Mitochondrial dysfunction in patients with primary congenital insulin resistance


1Wolfson Brain Imaging Centre, University of Cambridge, Cambridge, United Kingdom, 2Wellcome Trust Clinical Research Facility, Addenbrooke’s Hospital, Cambridge, United Kingdom, 3Siemens AG Healthcare Sector, Erlangen, Germany, 4Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom, 5Dept. of Biochemistry, University of Cambridge, Cambridge, United Kingdom, 6Cancer Research UK Cambridge Research Institute, Cambridge, United Kingdom, 7Magnetic Resonance and Image Analysis Research Centre, University of Liverpool, Liverpool, United Kingdom

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 31P-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 31P-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 T1 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=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 ankle. An accelerometer, an arterial catheter and a metabolic cart was attached to the subject who failed to perform the exercise to deplete PCr sufficiently. VO2 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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>INSR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>26.8 ± 4.8</td>
<td>26.8 ± 13.7</td>
<td>0.998</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>24.4 ± 4.0</td>
<td>23.1 ± 4.0</td>
<td>0.504</td>
</tr>
<tr>
<td>Glucose mmol/l</td>
<td>4.5 ± 0.3</td>
<td>4.5 ± 0.7</td>
<td>0.770</td>
</tr>
<tr>
<td>Insulin pmol/l</td>
<td>All &lt; 60</td>
<td>462 ± 267</td>
<td>-</td>
</tr>
<tr>
<td>ST VATP, mM/min</td>
<td>11.1 ± 1.9</td>
<td>10.1 ± 1.2</td>
<td>0.345</td>
</tr>
<tr>
<td>[ADP] μM</td>
<td>21.4 ± 8.3</td>
<td>29.0 ± 3.8</td>
<td>0.108</td>
</tr>
<tr>
<td>PCr recovery t1/2</td>
<td>17.0 ± 3.4</td>
<td>28.9 ± 5.2</td>
<td>0.001 **</td>
</tr>
<tr>
<td>corrected, s</td>
<td>38.6 ± 5.7</td>
<td>31.5 ± 5.6</td>
<td>0.037 *</td>
</tr>
<tr>
<td></td>
<td>20.6 ± 6.0</td>
<td>28.9 ± 7.1</td>
<td>0.032 *</td>
</tr>
</tbody>
</table>

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

Key results for both controls and INSR patients are shown in Table 1. There was no significant difference in VATP, measured using ST, between groups (p=0.345). However, there was a highly significant difference in the t1/2 for PCr recovery (Fig 1), that persisted even after correcting for differences in VO2 max (p=0.032).

No significant differences were found between Pi/ATP, Pi/ATP, PME/ATP or Pi/PCr ratios in the calf muscle.

No significant correlation was found between ST VATP and t1/2 (Fig 2, p=0.586).

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 t1/2 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.

References

Acknowledgements
We thank the study participants. We are grateful to Dr Craig Buckley for helpful discussions. This work was supported by the Medical Research Council, Welcome Trust, NIHR Cambridge Biomedical Research Centre and MRC CORD.