

¹³C MRS to monitor creatine uptake and clearance and for the direct detection of the phosphocreatine to creatine ratio in human skeletal muscle.

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Synopsis

Oral creatine intake is common practice in sports and medicine, but difficult to evaluate in vivo. To monitor the dynamic uptake and clearance of creatine in human skeletal muscle we introduce a new approach using ¹³C MRS and a 5 day supplementation regime with ¹³C-4 labelled creatine. The ¹³C creatine signals in the gastrocnemius muscle increased by a factor of 2, to a stable level in this period, while total creatine (assessed by ³¹P MRS) increased about 5%. The ratio Cr/TCr was found to be 0.28. Only after more than 3 months the ¹³C signals had returned to control levels.

Introduction: Creatine (Cr), phosphocreatine (PCr) and the creatine kinase reaction have important roles in health and disease. Oral creatine supplementation (*Cr-sup*) is commonly applied in sports and also used to improve clinical symptoms in several diseases[1]. Although oral Cr intake has been shown to increase the total Cr (TCr) level in skeletal muscle [e.g.1,2,3], up till now no method has been available to monitor the dynamic uptake and clearance of the extra creatine. In this pilot study, we used ¹³C MRS in combination with oral intake of ¹³C-4-creatine for this purpose.

Aims: To test if it is possible in humans (a) to measure the dynamic uptake and washout of ¹³C-4-creatine in skeletal muscle, (b) to measure separate ¹³C-MR signals of Cr and PCr, (c) to monitor interconversion in ¹³C MR spectra between labelled Cr and PCr during exercise.

Methods: One healthy male volunteer (53 yrs old) on a low creatine diet ingested 20 gr creatine/day for 5 days in four 5-gr doses at equally spaced intervals throughout the day, with 250 ml of a standard carbohydrate solution to augment skeletal muscle creatine accumulation [3]. 10% of the creatine was ¹³C-labeled at the guanidino carbon. The subject gave informed consent and the local ethical committee approved the study.

MRS measurements were performed on a 1.5 T MR system (Magnetom Vision, Siemens Erlangen) before, during and after the 5-day period of creatine intake, using a ¹³C surface coil of 13-cm diameter together with a circular polarized ¹H coil consisting of two slightly overlapping 15-cm loops for decoupling and shimming. The subject was placed with the gastrocnemius muscle of the right leg on top of the ¹³C surface coil. ¹³C-MR spectra were obtained with an adiabatic pulse for excitation, and continuous wave proton decoupling at 30 W during the first 300 ms of the data acquisition period of 819.2 ms (4k data points). Number of scans and repetition time (Tr) varied: 900 scans with TR = 1 s for detection of the uptake of ¹³C-labeled creatine (Fig.1; spectra zoomed between 154–160 ppm); 150 scans with TR = 8 s for the measurement of a fully relaxed spectrum (Fig.2); and 60 scans with TR = 2 s during an isometric exercise (Fig.3) to obtain a time resolution of 2 minutes. During the acquisition of the latter also 250 ms proton irradiation at 15W was used for NOE enhancement. During the exercise a static force of approximately 600 N was applied at the ball of the right foot for 6 minutes. In addition also unlocalized ³¹P-MRS measurements were performed with the right leg positioned on a 8 cm ³¹P surface coil using a 45° adiabatic excitation pulse. Spectra were obtained without NOE enhancement or proton decoupling using 48 averages with a TR of 12 sec. MRUI was used to determine peak areas of Cr and PCr in the ¹³C MR spectra and of Pi, PDE, PCr and ATP in the ³¹P MR spectra.

Fig. 1

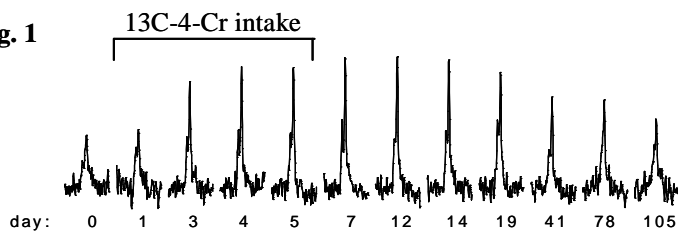


Fig. 2

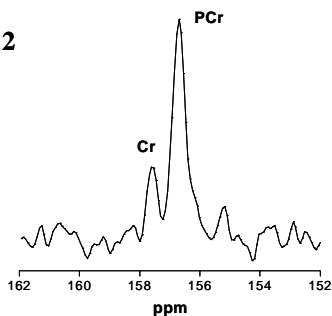
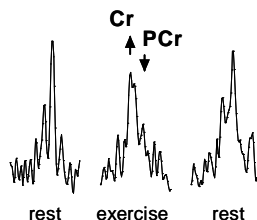


Fig. 3



Results and Discussion

As expected during the 5 day *Cr-sup* the PCr/ATP ratio (~ 4.4) in the gastrocnemius muscle increased by about 5% with respect to control [2]. The total ¹³C creatine signal increased by a factor of 2 (Fig 1). This is only slightly more than expected from the total creatine increase indicating little Cr turnover in this period. Within 4 days of *Cr-sup* the ¹³C creatine level reached a plateau. Two weeks after cessation of *Cr-sup* there is little decline in the Cr signal, again indicating minimum turnover in this subject. Even after 3 months ¹³C Cr is still elevated. Separate ¹³C signals were observed for PCr and for Cr (Fig 2). At relaxed conditions we found a Cr/TCr ratio of 0.28 ± 0.01 (n=3) which is similar to that found in mouse muscle (0.24) despite that in the latter muscle the PCr/ATP ratio is only 2.9 [4]. The assignments were confirmed by performing an exercise (Fig 3) and also by ¹³C saturation transfer which clearly reflected exchange between PCr and Cr. Calculations of the Cr/TCr ratio from generally accepted muscular ATP and/or total creatine values and the PCr/ATP ratio are lower than the Cr/TCr ratio obtained in this preliminary study.

Conclusion

A ¹³C MRS method is introduced allowing to study the kinetics of Cr uptake and washout, and the direct simultaneous observation of total creatine, PCr and Cr in human tissue. This provides a new window on the in vivo levels of some important metabolites in the CK reaction, and on creatine metabolism and supplementation in individual subjects.

References:

[1] Wyss M & Schulze A, Neuroscience 2002;112:243-60; [2] Vandenberghe K, Goris M, Van Hecke P, et al., J Appl Physiol. 1997;83:2055-63; [3] Green AL, Hultman E, Macdonald IA, et al., Am. J. Physiol. 1996;271:E821-26; [4] In t Zandt H, de Groof, A, et al., J Physiol. 2003 (in press).