-hyperinsulinemic clamps, insulin increased muscle ATP turnover rate increased by 23% (8.1 ± 0.9 vs. 10.0 ± 0.9 $\mu mol \cdot g^{-1} \cdot min^{-1}$, $p = 0.025$). Overall, muscle ATP turnover rates correlated positively with muscle glycogen synthesis rates ($r_s = 0.46$, $p = 0.005$; Figure 4).

Plasma and Breath ^{13}C Enrichments

Plasma enrichment of ^{13}C-glucose increased steadily during the [1-^{13}C] glucose infusion in all the clamps. In both diabetic and control groups the ^{13}C APE in expired breath increased steadily during isoglycemia (0.14 ± 0.01 to 0.36 ± 0.07 at 90 and 150 min, $p < 0.01$; 0.23 ± 0.04 to 0.44 ± 0.05 at 90 and 150 min). The same pattern was observed during hyperglycemia

(0.28 ± 0.02 to 0.50 ± 0.03 and 0.44 ± 0.07 to 0.72 ± 0.08 respectively, $p = 0.003$). In order to compare rates of glucose oxidation corrected for plasma glucose enrichment, the ratio of breath to plasma ^{13}C APE was examined. This index of whole-body glucose oxidation rate was higher during the hyperglycemia study compared to the isoglycemia study in both diabetic (2.53 ± 0.17 vs. 3.32 ± 0.21, $p = 0.001$) and control subjects (2.87 ± 0.24 vs. 5.06 ± 0.84, $p = 0.008$).

DISCUSSION

The study design was successful in using acute hyperglycemia to increase the rate of glycogen synthesis in muscle of type 2 diabetes subjects to that of the non-diabetic control group at isoglycemic hyperinsulinemia. Hyperglycemia increased muscle glycogen synthesis rate by 2.2-fold in the diabetic subjects and by 2.5-fold in the control subjects with a simultaneous increase in muscle ATP turnover rates (1.2-fold for both). Muscle ATP turnover rates were found to be positively correlated with muscle glycogen synthesis rates.

Hyperglycemic clamp studies have previously demonstrated that acute hyperglycemia, by the mass-action effect of glucose, can stimulate oxidative and non-oxidative glucose disposal (51, 52). Vaag and colleagues showed that fasting and insulin-stimulated glucose oxidation, glucose storage and muscle glycogen synthase activation were all fully normalized during hyperglycemia in type 2 diabetic patients (47). Rate of insulin-stimulated glucose disposal in type 2 diabetic subjects was shown to be 57% greater during hyperglycemia compared with euglycemia, and the major part (89%) of the increase in glucose metabolism during

312 hyperglycemia was due to an increase in non-oxidative glucose metabolism (11). In type 2
313 diabetes, intra-myocellular glucose levels are similar to control values and insulin brings
314 about a lesser rise in glucose-6-phosphate (7).
315
316 The present results suggest that muscle ATP turnover rate depends upon the metabolic
317 state rather than reflecting a primary defect of type 2 diabetes. Other recent studies support
318 this interpretation. A high fat diet decreased ATP turnover rate in muscle in rodents (19).
319 Raising FFA levels in young healthy individuals for more than 6 h decreased insulin-
320 stimulated muscle ATP production (3). Prolong fasting of ~60 hours in humans lowered
321 insulin-stimulated glucose uptake in association with elevated plasma FFA and overall
322 reduction in intrinsic mitochondrial functional capacity of skeletal muscle (14). Despite early
323 reports of studies showing an association between mitochondrial dysfunction and insulin
324 resistance in type 2 diabetic patients, there are increasing instances of discordance between
325 the two. ATP turnover rates were found not to relate to insulin resistance in offspring of
326 mothers with type 2 diabetes (35). Improvement in insulin sensitivity through either calorie
327 restriction (46) or pharmacological agents such as thiazolidinedione (41) in both insulin-
328 resistant states and type 2 diabetic patients have been observed without any accompanying
329 changes in mitochondrial function in muscle. Using post-exercise phosphocreatine re-
330 synthesis rate as an alternative method to assess mitochondrial function, rosiglitazone and
331 pioglitazone were found to have opposite effects on mitochondrial function although both
332 improved insulin sensitivity (36). Using the same technique, no defect was detectable in
333 either early or late stage type 2 diabetes compared with exercise-matched normoglycaemic
334 controls (1, 8). This is in contrast to other studies which have reported reduced
335 phosphocreatine recovery in type 2 diabetes subjects compared with matched controls (23, 34,

40). First-degree relative of type 2 diabetes patients showed *ex vivo* observations of decreased mitochondrial capacity and it has been suggested that gradual changes in mitochondrial may occur (34). The importance of matching for physical activity must be noted, as lower muscle ATP turnover rates have been reported in studies comparing type 2 diabetic subjects with non-activity matched controls (31). It should also be noted that different exercise protocols used in the phosphocreatine recovery method may account for the differences reported (8). Furthermore, observation by Schrauwen-Hinderling and coworkers that muscle ATP turnover rates are inversely proportional to fasting blood glucose is critical in that abnormal ATP turnover cannot be an early feature explaining the onset of the condition (34, 40). Ex-vivo studies of mitochondria have also shown discordance between mitochondrial function and insulin sensitivity in offspring of mothers with type 2 diabetes (15). In earlier observations by Kelley *et al*, obese and type 2 diabetic subjects had smaller mitochondria and increased numbers of damaged mitochondria (18). In addition, mitochondrial surface area was positively correlated with insulin-stimulated glucose disposal (18). Subsarcolemmal fraction of mitochondria content has been found to be lower in type 2 diabetes, although this could possibly be due to decreased levels of physical activity of the subjects (38). On the other hand, intramyofibrillar mitochondrial content has been observed not to differ between type 2 diabetes and weight matched control subjects even though it was lower compared to lean insulin-sensitive controls (6). Although the present data relate to observations during established type 2 diabetes, overall the concept of mitochondrial dysfunction as a primary abnormality in type 2 diabetes is not well supported.

In vivo skeletal muscle ATP turnover rate has typically been observed over considerably longer periods of time to achieve a summed single measurement (3, 45). The time period over

which ATP turnover rate is measured is important. Brehm and colleagues did not observe any change in muscle ATP turnover rate in vivo during 3 hours of elevated FFA levels despite observing marked reduction in whole-body glucose disposal (2). The authors only observed a decrease in insulin-stimulated ATP turnover rate when FFA levels were elevated for more than 4 hours (3). In the present study the time resolution of the technique allowed acquisition of data over 30 minutes time periods. This revealed a gradual fall in 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 hours) and 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 (IMCL) 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 have shown 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 the dependence of muscle ATP turnover rate upon intracellular substrate supply and that ATP turnover rates are not limited by the fact of the type 2 diabetes but respond to metabolic conditions.

Previous work had suggested that acute elevation of insulin to post-prandial levels would increase muscle ATP turnover rate in healthy normoglycemic controls but not in the 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 proceeded to conduct a

sub-study 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, and this short study has now been published (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 occurring 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 hours of insulin stimulation. This is in contrast to findings of 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 upon any measurable increase in muscle ATP turnover rate. It is likely that processes other than insulin's effect on glucose metabolism, such as upon mitochondrial fusion and proliferation (26, 50) or mitochondrial protein synthesis (43), may affect muscle ATP turnover rate on a timescale 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 amongst ATP turnover rates at different glycogen synthesis rates observed during iso- and hyperglycemia (Figure 4). The reason for this is unclear, as is the proportion of ATP utilization related to glycogen synthesis. Bajpeyi *et al* also reported a broad range of maximal ATP synthetic rate, determined from the rate of phosphocreatine recovery, within type 2 diabetes subjects whereby 52% had maximum ATP synthesis rates

that were within the range observed in healthy sedentary controls and 24% had overlapped with the active control group (1).

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 amongst the diabetic subjects. As 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 $mg \cdot kg_{ffm}^{-1} \cdot min^{-1}$ in control and diabetic subjects during the isoglycemic-hyperinsulinemic clamps; and 0.04 ± 0.30 and -0.17 ± 0.32 $mg \cdot kg_{ffm}^{-1} \cdot min^{-1}$ in control and diabetic subjects during the hyperglycemic-hyperinsulinemic clamps respectively. By adding 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) $mg \cdot kg_{ffm}^{-1} \cdot min^{-1}$ respectively. Hence, consideration of glucose endogenous production does not change the interpretation of the primary results.

The present data demonstrate that normalizing the rate of glycogen synthesis by hyperglycemia is associated with 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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References

1. **Bajpeyi S, Pasarica M, Moro C, Conley K, Jubrias S, Sereda O, Burk DH, Zhang Z, Gupta A, Kjems L, and Smith SR**. Skeletal muscle mitochondrial capacity and insulin resistance in type 2 diabetes. *J Clin Endocrinol Metab* 96: 1160-1168, 2011.
2. **Brehm A, Krssak M, Schmid AI, Nowotny P, Waldhausl W, and Roden M**. Acute elevation of plasma lipids does not affect ATP synthesis in human skeletal muscle. *Am J Physiol Endocrinol Metab* 299: E33-38, 2010.
3. **Brehm A, Krssak M, Schmid AI, Nowotny P, Waldhausl W, and Roden M**. Increased lipid availability impairs insulin-stimulated ATP synthesis in human skeletal muscle. *Diabetes* 55: 136-140, 2006.
4. **Calvo JA, Daniels TG, Wang X, Paul A, Lin J, Spiegelman BM, Stevenson SC, and Rangwala SM**. Muscle-specific expression of PPARgamma coactivator-1alpha improves exercise performance and increases peak oxygen uptake. *J Appl Physiol* 104: 1304-1312, 2008.
5. **Chanseaume E, and Morio B**. Potential mechanisms of muscle mitochondrial dysfunction in aging and obesity and cellular consequences. *Int J Mol Sci* 10: 306-324, 2009.
6. **Chomentowski P, Coen PM, Radikova Z, Goodpaster BH, and Toledo FG**. Skeletal muscle mitochondria in insulin resistance: differences in intermyofibrillar versus subsarcolemmal subpopulations and relationship to metabolic flexibility. *J Clin Endocrinol Metab* 96: 494-503, 2011.
7. **Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, Inzucchi S, Dresner A, Rothman DL, and Shulman GI**. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med* 341: 240-246, 1999.
8. **De Feyter HM, van den Broek NM, Praet SF, Nicolay K, van Loon LJ, and Prompers JJ**. Early or advanced stage type 2 diabetes is not accompanied by in vivo skeletal muscle mitochondrial dysfunction. *Eur J Endocrinol* 158: 643-653, 2008.
9. **DeFronzo RA, Tobin JD, and Andres R**. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237: E214-223, 1979.
10. **Forsen F, and Hoffman RA**. Study of moderately rapid chemical exchange reactions by means of nuclear magnetic double resonance. *J Chem Phys* 39: 2892-2901, 1963.
11. **Franssila-Kallunki AI, Eriksson JG, and Groop LC**. Time-dependent effect of hyperglycemia and hyperinsulinemia on oxidative and non-oxidative glucose metabolism in patients with NIDDM. *Acta Endocrinol (Copenh)* 127: 100-106, 1992.
12. **Handschin C, Choi CS, Chin S, Kim S, Kawamori D, Kurpad AJ, Neubauer N, Hu J, Mootha VK, Kim YB, Kulkarni RN, Shulman GI, and Spiegelman BM**. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J Clin Invest* 117: 3463-3474, 2007.
13. **He J, Watkins S, and Kelley DE**. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes* 50: 817-823, 2001.
14. **Hoeks J, van Herpen NA, Mensink M, Moonen-Kornips E, van Beurden D, Hesselink MK, and Schrauwen P**. Prolonged fasting identifies skeletal muscle mitochondrial dysfunction as consequence rather than cause of human insulin resistance. *Diabetes* 59: 2117-2125, 2010.
15. **Irving BA, Short KR, Nair KS, and Stump CS**. Nine Days of Intensive Exercise Training Improves Mitochondrial Function But Not Insulin Action in Adult Offspring of Mothers with Type 2 Diabetes. *J Clin Endocrinol Metab* 2011.
16. **Jue T, Rothman DL, Shulman GI, Tavitian BA, DeFronzo RA, and Shulman RG**. Direct observation of glycogen synthesis in human muscle with 13C NMR. *Proc Natl Acad Sci U S A* 86: 4489-4491, 1989.
17. **Kacerovsky-Bielesz G, Chmelik M, Ling C, Pokan R, Szendroedi J, Farukuoye M, Kacerovsky M, Schmid AI, Gruber S, Wolzt M, Moser E, Pacini G, Smekal G, Groop L, and Roden M**. Short-term

exercise training does not stimulate skeletal muscle ATP synthesis in relatives of humans with type 2 diabetes. *Diabetes* 58: 1333-1341, 2009.

18. **Kelley DE, He J, Menshikova EV, and Ritov VB**. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 51: 2944-2950, 2002.

19. **Laurent D, Yerby B, Deacon R, and Gao J**. Diet-induced modulation of mitochondrial activity in rat muscle. *Am J Physiol Endocrinol Metab* 293: E1169-1177, 2007.

20. **Lebon V, Dufour S, Petersen KF, Ren J, Jucker BM, Slezak LA, Cline GW, Rothman DL, and Shulman GI**. Effect of triiodothyronine on mitochondrial energy coupling in human skeletal muscle. *J Clin Invest* 108: 733-737, 2001.

21. **Lim EL, Hollingsworth KG, Smith FE, Thelwall PE, and Taylor R**. Inhibition of lipolysis in type 2 diabetes normalizes glucose disposal without change in muscle glycogen synthesis rates. *Clin Sci (Lond)* 2011.

22. **Lim EL, Hollingsworth KG, Thelwall PE, and Taylor R**. Measuring the acute effect of insulin infusion on ATP turnover rate in human skeletal muscle using phosphorus-31 magnetic resonance saturation transfer spectroscopy. *NMR Biomed* 23: 952-957, 2010.

23. **Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, van de Weijer T, Sels JP, Schrauwen P, and Hesselink MK**. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes* 59: 572-579, 2010.

24. **Menshikova EV, Ritov VB, Toledo FG, Ferrell RE, Goodpaster BH, and Kelley DE**. Effects of weight loss and physical activity on skeletal muscle mitochondrial function in obesity. *Am J Physiol Endocrinol Metab* 288: E818-825, 2005.

25. **Mignault D, St-Onge M, Karelis AD, Allison DB, and Rabasa-Lhoret R**. Evaluation of the Portable HealthWear Armband: a device to measure total daily energy expenditure in free-living type 2 diabetic individuals. *Diabetes Care* 28: 225-227, 2005.

26. **Molina AJ, Wikstrom JD, Stiles L, Las G, Mohamed H, Elorza A, Walzer G, Twig G, Katz S, Corkey BE, and Shirihai OS**. Mitochondrial networking protects beta-cells from nutrient-induced apoptosis. *Diabetes* 58: 2303-2315, 2009.

27. **Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, and Groop LC**. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34: 267-273, 2003.

28. **Naressi A, Couturier C, Castang I, de Beer R, and Graveron-Demilly D**. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. *Comput Biol Med* 31: 269-286, 2001.

29. **Pagel-Langenickel I, Bao JJ, Pang LY, and Sack MN**. The Role of Mitochondria in the Pathophysiology of Skeletal Muscle Insulin Resistance. *Endocr Rev* 31: 25-51, 2010.

30. **Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, and Mandarino LJ**. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A* 100: 8466-8471, 2003.

31. **Petersen KF, Dufour S, Befroy D, Garcia R, and Shulman GI**. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 350: 664-671, 2004.

32. **Petersen KF, Dufour S, and Shulman GI**. Decreased insulin-stimulated ATP synthesis and phosphate transport in muscle of insulin-resistant offspring of type 2 diabetic parents. *PLoS Med* 2: e233, 2005.

33. **Phielix E, and Mensink M**. Type 2 Diabetes Mellitus and Skeletal Muscle Metabolic Function. *Physiology & Behavior* 94: 252-258, 2008.

34. **Phielix E, Schrauwen-Hinderling VB, Mensink M, Lenaers E, Meex R, Hoeks J, Kooi ME, Moonen-Kornips E, Sels JP, Hesselink MK, and Schrauwen P**. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. *Diabetes* 57: 2943-2949, 2008.

35. **Prikoszovich T, Winzer C, Schmid AI, Szendroedi J, Chmelik M, Pacini G, Krssak M, Moser E, Funahashi T, Waldhausl W, Kautzky-Willer A, and Roden M**. Body and liver fat mass rather than muscle mitochondrial function determine glucose metabolism in women with a history of gestational diabetes mellitus. *Diabetes Care* 34: 430-436, 2011.

36. **Rabol R, Boushel R, Almdal T, Hansen CN, Ploug T, Haugaard SB, Prats C, Madsbad S, and Dela F**. Opposite effects of pioglitazone and rosiglitazone on mitochondrial respiration in skeletal muscle of patients with type 2 diabetes. *Diabetes Obes Metab* 12: 806-814, 2010.

37. **Ravikumar B, Gerrard J, Dalla Man C, Firbank MJ, Lane A, English PT, Cobelli C, and Taylor R**. Pioglitazone decreases fasting and postprandial endogenous glucose production in proportion to decrease in hepatic triglyceride content. *Diabetes* 57: 2288-2295, 2008.

38. **Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, and Kelley DE**. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes* 54: 8-14, 2005.

39. **Rizza RA, Mandarino LJ, and Gerich JE**. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 240: E630-639, 1981.

40. **Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Jeneson JA, Backes WH, van Echteld CJ, van Engelshoven JM, Mensink M, and Schrauwen P**. Impaired in vivo mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects. *Diabetologia* 50: 113-120, 2007.

41. **Schrauwen-Hinderling VB, Mensink M, Hesselink MK, Sels JP, Kooi ME, and Schrauwen P**. The insulin-sensitizing effect of rosiglitazone in type 2 diabetes mellitus patients does not require improved in vivo muscle mitochondrial function. *J Clin Endocrinol Metab* 93: 2917-2921, 2008.

42. **Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, and Shulman RG**. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 322: 223-228, 1990.

43. **Stump CS, Short KR, Bigelow ML, Schimke JM, and Nair KS**. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc Natl Acad Sci U S A* 100: 7996-8001, 2003.

44. **Szendroedi J, and Roden M**. Mitochondrial fitness and insulin sensitivity in humans. *Diabetologia* 2008.

45. **Szendroedi J, Schmid AI, Chmelik M, Toth C, Brehm A, Krssak M, Nowotny P, Wolzt M, Waldhausl W, and Roden M**. Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. *PLoS Med* 4: e154, 2007.

46. **Toledo FG, Menshikova EV, Azuma K, Radikova Z, Kelley CA, Ritov VB, and Kelley DE**. Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. *Diabetes* 57: 987-994, 2008.

47. **Vaag A, Damsbo P, Hother-Nielsen O, and Beck-Nielsen H**. Hyperglycaemia compensates for the defects in insulin-mediated glucose metabolism and in the activation of glycogen synthase in the skeletal muscle of patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35: 80-88, 1992.

48. **Vanhamme L, Van Huffel S, Van Hecke P, and van Ormondt D**. Time-domain quantification of series of biomedical magnetic resonance spectroscopy signals. *J Magn Reson* 140: 120-130, 1999.

49. **Wolfe RR**. Tracers in metabolic research: radioisotope and stable isotope/mass spectrometry methods. *Lab Res Methods Biol Med* 9: 1-287, 1984.

588 50. **Yang JY, Yeh HY, Lin K, and Wang PH**. Insulin stimulates Akt translocation to mitochondria:
589 implications on dysregulation of mitochondrial oxidative phosphorylation in diabetic myocardium. *J*
590 *Mol Cell Cardiol* 46: 919-926, 2009.
591 51. **Yki-Jarvinen H**. Acute and chronic effects of hyperglycaemia on glucose metabolism.
592 *Diabetologia* 33: 579-585, 1990.
593 52. **Yki-Jarvinen H, Young AA, Lamkin C, and Foley JE**. Kinetics of glucose disposal in whole body
594 and across the forearm in man. *J Clin Invest* 79: 1713-1719, 1987.
595
596

597 **FIGURE LEGENDS**
598
599
600 **Figure 1.** Schematic representation of experimental protocol.
601
602
603 **Figure 2**. Time course of (A) plasma glucose and (B) plasma insulin concentrations during
604 the two experimental conditions: isoglycemia in control (○) and in diabetic (●) subjects;
605 hyperglycemia in control (Δ) and in diabetic (▲) subjects. Values are means ± SE. $^{*}p < 0.01$
606 control vs. diabetes.
607
608 **Figure 3.** (A) Glucose disposal rate during the final 30 mins of the clamps, (B) muscle
609 glycogen synthesis rate between 70-150 min and (C) muscle ATP turnover rate between 90-
610 120 min in the isoglycemic- and hyperglycemic-hyperinsulinemic clamps. Values are means
611 ± SE. $^{*}p < 0.05$ and $^{**}p < 0.01$.
612
613 **Figure 4.** Positive correlation between muscle glycogen synthesis rate between 70-150 min
614 and muscle ATP turnover rate ($r_s = 0.46$, $p = 0.005$).

615 **TABLE LEGENDS**

616

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

618

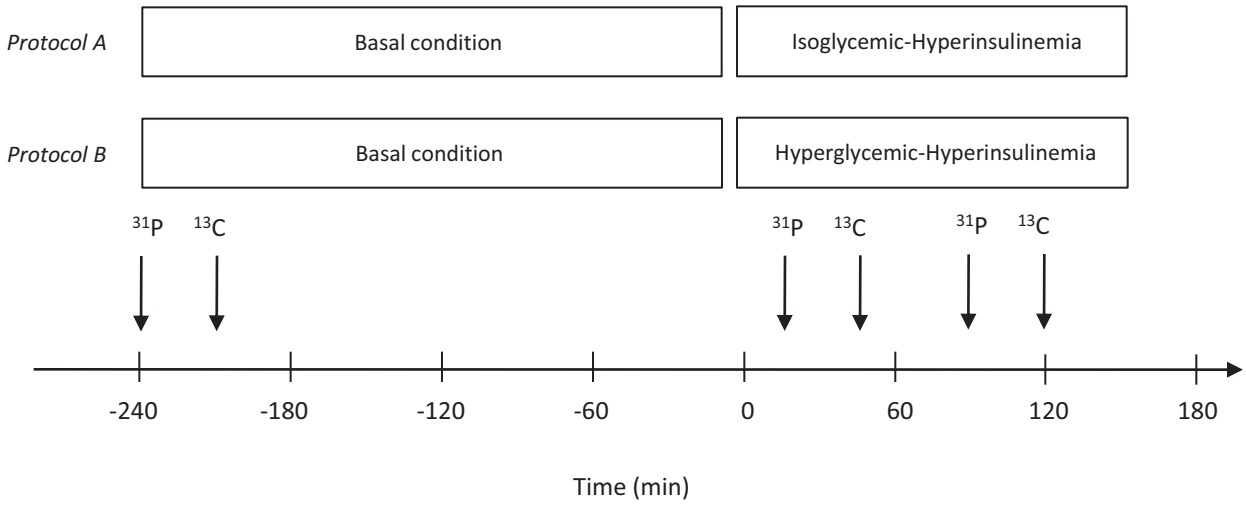
619

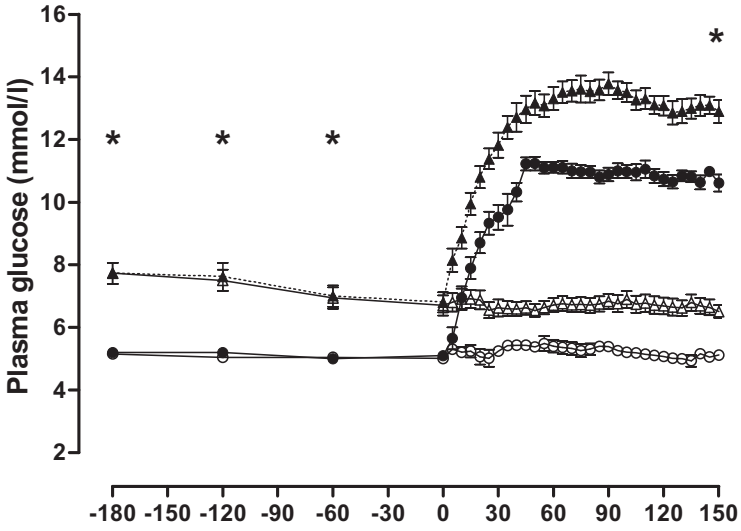
620 **Table 2.** Muscle ATP turnover rates ($\mu mol \cdot g^{-1} \cdot min^{-1}$)

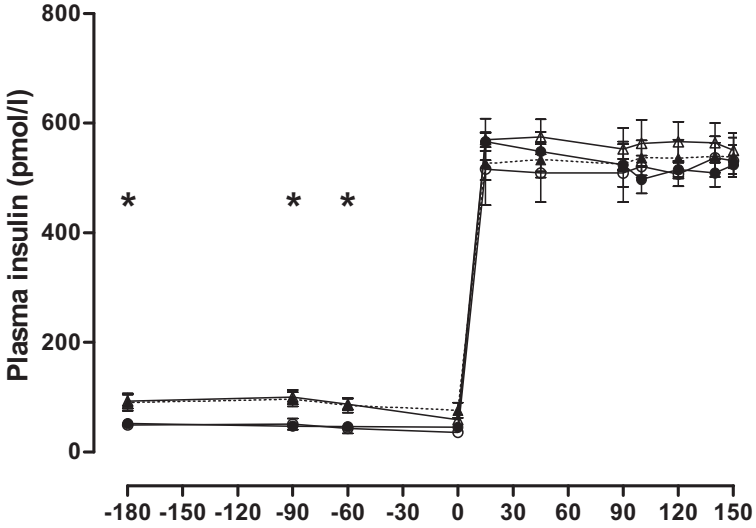
621

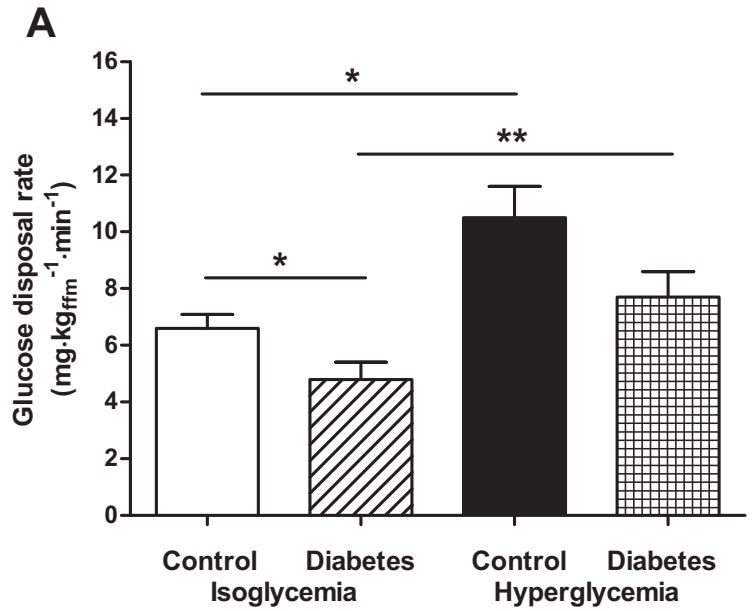
622

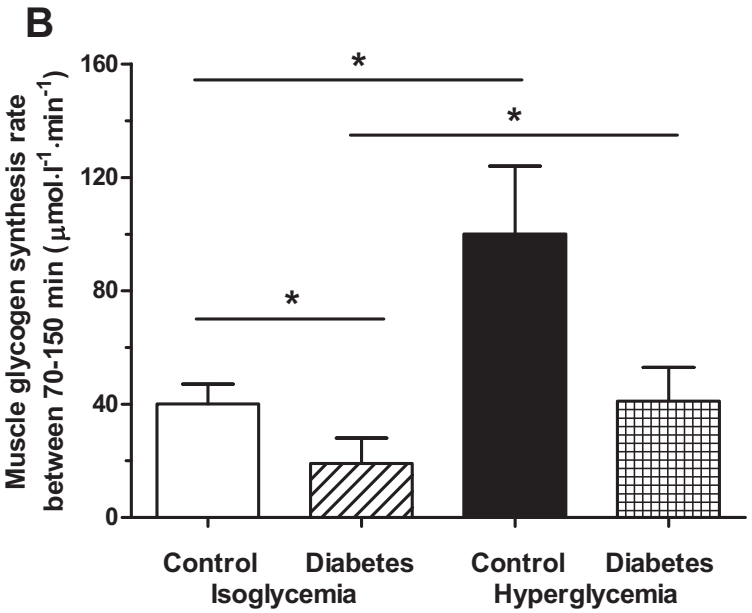
623

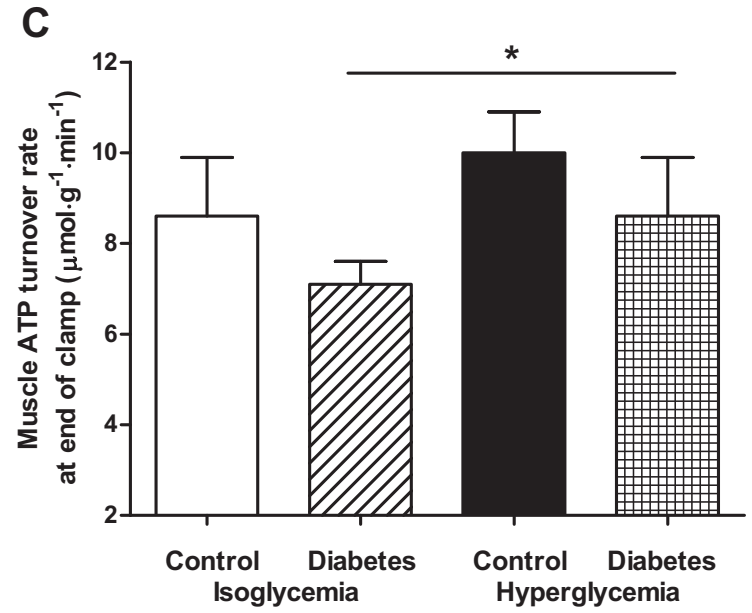

Protocol A
Basal condition
Isoglycemic-Hyperinsulinemia
Protocol B
Basal condition
Hyperglycemic-Hyperinsulinemia
31P
13C
31P
13C
31P
13C
-240
-180
-120
-60
0
60
120
180
Time (min)

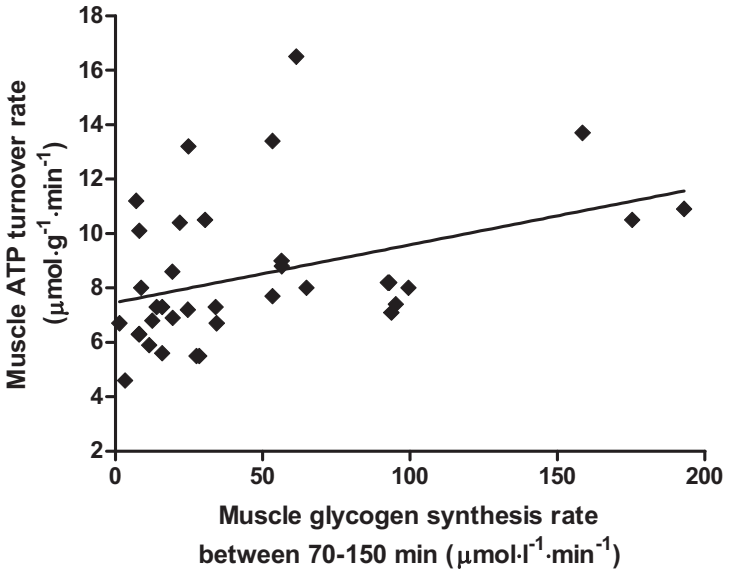

Plasma glucose (mmol/l)
16
14
12
10
8
6
4
2
*
*
*
*
-180 -150 -120 -90 -60 -30 0 30 60 90 120 150


Plasma insulin (pmol/l)
800
600
400
200
0
*
*
*
-180 -150 -120 -90 -60 -30 0 30 60 90 120 150


A
Glucose disposal rate
(mg·kg$_{ffm}^{-1}$·min^{-1})
16
14
12
10
8
6
4
2
0
*
**
*
Control
Diabetes
Control
Diabetes
Isoglycemia
Hyperglycemia


B
Muscle glycogen synthesis rate
between 70-150 min (µmol.l^{-1}·min^{-1})
160
120
80
40
0
*
*
*
Control
Diabetes
Control
Diabetes
Isoglycemia
Hyperglycemia


C
Muscle ATP turnover rate
at end of clamp (µmol·g^{-1}·min^{-1})
12
10
8
6
4
2
*
Control
Diabetes
Control
Diabetes
Isoglycemia
Hyperglycemia


Muscle ATP turnover rate
(μmol·g-1·min-1)
18
16
14
12
10
8
6
4
2
0
50
100
150
200
Muscle glycogen synthesis rate
between 70-150 min (μmol·l-1·min-1)

	T2DM (7M, 3F)	Control (6M, 2F)	*p value*
Age (years)	57 ± 2	53 ± 3	*0.3*
BMI (kg/m^2)	28.7 ± 1.2	28.1 ± 1.1	*0.8*
Fat mass (kg)	26.1 ± 2.0	26.4 ± 1.7	*0.9*
Fat free mass (kg)	54.7 ± 3.8	58.5 ± 3.6	*0.5*
Fasting glucose (mmol/l)	7.7 ± 0.3	5.1 ± 0.1	*0.001*
Fasting insulin (pmol/l)	93 ± 14	49 ± 6	*0.026*
HbA_{1c} (%)	6.6 ± 0.2	5.4 ± 0.1	*0.001*
Fasting triglyceride (mmol/l)	1.6 ± 0.2	1.4 ± 0.2	*0.5*
Mean daily energy expenditure (cal)	2455 ± 198	2248 ± 76	*0.4*
Mean daily steps taken	6160 ± 385	5701 ± 288	*0.4*

Values are means ± SE. T2DM, type 2 diabetes. BMI, body mass index.

	Muscle ATP turnover rates		
Time	Baseline	15-45 min	90-120 min
Isoglycemia clamps:			
Control	8.6 ± 0.7	9.3 ± 1.1	8.6 ± 1.3
Diabetes	8.6 ± 0.8	7.7 ± 0.7	7.1 ± 0.5
Hyperglycemia clamps:			
Control	8.1 ± 0.9	8.9 ± 0.7	10.0 ± 0.9*
Diabetes	8.7 ± 0.7	8.1 ± 0.6	8.6 ± 0.7

Values are means ± SE. * $p < 0.05$ vs. baseline.