Normal *in vivo* skeletal muscle mitochondrial function in subjects with impaired glycemic control and in long-standing, insulin treated type 2 diabetes patients

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Introduction

Recent studies support the concept that skeletal muscle mitochondrial dysfunction plays a key role in the pathogenesis of type 2 diabetes (T2D) [1,2]. Mitochondrial dysfunction might lead to decreased lipid utilization, resulting in the accumulation of intramyocellular lipid (IMCL). IMCL metabolites can interfere with the insulin-signaling cascade, leading to insulin resistance and possibly T2D. However, it is not known whether the decreased mitochondrial function observed in T2D patients is the consequence of an inactive lifestyle or that it is associated with the progression of the disease itself. We compared skeletal muscle oxidative capacity and IMCL levels measured with ^{31}P and ^{1}H MRS, respectively, in long-standing, insulin treated T2D patients (T2D), subjects with impaired glycemic control (Pre-T2D) and subjects with normal glucose tolerance (NGT), all matched for sex, age and body mass index (BMI).

Subjects and methods

All data are expressed as means ± SEM. To determine whether there were differences between groups, statistical analyses were performed using SPSS 14.0. The non-parametric Kruskall-Wallis H test was performed to test for overall differences between groups ($p < 0.05$). The Mann-Whitney U test was used for pair-wise comparisons between groups applying the Bonferroni correction ($p < 0.017$).

Subjects' plasma glucose and insulin levels were determined during an oral glucose tolerance test (OGTT) and based on WHO definitions subjects were classified as NGT, Pre-T2D or T2D. Twelve healthy male subjects were included in the NGT group, 11 male subjects in the Pre-T2D group (2 subjects with impaired glucose tolerance, 5 with impaired fasting glucose and 4 previously undiagnosed T2D patients with $HbA_1c < 6\%$) and 11 long-standing, insulin treated T2D patients in the T2D group (duration of T2D: 12.1 ± 2.1 years, all > 5 years; duration of insulin therapy: 7.0 ± 2.4 years). They were matched for sex, age and BMI (age (years): 55 ± 2, 59 ± 1 and 59 ± 2, p=n.s.; BMI (kg/m^2): 32.7 ± 1.3, 32.4 ± 1.1 and 32.2 ± 1.2, p=n.s., in NGT, Pre-T2D and T2D subjects, respectively). Maximal workload capacity (Wmax) and maximal oxygen uptake capacity (VO_2max) were determined during an incremental exhaustive exercise test on a cycle ergometer. Wmax and VO_2max were similar in NGT and Pre-T2D subjects, but lower for the T2D patients (Wmax (W/kg): 2.6 ± 0.1, 2.4 ± 0.1 and 1.6 ± 0.1; VO_2max (ml/kg/min): 33.9 ± 1.6, 32.0 ± 1.7 and 24.3 ± 1.4, in NGT, Pre-T2D and T2D subjects, respectively). Habitual physical activity level was assessed with a questionnaire and expressed in a scale of the energy cost of various physical activities in multiples of the resting metabolic rate (MET) (19.6 ± 2.1, 19.3 ± 2.8 and 13.3 ± 1.3, p=n.s., in NGT, Pre-T2D and T2D subjects, respectively).

MRS measurements were performed with a 1.5 T whole-body scanner (Gyroscan S15/ACS, Philips Medical Systems, Best, The Netherlands). ^{31}P MR spectra were measured with a 6-cm diameter surface coil placed over the *M. vastus lateralis* using a repetition time of 3 s and 2 scans per spectrum (6 s time resolution) during a rest-exercise-recovery protocol. The intracellular pH and phosphocreatine (PCr), inorganic phosphate (Pi), adenosine diphosphate (ADP) and phosphodiester (PDE) concentrations were determined at rest and at the end of an incremental single-leg extension exercise. The intensity of the exercise protocol was adjusted to reach a similar end-exercise pH for all subjects. Recoveries of PCr and ADP were fitted to mono-exponential functions. Results are expressed as the metabolite's time constant of recovery, i.e. τ_{PCr} and τ_{ADP}. The maximum aerobic capacity (Q_{max}) was calculated as described by Kemp *et al.* [3]. IMCL was measured with single-voxel ^{1}H MRS using the PRESS sequence with CHESS water suppression (voxel size 10×10×15 mm^3, TR/TE 1500/35 ms). For each subject, 5 voxels were measured at different positions within the *M. vastus lateralis*. IMCL was expressed as a percentage of the water signal measured in the same voxel without water suppression.

Results

At rest, concentrations of the ^{31}P metabolites were similar for the NGT, Pre-T2D and T2D subjects (Table 1). The end-exercise PCr and Pi concentrations were not significantly different for the three groups, but the end-exercise ADP concentration was lower in the T2D patients (Table 1). However, most importantly, the end-exercise pH was not different for the three groups, which is a prerequisite for comparing τ_{PCr}. Figure 1 illustrates the mono-exponential fit of the PCr recovery for one subject and Figure 2 shows the results for all subjects. The parameters for oxidative capacity determined from the recovery phase, i.e. τ_{PCr}, τ_{ADP} and Q_{max}, were not significantly different between NGT, Pre-T2D and T2D subjects (Table 1). IMCL levels were not significantly different between groups (1.30 ± 0.11, 1.63 ± 0.20 and 1.86 ± 0.23 % of the water signal in NGT, Pre-T2D and T2D subjects, respectively).

Discussion

Skeletal muscle mitochondrial function as determined by τ_{PCr}, τ_{ADP} and Q_{max} did not differ between NGT and Pre-T2D subjects matched for sex, age and BMI and with similar habitual physical activity levels. Therefore, skeletal muscle mitochondrial dysfunction does not seem to play a role in the early stages of the development of insulin resistance and/or T2D. Skeletal muscle mitochondrial function was also not impaired in long-standing, insulin treated T2D patients compared to NGT and Pre-T2D subjects, corroborating that mitochondrial dysfunction is probably not involved in the pathogenesis of T2D.

Conclusion

In vivo skeletal muscle mitochondrial function as measured by post-exercise ^{31}P MRS is not impaired in subjects with impaired glycemic control or in long-standing, insulin treated T2D patients.

Table 1 ^{31}P MRS parameters during rest, end of exercise and recovery.

		NGT	Pre-T2D	T2D
Rest	**[PCr] (mM)**	36.9 ± 0.8	37.6 ± 0.7	37.3 ± 1.2
	[Pi] (mM)	4.7 ± 0.2	5.0 ± 0.2	4.6 ± 0.2
	[ADP] (μM)	10.0 ± 0.1	10.4 ± 0.2	10.1 ± 0.1
	[PDE] (mM)	5.9 ± 0.2	6.0 ± 0.3	5.8 ± 0.4
	pH	7.06 ± 0.004	7.08 ± 0.007	7.06 ± 0.005
End-exercise	**[PCr] (mM)**	16.9 ± 1.0	16.5 ± 0.9	20.2 ± 1.3
	[Pi] (mM)	23.5 ± 1.4	24.2 ± 1.1	19.8 ± 1.2
	[ADP] (μM)	65.3 ± 4.9	70.7 ± 3.3	46.8 ± 2.8*#
	pH	6.90 ± 0.03	6.92 ± 0.03	6.90 ± 0.04
Recovery	**τ_{PCr} (s)**	44.6 ± 2.8	42.1 ± 2.0	49.4 ± 5.5
	τ_{ADP} (s)	19.1 ± 1.1	16.7 ± 1.5	22.5 ± 2.9
	Q_{max} (mM/s)	0.68 ± 0.04	0.73 ± 0.03	0.61 ± 0.05

* significantly different from NGT, # significantly different from Pre-T2D

References

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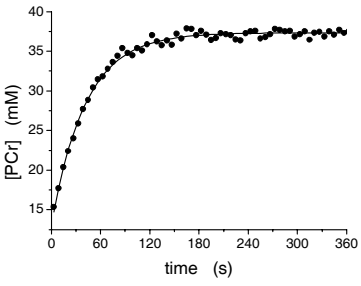

Figure 1 PCr recovery curve for one subject. A mono-exponential function (dark lines) was fit to the actual data (filled circles) obtained every 6 s. The time constant for PCr recovery was 41.8 s.

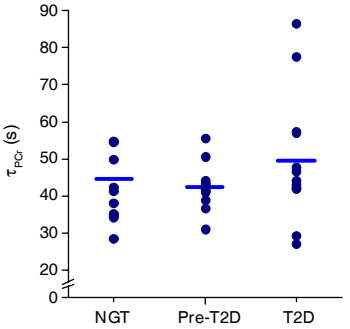

Figure 2 τ_{PCr} in NGT, Pre-T2D and T2D subjects. Bars indicate the average values for the three groups.