Is free carnitine visible in ^{1}H-MR spectra of skeletal muscle?

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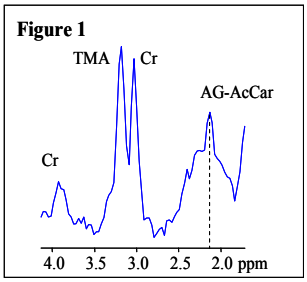
Introduction

Intramyocellular carnitine (Car) not only facilitates the transport of fatty acids into mitochondria, but also provides a buffering function for acetyl-groups through the formation of acetylcarnitine (AcCar) during conditions of excess acetyl-CoA production – e.g. during intense exercise[1]. The acetyl-group of AcCar (AG-AcCar) gives rise to a narrow peak at 2.13ppm in ^{1}H MR spectra of skeletal muscle[2]. The trimethylammonium (TMA) group of both, Car and AcCar, is thought to contribute to the TMA-peak. It would be expected that this peak remains unaffected by the buildup of AcCar since studies using muscle biopsies showed that the production of AcCar is mirrored by a concomitant decrease of free Car[3]. However, in previous single voxel and spectroscopic imaging (SI) studies we observed an increase in TMA after prolonged exercise, and we hypothesized that this increase was due to the production of AcCar[2, 4]. For the present evaluation, we reanalyzed the data of a previous exercise study[4], and now also included fitting of AG-AcCar, which has not been performed previously in SI. The aim of the study was to assess if the increase of TMA is at least partly due to an exercise-related production of AcCar and to test the hypothesis that free Car is not visible in MR spectra of muscle.

Subjects and methods

Subjects and data acquisition have been described in more detail previously[4]. Briefly: 8 trained cyclists performed a cycle exercise on an ergometer (3h, ~50% of maximal aerobic power) and 8 trained runners performed a running exercise on a treadmill (3h, ~50% of maximal aerobic speed). SI was performed before and immediately after exercise in thigh or calf, for cyclists and runners, respectively on a 1.5T system (SIGNA, GE). Details of the 2D SI sequence were as follows: transverse orientation, PRESS volume pre-selection, TR: 1.2 s, TE: 35 ms, water presaturation, matrix: 36x36 (circular sampling), FOV: 20x20 cm. Postprocessing involved: spatial zerofilling and apodization, as well as lipid extrapolation. For the present evaluation AG-AcCar at 2.13 ppm was included in the fit algorithm and the spectra were fitted using TDFDFIT[5]. Statistical analysis: Linear regression was performed by total least-squares approximation for data with errors in both dimensions with the change in TMA (ΔTMA) and ΔAG-AcCar as variables (software by Craig Markwardt, 2009).

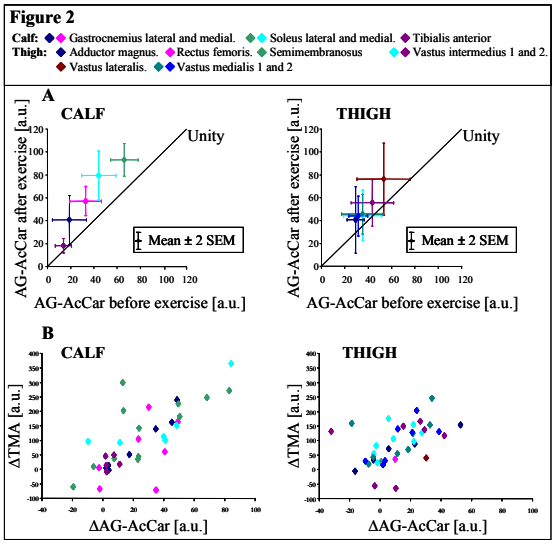
Results



- **Fig. 1** displays the post-exercise spectrum from one volunteer in m. soleus, averaged over 7 voxels. The AG-AcCar peak at 2.13 ppm was clearly visible.
- As demonstrated previously, the TMA signal increased significantly after exercise ($p<0.001$ for calf and thigh).
- The peak at 2.13 ppm assigned to AG-AcCar also increased significantly ($p<0.001$ for calf and thigh, **Fig. 2A**).
- ΔTMA was correlated with ΔAG-AcCar in calf ($R^2=0.55$, $p<0.001$), with the slope of the regression line 4.2 (SD: 0.8) and in thigh ($R^2=0.27$, $p<0.001$), with the slope found to be 4.0 (0.8) (**Fig. 2B**).
- Furthermore the shift of TMA's frequency was significantly correlated with the increase of AG-AcCar in the calf ($R^2=0.30$, $p<0.001$), but not the thigh ($R^2=0.04$, $p=0.16$).

Discussion

The present data show that in skeletal muscle the exercise-induced increase in TMA is strongly correlated with the production of AG-AcCar. It is therefore very probable that the increase in AcCar at least partly contributes to elevated TMA.



Since from muscle biopsies it is known that total Car remains unchanged after exercise[3] the present findings suggest that free Car is not visible (or at least several-fold less visible than acetylated Car) in muscle ^{1}H MR spectra and does therefore not contribute to the TMA peak. A possible explanation might be that "free carnitine" is actually membrane-bound or attached to a protein, thereby decreasing its T_2. Alternatively, free Car might be restricted to a cellular compartment that is not NMR visible.

If the increase of TMA was solely due to AcCar one would expect the area of TMA to increase 3 times more than AG-AcCar (3 to 1 ratio of protons). In the present analysis the confidence interval for the slope of the regression line is slightly above 3 which may imply that other metabolites (e.g. acylcarnitines of different chain lengths) might contribute to the increase in TMA. However, for the following reasons it is still possible that AcCar alone is responsible for the elevated TMA-signal: 1) Pre-exercise AG-AcCar might be overestimated (small signals, fit restricted to positive values) consequently ΔAG-AcCar might be underestimated. 2) ΔTMA could be overestimated (overlap with creatine's satellite peak). 3) On the other hand, it is also possible that the T_1 of the produced TMA is smaller than that of AG-AcCar (note the short TR).

The TMA peak of AcCar is shifted slightly upfield compared to the main TMA peak of muscle[2]. An exercise-induced shift of TMA's frequency corroborates an increased contribution of AcCar in calf, while an overall poorer spectral quality renders this relationship non-significant in thigh muscles.

In conclusion, as previously suggested for the visibility of creatine / phosphocreatine resonances in muscle after exercise[6], also Car / AcCar apparently demonstrate different visibilities in muscle spectra, which may serve as an interesting feature to measure muscle energetics.

References: (1) Harris RC et al. *J Appl Physiol.* 1987;63:440-442. (2) Kreis R et al. *NMR Biomed.* 1999;12:471-476. (3) Stephens FB et al. *J Physiol.* 2007;581:431-444. (4) Zehnder, M et al. *Proceedings of ISMRM.* 2005; 13: 798. (5) Slotboom J et al. *Magn Reson Med.* 1998;39:899-911. (6) Kreis R et al. *J Magn Reson.* 1999;137:350-357.

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