Mitochondrial Capacity and Oxygenation Measured by ^{31}P MRS and Near Infrared Spectroscopy in Healthy and Diabetic Subjects

J. Fulford[1], R. Sriraman[2], C. Ball[2], J. Tooke[2], N. Armstrong[1], A. Shore[2]

[1]CHERC, University of Exeter, Exeter, Devon, United Kingdom, [2]Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, Devon, United Kingdom

Introduction

Type 2 diabetes results from a combination of insulin resistance and beta cell dysfunction. Insulin resistance that is an abnormal glucose uptake into tissue for a given level of insulin has many causal pathways. Resistance to the actions of insulins have been reported in intracellular pathways as well as in the delivery of insulin and glucose to tissue. One potential contributing pathway is a defect in mitochondrial fatty acid oxidation, which in turn lead to increases in intracellular fatty acid metabolites (fatty acyl CoA and diacylglyerol) that disrupt insulin signalling and reduce ATP synthesis. Oxidative ATP synthesis is a function of three factors: mitochondrial volume and competence, oxygen supply and the metabolic driving force change in ADP concentration. The first two factors effectively equate to "mitochondrial capacity" and are reflected by the rate of post exercise PCr recovery. In conjunction, post exercise deoxyhaemoglobin recovery reflects the rate at which oxygen supply exceeds the oxygen demand of recovering muscle, which equates to the mitochondrial oxygenation status. By using ^{31}P MRS and near infrared spectroscopy (NIRS) during an exercise protocol, it is possible to measure mitochondrial capacity and oxygenation status in 'normal' and diabetic populations, potentially providing evidence for the functional basis of insulin resistance (1).

Experimental Protocol

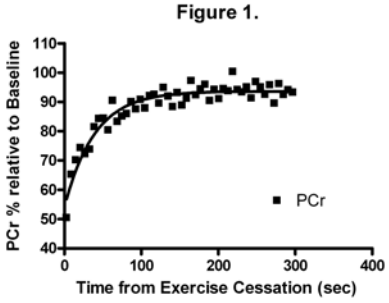
On their first visit, the subjects were given the opportunity to practice the exercise protocol within a to-scale dummy scanner, to ensure familiarity both with the task and the scanner environment. One a second visit the exercise protocol was performed within a 1.5T Philips Gyroscan Clinical Intera system at the Peninsula Magnetic Resonance Research Centre at the University of Exeter. A 6cm ^{31}P transmit /receive surface coil was placed within the subject bed and the subject asked to lie upon it in a prone position such that the coil was centred at thigh level over the quadriceps muscle, with strapping placed at the back, hip and knee to ensure no movement occurred during the protocol. Initially, fast field echo (FFE) images were acquired to ensure the muscle was positioned correctly relative to the coil. An automatic shimming protocol was undertaken, specifically, within a volume that defined the quadriceps muscle to optimise the signal from the muscle under investigation and matching and tuning of the coil was carried out. Subsequently, an unsaturated ^{31}P spectra was acquired to allow T1 correction for those spectra obtained during the exercise protocol. Subjects were then required to perform knee extension and flexion exercise with their right leg against a pulley system, to which a mass equal to 80% of the maximum amount they could achieve during the training visit, was attached, via a strap fitted to the foot. Exercise was carried out at a metronomic frequency of 0.66 Hz, with all force and power measurements recorded via a non-magnetic strain gauge and shaft encoder present within the pulley system. To ensure a consistent work rate and muscle position relative to the coil during exercise, the subject was visually queued, with associated feedback, via a display visible within the scanner consisting of 2 vertical bars, one which moved at a constant frequency of 0.66 Hz triggered via the scanner, and one which displayed the relative foot position via a sensor present within the pulley. Thus, the subject was required to match the movements of these 2 bars. During the entire protocol, phosphorous spectra were acquired every 1.5 seconds, with a spectral width of 1500 Hz, and 512 data points. Phase cycling, with 4 phase cycles was employed, leading to a spectra acquired every 6 s, with all scanning conforming to NRPB guidelines (2). The exercise protocol consisted of 1 minute of baseline measurements, 5 minutes of exercise and 9 minutes of recovery during which time spectra were continuously obtained. During the exercise protocol near infrared spectroscopy was also undertaken on the quadriceps muscle to measure tissue oxygenation. The instrument (NIRO 300, Hamamatsu Photonics K.K.Japan) employed an LED, producing light at wavelengths of 760nm and 840nm, with sampling at an approximate depth of 2.5 cm via a photodiode detector.

^{31}P spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 2) software package employing the AMARES fitting algorithm (3,4). Spectra were fitted assuming the presence of the following peaks: inorganic phosphate (Pi), phosphodiester, phosphocreatine (PCr), α-ATP (2 peaks, amplitude ratio 1:1), γ-ATP (2 peaks, amplitude ratio 1:1) and β-ATP (3 peaks, amplitude ratio 1:2:1) allowing the calculation of d[PCr]/dt and d[Pi]/dt during exercise and recovery. pH was calculated from the chemical shift of Pi relative to PCr. The recovery of PCr at the cessation of exercise was fitted to an exponential curve within Prism software (GraphPad Software Inc, San Diego, California, USA) such that $PCr=B+C(1-\exp^{-t/tau})$ where B and C are constants, t is the time elapsed since the end of exercise and tau is the PCr recovery rate. A NIRS notional rate constant, t_{NIR} was calculated =ln2/half time to reach baseline value (deoxyhaemoglobin) following cessation of exercise.

Subjects

8 healthy volunteers (Age: 55 ± 11 years, BMI: 27 ± 2) and 8 diabetic subjects (Age: 64 ± 9 years, BMI: 29 ± 4), whose glycaemic control was maintained by diet and/or oral hypoglycaemics gave written consent to participate in the study which was approved by the Local Research and Ethics Committee.

Results



No significant differences were found between the two groups for their baseline measurements of pH and Pi/PCr. An example of a typical PCr recovery response is illustrated in Figure 1. Large differences were obtained between healthy and diabetic subjects for the PCr recovery rate constant, tau, the NIRS rate constant t_{NIR} and the ratio of tau/t_{NIR}. Healthy subjects had an average value for tau of 38.9 ± 16.7 sec compared with 75.8 ± 42.5 sec for diabetic subjects (p=0.014). For t_{NIR}, values of 0.48 ± 0.31 $mins^{-1}$ and 0.21 ± 0.05 $mins^{-1}$ were obtained (p=0.033), whereas for tau/ t_{NIR}, values of 1.51 ± 0.73 and 2.18 ± 0.52 were obtained (p=0.052) for healthy and diabetic subjects respectively.

Discussion

There was a wide range of values obtained from individual subjects, this is in part explained by the wide range of subject characteristics, in terms of age and physical fitness. However, even given the subject variation, the study provides support to the hypothesis that diabetic subjects, compared with healthy ones, have both impaired mitochondrial capacity, as shown by a larger PCr rate constant and oxygenation, as illustrated by a smaller NIRS rate constant.

References

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