Assessment of acetylcarnitine in individuals with type 1 diabetes after exercise in eu- and hyperglycemia using ^{1}H MR spectroscopy in skeletal muscle

A. Boss[1], C. Stettler[2,3], M. Ith[1,4], S. Jenni[2,5], C. Boesch[1], and R. Kreis[1]

[1]Department of Clinical Research, University of Bern, Bern, Switzerland, [2]Division of Endocrinology, Diabetes and Clinical Nutrition, Inselspital, Bern, University Hospital and University of Bern, Bern, Switzerland, [3]Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland, [4]Institute for Diagnostic, Interventional and Pediatric Radiology, University Hospital and University of Bern, Bern, Switzerland, [5]International Center for Circulatory Health, Imperial College, London, Switzerland

Introduction / Background

^{1}H magnetic resonance spectroscopy (MRS) of the skeletal muscle allows for the non-invasive assessment of acetylcarnitine (AC) accumulation during and after exercise[1]. AC increases when the production of acetyl-CoA by the pyruvate dehydrogenase complex exceeds its utilization by the TCA-cycle[2]. The transfer of the acetyl-group to carnitine regenerates free CoA which allows intra-mitochondrial CoA-dependent reactions to proceed. It was shown with muscle biopsies that different diets and thus altered substrate availability not only affect PDC-activity but also AC-concentrations[3]. So far, ^{1}H-MRS has not been used to follow the influence of substrate availability upon the concentration of AC. Therefore, we re-analyzed muscle spectra of a study set up to investigate the effects of euglycaemia vs. hyperglycaemia on substrate metabolism during prolonged exercise in type 1 diabetic subjects[4], where the original endpoints had been intramyocellular lipids (IMCL) and glycogen.

Methods

Details of this randomized, single-blinded cross-over trial have been described before[4]. Briefly, 7 physically active type 1 diabetic males (33.5 ± 2.4 years, BMI: 24.3 ± 0.4 kg/m^2) performed prolonged ergometer exercise (120min, 55 - 60% VO_2max) twice; on one occasion in euglycaemia (5 mmol/L), on the other in hyperglycaemia (11 mmol/L). Insulin infusion was kept constant and equal during both conditions (median: 7mU m^{-2} min^{-1}). Fuel metabolism before and during exercise was assessed by a combination of indirect calorimetry and tracer methods (D-[U-^{13}C]glucose, D-[6-6-$^2H_2$]glucose). Furthermore ^{1}H-MRS and ^{13}C MRS (1.5T SIGNA, GE Medical) were applied for the assessment of exercise-induced depletion of IMCL and glycogen.

For the present evaluations, ^{1}H-MRS spectra obtained from vastus intermedius (ROI: 11 x 12 x 18 mm^3, PRESS localization, TR=3s, TE=20ms, 2000 Hz, 1024 pts, 2x128 acquisitions, water presaturation) were analyzed using a fit-strategy optimized for the AC-peak using TDFDFit[5] (Voigt lines, bounded parameter space, prior knowledge with regard to frequency and linewidths of AC linked to the creatine resonance). AC content was expressed in absolute arbitrary units relative to the water signal intensity obtained from separate reference scans with varying TE). ^{1}H-MRS spectra were obtained before and ~ 60 min after completion of exercise (same timing in both conditions). Statistical analysis: ANOVA for AC and IMCL with factors: eu- vs. hyperglycemia, pre- vs. post-exercise, and the mutual interaction.

Results

- **Fig. 1** portrays the main results in the form of the summed spectra pre-and post exercise and the respective differences, for both euglycaemia and hyperglycaemia. The acetyl-group of AC gives rise to a peak at 2.13 ppm, while the trimethylammonium (TMA) protons in carnitine show up at 3.2 ppm, both well visible as positive features in the difference spectra, while IMCL produces negative peaks, because it was partially depleted during exercise.
- As reported previously[4], fat oxidation was higher in euglycaemia than in hyperglycaemia (49.4 ± 4.8 vs. 30.6 ± 4.2 % of total energy expenditure, $p<0.05$, paired t-test). IMCL was partly depleted after exercise (-16%) and its use was higher in euglycaemia, though not significantly.
- AC was produced with exercise ($p<0.0001$) and the increase was significantly higher in euglycaemia ($p=0.0003$ for the interaction). The AC production in exercise is presented for all subjects in **Fig. 2**.

Fig. 1

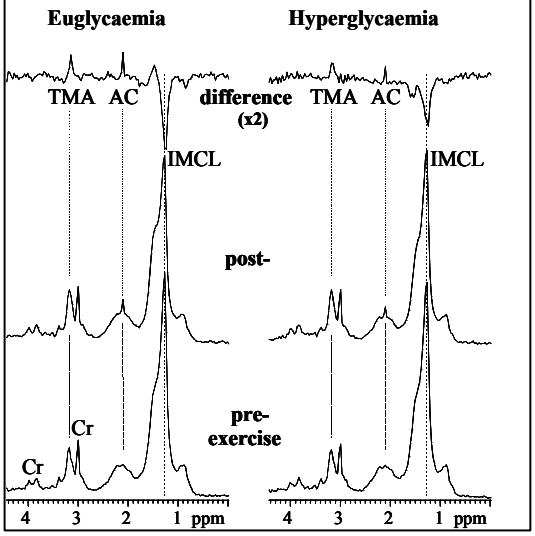
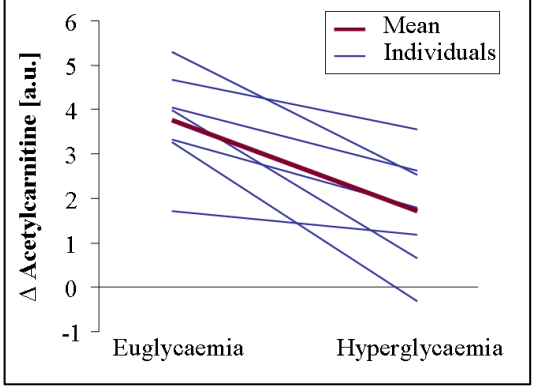


Fig. 2



Discussion

We have shown that the influence of substrate availability upon exercise-induced AC accumulation can be assessed non-invasively by ^{1}H-MRS. In particular, post-exercise AC in vastus intermedius of type 1 diabetic subjects was found to be more elevated in euglycaemia than in hyperglycaemia. This was accompanied by increased fat oxidation and IMCL use during euglycaemia. This is contrary to initial expectations, where we had speculated that AC production would be higher in hyperglycaemia, because a presumed increased glycolytic flux and pyruvate availability should stimulate pyruvate dehydrogenase complex activity[6]. However, AC production in muscle is multifactorial and, for instance, it has been shown in biopsies from resting muscle that high levels of AC coincide with high rates of β-oxidation[3]. In order to follow glycogen metabolism, ^{1}H-MR spectra were acquired after ^{13}C-MRS in this study, resulting in a delay of about 60 minutes after exercise for the measurements of AC. Therefore, it can be speculated that either AC accumulated[1] differently after exercise, or that AC might have decreased more rapidly after exercise in hyperglycaemia. From the MR-methodological point-of-view, it should be mentioned that the consistent observation of a TMA peak in the difference spectra confirms the idea[1] that the carnitine moiety becomes more NMR-visible upon acetylation. This demonstrates the importance of local molecular environment for the understanding of ^{1}H MRS of muscle.

References

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