The effect of two β-alanine dosing protocols on muscle carnosine synthesis and washout measured by ^{1}H-MR spectroscopy

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Introduction: Carnosine (ß-alanyl-L-histidine) is a cytoplasmic dipeptide found in high concentrations in skeletal muscle, particularly in type II muscle fibers. Chronic (~4 weeks) ß-alanine (BA) supplementation (~5g BA/day) has been shown to increase muscle carnosine contents by >50%, which can contribute ~15% towards total intracellular muscle buffering capacity. However, the optimal BA dosing regime to augment intramuscular carnosine contents remains to be clarified. It has been shown that ^{1}H-MRS can follow carnosine levels in muscle non-invasively [1, 2, 3].

Purpose: To compare two different 8-weeks BA dosing regimes on the time-course of muscle carnosine loading and subsequent 8-weeks wash-out.

Methods: This placebo-controlled, double-blind clinical trial included healthy males (25.0±4.5 yrs) randomized into three groups utilizing three BA dosing regimes, all doses being split daily by half, morning and evening (controlled-release CarnoSyn™ tabs; NAI, Inc) as follows:
(1) Placebo (PL): 0g BA/day for 8 weeks (n=10);
(2) Slow-Gradual (SG): 1.6g BA/day for 8 weeks (n=11); and
(3) Load-Maintain (LM): 3.2g BA/day for 4 weeks, followed by 1.6g BA/day for 4 weeks (n=10).

A time-course of carnosine concentrations in both tibialis anterior (TA) and gastrocnemius (GA) muscles was measured by means of ^{1}H-magnetic resonance spectroscopy at weeks 0, 2, 4, 8, 12 and 16, using a 3 Tesla MR system (TRIO SIEMENS). Following the acquisition of a localizer series by the body coil for the adjustment of the spectroscopy voxel relative to the tibia plateau, a high-resolution imaging series of the calf (Fast spin echo, echo train 19, TR = 2660 ms, TE 13 ms, slice 4 mm, pixel 0.625mm*0.625mm) was acquired for the definitive placement of the MRS voxel. A standard flexible surface coil was used to obtain the high-resolution images and subsequent PRESS spectra (TR = 3000 ms, TE = 30 ms). The default voxel size was set to 18x18x30 mm (LR, AP, HF), but was adjusted in LR and AP direction in case of small TA or GA muscles or fatty infiltrations. 96 scans with the central frequency at the carnosine-H_2 position (8.0 ppm) were followed by an unsuppressed water scan (n=1) with the central frequency shifted to the water position in order to correct for the chemical shift displacement and to acquire exactly the same voxel for the metabolites and the water standard. Quantitation of the carnosine-H_2 and the water resonance was done in jMRUI-3.0 [4]. During the measurement the leg was fixed in all three directions by a home-built fixation device.

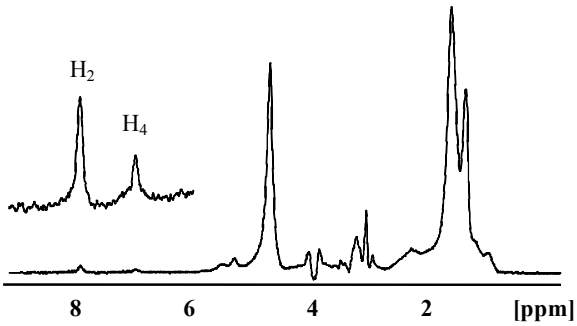

Fig.1: ^{1}H-MR spectrum of m.tibialis anterior, insert shows carnosine-H_2 and -H_4 resonances

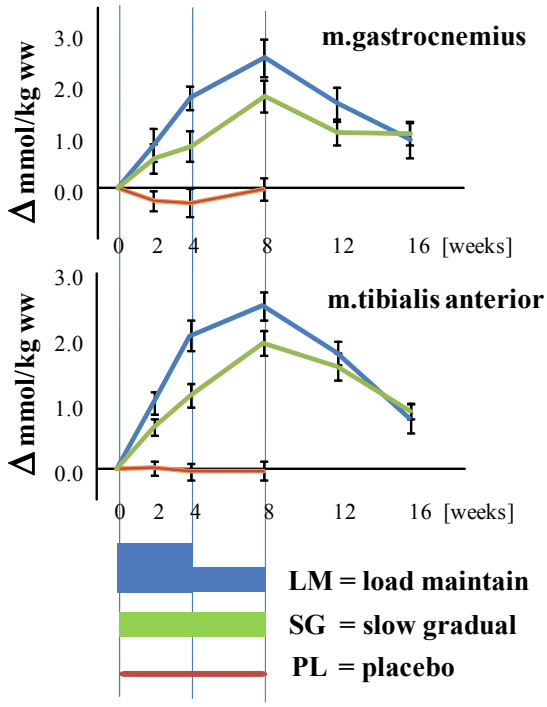

Fig.2: time course of carnosine during treatment and washout (average +/- sem)

Results: In the ^{1}H-spectrum of the tibialis anterior muscle (Fig.1), the carnosine-H_2 resonance at 8.0 ppm is less affected by dipolar coupling, resulting in broadening and T_2 shortening, than the carnosine-H_4 resonance [3]. As shown in Fig.2, there was no significant increase in muscle carnosine in the placebo trial of TA or GA. During the first 4 weeks with the different dosages, the increase for LM (TA 2.04 mmol/kg ww, GA 1.75 mmol/kg ww) was about twofold the increase with SG (TA 1.12 mmol/kg ww, GA 0.80 mmol/kg ww). During the identical dosage for weeks 5 to 8, SG lead to a slightly higher additional increase (TA 0.81 mmol/kg ww, GA 0.98 mmol/kg ww) than for LM (TA 0.46 mmol/kg ww, GA 0.77 mmol/kg ww). At week 8, the two dosage regimes were still different with (TA +34%, GA +20%) after SG and (TA +44%, GA +29%) after LM. From weeks 8 to 16, the decay rates were between 0.18 mmol/kg ww and 0.43 mmol/kg ww per week, i.e. the muscular carnosine levels were still above the basis values after 8 weeks wash-out. Flushing symptoms were recorded; they were similar and trivial in all 3 groups.

Conclusions: A clear dose-response was found in that 3.2g BA/day resulted in ~2-fold greater increase in muscle carnosine synthesis in both TA and GA muscle, as compared to 1.6g BA/day over 4 weeks, with type I fibers (TA) being more responsive to loading. A higher BA supplementation loading phase, followed by a lower maintenance phase, is an effective way to increase muscle carnosine.

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