A comparison of different methodologies to study skeletal muscle mitochondrial function in Type 2 diabetes patients

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Introduction

Skeletal muscle mitochondrial function is now frequently being studied in T2D patients (1) using various experimental techniques ranging from whole-body measurements to *in vitro* analyses, representing different aspects of the respiratory system. We evaluated the interrelationship of the different parameters generally used to describe mitochondrial function in a group of T2D patients to elucidate which methods preferably should be used to evaluate the outcome of interventions focused on mitochondrial function.

Materials & Methods

Eleven male T2D patients were selected (mean ± SE: age: 59.1 ± 2.3 years, BMI: 32.2 ± 1.2 kg/m^2). Subjects were diagnosed with T2D over 5 years and were on exogenous insulin treatment for at least 24 months. Patients using thiazolidinediones and/or β-blockers shorter than 6 months, subjects with impaired liver function, renal failure, severe retinopathy or a history of severe cardiovascular problems were excluded from participation.

Maximal whole-body oxygen capacity (VO_{2max}) was measured during an incremental exercise test until exhaustion on a cycle ergometer using a ramp protocol.

Blood and muscle biopsy samples were collected after an overnight fast preceded by a standardized meal (35.2 ± 1.8 kJ/per kg body weight). Muscle biopsy samples were collected from the *M. vastus lateralis* by the percutaneous needle-biopsy technique. Muscle samples were used for immuno-histochemical staining analyses including muscle fiber type determination using antibodies against human myosin heavy chain (MHC) type-I and type-IIa. Muscle fiber-type specific oxidative capacity was determined by measuring SDH activity using histochemical analyses.

^{31}P MRS of the *M.vastus lateralis* was performed using a 1.5-Tesla whole-body magnet (Gyroscan S15/ACS, Philips Medical Systems, Best, the Netherlands) and a 6-cm diameter surface coil. Spectra were acquired during a rest-exercise-recovery protocol (repetition time of 3 s, 2 scans/spectrum, time resolution of 6 s) and were fitted using the nonlinear least squares algorithm AMARES in the jMRUI software package. The subjects performed a single-leg extension exercise with increasing workload resulting in a sufficient phosphocreatine (PCr) depletion without severe muscle acidification. PCr and ADP recoveries were fitted to mono-exponential functions resulting in the metabolites' time constants of recovery, i.e. τ_{PCr} and τ_{ADP}. Initial PCr recovery rate (V_{PCr}) and maximum aerobic capacity (Q_{max}) were calculated as described by Kemp et al. (2).

Results

Whole body VO_{2max} was on average 24.3 ± 1.4 ml/kg/min. No significant differences in SDH-activity between type-I and type-IIa muscle fibers were observed (T-test, $p > 0.05$), whereas type-IIx muscle fiber SDH-activity was lower compared to the type-I and type-IIa muscle fibers (t-test, $p < 0.05$).

Figure 1 shows typical ^{31}P spectra from a subject's *M. vastus lateralis* at rest and at the end of exercise, respectively. At the end of exercise, the average PCr depletion for all subjects was 45.5 ± 2.3% and the intracellular pH was 6.90 ± 0.04. Figure 2 illustrates an example of both raw data and mono-exponential fits of PCr and ADP recoveries. All ^{31}P MRS markers of mitochondrial function displayed a rather wide range of values, with e.g. τ_{PCr} ranging from 27.2 to 86.6 s. Table 2 shows Pearson's correlation coefficients for the different markers of mitochondrial function. All ^{31}P MRS markers of mitochondrial function revealed a strong correlation with whole-body VO_{2max}. Similar relationships were found for most of the ^{31}P MRS parameters and *in vitro* parameters such as type-I SDH-activity, total muscle SDH-activity and percentage of type-I muscle fibers. Whole-body VO_{2max} was positively correlated with SDH-activity of both type-I and type-IIa muscle fibers.

Conclusion

Significant correlations exist between markers of oxidative capacity as measured by post-exercise ^{31}P MRS, whole-body oxygen uptake and skeletal muscle fiber-type specific oxidative enzyme activity measured from muscle biopsies. These parameters all seem to provide good estimates of mitochondrial function implying that these different methods all can be used to evaluate mitochondrial function in T2D patients.

References

1 Kelley DE et al., Diabetes 2002; 5:2944-50.

2 Kemp GJ et al., Magn Reson Med 1994; 31:248-58.

Table 1. Pearson's correlation matrix among ^{31}P MRS markers of mitochondrial function, whole body oxygen uptake and histochemical muscle fiber analysis

Variable	VO_{2max} per kg BW	SDH Type I	SDH Type IIa	Total SDH	%Type I	%Type IIa
τ_{PCr} (s)	-0.70*	-0.70*	0.03	-0.48	-0.75*	0.83**
τ_{ADP} (s)	-0.90**	-0.76**	0.38	-0.70*	-0.45	0.57
V_{PCr} (mM/s)	0.74*	0.59	0.18	0.47	0.60	-0.59
Q_{max} (mM/s)	0.65*	0.51	0.07	0.38	0.63*	-0.67*
VO_{2max} per kg BW	-	0.77**	0.62*	0.76**	0.21	-0.41

Muscle fiber type composition (% type I and IIa) based on number of fibers.
* $p<0.05$, ** $p<0.01$, all correlations are based on n=11.

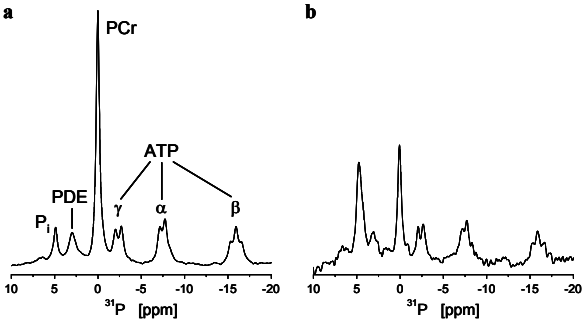

Figure 1. Typical *M. vastus lateralis* ^{31}P MR spectra for one subject at rest (panel a, number of scans = 60) and at the end of exercise (panel b, number of scans = 2). P_i indicates inorganic phosphate; PDE, phosphodiesters; PCr, phosphocreatine; and γ, α, β indicate the three phosphate groups of ATP. For this subject the PCr depletion at the end of exercise was 47.6% and the intracellular pH was 6.95.

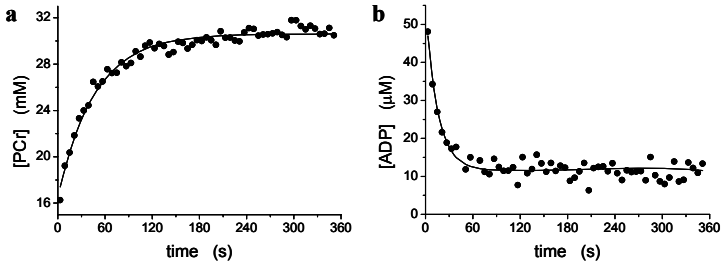

Figure 2. PCr (panel a) and ADP (panel b) recovery curves for an individual subject (the same subject as in Figure 1). Mono-exponential functions (dark lines) were fit to the actual data (filled circles) obtained every 6 s. The time constants for PCr and ADP recovery were 46.9 and 15.6 s, respectively.