

Increased daily walking improves lipid oxidation without changes in mitochondrial function in Type 2 diabetes

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Running title: *Walking, metabolism and Type 2 diabetes*

The study was funded by the Wellcome Trust (grant number 073561) and Dr Trenell is supported by a Diabetes UK RD Lawrence Fellowship.

Received 11 February 2008 and accepted 10 May 2008.

Objective: To determine whether increased daily physical activity improves mitochondrial function and/or lipid oxidation in Type 2 diabetes.

Research Design And Methods: Volunteers with (n=10) and without (n=10) Type 2 diabetes were matched for habitual physical activity, age, sex and weight. Basal and maximal mitochondrial activity, physical activity and resting substrate oxidation were measured at baseline, and after 2 and 8 weeks of increased physical activity.

Results: Baseline physical activity (6450 ± 851 vs. 7638 ± 741 steps/day), basal ATP use (12 ± 1 vs. 12 ± 1 $\mu\text{mol/ml/min}$), phosphocreatine recovery from exercise (31 ± 5 vs. 29 ± 3 sec) and basal lipid oxidation (0.57 ± 0.07 vs. 0.65 ± 0.06 mg/kg body weight/min) were similar in people with and without Type 2 diabetes. There was a significant increase in physical activity after 8 weeks (12322 ± 1979 vs. 9187 ± 1159 steps/day respectively). Following increased physical activity there were no changes in basal ATP use or phosphocreatine recovery after exercise in either group. Basal lipid oxidation increased after 8 weeks of increased physical activity in people with Type 2 diabetes (0.79 ± 0.08 mg/kg/min) but not people without (0.68 ± 0.13 mg/kg body weight /min).

Conclusions: Resting and maximal ATP turnover are not impaired in people with well-controlled Type 2 diabetes compared with controls matched for physical activity as well as age and weight. Increased unsupervised daily physical activity is sustainable and improves lipid oxidation independent of change in mitochondrial activity in people with Type 2 diabetes.

The potential role of the mitochondria in the development of insulin resistance and the development of Type 2 diabetes has recently attracted much interest. Muscle biopsies taken from people with Type 2 diabetes demonstrate smaller mitochondria and lower activities of oxidative enzymes compared to those of lean individuals without diabetes [1]. Insulin resistant people with a family history of diabetes have reduced basal mitochondrial activity in skeletal muscle compared to insulin sensitive individuals [2]. These observations, in combination with others [3-6], raise the possibility that mitochondrial defects could underlie Type 2 diabetes. Defects in oxidative function could possibly help explain the impaired fatty acid oxidation [7] and elevated intra-myocellular lipid [8] characteristic of impaired insulin action and Type 2 diabetes. The elevated intramuscular lipid may affect insulin signalling in skeletal muscle [5], exacerbating insulin resistance.

However, other studies have not observed abnormalities in basal mitochondrial activity in skeletal muscle of people with Type 2 diabetes [9]. Recent biopsy work has also shown that differences in oxidative enzymes between people with and without Type 2 diabetes disappear when corrected for mitochondrial density [10]. These data raise the possibility that Type 2 diabetes is associated with normal mitochondrial function, but that the mitochondrial capacity is reduced. This is an important differentiation as it holds implications for the therapeutic approach to Type 2 diabetes.

People with Type 2 diabetes are more sedentary than those without diabetes [11]. It is clear that reversing this sedentary lifestyle with physical activity and / or exercise can produce significant improvements in long term glucose control [12]. These benefits could be mediated, at least in part, by changes in mitochondrial function [13]. In people with

Type 2 diabetes, moderate-intensity exercise combined with moderate weight loss produced a significant improvement in insulin sensitivity and mitochondrial density [14]. However, such moderate intensity exercise programs are difficult to implement and usually require close supervision. In contrast, unsupervised walking has been shown to produce significant improvements in long term glucose control and is a sustainable behaviour over long periods of time (2 years) [15]. Little is known about how low intensity physical activity interventions such as walking influence muscle metabolism in people with Type 2 diabetes.

This study was designed to: 1) determine whether there are differences in basal and stimulated mitochondrial activity in people with Type 2 diabetes compared with physical activity matched controls, and 2) establish whether an increase in daily physical activity is associated with changes in mitochondrial ATP turnover and changes in lipid oxidation.

PARTICIPANTS AND METHODS

Subject Information and Initial Testing: Sedentary people with Type 2 diabetes (>2yr duration, HbA_{1c} <7.5%, stable control on either diet or diet + sulphonylurea and/or metformin) ($n = 10$) and age, weight and physical activity matched people without Type 2 diabetes ($n = 10$) were recruited. Volunteers with heart, liver, kidney, diabetic foot disease or undertaking a physical activity programme were excluded. Participants were assessed before and after 2 and 8 weeks of increased physical activity. At each time point physical activity, resting substrate oxidation, fasting plasma glucose and HbA_{1c} were assessed. Basal and maximal ATP use and intra-myocellular lipid (IMCL) were quantitated using magnetic resonance techniques. For all metabolic evaluations, participants were transported to the magnetic

resonance facility by taxi and data collected in the fasted state. Participants provided informed consent to join the study and the study was approved by the local Research Ethics Committee.

Magnetic Resonance Acquisition: Magnetic resonance data were acquired using a 3T Achieva scanner (Philips, Best, NL) with a in-built body coil used for imaging, a 14cm diameter surface coil for phosphorus spectroscopy and a 10cm diameter pair of flexible coils (Philips, Best, NL) for proton spectroscopy

Resting ATP Flux: This technique has previously been described in non-technical terms [16]. In brief, a saturation transfer sequence to measure transfer of magnetisation between γ -ATP and inorganic phosphate (Pi) [17]. The steady-state magnetization of Pi was measured during selective irradiation of γ -ATP, M_z , and compared with the equilibrium Pi magnetization with the irradiation placed symmetrically downfield from the Pi frequency (TR = 25s, bandwidth 3000Hz, 2048 points, 16 averages), M_0 . The fractional reduction of Pi magnetization upon saturation of γ -ATP, $(M_0 - M_z)/M_0$, was used to calculate the pseudo-first order rate constant using the Forsen-Hoffman equation [18]. T_1^* was measured using an inversion recovery experiment ($\tau_1 - 180^\circ - \tau_2 - 90^\circ$ - acquire, TR = 25s, 4 averages) while saturation of γ -ATP was performed during the delay times τ_1 and τ_2 . Broadband proton-decoupling was used. Eight variable τ_2 time delays were used ranging from 635ms to 9035ms. The intra-day variability of the method is 6.5% and inter day variation 8.0%.

Maximal ATP Generation: Plantar flexion exercise at 30% of the maximum voluntary contraction was performed in the MRI scanner on a custom built device. The study protocol consisted of 3minutes of plantar flexion at 2Hz and 3minutes of rest and changed pH levels as little as possible [19]. Phosphorus spectra were collected at 10s

intervals throughout exercise (NS=2, bandwidth = 3000Hz, 2048 points, broadband decoupling and NOE).

Intramyocellular Lipid: Proton magnetic resonance spectroscopy was acquired using a localized PRESS sequence (voxel size 15x15x20mm) in soleus with water suppression (TR/TE/NSA = 3000ms/37ms/32, 2048 samples, bandwidth 2000Hz). 16 unsuppressed averages were collected for reference.

Quantitation of Spectra: Analysis of all spectra was performed with jMRUI (version 3.0) [20] using AMARES [21] with custom prior knowledge. PCr concentrations were calculated by measuring PCr relative to β ATP, correcting for magnetic saturation, and assuming a resting [ATP] of 8.2 mmol.l⁻¹ [19]. Maximal ATP production was assessed from post-exercise PCr kinetics [19]. Proton spectra were analysed for IMCL and expressed relative to the water reference peak.

Physical Activity: Physical activity was assessed over 3 days using a validated multi-sensor armband [22] (SenseWear, Bodymedia, Pittsburgh, USA). Physical activity goals were set, with participants targeting 45minutes walking extra per day, and the benefits to glucose control (diabetes group) and long term well being (control group) discussed. Participants were also provided with a pedometer, recorded the pedometer reading and received period phone calls from the research team.

Indirect Calorimetry: Expired gases were collected from a constant-flow hood calorimeter (Deltatrac, Datex Ohmeda, Hertfordsire, UK) over 30minutes. Substrate oxidation rates and energy expenditure were calculated from oxygen consumption and carbon dioxide production values using stoichiometric equations [23].

Whole Blood Glucose and Plasma Insulin: Whole blood glucose was measured (YSI glucose analyser, YSI Inc, Ohio, USA). Plasma insulin was measured using enzyme-

linked immunosorbent assay kit (DAKO, Ely, UK). HbA_{1c} was measured using high performance liquid chromatography (TOSOH Corporation, Tokyo, Japan).

Statistical Analysis: Statistical calculations were performed using SPSS version 11 (SPSS inc., Chicago, Ill, USA). Two-way analysis of variance (time and treatment) was used to assess metabolic and physiological differences between groups. Statistical significance was accepted at $P < 0.05$. Data are presented as mean with standard error of the mean unless otherwise stated.

RESULTS:

Baseline Group Description: The characteristics of the Type 2 diabetes and control groups are given in Table 1. Groups were matched for age, sex and weight. The diabetes group were shorter than the control group, resulting in a higher BMI. Habitual physical activity was similarly low in both groups. Participants with Type 2 diabetes had good glucose control, demonstrated by a mean HbA_{1c} of $6.7 \pm 0.3\%$ and a fasting whole blood glucose concentration of 7.1 ± 0.4 mmol/l.

Physical Activity: The physical activity monitors showed that both groups had low baseline activity levels. There was no significant difference in the number of steps taken per day between the diabetes and control groups at baseline (Figure 1). Following counselling, both groups demonstrated a sustained increase in the number of steps taken per day ($P < 0.05$, Figure 1). At 8-weeks, both groups undertook more steps than baseline, however the control group had reduced the number of steps from the 2 week point ($P < 0.05$, Figure 1).

Resting Skeletal Muscle Metabolites: Skeletal muscle metabolites were similar between the two groups (Table 2). Resting skeletal muscle ADP concentrations were similar between people with or without

diabetes and did not change after 2 weeks or 8 weeks of increased physical activity (Table 2). There were no differences in resting skeletal muscle pH between people with and without diabetes and this was not influenced by 2 and 8 weeks of increased physical activity (Table 2). Similarly, the ratio of IMCL to water were comparable between people with and without diabetes at baseline and also did not change after 2 and 8 weeks of increased physical activity (Table 2).

Mitochondrial activity: The Type 2 diabetes and control groups showed similar basal and maximal ATP turnover rates (Table 3). There was no relationship between basal and maximal ATP turnover (R^2 0.194, $P > 0.05$). Both basal and maximal ATP turnover remained constant following 2 or 8 weeks of increased physical activity in either the Type 2 diabetes or control group (Table 3). End-exercise ADP and PCr use during exercise were similar (Table 3), ensuring that the recovery modelling of phosphocreatine was completed from similar metabolic starting points.

Indirect Calorimetry: People with and without Type 2 diabetes showed similar levels of carbohydrate (2.82 ± 0.19 vs. 2.61 ± 0.17 mg/kg body weight/min) and lipid oxidation (Figure 1). There were no changes in basal energy expenditure at any time point. Following 8 weeks of increased physical activity, the diabetes group show an increase in rate of lipid oxidation (Figure 1) and a decrease in rate of carbohydrate oxidation (to 2.26 ± 0.022 mg/kg body weight/min).

Plasma glucose, insulin and HbA_{1c}: People with Type 2 diabetes had a higher HbA_{1c} ($P < 0.01$, Table 2) and fasting plasma glucose levels ($P < 0.01$, Table 2). Insulin sensitivity, as assessed using HOMA, was lower in people with Type 2 diabetes than in controls ($P < 0.05$, Table 2). In the Type 2 diabetes group there was a non-significant trend towards lower fasting plasma glucose ($P=0.08$, Table 2).

Anthropometric Measurements:

There were no differences in weight between people with and without Type 2 diabetes (Table 1). There was no change in weight in the Type 2 diabetes group after 2 weeks, although after 8 weeks there was a significant decrease relative to both baseline and 2 weeks ($P < 0.05$, Table 2). Controls showed no change in weight after 2 or 8 weeks of increased physical activity (Table 2).

DISCUSSION

We observed no abnormality in mitochondrial function in people with well-controlled Type 2 diabetes compared with physical activity, age and weight-matched controls. There was no relationship between basal (fasted) and maximal (recovery from exercise) ATP synthesis, suggesting that the factors influencing basal and maximal ATP synthesis are different. The physical activity intervention markedly increased the number of steps taken per day during the 8 week intervention. Fasting lipid oxidation was increased, but there was no change in ATP turnover nor maximal ATP production.

The observation of no abnormality in basal ATP flux contrasts with recent studies. The seminal study indicating the possible importance of mitochondrial function in development of Type 2 diabetes observed basal ATP flux in extreme phenotypes of insulin sensitivity [2]. From 150 screened subjects, the most and least sensitive people underwent assessment of basal ATP flux and intra-myocellular lipid using magnetic resonance techniques. The 14 least insulin sensitive subjects (with a family history of diabetes) had lower basal ATP flux and higher intramuscular lipid levels than the 10 most insulin-sensitive people. It was suggested that impaired mitochondrial function reduces the ability to oxidise lipid, with accumulation of intramuscular lipid impeding insulin signalling [5], forming a pathway from impaired mitochondrial function to the development of Type 2

diabetes. A further study demonstrated impaired ATP flux in people with type 2 diabetes, but the control subjects were unmatched for habitual physical activity [3]. The present data agree with a recent study reporting no difference in basal ATP use between age, weight and physical activity matched subjects with and without Type 2 diabetes using saturation transfer MR [9]. This study was only able to demonstrate differences in ATP flux in people with Type 2 diabetes under insulin stimulated conditions. The observation of no abnormality in basal ATP flux in diabetes implies that abnormal basal mitochondrial function is unlikely to be a primary causative factor in Type 2 diabetes.

In contrast to basal ATP synthesis rates which are primarily influenced by steady state energy demand, the recovery of PCr from exercise is a robust measure of maximal oxidative ATP turnover [19]. This also reflects the recovery from muscular activity which happens frequently throughout the waking day, and any abnormality could bring about marked differences in muscle metabolism which may be associated with Type 2 diabetes. The present data show no differences in the recovery of PCr from exercise between people with and without Type 2 diabetes when controlled for habitual physical activity, weight and age. To our knowledge, the present study is the first report to include both measures of basal and maximal ATP turnover in people with or without diabetes. No correlation was found between the basal and maximal ATP turnover rates. The lack of relationship between basal and maximal ATP turnover supports the concept that ATP turnover in these tests is determined by different factors. In the fasted, non-exercise state, the level of insulin stimulated glucose uptake is likely to dominate requirement for ATP synthesis. This suggestion is based on studies which show a robust relationship between ATP synthesis and insulin stimulated glucose uptake in

skeletal muscle [9]. In the post-exercise stimulated state, the ability to supply and use oxygen are likely to dominate the rate of ATP turnover. The lack of correlation between the basal and maximal measures of ATP synthesis highlights the importance of examining separately these differing states.

A previous study reported maximal ATP turnover to be reduced in people with Type 2 diabetes compared to a BMI-matched control group [3]. However, this study did not objectively control for differences in habitual physical activity, a factor which can influence mitochondrial function [13]. People with Type 2 diabetes tend to be less physically active than people without diabetes [11]. Indeed, the reported PCr recovery data post exercise of people with Type 2 diabetes [3] are comparable to both the Type 2 diabetes and control groups in the present study, raising the likelihood that the differences in maximal ATP turnover may lay in differences in habitual physical activity. Recent support has also been given to the idea that Type 2 diabetes is not necessarily associated with impaired mitochondrial function, but may reflect differences in mitochondrial volume. Direct analyses of mitochondria from biopsies taken from people with Type 2 diabetes show that any apparent defect in mitochondrial ATP production disappears when corrected for mitochondrial density [10]. The reduced oxidative capacity accompanying Type 2 diabetes may be the result of a deconditioning phenomenon [24].

The present study shows that walking an extra 45 minutes per day over an 8-week period is an insufficient stimulus to induce detectable mitochondrial biogenesis. The physical activity was deliberately chosen to be of low intensity, as walking has been shown to be achievable and sustainable by people with Type 2 diabetes [15, 25]. More intensive and prolonged physical activity and diet does change mitochondrial density and aerobic capacity [14]. These changes correlate

well with improvements in long-term glucose control and fasting insulin sensitivity. Other biopsy data suggests that the beneficial effect of exercise and moderate weight loss upon mitochondrial density is modest [26]. However, the *in vitro* function of mitochondria is improved, with a disproportionate increase in electron transfer chain activity following intervention. The improvements of mitochondrial function accompanying exercise are not replicated by weight loss alone [27], stressing the importance of exercise in modifying oxidative capacity and maintaining metabolic flexibility. Further work is required to define the long term effects of practically sustainable physical activity on mitochondrial function in muscle.

In Type 2 diabetes changes in mitochondrial capacity are intertwined with changes in lipid oxidation [7]. The present data demonstrate physical activity induced enhancement of resting lipid oxidation, independent of intramuscular lipid levels. Type 2 diabetes is characterised by both abnormal lipid storage and oxidation, with glucose control commonly reported to be negatively related to intramuscular lipid content [2, 8, 28] via an effect on insulin action [5]. Walking for an extra 45 minutes each day increases skeletal muscle mRNA expression of genes implicated in glucose and lipid metabolism [25]. The cumulative effect of a sustained increase in lipid oxidation and decrease in IMCL would be expected to improve blood glucose control [15]. The effects of increased physical activity upon circulating triglyceride turnover, and the consequential influence upon insulin action, remains to be determined.

An important aspect of this study was that people with Type 2 diabetes were able to sustain a more physically active lifestyle without supervision and this would be expected to influence metabolic risk and glucose control [29]. The study was not

powered to detect changes in glucose control in the basal state as this was not a primary objective. However, measures of glucose control were lower following increased physical activity. Other dynamic testing methods may have been more sensitive to changes in insulin sensitivity than HOMA. These data are in line with larger better powered studies of walking interventions [12]. No change in serum triglyceride occurred in either group (data not shown). It is also possible that the different motivations for taking part in this study produced differences in the physical activity behaviour. It is notable that the diabetic individuals sustained the level of physical activity better than the non-diabetic control group. The challenge ahead is to better understand how we can engage people with Type 2 diabetes in reducing sedentary periods as well as to define how the underlying physiological mechanisms produce these benefits.

The magnetic resonance methods applied here, and previously [2, 3, 9, 17], are not without limitation. As the mitochondria are not isolated, ATP production may be limited by external factors, such as the supply of oxygen or ATP demand. However, with these limitations noted, it is clear that these non-invasive techniques provide a patient friendly methodology which can be applied serially and complements more detailed *in vitro* techniques.

In summary, resting and maximal ATP turnover are not impaired in people with well-controlled Type 2 diabetes, when compared with controls matched for physical activity as well as age and weight. Increased daily physical activity in the form of walking is sustainable and improves lipid oxidation independent of mitochondrial activity in people with Type 2 diabetes.

ACKNOWLEDGEMENTS:

The authors are most grateful to the volunteers; Sister Jean Gerrard; Louise Morris and Carol Smith.

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Table 1 Baseline physical and metabolic characteristics of subjects.

	Type 2 Diabetes	Control
Age (yrs)	59 (2)	56 (2)
Weight (kg)	91 (4)	88 (4)
BMI	33 (1)*	30 (1)
Energy Expenditure (cal/day)	2319 (112)	2460 (146)
Total Steps Day	6450 (851)	7638 (741)
Systolic Blood Pressure (mmHg)	143 (2)*	134 (5)
Diastolic Blood Pressure (mmHg)	87 (2)	85 (3)
HbA _{1c} (%)	6.7 (0.3)**	5.7 (0.1)
Fasting Glucose (mmol/l)	7.1 (0.4)**	5.5 (0.2)
Insulin (mu/l)	13.9 (3.5) **	7.6 (1.5)

*significantly different from control ($P < 0.05$)

** significantly different from control ($P < 0.01$)

Table 2 Magnetic resonance spectroscopy measurements on muscle in people with and without Type 2 diabetes before and after 2 and 8 weeks of increased physical activity.

<i>Resting Data</i>	Type 2 Diabetes			Control		
	Baseline	2 weeks	8 weeks	Baseline	2 weeks	8 weeks
ADP ($\mu\text{mol/l}$)	9.54 (0.17)	9.75 (0.13)	9.47 (0.12)	9.38 (0.18)	9.43 (0.13)	9.33 (0.12)
pH	7.04 (0.01)	7.05 (0.01)	7.04 (0.01)	7.03 (0.01)	7.03 (0.01)	7.03 (0.01)
IMCL (relative to H ₂ O)	0.032 (0.006)	0.035 (0.009)	0.029 (0.003)	0.037 (0.005)	0.037 (0.004)	0.038 (0.005)
<i>End Exercise Data</i>						
End exercise ADP ($\mu\text{mol/l}$)	33.3 (5.1)	33.4 (4.9)	36.8 (4.8)	34.8 (3.7)	33.9 (4.7)	38.9 (3.8)
PCr use during exercise (% resting)	25 (4)	27 (5)	29 (4)	27 (3)	25 (3)	29 (3)
<i>Glucose Control</i>						
HbA _{1c} (%)	6.7 (0.3) *	6.7 (0.3) *	6.6 (0.2) *	5.7 (0.1)	5.7 (0.1)	5.6 (0.1)
Fasting Glucose (mmol/l)	7.1 (0.4) *	7.0 (0.4) *	6.5 (0.5)	5.5 (0.2)	5.7 (0.2)	5.5 (0.2)
Insulin (mu/l)	13.9 (3.5)	13.1 (2.2)	11.7 (2.3)	8.1 (1.4)	10.2 (3.0)	7.6 (1.5)
HOMA	4.4 (1.1) *	4.2 (0.8) *	3.4 (0.8) *	2.0 (0.4)	2.1 (0.4)	1.9 (0.4)

PCr: phosphocreatine, IMCL; Intra-myocellular Lipid.

* significantly different from people without diabetes ($P < 0.05$).

Figure 1 Number of Steps Walked, Basal ATP Flux and Basal Lipid Oxidation by people with (closed squares) and without (closed circles) Type 2 diabetes at baseline and after 2 and 8 weeks of increased physical activity. † significantly different from baseline; ‡ significantly different from 2 weeks ($P < 0.05$).

