

The bone-derived components from adult chickens provide a protective action against impaired bone metabolism in mice lacking vitamin D action.

Tamao Nishiura ^{1,2}, Niko Koyama ², Kanami Akasaki ², Satoshi Kotoura ¹, Masato Nakane ¹, Ritsuko Masuyama ²

¹ Marudai Food Co., Ltd., ² Ritsumeikan University Graduate School of Gastronomy Management, Japan

Objectives: In the Japanese meat processing industry, hens that have finished laying eggs called adult chickens are used as raw materials for processing. During the processing of minced adult chicken meat in a meat-bone separator, a large amount of bone-derived components remains in the product due to the characteristics of the methods. Accordingly, the massive amount of components derived from the bone characterizing the adult chicken is contained. One of the characteristics of the bones of adult chickens is the medullary bone, which is regarded as a temporary regulator of calcium storage in the body. In particular, it forms a reticular structure for storing calcium and often appears in the endocortical domain of long bones such as the femur and tibia. Hens lay eggs almost daily at peak times, requiring the immediate mobilization of large amounts of calcium from the body for eggshell formation. The calcium stored in the medullary bone is therefore utilized as materials of eggshell in addition to the intake. Thus, remarkable bone metabolism in which frequent changes in bone formation and bone resorption are operated in the laying hen's body during the egg-laying cycle, and considered that medullary bone contributes to calcium homeostasis. In this study, we hypothesized that medullary bone-derived components from adult chickens would affect systemic calcium metabolism, and we tested whether the administration of bone-derived components from adult chickens can improve bone metabolism in animals with impaired calcium absorption and homeostasis.

Materials and Methods: The calcium absorption in the intestine is promoted by vitamin D action initiated from the binding of vitamin D to the vitamin D receptor. Systemic vitamin D receptor knockout mice (VDRKO) lack this vitamin D-dependent calcium absorption, resulting in severely reduced blood calcium levels and disruption of calcium homeostasis in the body. As this is regarded as an appropriate model to evaluate a change of calcium homeostasis by the dietary factor. We investigated the effects of bone-derived components from adult chicken on calcium and bone phenotype in VDRKO. Each meat sample of the young chickens with no egg-laying experience and the adult chickens were prepared by pressing meat parts including bones in a meat-bone separator, mincing, heat-sterilizing, and freeze-drying. These samples were used as test diets (adult chicken bone-containing diet and young chicken bone-containing diet) by replacing 25% of the diet weight, other formulations except for protein source were based on AIN93G respectively. The composition of the chicken protein was reproduced with amino acid powder and these mixtures were supplemented to adjust protein levels among the diets. The carbohydrate, lipid, and protein levels of all diets were adjusted to a constant level. The calcium content was aligned with that of the adult chicken bone-containing diet, which was used as the control diet. After weaning, wild-type mice (WT) and VDRKO were divided into three dietary groups and received one of each diet during 4 weeks from 5 weeks of age. At the end of the experimental period, blood samples were collected and measured the concentrations of calcium, phosphorus, and the parameters related to bone metabolism. Femurs were used for fracture toughness analysis with the three-point bending method. Tissue sections were cut from the tibia and osteoclast morphometry was performed after TRAP staining.

Results and Discussion: Hypocalcemia, characteristic of VDRKO, was observed only in the young chicken bone-containing group (young chicken group) and control diet group and improved in the adult chicken bone-containing diet group (adult chicken group) ($P<0.05$) to the same level as WT. This result contributed to the improvement of blood parathyroid hormone (PTH) levels. It was observed severe hyperparathyroidism in the VDRKO young chicken and control diet groups but normalized in the adult chicken group ($P<0.01$). In the femur fracture toughness analysis, there was a decrease in bone strength in the VDRKO young chicken and control diet groups, but the adult chicken group recovered to the same level as WT ($P<0.01$). In osteoclast morphometry of the tibia, osteoclast function parameters such as osteoclast number ($P<0.01$) and osteoclast resorption surface ($P<0.05$) were significantly increased in the VDRKO adult chicken group compared to the young chicken group. The WT adult chicken group also showed a tendency to improve bone metabolism, including a decrease in blood PTH concentration and an increase in bone fracture strength, compared to the young chicken group and the control diet group.

Conclusions: Administration of a diet containing adult chicken bones to VDRKO normalized calcium homeostasis and bone strength, suggesting that components derived from adult chicken bones may contain complementary factors for vitamin D action that have been shown to improve bone metabolism.

Key words: Adult chicken, Bone metabolism, Vitamin D, Medullary bone, VDRKO