Effects of carnosine synthase deficiency on exercise performance and behavior in aged mice

Ai Egusa [1], Nobuhiro Nakao [2], Nobuya Yanai [3], Kenichiro Sato [3], Toshihide Nishimura [4]

[1] Department of Food Science and Technology, Nippon Veterinary and Life Science University, [2] Department of Animal Science, Nippon Veterinary and Life Science University, [3] Research Division, Tokai Bussan Co., Ltd., [4] Department of Nutrition, Kagawa Nutrition University, Japan

Objectives: Imidazole-containing dipeptides (IDs)—carnosine, anserine, and balenine—are found predominantly in the skeletal mus- cle of vertebrates. They exhibit physiological effects including antioxidant, antiglycation, and antifatigue effects. Many studies have been conducted on the effects of carnosine on improving exercise performance both in vitro and in vivo. Intramyocellular buffering, calcium release of sarcoplasmic reticulum, and calcium sensitivity of contractile apparatus are thought to be involved in this mechanism. However, recently it was reported that carnosine synthase knockout (KO) mice show no differences in skeletal muscle weight and muscle contraction compared with wild-type (WT) mice. To bridge this difference in findings between previ- ously reported results and KO mice, we investigated the effects of endogenous IDs on skeletal muscle, exercise performance, behavior in home cages, and metabolites in muscle using newly created carnosine synthase gene-deficient mice. In addition, we com- pared the effects of different genotypes on aging.

Materials and Methods: CARNS1 is a member of the ATP-grasp enzyme family and requires ATP for enzymatic reactions. Previ- ously reported KO mice were generated by removing sequences from Exon 3 to 10. However, as the unidentified sequence is en- coded in the reverse direction from Exon9 to Intron3, only Exon10 was removed in the newly prepared KO mice. In the targeting vector, inserted a cassette containing *En2 SA, IRES, LacZ, polyA* and the autonomous promoters *hBactP* and *Neo* into Intron9 of CARNS1, and inserted the *loxP* flanking sequence at both ends of Exon10 and in front of *hBactP* (KOMP-CSD, ID: 45781, PRP- GS00167-A-H12). Mice specifically designed for Exon10 to splicing were abbreviated as Carns1 to distinguish them from previ- ously reported KO mice. Female WT and Carns-KO mice were used at 30, 50, and 90 weeks of age. The mice was maintained at 22°C ± 2°C under a 14-:10-h light/dark cycle with free access to water and food (CMF; Oriental Yeast Co., Japan). Exercise perfor- mance was evaluated using a small animal grip strength-measuring device (GPM 101; Melquest Co., Japan) and drum-type exer- cise load device (FWS 1504; Melquest Co.). A 24-h video monitoring system (Phenotyper; Sophia Scientific Co., Japan) was used to evaluate the daily activities of mice in the home cage. After each test period, the anterior tibialis muscle was collected and ana- lyzed using CE-TOFMS for metabolomic analysis. The animal experiments were designed in compliance with animal use guide- lines and approved by the Animal Experimentation Approval Committee of Nippon Veterinary and Life Science University (Ap- proval numbers: 2019K-80 and 2020K-62).

Results and Discussion: There was no difference in body weight between WT and Carns-KO mice at all ages tested. In contrast, Carns-KO mice presented a significant reduction in anterior tibial and soleus muscle weights with aging. Furthermore, Carns-KO mice showed a significant decrease in exercise performance, by approximately 20% at 30 and 50 weeks of age, compared with WT mice. Similarly, the level of activity in the home cage was significantly reduced by 10% 20% in Carns-KO mice compared with that in WT mice of the same age. Thus, systemic ID deficiency suppresses not only forced exercise performance but also spontaneous exercise performance. The results of the metabolomic analysis of skeletal muscle showed that the amount of branched chain amino acids in Carns-KO mice was significantly lower than that in WT mice and that it decreased with aging. Leucine promotes muscle synthesis, and its decrease in the skeletal muscle might have contributed to the decrease in the weight of anterior tibial muscle. Additionally, because the amount of glutathione and NAD+ decreased in 90-week-old KO mice, it was considered that the oxidation level of skeletal muscle cells increased. These results revealed that ID affects muscle weight and exercise performance, especially during aging.

Key words: carnosine, Imidazole Dipeptide, Knock-out mouse, Exercise performance