Effect of suppression of free fatty acids on ATP turnover and inorganic phosphate uptake in Type 2 diabetes studied by 31P-MRS during an isoglycaemic-hyperinsulinaemic clamp

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Table 1 : Subject characteristics	
	T2D *n = 7*
Age (yrs)	56.7 ± 4.3
Weight (kg)	80.2 ± 15.7
BMI (kg/m^2)	28.8 ± 3.8
Daily energy expenditure (cal/day)	2452 ± 599
HbA1c (%)	6.6 ± 0.6

Introduction: Type 2 diabetes is a disorder of both glucose and lipid metabolism. Central to this is insulin resistance which correlates with intramyocellular lipid content and plasma free fatty acids. Most recently, evidence is raised to link abnormal fatty acid accumulation in muscle with reduced mitochondrial activity. This defect in mitochondrial function could be a primary, genetically determined effect or secondary effect of the fatty acid accumulation. However, understanding the mechanisms underlying this relationship is important, as it has implications for identifying targets for treatment for Type 2 diabetes. Magnetic resonance spectroscopy provides a non-invasive way to observe under physiological conditions real-time ATP synthesis rate as a direct measurement of mitochondrial activity. This provides a technique to study the link between fatty acid metabolism and skeletal muscle oxidative capacity in the pathogenesis of insulin resistance. Short term elevation of circulating lipids via a lipid infusion study [1] has shown that insulin stimulated ATP synthesis is reduced in skeletal muscle in insulin-sensitive humans: the question arises whether this is due to a decreased ability to produce ATP or a decreased stimulation of energy consumption within the muscle. This can be answered by administering acipimox, which is a suppressor of non-esterified free fatty acids (NEFA) and hence may be able to improve ATP turnover rate in type 2 diabetes in the latter case.

Methods: *Patients:* 7 patients (5m:2f) with well-controlled type 2 diabetes (T2D) were recruited (Table 1 gives the subject characteristics). All subjects were brought in for two study days. Subjects were fasted overnight and glucose/hormone infusions given via a catheter in an antecubital vein. Basal blood samples were collected (Table 2) and muscle ATP synthesis flux was measured by MR spectroscopy before acipimox 250mg or methyl-cellulose placebo of identical appearance was given orally in a single blind fashion at t = -180 min. Further ATP flux measurements were acquired before the start of an isoglycaemic-hyperinsulinaemic clamp (t = 0 min). Endogenous insulin secretion was suppressed by infusion of somatostatin (0.06 µg/kg/min) initiated 5 min before insulin infusion and continued for the duration of the clamp. A condition of standardised hyperinsulinaemia (~400pmol/l) during constant plasma glucose concentration (~6 mmol/l) was created by administering insulin in a priming and continuous infusion of 240 pmol/m^2/min for 120 mins. Fasting isoglycemia was maintained by adjusting the rate of a 20% [1-^{13}C]-enriched glucose infusion based on plasma glucose measurements performed at 5 minute intervals. Two further measurements of ATP synthesis flux were taken (at t = +20 and t = +120).

MRS acquisition: MRS data were acquired using a 3T Intera Achieva scanner (Philips, Best, NL) with a 14cm diameter surface coil for acquisition of phosphorus data. *ATP synthase flux*: A saturation transfer sequence was used to measure transfer between γ-ATP and inorganic phosphate in the gastrocnemius and soleus. The steady-state magnetization of P_i was measured during selective continuous irradiation of the γ-ATP resonance, M_z, and compared to the equilibrium Pi magnetization with the selective irradiation placed symmetrically downfield from the P_i frequency, M_o (TR = 25s). The fractional reduction of Pi magnetization upon saturation of γ-ATP, $(M_o\text{-}M_z)/M_o$, was used to calculate the pseudo-first order rate constant using the Forsen-Hoffman equation, $k_1 = [(M_o\text{-}M_z)/M_o](1/T_1^*)$ where T_1^* is the spin-lattice relaxation time for P_i during saturation of γ-ATP. Measurement of the Pi concentration then yields the rate, assuming [ATP] = 8.2 mM.

Results: (i) Acipimox effectively suppresses NEFA in the patients (figure 1a). (ii) The measured ATP flux declines during the clamp with placebo from 10.2 ± 3.4 µmol/g/min to 7.6 ± 2.2 µmol/g/min at 90 minutes ($p < 0.03$, Wilcoxon signed rank) whereas the ATP flux during the clamp with acipimox maintains a constant value (9.2 ± 1.0 µmol/g/min at baseline, vs 9.0 ± 1.7 µmol/g/min at 90 minutes (ns). Figure 1b shows these data expressed as average percentage change for the cohorts during the placebo and acipimox trials. (iii) The Pi concentration increases steadily across the time of the clamp on both days, but the trend of the rise is faster on the acipimox day and by 90 minutes, the overall increase is significantly greater with acipimox (Figure 1c : 53% ± 18 vs 35% ± 10, $p < 0.05$,Wilcoxon signed rank).

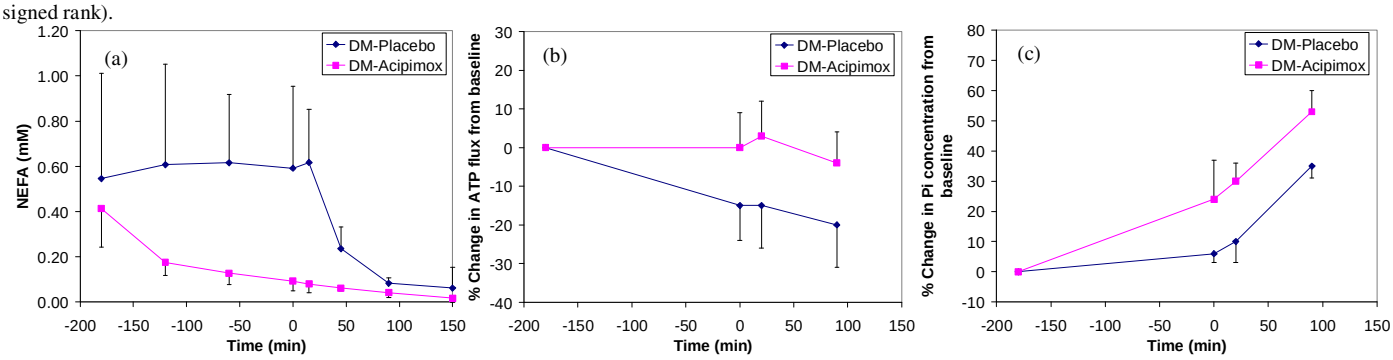

Figure 1 : (a) Non-esterified free fatty acid concentrations for the placebo and acipimox study days. The percentage change in (b) ATP turnover and (c) inorganic phosphate (Pi) conc just before (0 min) and during (20 min and 90 min) the isoglycaemic-hyperinsulinaemic clamp compared to basal (-180 min).

Discussion: (i) The change in the time course of the ATP flux between the placebo day and the acipimox day indicates that suppression of NEFA is removing the restricting effect of fatty acids on mitochondrial ATP turnover. (ii) The finding of declining ATP flux for diabetic patients under a placebo condition is not in agreement with other studies [eg 2]. However, the technique used here is likely to sample a greater proportion of the soleus muscle as well as gastrocnemius. The averaging of the phosphate concentration and rate constants of ATP turnover in the two compartments may lead to this apparent decline. (iii) The change in Pi concentration during the clamp under the placebo condition is in agreement with other studies [3,4]. It would appear the acipimox induced suppression of NEFA leads to a further significant increase in Pi. Previous work has shown that glucose transport processes are inhibited in the presence of enhanced NEFA [4].

Table 2 : Blood glucose and insulin measures on diabetic subjects			
	Time (mins)	Placebo study day	Acipimox study day
Fasting plasma glucose (mmol/l)	-180	6.91 ± 1.09	6.74 ± 0.98
	0	6.10 ± 0.97	5.76 ± 0.91
Fasting insulin (pmol/l)	-180	103 ± 45	101 ± 44
	0	70.4 ± 22.4	53.1 ± 19.5
End 30 min clamp: glucose (mmol/l)	90-120	6.09 ± 0.86	5.87 ± 0.81
End 30 min clamp: insulin (mmol/l)	90-120	607 ± 121	581 ± 90

Conclusion: Suppression of plasma FFA leads to enhanced insulin-stimulated ATP turnover in people with type 2 diabetes during an isoglycaemic-hyperinsulinaemic clamp, and also to increase the % change in inorganic phosphate over the period of the clamp. Through the use of carbon spectroscopy, this study will further determine whether the insulin-stimulated ATP turnover is secondary to the rate of glycogen synthesis in skeletal muscle. A matched control group is also being studied under placebo conditions.

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References: [1] Brehm *et al. Diabetes* 55:136 (2006), [2] Szendroedi *et al. PLoS* 4:154 (2007), [3] Petersen *et al.* PLoS 2:e233 (2005) [4] Roden *et al. Diabetes* 48:358-364 (1999)