Reproducibility of carnosine quantification in the calf muscle by 1H MRS at 7T and detection of its concentration changes following acute physical activity

Ivica Just Kukurova[1], Barbara Ukropcová[2,3], Marjeta Tušek Jelenc[1], Milan Sedliak[4], Marek Chmelik[1], Jozef Ukropec[2], Martin Kršťák[1,5], Siegfried Trattnig[1], and Ladislav Valkovič[1,6]

[1]High Field MR Centre, Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Vienna, Austria, [2]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia, [3]Faculty of Medicine, Comenius University, Bratislava, Slovakia, [4]Faculty of Physical Education and Sport, Comenius University, Bratislava, Slovakia, [5]Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria, [6]Institute of Measurement Science, Slovak Academy of Sciences, Bratislava, Slovakia

INTRODUCTION: Carnosine (β-alanyl–L-histidine) is a dipeptide involved in pH buffering, antioxidative, and anti-AGEs activity [1] of the human skeletal muscle. Reported values of its skeletal muscle physiological concentration range from 2 to 20mM [2], and were shown to depend on muscle fibre-type as well as age and gender [1]. Carnosine can be non-invasively detected in proton MR spectra (^{1}H MRS) where it is visible as two separate peaks raising from two protons on imidazole ring resonating at 8 ppm (C2) and 7 ppm (C4) at physiological pH [2]. Although muscle carnosine is often studied in relation to physical performance and endurance, only little is known about the immediate effect of acute physical exercise on its concentrations in the skeletal muscle. Therefore, the aim of this study was to establish a reproducible ^{1}H MRS measurement of carnosine in the soleus (SOL) and gastrocnemius medialis (GM) muscle and to test the changes of skeletal muscle carnosine concentration as an effect of one hour submaximal running exercise.

METHODS: All MR examinations were performed on a 7T Magnetom MR system (Siemens Healthcare, Erlangen, Germany) using 28-channel knee coil (QED, Mayfield Village, OH). Volunteers were laying in the supine position, with the widest part of the right calf placed in the centre of the coil in magnet isocenter. For carnosine detection by ^{1}H MRS in GM and SOL muscles, a STEAM sequence with a voxel size of ~20x12x20mm (4-6ml), TR=9s, TE=20ms, TM=20ms, 32 measurements, excitation pulse centered at 7.5 ppm and WS with BW=80Hz was applied. For the quantification, skeletal muscle water spectra were acquired in the same voxels, with the same parameters, but 4 measurements and the pulse centered at 4.7 ppm. To test the reproducibility of this protocol, five healthy volunteers (2f/3m, age 28.0±3.5y, BMI 21.4±2.1) underwent test-retest measurements including full repositioning and shimming. The effect of the submaximal one-hour running exercise on muscle carnosine levels in GM and SOL was studied in 7 healthy male, (age 34.3±3.8y, BMI 22.9±1.6, recreational runners/cyclists). After the baseline measurement (t_1) with the protocol described above, volunteers went for one hour street run (average distance: 11.4+/-0.4 km). Immediately after the exercise, entire MRS protocol was repeated twice. First carnosine spectra were acquired approximately 20 (GM) and 30 minutes (SOL) after the run (t_2). Second measurement was performed with additional 30 min delay (t_3). Separate spectral transients were phased, summed and fitted using the AMARES, jMRUI [3]. Absolute concentration of carnosine was calculated from the area under the C2 peak (8ppm) of carnosine, normalized to water peak intensity and corrected for relaxation effects, using previously assessed relaxation times [4]. Mean coefficient of variation (CV) was calculated to determine the repeatability of the carnosine quantification by our protocol. The effect of the acute exercise on the levels of the carnosine in the SOL and GM muscles was assessed with ANOVA.

Table 1: Mean values of carnosine concentration in the GM and SOL at three different time points (t_1, t_2 and t_3)

time point	GM	SOL
	[mmol/kg wet weight] mean±SD for n=7	
t1	7.6 ± 1.7	5.1 ± 1.4
t2	8.4 ± 2.2	5.6 ± 1.5
t3	8.1 ± 2.4	5.2 ± 1.7

RESULTS/DISCUSSION: The coefficient of variation for repeated measurements was 6.3% and 9.1% for SOL and GM, respectively. This is in good agreement with previous 3T study by Baguet et al. [5]. There were no statistically significant differences in the total carnosine concentration before and after exercise in any of the muscles (Table 1). However, physical activity did cause changes in the shape of the carnosine peak at 8 ppm. This was particularly prominent in the GM of 6 volunteers and much less in SOL (2 volunteers). The resonance line became wider (+40% in average) or got splitted into a double peak (Fig.1) after the run, so it was fitted by two separate peaks. The peaks were also shifted for up to 0.2 ppm 30 min after the exercise, especially in GM. The exercise induced shift tended to reduce during the recovery (50 min after exercise). Similar phenomenon was already observed by Damon et al [6] on stimulated frog muscle. As the chemical shift of the carnosine peak is pH sensitive [2], splitting and shift of the peaks is caused by pH changes in different compartments, represented by oxidative and glycolytic muscle fibres [6]. We propose that differences between carnosine peak shapes in GM and SOL in therefore appear due to different fiber-type content and could also be related to level of endurance training, which is known to increase content of Type I oxidative fibers and ratio of aerobic vs. anaerobic muscle metabolism.

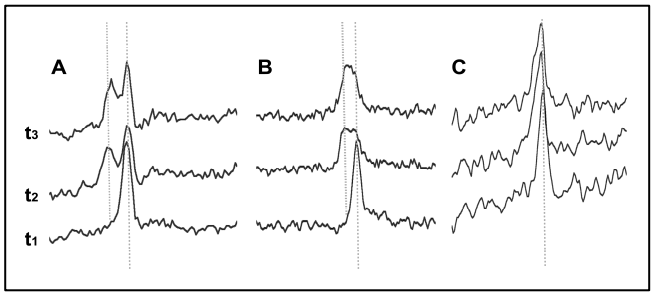

Fig.1: Examples of the carnosine peak at 8 ppm in the spectra during acute exercise experiment in three different time points (t_1, t_2 and t_3) in GM with the splitting of the peak (A) after the running, with widening the peak (B) and in SOL with single peak without shift (C) with reference lines to see the shift of the peaks in (A) and (B).

CONCLUSION: We have shown that the carnosine measurement by ^{1}H MRS at 7T is highly reproducible and that it holds great potential for investigation of differences in muscle metabolism. Even though the acute exercise does not change the absolute carnosine concentration levels, it is associated with changes in the shape of the carnosine peak in the MR spectra which might be related to different use of carnosine buffering capacity in oxidative and glycolytic muscle fibers leading to specific changes in the intramyofibrillar pH.

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