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The Effect of β -Alanine Supplementation on Carnosine and Histidine Content in the Hippocampus of 14-Month Old Rats

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ABSTRACT

Carnosine and histidine content in the hippocampus of 14-month-old male rats was examined following 30-days of β -alanine supplementation. All animals were provided identical diets, however 100-mg of β -alanine was mixed with glucomannan (80:20 blend) in the water of β -alanine supplemented animals. Hippocampal carnosine content was significantly greater ($p=0.005$) for β -alanine compared to control, while no differences ($p=0.438$) were noted in histidine content between groups. Results provide initial evidence that β -alanine supplementation increases carnosine content in the hippocampus of middle-aged rats, without compromising histidine content.

- β -alanine supplementation increases hippocampal carnosine content without compromising histidine content in middle-aged rats.

Key Words: Aging, Dietary Intervention, Nutrition, Brain, Middle-age, Health

INTRODUCTION

β -alanine supplementation has become one of the more popular supplements in the past 10-years, especially among strength/power athletes (Hoffman et al. 2018), but evidence is mounting regarding the potential health benefits among various population groups (Sale et al. 2013). β -alanine supplementation has been demonstrated to increase carnosine formation in myocardial (Blancquaert et al. 2016), skeletal (Church et al. 2017; Harris et al. 2006), and brain tissue (Hoffman et al. 2015; 2017). Carnosine is a dipeptide molecule consisting of the amino acids β -alanine and histidine, in which β -alanine is the rate limiting molecule in its synthesis (Dunnett and Harris 1999; Harris et al. 2006). Recently, Blancquaert and colleagues (2017) reported that prolonged β -alanine supplementation ($6 \text{ g} \cdot \text{day}^{-1}$ for 23 days) may reduce both plasma and muscle L-histidine content. A reduction in the intramuscular histidine pool may affect protein metabolism (Kriengsinyos et al. 2002) and impair motor response time (van Ruitenbeek et al. 2009). In contrast, recent investigations by others were unable to confirm any risk for depleting plasma or muscle histidine pool in young, college age participants (Church et al. 2017; Varanoske et al. 2017). One investigation examined two different β -alanine dosing strategies (i.e., $6 \text{ g} \cdot \text{day}^{-1}$ for 4 weeks and $12 \text{ g} \cdot \text{day}^{-1}$ for 2 weeks) and reported no change in muscle histidine levels, but did report a significant decline in plasma histidine concentrations in the $12 \text{ g} \cdot \text{day}^{-1}$ dose group only (Church et al. 2017). The other study reported no difference in muscle histidine content between men and women following 4-weeks of supplementing with $6 \text{ g} \cdot \text{day}^{-1}$ of β -alanine (Varanoske et al. 2017). It appears that extreme dosing regimens may reduce plasma histidine concentrations, however the physiological significance of this change with normal intramuscular histidine levels remains to be determined.

In consideration of the role that brain histidine may have in motor response time (van Ruitenbeek et al. 2009), a decrease in the brain histidine pool may have significant consequences regarding cognitive and motor function. Previous research has demonstrated significant elevations in carnosine content in various brain compartments (cortex, hippocampus, thalamus, hypothalamus, amygdala) following 30-days of β -alanine supplementation in 4-month old Sprague-Dawley rats (Hoffman et al. 2015; 2017). Interestingly, the histidine content across the brain was 86% higher in the animals consuming β -alanine than for animals consuming their normal diet (Hoffman et al. 2017). In addition, carnosine content was significantly correlated ($r = 0.75$) to histidine content in the hippocampus. Reductions in anxiety-like symptoms have been associated with elevations in carnosine levels in the hippocampus (Hoffman et al. 2015; Tomonaga et al. 2008). However, it has also been suggested that elevations in histidine can stimulate histaminergic neurons within the brain resulting in attenuating depressive-like symptoms (Tomonaga et al. 2008). Carnosinase the enzyme which degrades carnosine into histidine and β -alanine (Bellia et al. 2014), is elevated during aging (Bellia et al. 2009). Elevations in carnosinase in both circulation and in brain tissue are associated with significant reductions in carnosine synthesis and has been suggested to increase risk for disease (Bellia et al. 2009). In consideration of the limited study of β -alanine supplementation on brain carnosine content in older animals, the primary purpose of this study was to examine the effect of 30-days of β -alanine supplementation on hippocampal carnosine and histidine content in 14-month old rats.

METHODS

Animals

All experimental procedures were performed according to the principles and guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals. All treatment and testing procedures were approved by the Animal Care Committee of the Ben-Gurion University of the Negev, Israel (IL-26-06-2017). Fourteen-month-old male Sprague-Dawley rats were habituated to housing conditions for at least seven days. All animals were housed (two per cage) in a vivarium with stable temperature and a reversed 12-h light/dark cycle, with unlimited access to food and water.

Experimental design

Upon arrival to the research facility, all rats received a normal powder diet during a 7-day acclimation period. Rats were randomly assigned to one of two treatment groups: a vehicle-treated group (CTL; $n = 8$) in which rats were fed laboratory rat chow (Teklad Global 18% Protein Rodent Diet, Envigo Inc., Jerusalem, Israel) and water for 30 days or a β -alanine group (BA; $n = 6$), in which rats were fed the laboratory rat chow and provided β -alanine with glucomannan (80:20 blend) in a powder form mixed in their water. Glucomannan is a dietary fiber (Tester and Al-Ghazzewi 2013), that has been used to control drug and nutrient delivery (Shi et al. 2016). It was used in this study and others (Hoffman et al. 2015; 2017) to slow the absorption of β -alanine, and act similarly to a sustained-release formulation and minimize or prevent any adverse effects associated with paraesthesia. Rats were provided with 100 mg of the powder per kg of body mass. The primary ingredient of the rat chow was wheat, corn, wheat midds, soybean meal, corn gluten meal, and soy oil. The nutrient profile of the diet was 18.6%

protein, 6.2% fat and 3.1 kcal·g⁻¹. Diets were maintained until the end of the study. We have previously demonstrated that this dosing protocol can significantly increase brain carnosine levels in this specific species (Hoffman et al. 2015; 2017). Animals were handled once daily.

Tissue preparation

Animals were deeply anesthetized via an intraperitoneal injection of a ketamine and xylazine mixture (70 mg·kg⁻¹, 6 mg·kg⁻¹, respectively) and perfused transcardially with cold 0.9% physiological saline followed by 4% paraformaldehyde (Sigma-Aldrich) in 0.1 M phosphate buffer (pH 7.4). Brains were removed following perfusion and post-fixed in the same fixative for 12 h at 4°C. Brains were cryoprotected overnight in 30% sucrose in 0.1 M phosphate buffer at 4 °C. Brains were then frozen on dry ice and stored at -80 °C.

Brain Analysis

Brain hippocampal carnosine and histidine analysis was performed by liquid chromatography- electrospray ionization- tandem mass spectrometry (LC-ESI-MS/MS) by Heartland Assays (Ames, IA) using an Agilent 1200/6460 triple quadrupole LC/MS system (Santa Clara, CA). Standards and approximately 50 mg of tissue was processed using a EZ:faast™ (Phenomenex ,Torrance, CA) analysis kit for physiological amino acid by LC-MS (Badawy 2012). Briefly, the tissue with the internal standards were homogenized in cold PBS buffer. The homogenate was centrifuged 3,000 rpm for 15 min at 4°C. The supernatant was then processed by solid phase extraction followed by derivatization (propyl Chloroformate) of the amino acids and liquid-liquid extraction. Chromatographic separation of the derivatized amino acids was conducted on an EZ:faast amino acid analysis-mass spectrometry column (250 × 2.0 mm i.d., 4 µm). Ammonium format and formic acid in water and ammonium formate and

formic acid in methanol served as the eluents. A linear gradient was used as follows: 68-83% methanol for 0-13 minutes. Amino Acid and internal standards were optimized and data were collected using the Dynamic Multiple Reaction Monitoring (MRM) mode using Mass Hunter acquisition software (Agilent, Santa Clara, CA). The collected MRM data of unknown plasma samples were quantitated with the Mass Hunter Quantitation software (version B.4.01) from linear standard curves. Each sample was run in duplicate. The intra-assay coefficient of variation for carnosine and histidine in brain tissue was 3.7% and 0.5%, respectively. The average inter-assay variation was 15.2%. Limits of detection for these assays were 20 pmol·g⁻¹.

Statistical Analyses

Prior to any assessments, normality of distribution was verified with the Shapiro-Wilk test. No violations of sphericity were noted, and no corrections were necessary. Comparisons of hippocampal carnosine and histidine content between BA and CTL were performed using unpaired Student's *t* tests. Pearson's product-moment correlation was used to determine the correlation between histidine and carnosine content in the hippocampus. Data were analyzed using SPSS v23 software (SPSS Inc., Chicago, IL). An alpha level of $p < 0.05$ was used to determine statistical significance. All data are reported as means $\pm$ SD.

RESULTS

No significant differences were noted in weight gain between BA (23.1 ± 27.6 g) and CTL (23.5 ± 18.8 g). Hippocampal carnosine and histidine content can be seen in Figures 1 and 2, respectively. Carnosine content in the hippocampus was significantly greater ($p = 0.005$) for BA compared to CTL. However, no significant differences ($p = 0.438$) were noted in histidine

content of the hippocampus between BA and CTL. Further, no significant correlation was noted ($r = -0.12$) between hippocampal histidine and carnosine content.

[Place Figures 1 and 2 here]

DISCUSSION

The results of this study demonstrated that 30-days of β -alanine supplementation significantly increased carnosine content in the hippocampus of 14-month old Sprague-Dawley rats, without compromising the local histidine pool. The 89.5% difference in carnosine content between BA and CTL in these older rats was similar to that previously reported in young (i.e., 4-month old) animals in which a 76.4% difference was noted between the β -alanine supplemented and regular chow fed animals (Hoffman et al. 2017). Although it has been suggested that carnosinase the enzyme that degrades carnosine into β -alanine and histidine is elevated in older animals (Bellia et al. 2009), the evidence from this study indicates that β -alanine supplementation is still effective in increasing carnosine within the hippocampus of older rats. It is important to note that 14-month old rats are considered to be middle-aged rather than old (Sengupta 2013). However, by this age significant elevations in various inflammatory markers (e.g., glial fibrillary acidic protein, NF- κ B p50 and p65 subunits, TNF α and cyclooxygenase-2) have been reported in the hippocampus (Hoffman et al. 2019).

In contrast to previous research examining young rats (Hoffman et al. 2017), no differences were noted in the histidine content of the hippocampus between β -alanine supplemented and regular chow fed older rats. In the initial study investigating young animals the correlation between histidine and carnosine content was strong ($r = 0.75$), however, this was not supported by the present study. The difference between these studies is not clear, and may

simply be spurious in nature. In summary, this brief communication provides initial evidence that β -alanine supplementation can increase the carnosine content in the hippocampus of middle-aged rats, without compromising histidine content.

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Figure Legends

Figure 1: Hippocampal Carnosine Content. BA = β -alanine; CTL = Control group. * = Significant difference between groups. All data are presented as mean $\pm$ SD.

Figure 2: Hippocampal Histidine Content. BA = β -alanine; CTL = Control group. All data are presented as mean $\pm$ SD.

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