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Note

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Yoh-ichi Koyama[1,*], Arisa Hirota[1], Hidenori Mori[2], Hidenari Takahara[3], Kumiko Kuwaba[1], Masashi Kusubata[1], Youko Matsubara[1], Shohei Kasugai[4], Masato Itoh[5] and Shinkichi Irie[1]

[1] *Nippi Research Institute of Biomatrix, Nippi Inc., Tokyo 120–8601, Japan*
[2] *Department of Animal Science, School of Agriculture, Ibaraki University, Ibaraki 300–0332, Japan*
[3] *Molecular Biology and Biochemistry, Department of Applied Biological Resource Sciences,
School of Agriculture, Ibaraki University, Ibaraki 300–0332, Japan*
[4] *Masticatory Function Control, Graduate School, Tokyo Medical and
Dental University, Tokyo 113–0034, Japan*
[5] *Fuji Factory, Nippi Inc, Tokyo 120–8601, Japan*

Summary   Malnutrition, particularly protein undernutrition, contributes to the occurrence of osteoporotic fracture by lowering bone mass. In this study, the effects of dietary protein on bone mineral density and body weight in protein undernutrition were compared between gelatin and milk casein. When mice were fed for 10 wk with a low protein diet containing 10% casein or 6% casein+4% gelatin, there was no significant difference in the final body weight between the 6% casein+4% gelatin group and the 10% casein group. In contrast, bone mineral content and bone mineral density of the femur were significantly higher in the 6% casein+4% gelatin group than in the 10% casein group. Bone mineral content and bone mineral density did not differ significantly in 14% protein groups between 14% casein and 6% casein+8% gelatin. These results suggest that gelatin has differential effects on bone mineral density and body weight in protein undernutrition.
Key Words   bone mineral density, gelatin, milk casein, protein undernutrition

Malnutrition, particularly protein undernutrition, could be detrimental to the conservation of bone integrity with ageing, and reduced protein ingestion in hospitalised elderly patients contributes both to lower bone mineral density (BMD) of the femoral neck and to the high incidence of hip fracture. Nutritional supplementation normalising the protein intake will reduce the occurrence of osteoporotic fracture and improve clinical outcome after hip fracture (*1*). A number of protein sources have been used for protein supplementation including milk casein and gelatin. Milk casein contains all the essential amino acids. On the other hand, gelatin lacks tryptophan, an essential amino acid, and the amount of other essential amino acids is small (*2*). Nevertheless, it has been reported that the ingestion of gelatin has beneficial effects on osteoarthritis (*3*), nail defects (*4, 5*), hair growth (*6*), and the rate of metabolism (*7*). In this study, we investigated the effect of ingestion of gelatin and casein on BMD of mice. We have found that gelatin has differential effects on body weight and BMD in protein undernutrition.

Experimental

Animals and diets.   Male BALB/cA mice 6 wk old were purchased from Japan Clea Inc. (Tokyo, Japan) and maintained in the laboratory animal facilities of Ibaraki

University for 10 wk. Mice were divided into 4 groups so that the average body weight (23.8–24.1 g) did not differ significantly among groups. Each experimental group consisted of 14 mice that were divided into two cages (7 mice per cage). The protocol of this research on animals was approved by the animal welfare committee of Nippi Inc.

Basic powder diet was AIN-93M purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). AIN-93M contains 14% milk casein. Low protein diet was prepared by lowering the content of casein to 10% (abbreviated as 10C). Gelatin isolated from bovine bone was obtained from Nippi Inc. (Tokyo, Japan). 4% or 8% gelatin powder was added to the 6% casein diet to prepare a 10% or 14% protein diet (6C+4G and 6C+8G, respectively). Cornstarch was used to compensate for the decrease in weight of protein. Calcium content was 30 mg/100 g for casein and less than 4.5 mg/100 g for gelatin. Net protein content was 86% for casein and 83% for gelatin.

Measurement of bone mineral density.   Mice were killed by cervical dislocation. The right femur was isolated and kept in 70% ethanol until used. BMD was measured by dual-energy X-ray absorptiometry (DEXA) for small animals (DCS-600R, Aloka Co., Tokyo, Japan). From the proximal end to distal end, the femur was divided into 20 sections, and BMD of each section was measured. Bone mineral content (BMC) of the femur

* E-mail: ykoyama@nippi-inc.co.jp

represents the total of BMC at all sections. Diameter of the distal end and length of the femur were measured with an engineer's micrometer.

Statistics. F-test was employed to assess differences in standard deviation (SD). If SD differed significantly, Welch's *t*-test was employed to assess differences in the mean, whereas Student's *t*-test was used when SD did not differ significantly. $p < 0.05$ was considered significant.

Results and Discussion

Effects on body weight and total food intake are summarized in Table 1. Final body weight did not differ significantly between 10C and 6C+4G and between 14C and 6C+8G though body weight gain and %increase was slightly smaller in the gelatin-supplemented groups. Total food intake per mouse during 10 weeks of breeding did not differ evidently among the 4 groups. Table 2 summarizes the size, BMC and BMD of the femur. There was no significant difference in weight, length or thickness of the femur between 10C and 6C+4G. In contrast, BMC was significantly higher in 6C+4G than in 10C. BMD at the femoral neck (position 7) and at the middle of the femur (position 9) were also significantly higher in 6C+4G than in 10C. Weight, length and thickness of the femur in 14C and 6C+8G were similar to those in 10C or 6C+4G. BMC in 14C and 6C+8G were significantly higher than that of 10C and did not differ from that in 6C+4G. These results suggest that ingestion of gelatin has an up-regulating effect on BMC and BMD of the femur in protein undernutrition, and that this effect is not associated with an increase in femoral size.

Dietary ingestion of protein is essential to improve protein undernutrition. It is well-known that BMD increases when body weight becomes higher (8).

However, this is not the case in the present study because body weight did not differ significantly between 6C+4G and 10C but BMC and BMD were significantly higher in 6C+4G than 10C. Significantly higher BMC and BMD in 6C+4G can not be explained by a difference in calcium content or net protein content of a protein source, because calcium content was evidently higher in casein (30 mg/100 g) than in gelatin (less than 4.5 mg/100 g), and because net content of protein was slightly lower for gelatin (83%) than casein (86%). Therefore, the present study clearly suggests that gelatin has differential effects on BMD and body weight particularly in protein undernutrition.

In 14% protein groups (14C and 6C+8G), gelatin supplementation did not result in a higher BMC or BMD. BMC and BMD of 14C or 6C+8G were higher than those of 10C and comparable to those of 6C+4G. Thus, it seems possible that the effect of gelatin is evident in protein undernutrition but is not observed clearly when BMC or BMD is high with sufficient supplementation of proteins.

Milk casein contains all the essential amino acids, while gelatin lacks some essential amino acid (2). Therefore, it is unlikely that the effect of gelatin on BMD resulted from essential amino acids supplementation. Collagen contains a large amount of hydroxyproline, which is rarely found in other protein (2). However, hydroxyproline derived from orally administered gelatin does not seem to be utilized directly to synthesize hydroxyproline in femoral collagen, because hydroxyproline in collagen is post-translationally modified from proline and is not synthesized from orally administered hydroxyproline (9, 10). Thus, the effects of gelatin on BMD can not be attributed to a supplement of essential amino acids or hydroxyproline.

Oesser et al. have recently reported that orally admin-

Table 1. Effects on body weight and total food intake.

Group	Initial body weight (g)	Final body weight (g)	Body weight gain[a] (g)	% Increase[b]	Total food intake (g/mouse)
10C	24.0±1.3	32.6±2.7	8.6	36	143
6C+4G	24.1±0.7	31.8±1.8	7.7	32	144
14C	23.8±0.9	34.1±2.6	10.3	43	145
6C+8G	23.9±0.7	32.6±1.5	8.7	36	146

Values are Mean±SD for initial body weight and final body weight.
[a]: (mean of final body weight)−(mean of initial body weight), [b]: (body weight gain/mean of initial body weight)×100.

Table 2. Size, weight and mineral content of femur.

Group	Weight (mg)	Length (cm)	Thickness (cm)	BMC (mg)	BMD at section 7[a] (mg/cm^2)	BMD at section 9[b] (mg/cm^2)
10C	71.5±4.0	1.39±0.02	0.25±0.02	20.5±1.7	29.5±2.5	29.9±3.3
6C+4G	72.0±2.9	1.39±0.02	0.24±0.01	22.5±1.8*	31.4±2.7*	32.2±2.3*
14C	72.8±4.3	1.39±0.01	0.24±0.01	22.5±1.7	30.7±2.0	32.5±2.9
6C+8G	72.2±3.1	1.39±0.02	0.25±0.01	22.5±1.7	30.9±2.3	31.6±1.6

Values are Mean±SD.
BMC: total bone mineral content per femur, BMD: bone mineral density.
[a]: femoral neck, [b]: middle of the femur, * $p < 0.05$.

istered gelatin hydrolysate accumulates to cartilage specifically (11). Their study suggests that gelatin-derived peptides in blood circulate the body. Thus, it is possible that gelatin-derived peptides reached the femur and promoted the deposition of bone mineral or inhibited degradation of bone. Interaction of osteoblasts or osteoclasts with extracellular matrix plays an important role in bone remodeling. For example, expression of alkaline phosphatase in osteoblasts is inhibited by blocking the binding of collagen to $\alpha_2\beta_1$ integrin (12). Therefore, it seems possible that gelatin peptides, which were derived from orally administered gelatin, affect bone remodeling by binding to integrin of osteoblasts or osteoclasts, thus resulting in an increased BMC and BMD in the femur. Alternatively, gelatin peptides might inhibit the activity of enzymes. Actually, gelatin-derived peptides could be an inhibitor of angiotensin I-converting enzyme (13). It is tempting to speculate that gelatin peptides could inhibit a matrix metalloproteinase which cleaves bone collagen and subsequently suppresses degradation of bone. Further study is required to elucidate the mechanism of BMD enhancement by digested gelatin peptides.

In summary, the present study suggests that gelatin has an up-regulating effect on BMC and BMD, whose mechanism may differ from that of casein in protein undernutrition.

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