

## Absorption and Effectiveness of Orally Administered Low Molecular Weight Collagen Hydrolysate in Rats

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Collagen, a major extracellular matrix macromolecule, is widely used for biomedical purposes. We investigated the absorption mechanism of low molecular weight collagen hydrolysate (LMW-CH) and its effects on osteoporosis in rats. When administered to Wistar rats with either [<sup>14</sup>C]proline (Pro group) or glycyl-<sup>14</sup>C]prolyl-hydroxyproline (CTp group), LMW-CH rapidly increased plasma radioactivity. LMW-CH was absorbed into the blood of Wistar rats in the peptide form. Glycyl-prolyl-hydroxyproline tripeptide remained in the plasma and accumulated in the kidney. In both groups, radioactivity was retained at a high level in the skin until 14 days after administration. Additionally, the administration of LMW-CH to ovariectomized stroke-prone spontaneously hypertensive rats increased the organic substance content and decreased the water content of the left femur. Our findings show that LMW-CH exerts a beneficial effect on osteoporosis by increasing the organic substance content of bone.

**KEYWORDS:** Absorption; collagen tripeptide; low molecular weight collagen hydrolysate; osteoporosis; OVX; SHRSP

### INTRODUCTION

Collagen is one of the major macromolecules constituting the extracellular matrix. Gelatin, the denatured and partially hydrolyzed form of collagen, has been widely used in various food and nonfood products. The nutritional values of collagen and gelatin are low because they are rich in nonessential amino acids, such as glycine (Gly), proline (Pro), and hydroxyproline (Hyp). However, these proteins have been widely used for biomedical purposes. A number of studies on both humans and animals have shown positive immunotherapeutic effects of orally administered collagen and collagen hydrolysate on collagen-induced rheumatoid arthritis (1, 2), osteoarthritis, and joint disorders (3–6). These studies suggest that oral tolerance to collagen hydrolysate suppresses immune responses to autoimmune and inflammatory diseases, although the underlying mechanisms have not been completely elucidated. Collagen triple helix has a highly repeated Gly-X-Y sequence, in particular the Gly-Pro-Hyp sequence. Collagen-derived small peptides also have a variety of physiological functions, such as bone marrow cell differentiation (7, 8), chemotaxis (9–11), angiotensin-converting enzyme inhibition (12–14), and platelet aggregation (15).

It has been suggested that orally administered collagen peptides have beneficial effects on bone metabolism. Adam et al.

showed that the oral intake of collagen hydrolysate with calcitonin exhibited a greater inhibitory effect on bone resorption in osteoporotic patients than did calcitonin alone (16). In mice, the oral ingestion of gelatin increased bone mineral density under protein-undernutrition conditions (17). In both growing and calcium-deficient rats, orally administered collagen peptides exerted beneficial effects on bone mineral density (18). Furthermore, studies have reported that the oral administration of tripeptides derived from collagen accelerated bone healing in rats (19, 20). Several reports have suggested that type I collagen interacts with  $\alpha 2\beta 1$  integrin receptors on cell membranes, thereby inducing the differentiation of bone-marrow cells into osteoblasts (21–23).

Although collagen has been used in therapeutic applications for a long time, the absorption mechanism is not well understood. Prior to absorption, peptides generally undergo proteolytic digestion in the gastrointestinal tract. However, Oesser et al. reported that orally administered collagen hydrolysate can be absorbed from the intestine in a high molecular weight form and be preferentially accumulated in the cartilage tissue in mice (24). We previously reported that several collagen-derived small peptides were detectable in human serum and plasma after the oral ingestion of collagen hydrolysates (25). Pro-Hyp, the major constituent of peptides in plasma derived from chicken type I collagen hydrolysate, exerted a stimulating effect on the growth and migration of skin fibroblasts (26). In the present study, we

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further investigated the absorption of low molecular weight collagen hydrolysate (LMW-CH) in rats by using radioactive tracers. We evaluated the distribution of radioactivity in the body and analyzed the radioactive peptides in the plasma. Additionally, we investigated the effect of the oral administration of LMW-CH on the bone composition of ovary-extracted (OVX) stroke-prone spontaneously hypertensive rats (SHRSPs). We describe here new findings on the absorption mechanism of collagen hydrolysate and its beneficial effects on osteoporosis.

## MATERIALS AND METHODS

**Experimental Animals.** Wistar rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan). SHRSP-Izumo strain rats were obtained from inbred colonies maintained in Tohoku University. The rats were reared with free access to a commercial diet (F-2; Funabashi Farms Co. Ltd., Chiba, Japan) and water. All animals were housed under controlled conditions in air-conditioned rooms at 23 °C, 50% ± 5% humidity, and a 12 h light–dark cycle. All procedures performed involving the animals and the animals' care, were in conformity with the institutional guidelines of Tohoku University.

**Preparation of Collagen Hydrolysate and Qualitative Analysis.** Chicken-foot collagen hydrolysate was prepared from chicken legs, as described previously (14, 25). LMW-CH was obtained by treating the collagen hydrolysate with proteases, as described previously (14). All preparations were of food grade and were obtained from a commercial source (Nippon Meat Packers; Osaka, Japan).

The average molecular weight of LMW-CH was determined by gel-filtration chromatographic analysis. LMW-CH was dissolved in water at a concentration of 10 mg/mL, and the solution was diluted using nine volumes of 30% acetonitrile containing 0.1% trifluoroacetate. The sample was centrifuged at 22000g for 5 min at 4 °C and filtered to remove debris. The sample was then injected into a high-performance liquid chromatograph system with a Superdex peptide column (GE Healthcare Life Sciences; Uppsala, Sweden) and run at a flow rate of 0.5 mL/min for 60 min with isocratic 30% acetonitrile containing 0.1% trifluoroacetate. Elution was monitored by absorbance at 214 nm.

**Administration of LMW-CH and Radiolabeled Tracers.** Forty-eight Wistar rats (5 weeks old) were fed on a commercial diet and distilled water for a week; the rats were equally divided into two groups: a Pro group and a collagen tripeptide (CTp) group. All rats were deprived of food for 14 h. The Pro and CTp groups were provided by gastric intubation 288 mg of LMW-CH with 60 kBq of L-[U-<sup>14</sup>C]Pro (GE Healthcare Life Sciences; Uppsala, Sweden; 9.47 GBq/mmol) or an equivalent amount of the LMW-CH with 570 kBq Gly-[U-<sup>14</sup>C]Pro-Hyp (GE Healthcare Life Sciences; 9.88 GBq/mmol), respectively. Blood, liver, kidney, spleen, cartilage, femurs, tibias, brain, skeletal muscle, and skin were collected before administration and at 0.5 h, 1 h, 3 h, 6 h, and 14 days after administration (each *n* = 4).

**Measurement of Radioactivity in Tissue Samples.** Each aliquot of these tissues was minced with scissors and dissolved in 1 mL of soluen-350 (Packard; Meriden, CT, USA). The radioactivity was counted using 7 mL of scintillation cocktail (Hyonic Fluor; Packard, Meriden, CT, USA). Blood samples (100 μL) were added to 400 μL of soluen-350/isopropanol mixture solution (1:1) and dissolved at 50 °C for 30 min. Then, the samples were decolorized using 500 μL of 30% H<sub>2</sub>O<sub>2</sub>, and the radioactivity was counted using 7 mL of scintillation cocktail.

**Thin-Layer Chromatographic Analysis of Blood and Skin Samples.** Plasma was separated from each blood sample by centrifugation at 1500g for 30 min at 4 °C. Proteins were removed from the plasma by adding three volumes of cold ethanol and centrifuging at 1500g for 30 min at 4 °C. The supernatant was collected and saved for further analysis. Samples were spotted onto a thin-layer chromatography (TLC) plate (FUNACEL SF; Funakoshi Co., Ltd., Tokyo, Japan) with standards and developed in *n*-butanol/acetic acid/water (20:3:7). Spots were detected with ninhydrin solution (ninhydrin splay; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The radioactivity was detected by using a BAS-MS IP plate (Fujifilm Co., Tokyo, Japan) and an FLA-2000 image analyzer. The density of the spot was quantified by using the ImageGauge software (Fujifilm).

**Table 1.** Amino Acid Composition of LMW-CH

amino acid	weight ratio (%)
glycine	21.3
hydroxyproline	12.4
proline	12.0
alanine	8.9
arginine	8.8
glutamic acid/glutamine	11.1
lysine	3.5
aspartic acid/asparagine	5.7
leucine	3.0
serine	2.5
phenylalanine	2.2
threonine	2.0
valine	1.9
isoleucine	1.3
hydroxylysine	1.2
methionine	0.9
histidine	0.8
tyrosine	0.5
cysteine	0
tryptophan	0

Skin tissues (14 days after the administration) were added to 10 volumes of 6 M HCl and hydrolyzed at 110 °C for 24 h. Then the samples were dried under an N<sub>2</sub> stream, dissolved in 50% ethanol, dried again, and dissolved in 50% ethanol. The samples were analyzed using TLC, as described earlier.

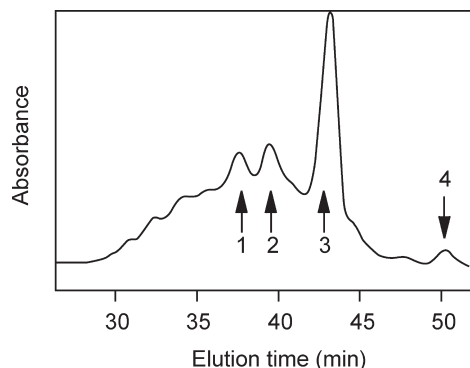
**Administration of LMW-CH to Ovariectomized Rats.** Male SHRSP-Izumo (8 weeks old) rats were equally divided into three groups: control, OVX, and OVX with LMW-CH (OVX-CH) (each *n* = 8). The rats underwent either ovariectomy (the OVX and OVX-CH groups) or a sham operation (the control group) using a dorsal approach under anesthesia with ketamine (Ketalar; Sankyo; Japan) and xylazine (Selactar; Bayer AG; Leverkusen, Germany). One week after the operation, rats in each group were fed on a commercial diet (F-2); the control and OVX groups were provided distilled water, and the OVX-CH group was provided distilled water containing 10% LMW-CH. The amount of water consumed by each rat was measured every day. During the 20 weeks of the experimental period, the body weight of each rat was measured once a week. At the end of the experimental period, blood was collected from the abdominal aorta under diethyl ether anesthesia. The plasma was separated immediately by centrifugation and stored at –20 °C until further analysis. The liver, kidney, adrenal gland, spleen, and uterus were excised and weighed.

**Bone Composition Analyses.** The left femur and tibia were carefully removed and weighed. The water content of samples was determined by drying at 105 °C for 24 h. Bone ash content was determined by ashing at 650 °C for 24 h. The ash was weighed and then dissolved in 10 mL of 6 M HCl, and the phosphorus content was determined by the molybdenum blue method using P-test Wako (Wako Pure Chemical Industries). The organic substance content of samples was calculated by subtracting the ash weight from the dried weight.

**Statistical Analysis.** Statistical significance was evaluated by using ANOVA, along with the Bonferoni test. A *p* value of less than 0.05 was considered statistically significant. Statistical analysis was performed using the Prism 4 software (GraphPad Software Inc., La Jolla, CA, USA).

## RESULTS AND DISCUSSION

**Preparation of LMW-CH.** Avian foot proteins predominantly consist of type I collagen. The composition of LMW-CH prepared from chicken feet was as follows: protein, 92%; fat, 0%; ash, 2%; and carbohydrate, 6%. **Table 1** shows the amino acid composition of the LMW-CH. Nearly 100% of the amino acids of the LMW-CH was derived from collagen, and its composition was almost the same as that of chicken type I collagen (25). **Figure 1** shows the elution profile of the LMW-CH, as analyzed by Superdex peptide chromatography. The LMW-CH mainly



**Figure 1.** Gel filtration profile of LMW-CH. LMW-CH prepared from chicken feet was analyzed using Superdex peptide. Elution was monitored by absorbance at 214 nm. Arrows indicate elution positions of standard peptides. Peak 1, nonapeptide; peak 2, hexapeptide; peak 3, tripeptide; peak 4, amino acid.

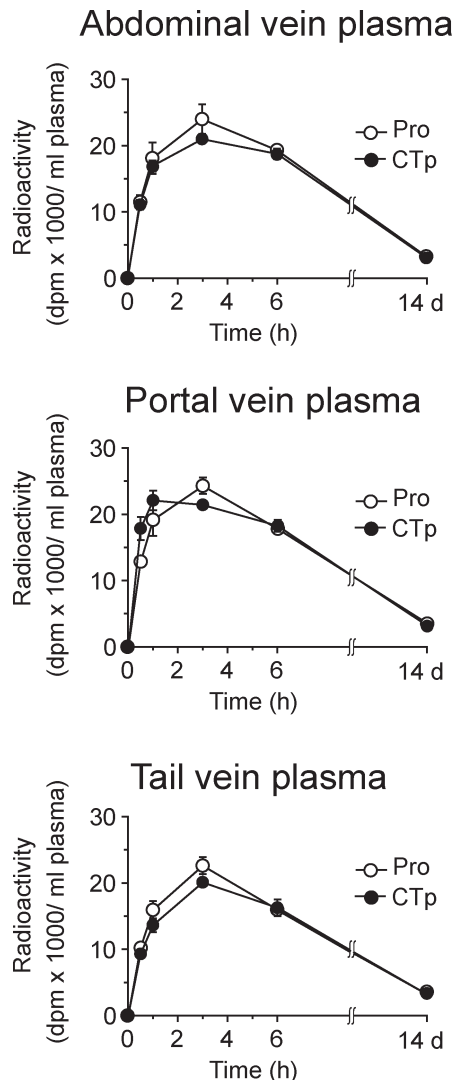
constituted tripeptides, a considerable amount of longer oligopeptides, and a small portion of amino acids. The average molecular weight of the LMW-CH was approximately 800 Da.

**Absorption and Organ Distribution of  $^{14}\text{C}$ -Labeled LMW-CH.** Because the LMW-CH contains a high amount of the tripeptide Gly-Pro-Hyp, which is the main peptide sequence of chicken type I collagen, we used Gly- $^{14}\text{C}$ Pro-Hyp as a tracer for the tripeptide and compared its absorption with  $^{14}\text{C}$ Pro, which we used as a tracer for amino acids. In this experiment, we administered LMW-CH to Wistar rats together with either  $^{14}\text{C}$ Pro (Pro group) or Gly- $^{14}\text{C}$ Pro-Hyp (CTp group) and determined the distribution of radioactivity in the plasma and organs. **Figure 2** shows the time-course appearance of radioactivity in the plasma. In both groups, a rapid increase of radioactivity was observed in plasma from the portal vein, abdominal aorta, and tail vein; the radioactivity reached maximal concentrations 3 h after administration. Subsequently, the radioactivity declined to a nearly undetectable level.

The distribution of radioactivity in the tissues from both groups is shown in **Figure 3**. In both groups, a major portion of the radioactivity was detected in the liver. With respect to the brain, femur, and muscle tissues, radioactivity increased more rapidly in the Pro group than in the CTp group. In contrast, kidney radioactivity was significantly higher in the CTp group than in the Pro group at 0.5 to 3 h after administration. No significant difference was observed between the Pro and CTp groups regarding other organs. These observations suggest that Gly-Pro-Hyp was partly absorbed into blood in the peptide form and accumulated in the kidneys of rats.

Oesser et al. reported that in mice, orally administered, high molecular weight collagen hydrolysate was absorbed by the intestine, and preferentially accumulated in cartilage tissue, but not in the kidneys (24). Unlike the high molecular weight peptide, Gly-Pro-Hyp exhibited no cartilage-specific accumulation in rats. It is known that the intestine and kidney take up di- and tripeptides via  $\text{H}^+$ -coupled peptide transporters (27, 28). The brush-border membrane of the small intestine and kidney expresses PEPT1, whereas PEPT2 is predominantly expressed in the kidney itself (28–30). Therefore, it is likely that Gly-Pro-Hyp is directly taken up by the kidney via PEPTs and is excreted in the urine of rats.

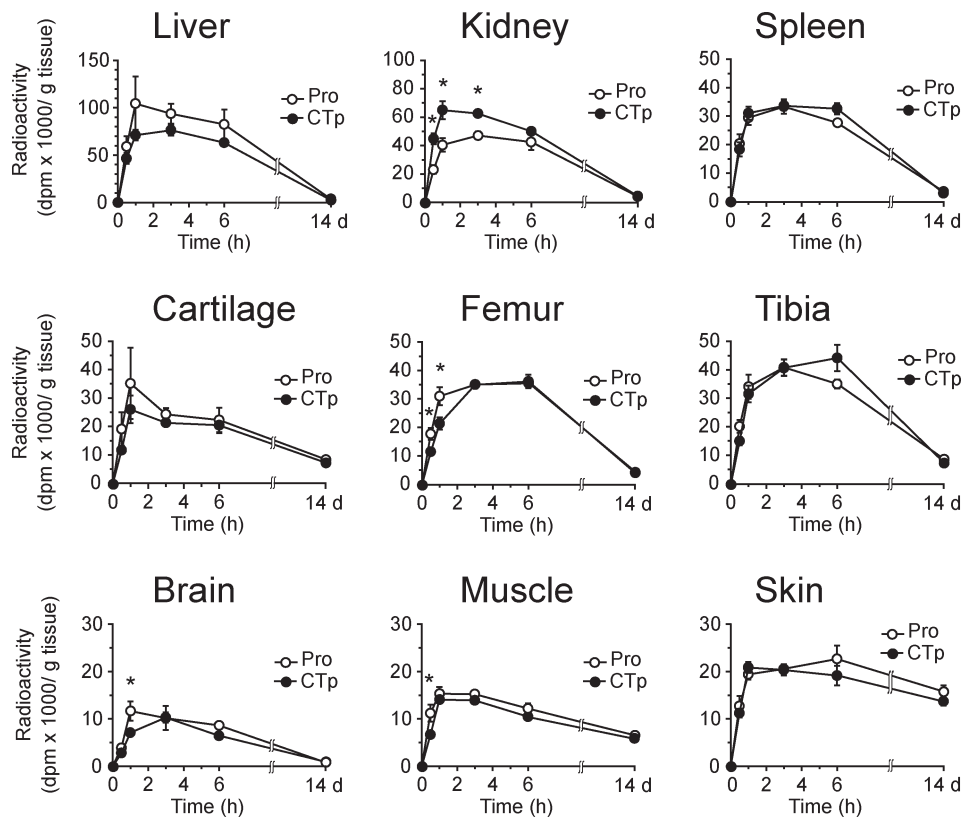
At 14 days after administration, radioactivity mostly disappeared from the organs, except for the skin, for which radioactivity persisted at a level 70% of that observed after 6 h. To confirm whether LMW-CH was utilized for the synthesis of skin proteins, we hydrolyzed the skin in the CTp group 14 days after



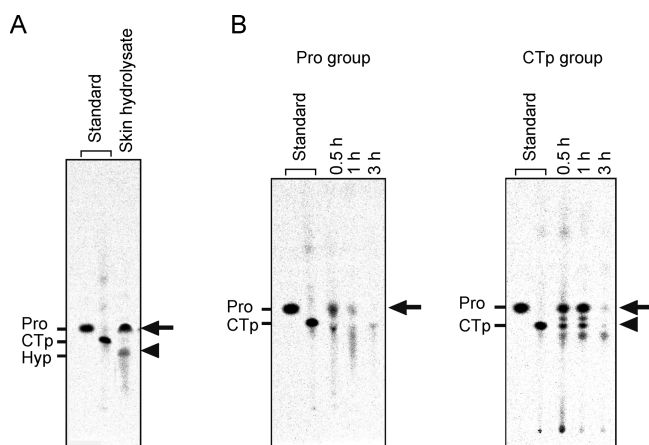
**Figure 2.** Time-course appearance of radioactivity in the plasma in rats administered LMW-CH and radioactive tracers. Wistar rats were administered LMW-CH, together with either  $^{14}\text{C}$ Pro (Pro group) or Gly- $^{14}\text{C}$ Pro-Hyp (CTp group). Blood was collected from the abdominal vein (top panel), the portal vein (middle panel), and the tail vein (bottom panel) of each rat before and at 0.5 h, 1 h, 3 h, 6 h, and 14 days after administration (each  $n = 4$ ). The radioactivity in each sample was adjusted with administered radioactivity and was normalized to the value in 1 mL of plasma. Values shown are the mean  $\pm$  SEM obtained from 4 rats.  $\circ$ , Pro group;  $\bullet$ , CTp group.

administration and analyzed the sample by TLC. **Figure 4A** shows the autoradiograph of the TLC profile. The hydrolyzed skin showed two spots corresponding to the Rf's of Pro and Hyp (indicated by the arrow and arrowhead, respectively). These findings indicate that orally supplemented LMW-CH can be absorbed and utilized for the synthesis of body proteins in rats.

**Gly-Pro-Hyp Present in Plasma As the Peptide Form.** The difference in tissue distribution between the Pro and CTp groups suggests the possibility that Gly-Pro-Hyp was absorbed in a form different from that of free amino acid. To confirm this possibility, we analyzed the radioactive peptides in the plasma from the portal vein using TLC. **Figure 4B** shows the autoradiograph of the TLC profiles. The TLC profiles were clearly different between the Pro and CTp groups. In the Pro group, no spot corresponding to the Rf of the tripeptide was detected, whereas the plasma in



**Figure 3.** Tissue distributions of radioactivity in rats administered LMW-CH and radioactive tracers. Liver, kidney, spleen, cartilage, femurs, tibias, brain, skeletal muscle, and skin were collected from rats in the Pro group and CTP group before and 0.5 h, 1 h, 3 h, 6 h, and 14 days after administration (each  $n = 4$ ). The radioactivity in each sample was adjusted with administered radioactivity and normalized to the value in 1 g of wet tissue. Values shown are the mean  $\pm$  SEM obtained from 4 rats.  $\circ$ , Pro group;  $\bullet$ , CTP group.



**Figure 4.** Autoradiograph of TLC profiles of skin (A) and plasma (B) in rats administered LMW-CH and radioactive tracers. (A) Skin in rats in the CTP group was collected 14 days after administration and analyzed by TLC, as described in Materials and Methods. The arrow and arrowhead show the spots corresponding to the Rf's of Pro and Hyp (0.39 and 0.27, respectively). (B) Plasma in rats in the Pro and CTP groups was collected at 0.5 h, 1 h, and 3 h after administration, from the portal vein, and analyzed by TLC. The arrow and arrowhead show the spots corresponding to the Rf's of Pro and Gly-Pro-Hyp (0.40 and 0.34, respectively).

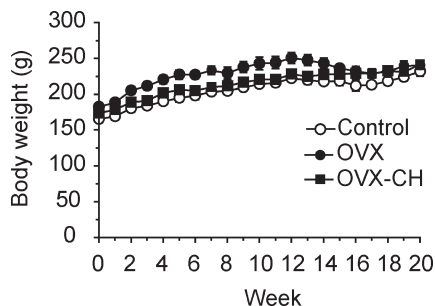
the CTP group showed an obvious spot corresponding to the tripeptide (Figure 4B, arrowhead). The plasma in the CTP group exhibited four spots: those corresponding to the Rf's of the Pro, dipeptides, and tripeptides, as well as an unidentified spot. In contrast, the TLC profile of the plasma in the Pro group showed

two spots, which corresponded to the Rf of Pro and to an unidentified spot (Figure 4B). These results indicate that Gly-Pro-Hyp was absorbed by the blood across the intestinal brush-border membrane in its intact form.

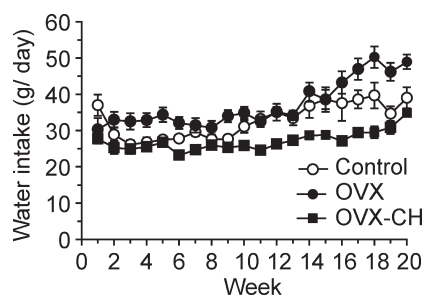
Previously, the authors reported that several food-derived collagen peptides were found in human blood after oral ingestion of collagen hydrolysate; the peptides constituted Pro-Hyp primarily as well as other di- or tripeptides in small portions (Ala-Hyp, Ala-Hyp-Gly, Pro-Hyp-Gly, Leu-Hyp, Ile-Hyp, and Phe-Hyp) (25). Additionally, by using porcine intestinal brush-border membrane vesicles, Aito-Inoue et al. reported that Gly-Pro-Hyp undergoes partial hydrolyzation to Pro-Hyp on the intestinal brush-border membrane before transcellular transport via PEPT1 (31). These differences may be attributable to the difference in substrate specificities between rat and porcine aminopeptidases. It is known that oligopeptides are frequently degraded in a short time by peptidase in blood. However, Gly-Pro-Hyp remained in rat plasma for several hours in its intact form (Figure 4B). In human blood, Pro-Hyp is not digestible by peptidase and is excreted into urine without degradation (31, 32). Thus, Gly-Pro-Hyp is considered to also be resistant to blood peptidases in rats.

**Oral Administration of LMW-CH to OVX-SHRSPs.** We further investigated the effect of collagen hydrolysate on osteoporosis by using ovariectomized SHRSP. Since ovariectomy diminishes endogenous estrogen, OVX-SHRSPs were used as models for postmenopausal women (33, 34). In this experiment, OVX-SHRSPs were administered either distilled water (i.e., the OVX group) or water containing LMW-CH (i.e., the OVX-CH group) for 20 weeks. Sham-operated SHRSPs were used as a control and were provided distilled water for 20 weeks.

**Figure 5** shows the alteration in the body weight of rats in each group. From 2 to 14 weeks, the OVX group exhibited significantly higher body weight than did the control group, which suggested an increase in body fat in the OVX group consequent to estrogen depletion. In contrast, the difference in body weights between the control and OVX-CH groups was not significant. The body weight of the OVX-CH group was significantly lower than that of the OVX group at 7 weeks and from 10 to 13 weeks. **Figure 6** shows the water intake during the experimental period. As compared with the control group, the OVX group showed significantly higher water intake from 18 to 20 weeks. The OVX-CH group showed the lowest water intake (significant differences versus the control group, at 6, 9, 10, 12, and from 14 to 20 weeks; versus the OVX group, at 1, 11, 12, and from 14 to 18 weeks).



**Figure 5.** Growth curves of rats in each group during the experimental period. SHRSPs were ovariectomized and administered distilled water (OVX group) or LMW-CH (OVX-CH group) for 20 weeks. Sham-operated SHRSPs were used as the control group and were provided distilled water for 20 weeks. The body weight of each rat was measured once per week. Values shown are the mean  $\pm$  SEM obtained from rats in the group (control group,  $n = 6$ ; OVX group,  $n = 6$ ; OVX-CH group,  $n = 8$ ).  $\circ$ , control group;  $\bullet$ , OVX group;  $\blacksquare$ , OVX-CH group. Significant differences at  $P < 0.05$ : control group versus OVX group, 2–14 weeks; control group versus OVX-CH group, none; OVX group versus OVX-CH group, 7 and 10–13 weeks.



**Figure 6.** Water intake of rats in each group during the experimental period. Water intake of each rat was measured once per day and averaged with data within a week. Values shown are the mean  $\pm$  SEM obtained from rats in the group (control group,  $n = 6$ ; OVX group,  $n = 6$ ; OVX-CH group,  $n = 8$ ).  $\circ$ , control group;  $\bullet$ , OVX group;  $\blacksquare$ , OVX-CH group. Significant differences at  $P < 0.05$ : control group versus OVX group, 18–20 weeks; control group versus OVX-CH group, 1, 11, 12, and 14–18 weeks; OVX group versus OVX-CH group, 6, 9, 10, 12, and 14–20 weeks.

**Table 2.** Effect of Ovariectomy and LMW-CH Administration on Organ Weight in SHRSP<sup>a</sup>

	liver (g)	spleen (g)	kidney (g)	adrenal glands (g)	uterus (g)	left femur (g)	left tibia (g)
control	8.78 $\pm$ 0.21 a	0.59 $\pm$ 0.06	1.64 $\pm$ 0.05 a	0.06 $\pm$ 0.00 a	0.38 $\pm$ 0.01 a	0.75 $\pm$ 0.02	0.52 $\pm$ 0.02
OVX	7.75 $\pm$ 0.28 b	0.65 $\pm$ 0.02	1.55 $\pm$ 0.07 a	0.05 $\pm$ 0.00 ab	0.06 $\pm$ 0.01 b	0.74 $\pm$ 0.02	0.55 $\pm$ 0.01
OVX-CH	8.98 $\pm$ 0.09 a	0.54 $\pm$ 0.02	2.16 $\pm$ 0.04 b	0.04 $\pm$ 0.00 b	0.12 $\pm$ 0.03 b	0.73 $\pm$ 0.01	0.58 $\pm$ 0.04

<sup>a</sup> Values shown are mean  $\pm$  SEM. Values within a column with different letters are significantly different ( $P < 0.05$ ).

The weights of organs in each group are shown in **Table 2**. In the OVX and OVX-CH groups, the uterus was atrophied. The OVX group had significantly smaller livers than did the control and OVX-CH groups. In contrast, the weight of kidneys was the highest in the OVX-CH group among the three groups. The adrenal glands in the OVX-CH group were significantly smaller as compared with those of the control group. Regarding the spleen, left femur, and left tibia, no significant differences were observed among the three groups.

As a consequence of estrogen depletion, ovariectomy atrophied the uteruses of SHRSPs and increased the rats' body weights. The administration of LMW-CH did not exert any effect on uterus weight in the OVX-SHRSPs, while it decreased the rats' body weight. However, LMW-CH significantly increased kidney weight in OVX-SHRSPs. Wu et al. reported that large amounts of collagen peptide induced renal hypertrophy in growing rats, although undesirable effects were not observed at low doses (18). Because the amount of LMW-CH used in the present study was nearly the same level as that used in their study, it is possible that the increased protein intake in the OVX-CH group caused the hypertrophy. However, the present study also showed that single-dose administration of LMW-CH with Gly-[<sup>14</sup>C]Pro-Hyp to Wistar rats caused an accumulation of radioactivity in their kidneys (**Figure 3**). It is known that SHRSPs develop severe hypertension and malignant nephrosclerosis. The possibility should be considered that the ingestion of a high amount of LMW-CH led to an accumulation of small peptides in the kidneys and promoted hypertrophy in OVX-SHRSPs. Further investigations are required to determine the optimal dose of supplementation with LMW-CH.

**LMW-CH Increased Organic Substance Content in Femurs.** To assess the osteoporosis status in rats, we focused on bone composition in femurs and tibias. Regarding the length and diameter of these bones, no significant difference was observed among the three groups (**Table 3**). **Table 4** shows the water, ash, and organic substance contents of the left femur and left tibia. The OVX-CH group showed the highest value of organic substance content ( $P < 0.05$ , versus the control and OVX groups) and the lowest water content ( $P < 0.05$ , versus the OVX group) of the left femur, among the three groups. Regarding the left tibia, the OVX-CH group also showed the highest organic substance content and the lowest water content, although the difference was not significant. These results indicate that the oral administration of LMW-CH increased the organic substance content of the bone in OVX-SHRSPs.

A number of reports have suggested that the administration of collagen peptide affects bone metabolism. In mice and rats, orally

**Table 3.** Effect of Ovariectomy and LMW-CH Administration on Length and Diameter of the Right Femur in SHRSP<sup>a</sup>

	length (mm)	longest diameter (mm)	shortest diameter (mm)
control	36.3 $\pm$ 0.2	5.1 $\pm$ 0.1	4.1 $\pm$ 0.0
OVX	36.0 $\pm$ 0.2	5.1 $\pm$ 0.1	4.1 $\pm$ 0.0
OVX-CH	35.7 $\pm$ 0.1	5.1 $\pm$ 0.1	4.1 $\pm$ 0.0

<sup>a</sup> The length and the longest and the shortest diameters of the right femur were measured. Values shown are mean  $\pm$  SEM.

**Table 4.** Effect of Ovariectomy and LMW-CH Administration on Bone Composition in SHRSP<sup>a</sup>

	left femur			left tibia		
	ash (%)	organic (%)	water (%)	ash (%)	organic (%)	water (%)
control	40.9 ± 0.3	26.1 ± 0.4 a	33.0 ± 0.7 ab	42.4 ± 2.0	27.1 ± 1.8	30.6 ± 1.0
OVX	38.9 ± 1.0	27.6 ± 1.0 a	33.5 ± 0.6 a	41.3 ± 1.5	27.6 ± 1.5	31.2 ± 1.4
OVX-CH	38.9 ± 0.8	31.2 ± 0.9 b	30.0 ± 1.2 b	43.0 ± 0.7	29.4 ± 0.9	27.6 ± 0.9

<sup>a</sup> Values shown are mean ± SEM. Values within a column with unlike letters are significantly different ( $P < 0.05$ ).

ingested collagen or collagen hydrolysate increased bone mineral density (17, 18). In the present study on OVX-SHRSPs, the administration of LMW-CH affected neither the length nor the diameter of the femur, but increased the organic substance content. Nomura et al. showed that administering sharkskin gelatin to ovariectomized rats increased not only the bone-mineral density of femurs but also the content of type I collagen and glycosaminoglycan in the epiphysis (35). The oral administration of LMW-CH may have a beneficial effect on osteoporosis by increasing the organic substance content of the bone.

#### ABBREVIATIONS USED

CTp, collagen tripeptide; Gly, glycine; Hyp, hydroxyproline; LMW-CH, low molecular weight collagen hydrolysate; OVX, ovary extracted; Pro, proline; SHRSP, stroke-prone spontaneously hypertensive rat; TLC, thin-layer chromatography.

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Received for review September 17, 2009. Revised manuscript received November 10, 2009. Accepted November 11, 2009.