Alaska Pollack Protein Prevents the Accumulation of Visceral Fat in Rats Fed a High Fat Diet

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Summary  In the first study (Study 1), 4-wk-old Sprague-Dawley (SD) rats were fed high fat diets containing casein, Alaska pollack, yellowfin tuna, or chicken as the protein source for 28 d. The purpose of this study was to compare the effect of Alaska pollack protein with other animal proteins (casein, yellowfin tuna, and chicken) on the prevention of visceral fat accumulation. We found that Alaska pollack protein was a more potent inhibitor of visceral fat accumulation than the other proteins ($p<0.05$). In the second study (Study 2), we determined the quantity of Alaska pollack protein needed to have an effect. To test this, 4-wk-old SD rats were fed diets containing different percentages of Alaska pollack proteins (0, 3, 10, 30 or 100%) to replace casein as the protein source for 28 d. The diets with 30 or 100% Alaska pollack protein as the protein source prevented visceral fat accumulation and elevated plasma adiponectin levels. Based on these findings, an inhibitory effect on the accumulation of visceral fats can be achieved by consuming a diet in which 30% or more of the total protein content comes from Alaska pollack.

Key Words  Alaska pollack protein, high fat diet, visceral adipose tissue, adiponectin

The aim of the present study was to determine the effect of different animal protein sources on visceral fat accumulation and adiponectin secretion in Sprague-Dawley (SD) rats.

In recent years, hypertension, hyperlipidemia and diabetes mellitus have emerged as worldwide problems. Obesity is well known as a major cause of these diseases. To reduce obesity, food or energy intake must be reduced and physical activity should be increased. However, many people find it difficult to reduce food intake or increase physical activity and often resort to anti-obesity treatments such as surgical procedures, drugs or supplements. Therefore, ingestible substances that have the potential to prevent the accumulation of visceral fat could be used to prevent obesity and associated diseases. In our previous animal study, we found that using Alaska pollack as the source of dietary protein prevented high fat diet-induced visceral fat accumulation. The aim of the present studies was to determine whether this effect was specific to Alaska pollack protein. Therefore, we compared the effects of Alaska pollack protein with that of yellowfin tuna and chicken protein on the prevention of visceral fat accumulation. In addition, because these proteins can be used as functional foods with specific health effects for humans, we also wanted to determine the relative dietary composition of Alaska pollack protein that was needed to exert this effect. Therefore, the second aim was to determine the optimal proportion of Alaska pollack meat to replace casein in the diet to prevent visceral fat accumulation.

MATERIALS AND METHODS

1. Preparation of protein samples and diets. Chicken (Gallus gallus domesticus) meat raised in Iwate Prefecture was purchased from a market. Meat of Alaska pollack (Theragra chalcogramma) from Alaska and yellowfin tuna (Thunnus albacares) from Shizuoka Prefecture were products of Nippon Suisan Kaisha, Ltd. The protein samples were freeze-dried, ground, and the fat component was removed using solvents. In Study 1, we used chloroform and methanol from Wako Pure Chemical Industries, Ltd. to remove the fat component from the protein samples. To remove the solvent residues, the samples were dried at room temperature in a draft chamber, and then heated at 80˚C for 4 h. In Study 2, ethyl acetate and ethanol from Wako Pure Chemical Industries, Ltd. were used to remove the fat component. The test diets used in the study were prepared by Research Diets, Inc. (New Brunswick, NJ, USA). Nutritional analysis of each protein sample and the test diets was performed by SRL, Inc. (Tokyo, Japan), and the analysis of total amino acids and fatty acids of each protein sample was performed by the Food Safety Research Center, Nippon Suisan Kaisha, Ltd.

Overall composition, amino acid composition and fatty acid composition of the different protein samples
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There were no differences in these values between Study 1 and Study 2. Analysis of the fatty acid composition revealed that the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content per 100 g of protein were 0.78 mg and 0.58 mg for the casein protein, 113.29 mg and 168.95 mg for the Alaska pollack protein, 24.93 mg and 155.30 mg for the yellowfin tuna protein, and 3.02 mg and 11.40 mg for the chicken protein, respectively. The total amount of DHA and EPA in the protein samples was confirmed to be no greater than 0.5%. Accordingly, the effects of these substances on the study results are considered to be negligible.

Table 1. Composition of the experimental diet (g).

|                | Control | HFc (3, 10, 30, 100%) | HFn | HFc (11001) |
|----------------|---------|----------------------|-----|-------------|
| Casein         | 200     | 200                  | 194, 180, 140, — | —  | —  |
| t-Cystein      | 3       | 3                    | 3   | 3           | 3   |
| Pollack        | —       | —                    | 6, 20, 60, 200   | —  | —  |
| Yellowfin tuna | —       | —                    | —   | 200         | —   |
| Fowl           | —       | —                    | —   | 200         | —   |
| Cornstarch     | 315     | 72.8                 | 72.8 | 72.8        |
| Maltoextrin 10 | 35      | 100                  | 100  | 100         |
| Sucrose        | 350     | 172.8                | 172.8 | 172.8       |
| Cellulose, BW200 | 50   | 50                   | 50   | 50          |
| Corn oil       | 25      | 25                   | 25   | 25          |
| Lard           | 20      | 177.5                | 177.5 | 177.5       |
| Mineral mix S10026 | 10 | 10                   | 10   | 10          |
| Dicalcium phosphate       | 13 | 13                   | 13   | 13          |
| Calcium carbonate      | 5.5    | 5.5                  | 5.5  | 5.5         |
| Potassium citrate+H2O   | 16.5  | 16.5                 | 16.5 | 16.5        |
| Vitamin mix V10001     | 10      | 10                   | 10   | 10          |
| Choline bitartrate     | 2       | 2                    | 2    | 2           |

HFc, high fat+casein group; HFn, high fat+Alaska pollack group; HFc, high fat+yellowfin tuna group; HFc, high fat+chicken group.

Table 2. Amino acid composition of protein sources (g/100 g).

|                | Casein | Pollack | Yellowfin tuna | Chicken |
|----------------|--------|---------|----------------|---------|
| Taurine        | 0      | 0.66    | 0.06           | 0.07    |
| Aspartic acid  | 5.62   | 8.62    | 7.94           | 7.96    |
| Threonin       | 3.48   | 3.65    | 3.58           | 3.59    |
| Serine         | 4.40   | 3.45    | 2.89           | 3.03    |
| Glutamic acid  | 20.61  | 13.95   | 12.59          | 13.81   |
| Glycine        | 1.67   | 3.83    | 3.75           | 3.70    |
| Alanine        | 2.72   | 5.32    | 4.86           | 4.97    |
| Valine         | 5.93   | 4.73    | 4.69           | 4.59    |
| Cystine        | 0      | 0.37    | 0.31           | 0.40    |
| Methionine     | 2.38   | 1.43    | 1.23           | 1.11    |
| Isoleucine     | 4.71   | 4.41    | 4.32           | 4.50    |
| Leucine        | 8.22   | 7.46    | 6.86           | 7.14    |
| Tyrosine       | 4.75   | 3.23    | 3.00           | 2.97    |
| Phenylalanine  | 4.32   | 3.69    | 3.32           | 3.48    |
| Lysine         | 7.03   | 8.53    | 7.94           | 8.04    |
| 1-Methylhistidine | 0  | 0.35    | 1.16           | 1.69    |
| Histidine      | 2.43   | 1.91    | 6.26           | 2.90    |
| Arginine       | 3.22   | 5.89    | 5.12           | 5.74    |
| AsparaginPe      | 0      | 5.51    | 5.21           | 5.69    |
| Proline        | 8.89   | 5.82    | 5.54           | 5.59    |

Table 3. Fatty acid composition of protein sources (mg/100 g).

|                | Casein | Pollack | Yellowfin tuna | Chicken |
|----------------|--------|---------|----------------|---------|
| Tetradecanoic acid         | 31.46  | 7.60    | 5.64           | 8.95    |
| Tetradecenoic acid         | 1.55   | 0.00    | 0.00           | 0.00    |
| Pentaenoic acid            | 3.21   | 1.29    | 4.70           | 1.28    |
| Hexadecenoic acid          | 84.04  | 113.97  | 168.20         | 254.42  |
| Hexadecenoic acid          | 4.65   | 9.85    | 20.09          | 34.07   |
| Heptadecanoic acid         | 1.66   | 2.14    | 6.85           | 1.98    |
| Heptadecenoic acid         | 0.72   | 0.68    | 0.94           | 0.93    |
| Octadecanoic acid          | 41.18  | 14.35   | 69.42          | 107.33  |
| Octadecenoic acid          | 74.38  | 70.24   | 103.22         | 436.86  |
| Octadecadecanoic acid      | 5.34   | 5.01    | 5.78           | 158.72  |
| Octadecatetraenoic acid    | 3.57   | 2.03    | 1.61           | 0.93    |
| Octadecetetraenoic acid    | 0.25   | 0.28    | 0.87           | 1.16    |
| Octadecatetraenoic acid    | 2.27   | 4.05    | 0.94           | 8.26    |
| Icosanoic acid             | 0.75   | 0.28    | 0.87           | 0.00    |
| Icosanoic acid             | 0.86   | 9.62    | 4.50           | 13.95   |
| Icosanoic acid             | 0.00   | 0.62    | 1.34           | 1.28    |
| Icosanoic acid             | 0.00   | 0.39    | 0.54           | 0.70    |
| Icosanoic acid             | 0.25   | 0.39    | 0.94           | 8.95    |
| Icosanoic acid             | 0.30   | 2.42    | 1.21           | 0.00    |
| Icosanoic acid             | 0.00   | 10.81   | 31.45          | 41.98   |
| Icosanoic acid             | 0.78   | 113.29  | 24.93          | 3.02    |
| Docosanoic acid            | 0.00   | 0.00    | 1.01           | 0.93    |
| Docosanoic acid            | 0.39   | 3.83    | 0.00           | 11.40   |
| Docosanoic acid            | 0.00   | 2.19    | 0.00           | 2.44    |
| Docosanoic acid            | 0.78   | 6.87    | 5.85           | 9.19    |
| Docosanoic acid            | 0.58   | 168.95  | 155.30         | 11.40   |
| Tetracosanoic acid         | 0.14   | 1.18    | 2.42           | 0.00    |

are shown in Tables 1, 2 and 3, respectively. There were no differences in these values between Study 1 and Study 2. Analysis of the fatty acid composition revealed that the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content per 100 g of protein were 0.78 mg and 0.58 mg for the casein protein, 113.29 mg and 168.95 mg for the Alaska pollack protein, 24.93 mg and 155.30 mg for the yellowfin tuna protein, and 3.02 mg and 11.40 mg for the chicken protein, respectively. The total amount of DHA and EPA in the protein samples was confirmed to be no greater than 0.5%. Accordingly, the effects of these substances on the study results are considered to be negligible. Table 4 shows the nutrition analysis of the test diet protein sources.
housing individually in aluminum cages (W25 × H18 cm) with a mesh bottom. During the acclimatization period, up to five animals were housed in single stainless-steel wire mesh cages (W37 × H18 cm) with a mesh bottom. During the 28 d feeding period, the animals were housed in a barrier animal room maintaining an ambient temperature of 24 ± 2°C and humidity of 55 ± 10% with an air exchange rate of 20 times per hour and a 12 h light (7:00 am–7:00 pm) and 12 h dark cycle. During the acclimatization period, up to five animals were housed in single stainless-steel wire mesh cages (W37 × D40 × H18 cm) with a mesh bottom. During the 28 d feeding period, the animals were housed individually in aluminum cages (W25 × D40 × H18 cm) with a flat plate bottom. The cages were replaced at least twice a week with clean cages that contained autoclaved bedding (made of fir shavings). All rats were allowed free access to Labo MR Stock diet from Nosan Corporation (Kanagawa, Japan) and chlorinated well water during the experiment.

Food consumption was also determined every 2 d during this period. On days 7, 14 and 21, blood samples were collected from orbital veins to determine plasma levels of insulin and adiponectin. After the 28 d feeding period, blood samples were collected without anesthesia from the caudal veins of animals fasted for 16 h to determine blood glucose levels as described above. Laparotomy was performed under isoflurane anesthesia and whole blood was collected and plasma samples were prepared. Liver and visceral adipose tissues (as for Study 1) were dissected and their wet weights were measured. These samples were stored at −80°C until used for biochemical analysis.

Study 2: As in Study 1, the body weights of the animals were measured before and after the 28-d feeding period, and food consumption was also determined during this period. On days 7, 14 and 21, blood samples were collected from orbital veins to determine plasma adiponectin levels.

The animal experiments were performed under contract by Japan SLC, Inc. (Shizuoka, Japan) and were approved by the Laboratory Animal Care and Use Committee of Japan SLC, Inc. Eighty male SD rats (SPF) aged 3 wk old, from Japan SLC, Inc. were used in this study. Initially, the rats were fed for 5 d to acclimatize them to the housing conditions; their physical conditions and suitability for the study were evaluated.

The animals were housed in a barrier animal room maintained at an ambient temperature of 24 ± 2°C and humidity of 55 ± 10% with an air exchange rate of 20 times per hour and a 12 h light (7:00 am–7:00 pm) and 12 h dark cycle. During the acclimatization period, up to five animals were housed in single stainless-steel wire mesh cages (W37 × D40 × H18 cm) with a mesh bottom. During the 28 d feeding period, the animals were housed individually in aluminum cages (W25 × D40 × H18 cm) with a flat plate bottom. The cages were replaced at least twice a week with clean cages that contained autoclaved bedding (made of fir shavings). All rats were allowed free access to Labo MR Stock diet from Nosan Corporation (Kanagawa, Japan) and chlorinated well water during the experiment.

Study 1: The composition of each test diet is shown in Table 1. After completion of the acclimatization period, the rats were assigned to the following five groups, keeping the mean weight of each group the same: the control group (n = 7), high fat + casein (HFc; n = 7), high fat + Alaska pollack (HFp; n = 8), high fat + yellowfin tuna (HFt; n = 8) and high fat + chicken (HFc; n = 8).

Study 2: As shown in Table 1, diets containing different percentages of Alaska pollack and casein protein were used. After completion of the acclimatization period, the rats were assigned to the following five groups keeping the mean weight of each group the same: high fat + 100% casein (HFc100; n = 8), high fat + 3% Alaska pollack (HFp3; n = 8), high fat + 10% Alaska pollack (HFp10; n = 8), high fat + 30% Alaska pollack (HFp30; n = 8), and high fat + 100% Alaska pollack (HFp100; n = 8).

Measurements and analysis procedures.

Study 1: The animals were weighed before the start and after the completion of the 28 d feeding period. Food consumption was also determined every 2 d during the feeding period. The weight of the food consumed was calculated by subtracting the new weight of the feeders from the known weight of the full feeders. After 28 d, blood samples were collected from the caudal veins of unanesthetized non-fasting animals. Blood glucose levels were determined using a simplified blood glucose monitoring system, One Touch Ultra (Johnson & Johnson, Tokyo, Japan). Then, laparotomy was performed under isoflurane anesthesia and whole blood was collected from the abdominal aorta. Blood samples were transferred into heparinized tubes and centrifuged to obtain plasma samples. The liver and adipose tissue around the mesentery, kidneys, and epididymides were dissected and their wet weights were measured. These samples were frozen in liquid nitrogen and stored at −80°C until used for biochemical analysis.

Study 2: As in Study 1, the body weights of the animals were measured before and after the 28-d feeding period, and food consumption was also determined during this period. On days 7, 14 and 21, blood samples were collected from orbital veins to determine plasma adiponectin levels.

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2. Animal treatment. The animal experiments were performed under contract by Japan SLC, Inc. (Shizuoka, Japan) and were approved by the Laboratory Animal Care and Use Committee of Japan SLC, Inc. Eighty male SD rats (SPF) aged 3 wk old, from Japan SLC, Inc. were used in this study. Initially, the rats were fed for 5 d to acclimatize them to the housing conditions; their physical conditions and suitability for the study were evaluated.

The animals were housed in a barrier animal room maintained at an ambient temperature of 24 ± 2°C and humidity of 55 ± 10% with an air exchange rate of 20 times per hour and a 12 h light (7:00 am–7:00 pm) and 12 h dark cycle. During the acclimatization period, up to five animals were housed in single stainless-steel wire mesh cages (W37 × D40 × H18 cm) with a mesh bottom. During the 28 d feeding period, the animals were housed individually in aluminum cages (W25 × D40 × H18 cm) with a flat plate bottom. The cages were replaced at least twice a week with clean cages that contained autoclaved bedding (made of fir shavings). All rats were allowed free access to Labo MR Stock diet from Nosan Corporation (Kanagawa, Japan) and chlorinated well water during the experiment.

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4. Biochemical analysis. Insulin and adiponectin levels in each plasma sample were measured using rat insulin ELISA Kit No. AKRIN-0108 from Shibuyagi (Gunma, Japan) and mouse/rat adiponectin ELISA Kit No. 410713 from Otsuka Pharmaceutical (Tokyo, Japan), respectively.

5. Statistical analysis. The results are expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used to test differences between the groups. p values < 0.05 were considered to be statistically significant.

RESULTS

Study 1

Table 5A shows the body weight before and after the feeding period, and the total energy intake. The mean body weight after the feeding period was significantly lower (p < 0.01) in the control (187.0 ± 29.6 g) and HFp (186.6 ± 60.5 g) groups than in the HFc (248.2 ± 40.0 g), HFt (283.3 ± 21.5 g) and HFc (275.8 ± 18.2 g) groups. Total energy intake during the feeding period was significantly greater (p < 0.05) in the HFc (1,928.1 ± 306.5 kcal), HFp (1,819.9 ± 360.7 kcal), HFt (2,116.3 ± 175.3 kcal) and HFc (2,085.8 ± 222.5 kcal) groups than in the control group (1,439.0 ± 185.7 kcal), but was not significantly different between the HFc, HFp, HFc and HFc groups. These results confirmed that food consumption under high fat dietary conditions was not affected by the type of protein.

| Test       | Casein | Pollack | Yellowfin tuna | Chicken |
|------------|--------|---------|----------------|---------|
| Energy (kcal) | 38.3   | 369     | 373            | 379     |
| Protein (g)  | 91.5   | 92.3    | 93             | 93.2    |
| Fat (g)      | 0.3    | 0.6     | 0.7            | 1.2     |
| Carbohydrate (g) | 2.6    | 0       | 0              | 0       |
| Water (g)    | 3.7    | 2       | 2.2            | 1.8     |
| Ash (g)      | 1.5    | 5.7     | 4.7            | 4.3     |

Table 4. Nutritional analysis of protein sources (per 100 g).
Blood glucose levels were similar in the control (128.7±17.3 mg/dL), HFc (128.7±11.7 mg/dL), HPc (128.6±30.1 mg/dL), HPt (139.1±18.2 mg/dL) and HFck (134.8±11.0 mg/dL) groups. Figure 1 shows the weights of the visceral adipose tissue in each group. Compared with the control group, the HFc, HPt and HFck groups, but not the HPc group, had significantly higher visceral adipose tissue weights, which were increased by 85.3, 108.1, 98.0 and 14.4%, respectively \( (p<0.01) \). The increase was significantly lower in the HPc group than in the HFc, HPt and HFck groups \( (p<0.05) \).

Figure 2 shows the plasma insulin levels. The plasma insulin levels were significantly higher \( (p<0.05) \) in the HFc (2.35±1.35 ng/mL), HPt (2.10±1.23 ng/mL) and HFck groups (1.97±0.49 ng/mL) than in the control group (1.42±0.91 ng/mL), by 66.0, 48.6 and 38.8%, respectively. In contrast, the plasma insulin level was significantly lower in the HPc group (0.81±0.70 ng/mL) than in the HFc, HPt and HFck groups (all \( p<0.03) \), and was even lower than in the control group.

Figure 3 shows the plasma adiponectin levels, which were significantly lower in the HPc group (0.70±0.85 ng/mL) and HFck, high fat+chicken group, \( p<0.05 \) in the HFc (1.76±0.70 μg/mL), HPt (1.74±0.06 μg/mL) and HFck (1.74±0.08 μg/mL) groups by 6.5, 7.6 and 7.2%, respectively, than in the HPc group (1.88±0.05 μg/mL).
Study 2

Table 5B shows the body weight before and after the feeding period and the total energy intake. Body weight at the end of the feeding period was significantly higher \((p<0.05)\) in the HFp10 group (318.4 \pm 31.0 g) than in the HFc100 group (291.7 \pm 16.3 g), but was not significantly different between the other groups. No statistically significant between-group difference in total energy intake was found.

Figure 4 shows the visceral adipose tissue weights in each group. The mean visceral adipose tissue weight was significantly lower in the HFp10 group (11.70 \pm 3.06 g) and HFp100 (12.06 \pm 1.63 g) groups than in the HFc100 group (15.10 \pm 2.25 g) by 22.5% \((p<0.03)\) and 20.2% \((p<0.01)\), respectively. This indicates that consumption of a diet comprising 30% or more of dietary protein as Alaska pollack protein decreases visceral fat accumulation.

The weight of visceral adipose tissues differed between Study 1 and Study 2. This may be due to the different methods used to remove the fat component in each study, which may have influenced the results.

The plasma insulin level was not significantly different between any of the high fat feeding groups. The plasma insulin level tended to be lower in the control group than the high fat feeding groups but this was not significant (data not shown).

Figure 5 shows plasma adiponectin levels on days 7, 14, 21 and 28 of the feeding period. Plasma adiponectin levels were significantly higher by 23.7% \((p<0.05)\) on day 21 and by 31.7% \((p<0.01)\) on day 28 in the HFp100 group than in the HFc100 group. This indicates that rats fed a high fat diet supplemented with Alaska pollack protein show increases in plasma adiponectin levels within 21 d.

DISCUSSION

The potential beneficial effects of fish consumption on human health and diseases are desirable. The trend towards an association between increased fish consumption and lower all-cause and coronary heart disease death rates was attributable to an inverse relationship between fish consumption and death from myocardial infarction. For men who consumed 35 g or more of fish per day, the relative risk of any death from myocardial infarction was 0.56 compared with the non-consumers (1). Zhang et al. (2) compared the fish consumption in 36 countries and found that Iceland and Japan have the highest fish consumption and the lowest mortality in both sexes from all causes, ischemic heart disease and stroke. In Study 1, although no statistically significant difference was found in total energy intake between the groups fed the high fat diets, rats in the HFp group had a significantly lower weight of visceral adipose tissue. In addition, we anticipated that
plasma insulin levels would be elevated in animals fed a high-fat diet and, therefore, insulin resistance would be increased (3). However, plasma insulin levels were significantly lower in the HFp group compared with the HFc, HFi and HFck groups. This result supports the findings of previous studies (4–6) that insulin resistance is reduced in animals fed a high fat containing cod meat. Adiponectin is one of the cytokines released from adipose tissues and exerts physiological effects associated with improvements in obesity. In this study, the plasma adiponectin levels were significantly higher in the HFp group. The increased plasma adiponectin levels in the group fed the Alaska pollack protein indicate that Alaska pollack protein influences fat metabolism. Our results support earlier findings that plasma adiponectin levels are significantly lower in obese than non-obese subjects (7) and suggest that Alaska pollack protein can prevent visceral fat accumulation, improve insulin resistance and increase plasma adiponectin levels. These effects differ from those of other protein sources such as meat from chicken or yellowfin tuna. It has also been reported that the level of total blood cholesterol (8–10), but not LDL, is increased in animals fed a high fat diet containing cod meat. Even though visceral adipose tissue weights were not measured in the previous studies, it appears that a diet containing cod protein prevented physiological damage induced by obesity. Soybean protein has been reported to work as a regulator of lipid metabolism (11). In that study, Wistar fatty and lean rats were fed a diet containing either soybean protein or casein for 40 d, and the increase in body weight was significantly reduced in rats fed the soybean protein diet compared with rats fed the casein diet. The plasma and liver cholesterol concentrations were also significantly higher in the fatty rats and lower in rats fed soybean protein than in those fed casein. Therefore, it is possible that similar mechanisms are involved in the effects shown by our study and by theirs.

Several neutraeutralcals such as catechin (12) and globin digests (13, 14), that prevent accumulation of visceral fat have been developed. These have mechanisms of action that vary widely and are not yet fully understood. The mechanism of catechins in animals involves the upregulation of hepatic β-oxidation gene expression and catabolism of accumulated fat (12). It is possible that these mechanisms differ from that of Alaska pollack protein. On the other hand, globin digests are considered to act through an oligopeptide (Val-Val-Tyr-Pro), which inhibits pancreatic lipase activity, to prevent fat digestion and fatty acid absorption in the intestine (13, 14). In the case of Alaska pollack protein, it is possible that peptides similar to those in globin digests are released either during the preparation of the test diets or during digestion in the intestinal tract.

We also demonstrated, in Study 2, that diets in which 30% or more of the protein is supplied by Alaska pollack protein prevented the accumulation of visceral fat in rats fed a high-fat diet. Thus Alaska pollack protein may be physiologically functional in humans when consumed daily.

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