Mitochondrial dysfunction in patients with primary congenital insulin resistance

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Introduction

Accumulating evidence strongly suggests that mitochondrial dysfunction is associated with insulin resistance and type 2 diabetes [1]. However, whether mitochondrial dysfunction results in ectopic fat accumulation in liver and skeletal muscle, and hence causes insulin resistance, or is a consequence of insulin resistance remains uncertain [1]. Here we approach this question by using ^{31}P-MRS at rest (using the saturation transfer technique), and post exercise (PCr recovery rate), in subjects with congenital severe insulin resistance due to loss-of-function mutations in the *INSR* gene.

Method

6 patients (5 female, 1 male) with mutations in the insulin receptor (*INSR*) and 11 age- and BMI-matched control subjects (8 female, 3 male) underwent ^{31}P-MRS examination using a 3T Siemens MAGNETOM Verio scanner following an overnight fast, and were transported by wheelchair on the morning of the scan.

Saturation Transfer (ST) measurement:

A 12 cm diameter RAPID surface coil was placed under the calf muscle. The steady-state Pi magnetisation was measured in the presence of selective saturation of the γATP resonance, and compared with a control (irradiation frequency symmetrical to the Pi peak), with parameters (TR=25s, NA=48). The T_1 of Pi under conditions of γATP resonance saturation was measured (7 TI's between 9-9000ms and an additional reference (IR flip=0), NA=16, TR_{eff}=15s). A fully relaxed spectrum (NA=16) was used for measurements of metabolite concentrations ([ATP] was assumed to be 8.2 mM). [ADP] was calculated using established methods [2], with the assumption that the total creatine pool (Cr + PCr) is 42.5mM.

PCr recovery rate post exercise:

The volunteers were placed supine and a 9 cm diameter surface coil attached to their right quadricep (1/3 distal). A weight was attached to their right ankle corresponding to 30% MVC, which was determined the previous day using a dynamometer chair set to the same angles of exercise as in the scanner. The exercise paradigm consisted of 1 min rest, 1 min knee extensions (0.5 Hz), then 4 min rest. This was then repeated to enable two PCr recovery measurements, which were then averaged. TR=2s, BW=5kHz, NS=360. The PCr recovery half time, $t_{1/2}$, was found using a 2 parameter monoexponential fit. VO_2 max was predicted [3] using heart rate response during a standardised ramped step test, that was completed on a separate day.

All spectra were analysed in jMRUI [4,5] and fitted using the AMARES [6] algorithm. Statistics were performed in SPSS.

Results

		Control	*INSR*	p-value
	Age, yrs	26.8 ± 4.8	26.8 ± 13.7	0.998
	BMI, kg/m^2	24.4 ± 4.0	23.1 ± 4.0	0.504
	Glucose mmol/l	4.5 ± 0.3	4.5 ± 0.7	0.770
	Insulin pmol/l	All < 60	462 ± 267	-
ST	V_{ATP} , mM/min	11.1 ± 1.9	10.1 ± 1.2	0.345
	[ADP] , μM	21.4 ± 8.3	29.0 ± 3.8	0.108
PCr recovery	$t_{½}$, s	17.0 ± 3.4	28.9 ± 5.2	<0.001 **
	VO_2 max, ml/kg/min	38.6 ± 5.7	31.5 ± 5.6	0.037 *
	$t_{½}$ corrected, s	20.6 ± 6.0	28.9 ± 7.1	0.032 *

Table 1. Measurements expressed as mean ± SD in controls and *INSR* patients. * p<0.05, ** p<0.01

All participants completed the scans apart from two *INSR* subjects who did not do the ST measurement due to claustrophobia, and one *INSR* subject who failed to perform the exercise to deplete PCr sufficiently. VO_2 max data were not taken in one control, but the remainder of their values are included. pH depletion during exercise was minimal and <<0.1.

Key results for both controls and *INSR* patients are shown in Table 1. There was no significant difference in V_{ATP}, measured using ST, between groups (p=0.345). However, there was a highly significant difference in the $t_{1/2}$ for PCr recovery (Fig 1), that persisted even after correcting for differences in VO_2 max (p=0.032).

No significant differences were found between Pi/ATP, PCr/ATP, PME/ATP nor Pi/PCr ratios in the calf muscle.

No significant correlation was found between ST V_{ATP} and $t_{1/2}$ (Fig 2, p=0.586).

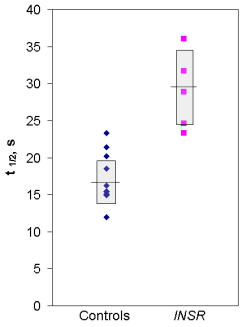

Fig 1 (left). $t_{1/2}$ values. Horizontal line indicates mean and the bar includes ± 2 sterr.

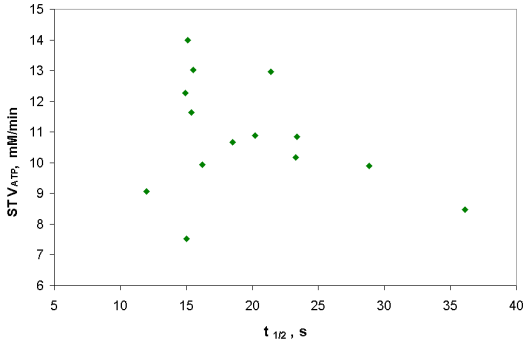

Fig 2 (above). ST V_{ATP} vs $t_{1/2}$ for both controls and INSR

Conclusion

PCr recovery post exercise is significantly slowed in the *INSR* patients suggesting that insulin resistance due to a well defined non mitochondrial primary defect in insulin signalling is nevertheless associated with evidence of mitochondrial dysfunction. This finding suggests that the association between mitochondrial dysfunction and insulin resistance previously reported in other conditions cannot necessarily be assumed to be unidirectional in its causation. Resting ATP synthesis rate measured from the saturation transfer method did not differ significantly between groups and did not correlate with the $t_{1/2}$ for PCr recovery. This is in agreement with recent findings in rats [7], that supports initial [8-9] and more recent [10] concerns over its validity in accurately measuring mitochondrial ATP synthesis rates (due to glycolytic components), and the physiological relevance of resting ATP synthesis as an index of muscle mitochondrial function.

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