1H-MRS detects differences of carnosine profile in skeletal muscle of rats fed with high-fat and placebo diets

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Introduction

Carnosine is a dipeptide consisting of β-alanine linked at the carboxyl terminus to histidine. These histidine containing peptides are found in high concentrations in the skeletal muscle (5-10mM) and to a lesser extent in neuronal tissue [1]. Various physiological functions have been associated with carnosine including pH buffering, metal chelation, free-radical scavenging and protein glycation inhibition [2]. β-alanine has been shown to be able to increase muscular carnosine content by about 40% in humans [1]. In this study, we have evaluated the upregulation of carnosine in rats fed with high fat and placebo diets.

Methods

In this IACUC-approved study, F344 rats on high fat diet [n=4] and placebo diet [n=2] (see Table 1) were scanned over a period of 8 weeks using a Bruker 7T ClinScan MRI/MRS scanner. Rats were anesthetized by 2.5% isoflurane during all experiments. The animals were maintained at 37°C by warm air circulation in the magnet. The rat's rectal temperature and respiration rate were maintained and monitored during all experiments. The MRI experiments included spin echo based T_1-weighted imaging on three planes for morphological evaluation. This was followed by a ^{1}H-MRS acquisition using a single voxel PRESS sequence with a VOI (3x3x3 mm^3 voxel ~ 27µl) covering on extensor digitorum longus (EDL) skeletal muscle with the following parameters: TR/TE = 4000/13 ms, with 128 averages, 1024 data points, SW=3500Hz. All the ^{1}H-MRS spectra were processed with FELIX software. Signal amplitudes of creatine at 3.03 ppm and carnosine imidazole C4 and C2 peaks (at 7.00 ppm and 8.00 ppm) were measured for all rats.

Results and Discussion

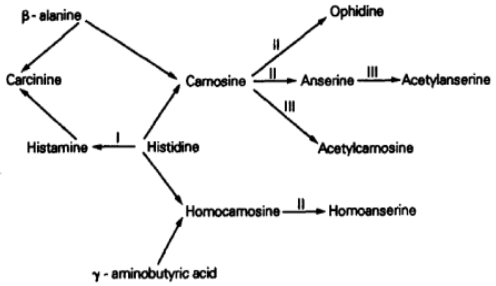


Fig.1. Pathways of metabolism of histidine-containing compounds. I, decarboxylation; II, methylation; III, acetylation. [6].

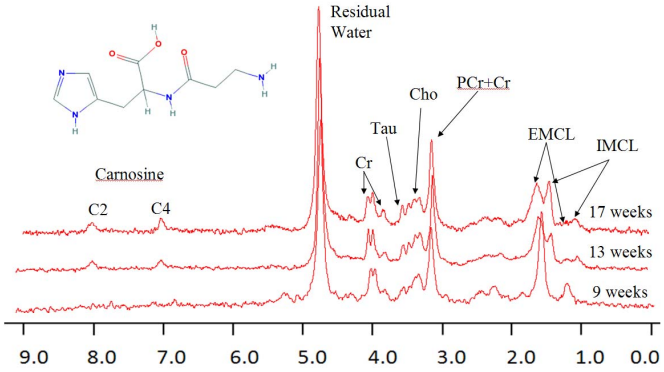


Fig.2. Shows the chemical structure of carnosine. ^{1}HMRS spectrum taken from the rats EDL compartment of skeletal muscle.

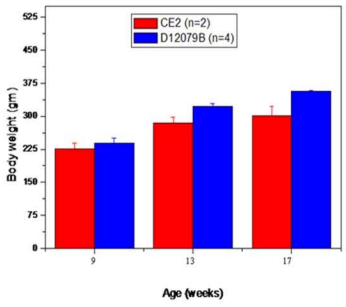
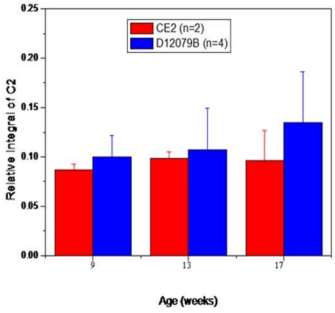


Fig.3. Histograms of rat body weights



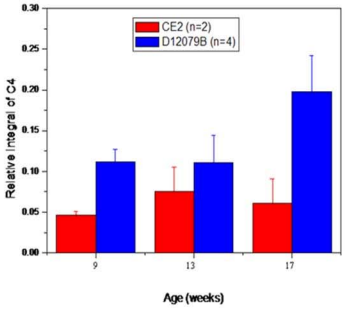


Fig.4. Histograms of relative integral of C2 and C4 peaks of the imidazole ring protons of carnosine.

	High fat	CE2
Water (%)	-	8.9
Protein (%)	17.8	25.4
Histidine (%)	0.5	-
Fat (%)	20.0	4.4
Fiber (%)	5.0	4.1
Crude ash (%)	-	6.9
Nitrogen free extract	49.9	50.3
Energy (kcal/g)	4.55	3.42

Table1. Comparison of High fat and control CE2 diets

In the skeletal muscle, carnosine formation is catalysed by the enzyme carnosine synthetase. The diagram in Figure 1 illustrates the metabolism of histidine-containing compounds. Due to the fact that carnosinase, the enzyme that splits the carnosine dipeptide into its constituents β-alanine and histidine, is found in little amounts in the skeletal muscles, the skeletal muscles have the highest concentrations of carnosine in animals. Figure 2 shows the skeletal muscle spectra for a high fat diet fed rat from 9-17 weeks of age. The maximum upregulation of carnosine is recorded when rats were 17 weeks old. Histidine supplementation has been shown to be able to boost the concentration of carnosine in skeletal muscles [3, 4]. Also, a histidine-free diet on rats has bore a trend where carnosine levels dip in muscle and olfactory bulb [5]. The increase in body weight for all rats is shown in Figure 3. Over a period of 13 weeks, the supplementation of high fat diet containing histidine (0.5%) apart from other amino acids caused a slight upregulation of carnosine content in the rats compared to their control which was fed CE2. Creatine to carnosine integrals revealed an increasing trend in carnosine levels over 8 weeks for rats on both diets. Figure 4 shows the upregulation of carnosine in both high fat and placebo diet fed rats. For rats given the high fat diet, the C2 peak ratios increased from 0.100 to 0.134 and C4 peak ratios increased from 0.111 to 0.197 across 8 weeks. In the control diet, C2 peak ratios increased from 0.087 to 0.096 and C4 peak ratios increased from 0.046 to 0.06 across 8 weeks (Fig.4). From our preliminary work both the C2 and C4 peaks are upregulated due to histidine in high fat diet. A higher dosage of histidine supplementation would have made the upregulation of carnosine content more pronounced.

Conclusion

Histidine supplementation upregulates carnosine content in the rat's EDL skeletal muscle and provides additional information on diet induced changes to metabolism. The further progression of rats to a state of obesity and/or diabetes could alter the metabolic profile and impact carnosine levels.

References

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