JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 10477–10482 10477
DOI:10.1021/jf902355m

Effect of Collagen Hydrolysates from Salmon and Trout Skins on the Lipid Profile in Rats

Masataka Saito,*,† Chikako Kiyose,§ Tomoyuki Higuchi,† Naoyuki Uchida,# and Hiramitsu Suzuki†

†Laboratory of Food Science and Technology, Kagawa Nutrition University, Chiyoda, Sakado, Saitama 350-0288, Japan, §Department of Applied Bioscience, Kanagawa Institute of Technology, Shimoogino, Atsugi, Kanagawa 243-0292, Japan, and #College of Bioresource Science, Nihon University, Kameino, Fujisawa, Kanagawa 243-0292, Japan

The effect of collagen hydrolysates from fish skins on lipid profile was assessed in rats administered chum salmon or rainbow trout collagen peptide. Single oral administration of soybean oil with or without one of either type of fish collagen peptide demonstrated that rat plasma triglycerides were significantly decreased 2 h later after the intake of oil and peptide mixtures ($p < 0.05$). The free and peptide forms of hydroxyproline derived from fish collagen peptides were statistically higher than those of zero time after oral administration. To test the effect of fish collagen peptides on continuous administration, rats were fed an AIN-93G purified diet containing 0.17% fish collagen peptide. The peptide groups had lower levels of plasma total lipids and triglycerides compared with the control group. However, the body, liver, and fat weights of rats were not significantly different between groups. These results suggest that fish collagen hydrolysates affect lipid absorption and metabolism in rats and may be useful in suppressing the transient increase of plasma triglycerides.

KEYWORDS: Fish skin; type I collagen; collagen hydrolysates; peptide; rat; triglyceride

INTRODUCTION

Functional food including collagen and collagen hydrolysates have recently and extensively appeared on the food market. Commercial collagen hydrolysates, which are called collagen peptide and contain large amounts of type I collagen derived from bovine, porcine, and chicken skins, are hydrolyzed with acid and then hydrolyzed with a food grade enzyme to further break down the molecular weight and are widely used as a protein additive in nutraceutical food and cosmetic applications. However, recent outbreaks of bovine spongiform encephalopathy, foot-and-mouth disease, and avian influenza virus have led to restrictions on the trade of collagen sources and products. Collagen peptides are thus being alternatively derived from fish skin, scale, and bone, and the processing of wastes from several fish species, as safe sources, has started (*1*−*4*).

Collagen is the most abundant structural protein and is present throughout the body. There are at least 27 or more different types of collagen in higher vertebrates (*5*). The major form of collagen is called type I collagen and is widely distributed in the skin, muscle, bone, and viscera of higher vertebrates. Lower vertebrates such as fish also have type I collagen, which makes up the scales, skin, bones, and swim bladder. Fish type I collagen, however, has unique structural features that distinguish it from the type I collagen seen in higher vertebrates such as humans, cattle, pigs, and chickens. For example, many fish have a specific type I collagen that includes a distinctive subunit that is not seen in higher vertebrates (*6*). It has been reported that the amino acid composition and amino acid sequence of fish type I collagens are quite different from those of higher vertebrates (*7*,*8*). Therefore, it is likely that the functionality of fish collagen as a food material is different from that of higher vertebrate collagen.

In general, animal and vegetal proteins are well-known as influences on cholesterol metabolism and experimental atherosclerosis (*9*). In animal experiments, the levels of total cholesterol and serum triglycerides are markedly suppressed by the ingestion of large amounts of gelatin, which is a mixture of water-soluble proteins derived primarily from collagen (*10*, *11*). With regard to the function of collagen peptide, Wu et al. have examined the safety and effectiveness of porcine collagen peptide in rats, focusing on bone metabolism (*12*). Interestingly, their results show that serum triglyceride concentration in rats, which were administered at 0.166 g/kg of body weight per day of collagen peptide from porcine skin, was significantly lower than that in the control group. These results suggest that the ingestion of collagen peptides may have a beneficial effect on lipid absorption and metabolism. However, there is little information available on the hypolipidemic effect of collagen peptide.

To obtain further information on the hypolipidemic effect of collagen peptide and on the functionality of fish type I collagen, normal Sprague−Dawley rats were each administered collagen peptide made from chum salmon or rainbow trout skin. Especially the influence of lipid absorption and metabolism in rats was examined by determining the plasma levels of lipids after administration.

*Corresponding author (telephone/fax +81-49-282-3729; e-mail msaito@eiyo.ac.jp).

Published on Web 10/16/2009

Table 1. Amino Acid Composition of Two Fish Collagen Peptides, Chicken Collagen Peptide, and Porcine Gelatin (Residues/1000)

	salmon	trout	chicken[a]	pig[a]
Asp	53	54	43	45
Hyp	55	54	100	88
Thr	20	23	17	15
Ser	51	50	27	34
Glu	79	81	79	78
Pro	104	95	116	128
Gly	369	369	350	353
Ala	100	89	108	91
Val	14	19	18	22
Cys	0	0	0	0
Met	12	15	4	4
Ile	10	11	10	9
Leu	16	21	24	24
Tyr	2	4	2	4
Phe	12	10	14	14
His	8	9	4	5
Hyl	10	7	10	7
Lys	22	19	23	25
Arg	65	70	50	55
total	1000	1000	1000	1000

[a] Chicken collagen peptide and porcine gelatin were produced by Nippon Meat Packers, Inc., and Sigma Chemical Co., respectively.

MATERIALS AND METHODS

Materials. Salmon collagen peptide from chum salmon, *Oncorhynchus keta*, was a gift from Ihara & Co., Ltd. (Tokyo, Japan). Trout collagen peptide from the skin of rainbow trout, *Oncorhynchus mykiss*, was a gift from Maruha Nichiro Holdings, Inc. (Tokyo, Japan). The protein purity of collagen peptide was >90%. Reagents for biochemical experiments were of the highest quality available from commercial vendors. Porcine skin gelatin was purchased from Sigma Chemical Co. (St. Louis, MO), and chicken collagen peptide was obtained from Nippon Meat Packers, Inc. (Osaka, Japan).

Molecular Weight Distribution and Amino Acid Analysis of Fish Collagen Hydrolysates by High-Performance Liquid Chromatography (HPLC). Molecular weight distributions of the two fish collagen peptide types were analyzed by gel filtration chromatography. A peptide sample, 0.01 mg, was dissolved in 0.01 M Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl and applied to a Superdex peptide 10/300GL column of 10 × 300 mm at a flow rate of 30 mL/h. The sample solution was centrifuged at 21880*g* for 10 min. The upper layer was collected and subjected to chromatography, with a molecular weight standard containing lysozyme (14300), insulin (5800), and substance P (1300). The effluent was monitored at 230 nm.

To determine the amino acid profile of the two fish collagen peptide types, the samples were hydrolyzed with 6 M hydrochloric acid (HCl) at 110 °C, in sealed tubes under reduced pressure, and then the amino acids were analyzed with an LC-VP amino acid analysis system (Shimadzu Co., Kyoto, Japan) equipped with a Shim-pack Amino-Lithium column and an RF-10AXL, following the method of postcolumn derivatization for amino acids with a fluorescent regent, *o*-phthalaldehyde (OPA), and sodium hypochlorite (*13*). The column was maintained at 39 °C, and amino acid derivatives were monitored by a fluorescence detector, with excitation and emission wavelengths set at 350 and 450 nm, respectively. The amino acid profile of the fish collagen peptides presented in **Table 1** shows a typical amino acid composition of fish type I collagen at about 36% glycine and 15% proline and hydroxyproline.

Single Oral Administration of Collagen Hydrolysates. The rats were maintained and treated according to the ethics committee guidelines of Kagawa Nutrition University for the care and use of laboratory animals. Sprague−Dawley International Genetic Standard rats (SD-IGS rats, 7-week-old males, Charles River Japan, Inc., Tokyo, Japan) were housed individually in a temperature-controlled room (25 ± 1 °C), with constant photoperiod (12/12, light/dark). They were given free access to water and to a control diet prepared in our laboratory and allowed to acclimatize for several days. The composition of diet was based on the AIN-93G purified diet and was composed of 10.0% sucrose, 15.2% glucose, 20.0% casein, 39.998% α-cornstarch, 5.0% cellulose, 3.5% mineral mixture, 1.0% vitamin mixture containing choline bitartrate, 0.3% L-cystine, 0.0014% *tert*-butylhydroquinone, and 5.0% soybean oil in mass percentage (*14*). Twelve rats were divided into three groups and were fasted for 14 h; then blood samples from the tail vein were collected at zero time. Rats in the control group (F) were each orally given 0.5 mL of soybean oil. Rats in the lipid and chum salmon (S′) and rainbow trout collagen peptide group (R′) were each simultaneously orally given 0.5 mL of soybean oil and 1.0 mL of fish collagen peptide (0.4 g/mL). Blood samples of the rats were collected at 2, 4, and 8 h, respectively, after a single oral administration. Plasma was prepared as a supernatant through a standardized protocol, after centrifugation at 880*g* for 10 min at 4 °C and stored at −80 °C until use.

Subsequently, 40 SD-IGS rats (7-week-old males) were initially fed, and then 4 rats scarified and the 36 remaining rats divided into 3 groups. These rats were fasted for 18 h previously and then administered the samples. Four rats were sacrificed immediately after the fasting period at zero time. Rats in the lipid group (LG) were each orally given 0.5 mL of soybean oil. The rats in the salmon collagen peptide group (PG) were each orally given 1.0 mL of chum salmon collagen peptide solution (0.4 g/mL). The rats in the lipid and collagen peptide group (LPG) were each simultaneously orally given 0.5 mL of soybean oil and 1.0 mL of salmon collagen peptide (0.4 g/mL). Four rats from each of these groups were sacrificed at 2, 4, and 8 h, respectively, after a single oral administration. After blood collection from the abdominal aorta, the plasma was separated and the livers were also removed and stored at −80 °C.

Continuous Oral Administration of Collagen Hydrolysates. Twelve SD-IGS rats (7-week-old males) were divided into three groups, including a control group (C) and accorded the AIN-93G formula, and two treatment groups containing 0.17% fish collagen peptide from either chum salmon skin (S) or rainbow trout skin (R). After 14 days, the rats were sacrificed and various organs were harvested.

Lipid Profile and Amino Acid Analysis. Determinations of triglycerides, total lipids, phospholipids, and total cholesterol in rat plasma were individually analyzed enzymatically using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Triglyceride levels in the liver were measured enzymatically using a triglyceride assay kit, after extraction of lipids by chloroform/methanol (1:1, v/v). As for amino acid analysis, 1 mL of plasma was deproteinized by the addition of the same volume of 10% trichloroacetic acid (TCA). The TCA fraction was freeze-dried and then dissolved in 500 μL of 0.25 M lithium citrate buffer (pH 2.2). The sample solution was centrifuged at 21880*g* for 10 min. The upper layer was collected and analyzed for free-form hydroxyproline, glycine, and proline. Total hydroxyproline, glycine, and proline were determined as 6 M HCl hydrolysates of the TCA fraction of rat plasma. Hydroxyproline, glycine, and proline levels in rat plasma were measured by the LC-VP amino acid analysis system mentioned above. The peptide form of each amino acid was estimated by subtracting the free form from the total in the HCl hydrolysates of the plasma TCA fraction.

Statistical Analysis. Data values were presented as mean ± standard deviation (SD). The significance of differences between groups at each time were determined by one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test. One-way ANOVA, followed by Dunnett's post hoc test, was used to evaluate the changes in triglycerides against zero time within each group. Statistical analyses were performed using KaleidaGraph ver. 3.6 for Windows (Hulinks Inc., Tokyo, Japan). Differences were considered to be significant at the level of $p < 0.05$.

RESULTS

Properties of Fish Collagen Hydrolysates. Molecular weight distributions of rainbow trout and chum salmon collagen peptides are analyzed by gel filtration chromatography in **Figure 1**. Both elution patterns were quite different, suggesting that the collagen peptides of trout and salmon had a wide distribution of molecular weight and average molecular weights of 3000 and 10000, respectively. Fish collagen peptides are analyzed for amino acid compositions, as listed in **Table 1**; fish collagen peptides are

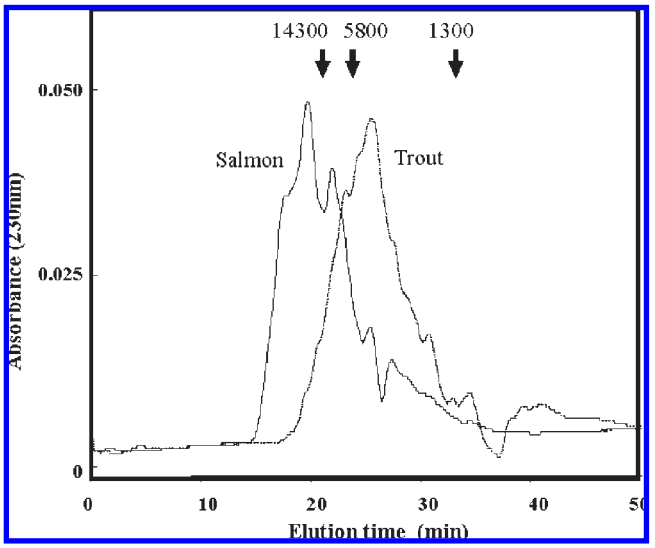

Figure 1. Elution patterns of gel filtration chromatogram of fish collagen peptides from salmon and trout skins. The arrows show the molecular weight standards containing lysozyme (14300), insulin (5800), and substance P (1300).

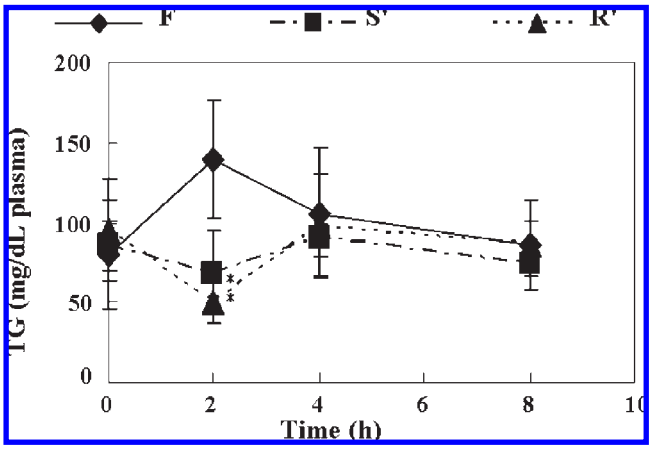

Figure 2. Changes in rat plasma triglycerides from the tail vein after a single oral administration of fish collagen peptides, chum salmon (S′) or rainbow trout collagen peptide group (R′). Values are means ± SD ($n = 4$). The significant difference from group F is shown by an asterisk ($p < 0.01$).

quite similar to type I collagen of the same species (*7*). Fish collagen peptides were obviously different from chicken peptide and porcine gelatin in the lower contents of imino acids, hydroxyproline and proline, and in the higher contents of amino acids, serine and methionine. Furthermore, salmon collagen peptide was characterized by a relatively high content of proline and alanine when compared to trout collagen peptide.

Changes in the Concentrations of Lipid Profile in Rat Plasma. As shown in **Figure 2**, plasma triglycerides from the tail vein in group F rise approximately 2-fold at 2 h after the single oral administration of trout or salmon collagen peptide in comparison with that of zero time. However, the triglycerides of group S′ and R′ at 2 h were statistically lower than that of group F ($p < 0.01$). In addition, the plasma triglyceride concentration from the abdominal aorta of LG after the single administration of salmon collagen peptide rose >2-fold at 2 h in comparison with that at zero time (**Figure 3**). The triglycerides of LPG at 2 h were statistically lower than that of LG. Although the triglyceride level of LPG increased at 4 h, it was lower than that of LG. Moreover, the triglyceride concentration of PG at 2 and 4 h after oral administration was significantly lower than that of zero time. Changes in total lipids are similar to the changes in triglycerides in **Figure 3**. Total cholesterol and phospholipid concentrations in plasma were not significantly different among each group, although at 4 and 8 h their concentrations slightly increased as compared with total lipids in LPG (**Figure 3**). There was no significant difference in triglyceride content in the rat livers among the three groups, LG, PG, and LPG, but the triglycerides of LPG at 2 and 8 h showed a higher tendency than those of LG and PG.

Concentrations of Amino Acid Free Form and Peptide Form in Rat Plasma. Changes in the concentrations of free and peptide forms of hydroxyproline, glycine, and proline are shown in **Figure 4**. The concentration of free hydroxyproline in PG at 2 h is statistically higher than that at zero time after oral administration. Subsequently, free hydroxyproline in PG at 8 h returned to the same level as that at zero time. Furthermore, the peptide form of hydroxyproline in PG was also higher than that at zero time after oral administration. Accordingly, it is clear that both free and peptide forms of hydroxyproline derived from fish collagen peptide have rapidly shifted from the intestine to the blood, and the concentration of free hydroxyproline in LPG at 2 h was also significantly higher than that at zero time. However, there was no significant difference in free hydroxyproline between PG and LPG at 2 h. The changes in free and peptide forms of glycine and proline were found to be the same as those of hydroxyproline.

Effect of Fish Collagen Hydrolysates on Growing Rat. Rats were divided into three groups for continuous administration according to diet composition: the AIN-93G control diet (C), the 0.17% chum salmon collagen peptide diet (S), or the rainbow trout collagen peptide diet (R). There is no significant difference in food intake, body weight, liver weight, and body fat between each groups, as listed in **Table 2**, whereas groups S and R had slightly higher levels of plasma glucose concentration compared with group C and their levels of plasma triglycerides and very low density lipoprotein triglycerides were lower than those of group C. The several lipid concentrations of group R were relatively lower than those of groups C and S. In particular, plasma triglycerides of the R group were significantly lower than that of group C ($p < 0.05$). There was no significant difference in triglyceride levels in the rat livers (**Table 2**).

DISCUSSION

Collagen hydrolysates made from fish skin have been used in food processing as a food material called marine collagen peptide in Japan. This study has examined the effect of fish collagen peptide ingestion on the lipid profile of SD-IGS rats. The plasma triglyceride concentration of groups F and LG at 2 h after a single oral administration rose remarkably in comparison with those of zero time (**Figures 2** and **3**). Interestingly, the triglyceride levels of groups S′, R′, and LPG at 2 h were statistically lower than those of groups F and LG, respectively. Additionally, the triglyceride concentration in PG at 2 and 4 h after administration was significantly lower than that at zero time. These results led to the conclusion that plasma triglyceride reduction was due to the intake of amino acids and/or low molecular weight peptides derived from fish collagen peptide absorbed from the intestine. Fish collagen peptide contains large amounts of glycine, proline, and hydroxyproline, which are modified by hydroxylation of proline, as shown in **Table 1**. To estimate the absorption rate of fish collagen peptide, the change in each amino acid of free and peptide forms in rat plasma when triglyceride concentration was reduced by fish collagen peptide ingestion was investigated

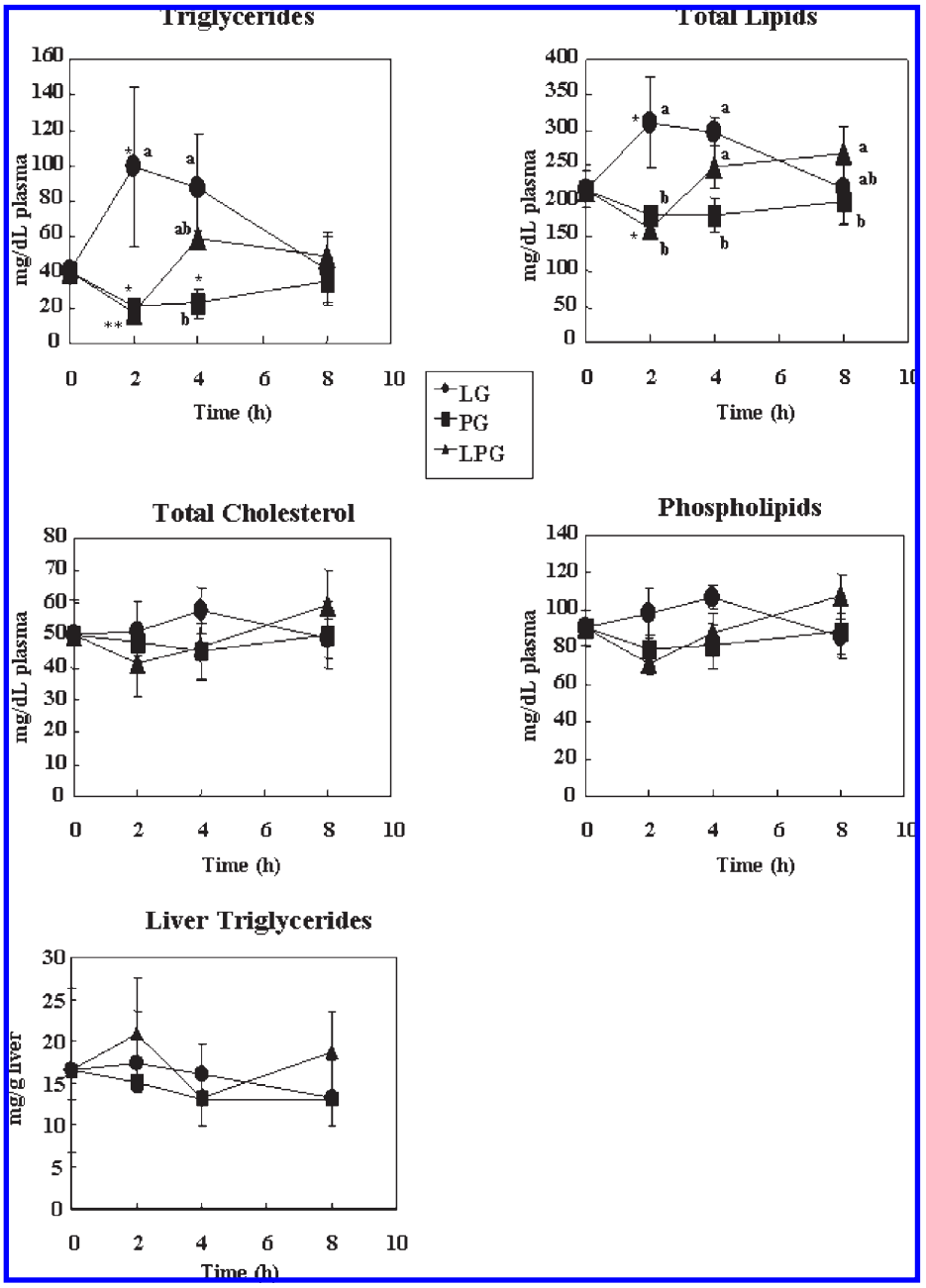

Figure 3. Changes in rat plasma lipids from the abdominal aorta and in liver triglycerides after a single oral administration of salmon collagen peptide. Values are means ± SD (n = 4). Changes are shown in the concentrations of plasma triglycerides, total lipids, phospholipids and total cholesterol, and liver triglycerides of lipid group (LG, ●), collagen peptide group (PG, ■), and lipid and collagen peptides (LPG, ▲). Different letters indicate significant differences between groups at each time ($p < 0.05$). Asterisks show changes in triglycerides against zero time within each group (∗, $p < 0.05$; ∗∗, $p < 0.01$).

(**Figures 3** and **4**). Furthermore, the correlations between plasma triglycerides and total hydroxyproline, glycine, and proline concentrations were calculated, and the results indicated that the concentration of triglycerides in rat plasma correlated negatively with plasma total hydroxyproline ($r = -0.63$, $p < 0.01$) and also correlated negatively with total glycine ($r = -0.53$, $p < 0.01$) and total proline ($r = -0.54$, $p < 0.05$). Therefore, it is clear that plasma triglyceride reduction owing to fish collagen peptide intake has a close relationship with the concentration of amino acid derived fish collagen peptides.

According to these results, two hypotheses are suggested, as follows; one is the direct effect on the delay or acceleration of triglyceride absorption by fish collagen peptide ingestion, and the other is the indirect effect on lipid metabolism by the amino acids and/or peptides derived from fish collagen peptide. There are reports that the intake of soybean protein isolate reduces triglycerides in rat plasma and liver (*15*−*17*). For example, Sugano et al. reported that triglyceride secretions in perfusates of the livers of rats administered soybean protein isolate were significantly lower than those of casein-fed rats (*18*). Moreover, Iritani et al. examined the effect of dietary soybean protein on lipogenic gene expression in rat liver and lipid markers in rat plasma (*19*). Plasma triglyceride concentrations were lower in soybean protein-fed rats than in those fed casein, and liver triglyceride concentrations were also low, in the same way as the plasma. In the present study, liver triglyceride contents of LPG at 2 and 8 h after a single oral administration of salmon collagen peptide were slightly higher than those of LG and PG (**Figure 3**). It is quite likely that the increase in lipid metabolic activity in the liver is induced by the ingestion of salmon collagen peptide.

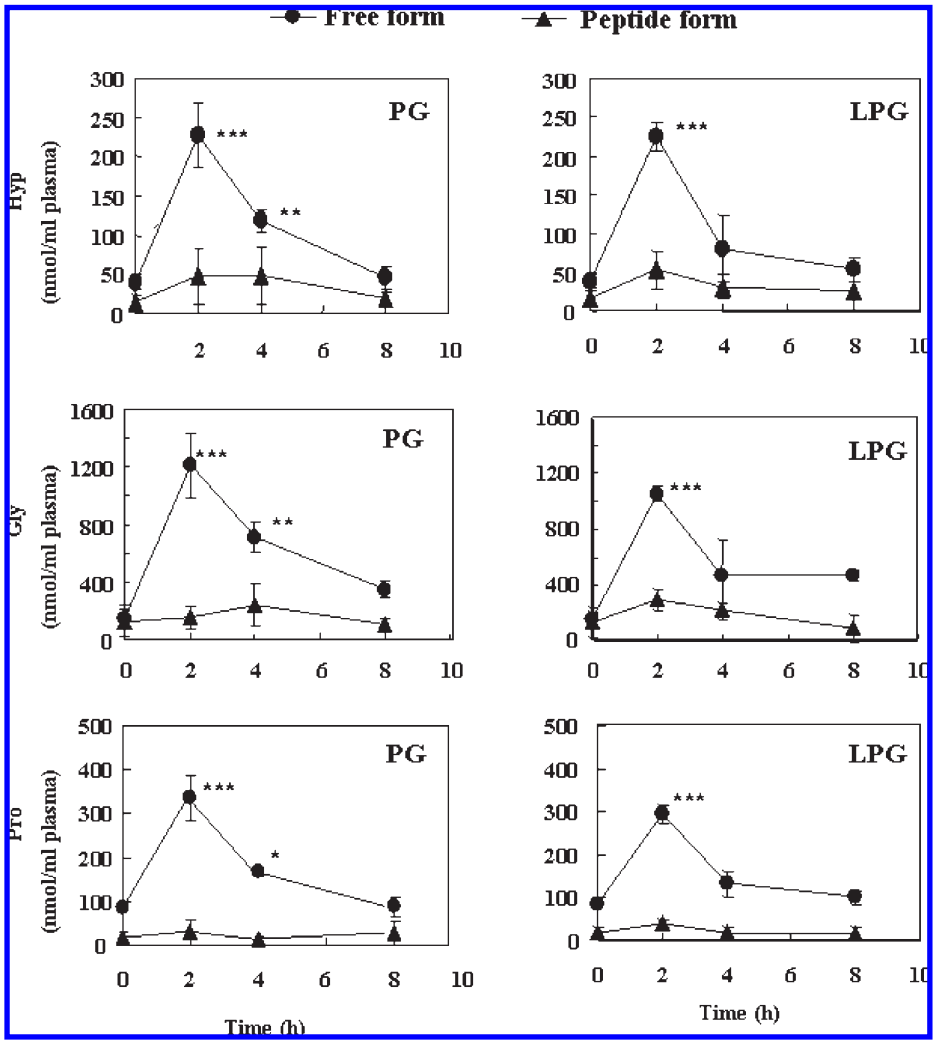

Figure 4. Free and peptide forms of the amino acids in rat plasma after a single administration of salmon collagen peptide. Values are means ± SD ($n = 3$). Collagen peptide group (PG) or lipid and collagen peptide group (LPG) showed the changes in concentrations of hydroxyproline, glycine, and proline free form (●) and hydroxyproline, glycine, and proline peptide form (▲), respectively. Significant difference from zero time is indicated by asterisks (∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$).

On the other hand, there was no significant difference in body weight, liver weight, and body fat between each group on the continuous administration of fish collagen peptides for 14 days (**Table 2**). The change in triglyceride content in the rat livers was not observable. We have been unable to find an increase of fat in rat feces after the continuous administration of fish collagen peptide in a few experiments (data not shown). From these composite results, it is presumed that plasma triglyceride reduction by fish collagen peptide intake may be not the same as the mechanism of that by soybean protein intake and is probably caused by the effect on lipid absorption and metabolism rather than the excretion of lipids and suppression of lipid digestion.

The distribution of molecular weight of trout collagen peptide was lower than that of salmon peptide, and the amino acid composition of each collagen peptide had a few differences (**Figure 1** and **Table 1**). It is reported that the structure and amount of food-derived peptide in human blood is different according to the collagen type and source (*20*). Therefore, plasma lipid reduction is assumed to depend on the molecular weight and structure of each collagen peptide. Until now, several food-derived collagen peptides have been identified in human blood after oral ingestion of collagen peptide (*21*, *22*), and it has been speculated that these peptides act as a biological trigger for collagen synthesis, the inhibition of angiotensin-converting enzyme, and platelet activation (*23*−*25*). In a recent study, oral intake of fish collagen peptide was reportedly associated with an improvement in the moisture content of stratum corneum (*26*). Over the past few years, we have determined the biochemical properties of some fish type I collagens to have different amino acid composition and amino acid sequence than those of higher vertebrates (*7*, *8*) and have found the novel function of fish collagen hydrolysates in animal experiments. It seems some amino acids and peptides derived from fish collagen may be greatly related to the temporary delay or acceleration of triglyceride absorption and metabolism and may be useful in suppressing the transient increase of plasma triglycerides. However, the long-term effect and the mechanism of most of the function of collagen peptide have not been elucidated in detail. Worldwide, collagen hydrolysates and gelatin alternatives made from some fish skin types have been rapidly used in food processing as popular food material (*27*, *28*). The characterization of the function of collagen hydrolysates and gelatin on lipid absorption and metabolism is of importance with respect to the search for dietary regimens that are able to reduce the increase in plasma triglycerides and that deliberately ingest essential lipids and lipid-soluble compounds. Experiments are now in progress to clarify

Table 2. Effect of Fish Collagen Peptides on Body Weight, Liver Weight, Liver Triglyceride, Body Fat, and Blood Parameters of Rats after Continuous Administration for 14 Days

	group C[a]	group S[a]	group R[a]
food intake (g)	322.4 ± 26.3	349.3 ± 21.2	344.8 ± 32.2
body weight (g)	349.8 ± 23.3	343.8 ± 14.6	344.8 ± 18.5
liver weight (g)	13.11 ± 0.91	12.92 ± 0.43	12.56 ± 0.89
liver triglyceride (mg/g of liver)	44.4 ± 19.0	72.0 ± 29.3	49.2 ± 5.70
body fat (g)	10.34 ± 1.97	10.68 ± 1.94	8.70 ± 1.82
blood parameters (mg/dL)			
triglycerides	102.5 ± 17.5	84.0 ± 21.9	63.5[b] ± 6.8
VLDL-TG	71.4 ± 13.1	42.4[b] ± 18.8	41.1[b] ± 10.7
LDL-TG	7.2 ± 0.5	5.4 ± 1.3	5.8 ± 1.4
HDL-TG	2.1 ± 0.5	2.1 ± 0.3	1.7 ± 0.2
total lipids	399.8 ± 47.9	394.3 ± 58.3	332.2 ± 20.9
total cholesterol	69.8 ± 11.5	79.8 ± 15.6	63.4 ± 4.7
VLDL-C	4.4 ± 0.8	3.4 ± 0.7	3.4 ± 0.8
LDL-C	11.6 ± 2.5	13.7 ± 4.5	11.9 ± 1.9
HDL-C	42.2 ± 6.7	49.5 ± 10.7	39.4 ± 3.9
phospholipids	135.5 ± 14.4	147.0 ± 18.8	123.8 ± 7.3
FFA (μequiv/L)	292.0 ± 81.5	522.7 ± 46.7	392.0 ± 84.7
glucose	237.9 ± 44.8	315.7 ± 86.9	299.4 ± 69.6
lipase (U/L)	6.5 ± 0.8	8.5 ± 2.4	6.8 ± 0.5

[a] Twelve SD-IGS rats were divided into three groups, including a control group (C) and two treatment groups containing 0.17% fish collagen peptide from either chum salmon skin (S) or rainbow trout skin (R). [b] Significant difference from group C at $p < 0.05$.

the effect of fish collagen hydrolysates on lipid profile in rats fed a high-fat diet for long-term administration.

LITERATURE CITED

(1) Kimura, S.; Miyauchi, Y.; Uchida, N. Scale and bone type I collagens of carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.* **1999**, *99B*, 473–476.

(2) Nomura, Y.; Sakai, H.; Ishii, Y.; Shirai, K. Preparation and some properties of type I collagen from fish scales. *Biosci., Biotehnol., Biochem.* **1996**, *60*, 2092–2094.

(3) Nagai, T.; Suzuki, N. Isolation of collagen from fish waste material−skin, bone, and fins. *Food Chem.* **2000**, *68*, 277–281.

(4) Morimura, S.; Nagata, H.; Uemura, Y.; Fahmi, A.; Shigematsu, T.; Kida, K. Development of an effective process for utilization of collagen from livestock and fish waste. *Process Biochem.* **2002**, *37*, 1403–1412.

(5) Prockop, D. J.; Kivirikko, K. I. Collagens: molecular biology, diseases, and potentials for therapy. *Annu. Rev. Biochem.* **1995**, *64*, 403–434.

(6) Kimura, S. The interstitial collagens of fish. In *Biology of Invertebrate and Lower Vertebrate Collagens*; Bairati, A., Garrone, R., Eds.; Plenum Press: New York, 1985; pp 397−408.

(7) Saito, M.; Takenouchi, Y.; N. Kunisaki, N.; Kimura, S. Complete primary structure of rainbow trout type I collagen consisting of α1(I)α2(I)α3(I) heterotrimers. *Eur. J. Biochem.* **2001**, *268*, 2817–2827.

(8) Saito, M. The structure and function of fish collagen. In *Current Topics in Biochemistry*; Ohyama, T., Ed.; Research Signpost: Trivandrum, India, 2006; pp 47−66.

(9) Carrol, K. K. Hypercholesterolemia and atherosclerosis: effects of dietary protein. *Fed. Proc.* **1982**, *41*, 2792–2796.

(10) Aust, L.; Poledne, R.; Elhabet, A.; Noack, R. The hypolipaemic action of a glycine rich diet in rats. *Nahrung* **1980**, *24*, 663–671.

(11) Oliveira, D. R.; Portugal, L. R.; Cara, D. C.; Vieira, E. C.; Alvarez-Leite, J. I. Gelatin intake increases the atheroma formation in apoE knock out mice. *Atherosclerosis* **2001**, *154*, 71–77.

(12) Wu, J.; Fujioka, M.; Sugimoto, K.; Mu, G.; Ishimi, Y. Assesment of effectiveness of oral administration of collagen peptides on bone metabolism in growning and mature rats. *J. Bone Miner. Metab.* **2004**, *22*, 547–553.

(13) Ishida, Y.; Fujita, T.; Asai, K. New detection and separation method for amino acids by high-performance liquid chromatography. *J. Chromatogr.* **1981**, *204*, 143–148.

(14) Reeves, P. G.; Nielsen, F. H.; Fahey, N. G.Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *1231*, 939–1951.

(15) Iritani, N.; Nagashima, K.; Fukuda, H.; Katsurada, A.; Tanaka, T. Effects of dietary proteins on lipogenic enzymes in rat liver. *J. Nutr.* **1986**, *116*, 190–197.

(16) Nimal Ratnayakea, W. M.; Sarwara, G.; Laffeya, P. Influence of dietary protein and fat on serum lipids and metabolism of essential fatty acids in rats. *Br. J. Nutr.* **1997**, *78*, 459–467.

(17) Tachibana, N.; Matsumoto, I.; Fukui, K.; Arai, S.; Kato, H.; Abe, K.; Takamatsu, K. Intake of soy protein isolate alters hepatic gene expression in rats. *J. Agric. Food Chem.* **2005**, *53*, 4253–4257.

(18) Sugano, M.; Tanaka, K.; Ide, T. Secretion of cholesterol, triglyceride and apolipoprotein A-I by isolated perfused liver from rats fed soybean protein and casein or their amino acid mixtures. *J. Nutr.* **1982**, *112*, 855–862.

(19) Iritani, N.; Hosomi, H.; Fukuda, H.; Tada, K.; Ikeda, H. Soybean protein suppresses hepatic lipogenic enzyme gene expression in wistar fatty rats. *J. Nutr.* **1996**, *126*, 380–388.

(20) Ohara, H.; Matsumoto, H.; Ito, K.; Iwai, K.; Sato, K. Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *J. Agric. Food Chem.* **2007**, *55*, 1532–1535.

(21) Iwai, K.; Hasegawa, T.; Taguchi, Y.; Morimatsu, F.; Sato, K.; Nakamura, Y.; Higashi, A.; Kido, Y.; Nakabo, Y.; Ohtsuki, K. Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *J. Agric. Food Chem.* **2005**, *53*, 6531–6536.

(22) Aito-Inoue, M.; Ohtsuki, K.; Nakamura, Y.; Park, E. Y.; Iwai, K.; Morimatsu, F.; Sato, K. Improvement in isolation and identification of food-derived peptides in human plasma based on precolumn derivatization of peptides with phenyl isothiocyanate. *J. Agric. Food Chem.* **2006**, *54*, 5261–5266.

(23) Oshima, G.; Shimabukuro, H.; Nagasawa, K. Peptide inhibitors of angiotensin I-converting enzyme in digests of gelatin by bacterial collagenase. *Biochim. Biophys. Acta* **1979**, *566*, 128–137.

(24) Kim, S. K.; Byun, H.G.; Park, P. J.; Shahidi, F. Angiotensin I converting enzyme inhibitory peptides purified from bovine skin gelatin hydrolysate. *J. Agric. Food Chem.* **2001**, *49*, 2992–2997.

(25) Knight, C. G.; Morton, L. F.; Onley, D. J.; Peachey, A. R.; Ichinohe, T.; Okumura, M.; Farndale, R. W.; Barnes, M. J. Collagen-platelet interaction: Gly-Pro-Hyp is uniquely specific for platelete Gp VI and mediates platelet activation by collagen. *Cardiovasc. Res.* **1999**, *41*, 450–457.

(26) Ohara, H.; Ito, K.; Iida, H.; Matsumoto, H. Improvement in the moisture content of the stratum corneum following 4 weeks of collagen hydrolysate ingestion. *Nippon Shokuhin Kagaku Kogaku Kaishi*. **2009**, *56*, 137–145.

(27) Karim, A. A.; Bhata, R. Gelatin alternatives for the food industry: recent developments, challenges and prospects. *Trends Food Sci. Technol.* **2008**, *19*, 644–656.

(28) Karim, A. A.; Bhata, R. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids* **2009**, *23*, 563–576.

Received for review July 8, 2009. Revised manuscript received September 19, 2009. Accepted September 30, 2009. This research was supported by a research grant from the High-Tech Research Center Project for private Universities: matching fund subsidy from Ministry of Education, Culture, Sports, Science and Technology (2008-2009).