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# Effects of Herring Roe on Plasma Lipid, Glucose, Insulin and Adiponectin Levels, and Hepatic Lipid Contents in Mice

Tomoyuki HIGUCHI[1], Nobuya SHIRAI[2] and Hiramitsu SUZUKI[1,3]

[1] *High Technology Research Center, and* [3] *Laboratory of Food Science and Technology, Kagawa Nutrition University, 3–9–21 Chiyoda, Sakado, Saitama 350–0288, Japan*

[2] *National Food Research Institute, 2–1–12 Kannondai, Tsukuba, Ibaraki 305–8642, Japan*

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***Summary*** We previously reported that lipids extracted from salted herring roe product (Kazunoko), which contains large amounts of cholesterol, phosphatidylcholine and *n*-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), decreased plasma lipid and glucose concentrations in mice. The aim of this study was to evaluate the effects of Kazunoko containing large amounts of protein on lipid and glucose metabolism in mice. Male Crlj:CD-1 (ICR) mice were fed three experimental diets containing lyophilized Kazunoko for 12 wk. The experimental diets were as follows: without Kazunoko (control diet); 1% Kazunoko (1% Kazunoko diet); and 4% Kazunoko (4% Kazunoko diet). Plasma total cholesterol, phospholipid and glucose concentrations tended to be lower in the 1% and 4% Kazunoko diet groups than in the control diet group. There were significant differences in plasma glucose concentration between the control and 4% Kazunoko diet groups ($p<0.05$). Plasma adiponectin concentrations in mice fed the 4% Kazunoko diet were also significantly higher than in those fed the control diet ($p<0.05$), but there were no marked differences in plasma insulin concentration among the three dietary groups. Hepatic total cholesterol and phospholipid contents tended to be lower in the 4% Kazunoko diet group than in control diet group. Plasma and hepatic *n*-3/*n*-6 ratios in the 1% Kazunoko diet and 4% Kazunoko diet groups were significantly higher when compared with those of the control diet group ($p<0.005$ and $p<0.0005$, respectively). These results suggest that ingestion of Kazunoko influences lipid and glucose metabolism in mice fed the Kazunoko diets, as compared with animals fed the control diet.

***Key Words*** herring roe, *n*-3 polyunsaturated fatty acid, plasma lipids, plasma glucose

Caviar, or salted sturgeon roe, is the most well-known salted fish roe product in the world. In Japan, various other salted fish roe products, such as Ikura (salmon roe), Tarako (pollack roe), Tobiko (flying fish roe) and Kazunoko (herring roe), have traditionally been consumed. Fish roe lipids include large amounts of *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs), such as eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) (*1*, *2*), which reportedly lower the risk of coronary heart disease (CHD) and metabolic syndrome by reducing serum triacylglycerol and increasing plasma total high-density lipoprotein (HDL) and HDL (*2*) cholesterol levels in human subjects (*3–7*). However, fish roe lipids are rich in cholesterol (*1*, *2*), and excess cholesterol intake reportedly increases the risks of CHD (*8*).

We previously reported that ingestion of Kazunoko lipids reduces plasma total cholesterol, triacylglycerol, phospholipid and glucose levels in mice (*9*). Kazunoko is a salted, yellow herring roe product that is consumed in blocks of 7–10 cm in length. Kazunoko contains large amounts of proteins in comparison with other fish roe products. Thus, Kazunoko may have an effect on lipid and glucose metabolism due to action of both lipids and proteins. We believe that utilization of fish roe is important for conservation of aquatic species, as worldwide demand for fish food products has increased markedly in recent years. The aim of this study is to clarify the effects of Kazunoko intake on plasma and hepatic lipids, and plasma glucose levels.

## MATERIALS AND METHODS

*Animals.* Male Crlj:CD-1(ICR) mice (age, 4 wk) were obtained from Charles River Japan, Inc. (Atsugi, Japan). Thirty mice were randomly divided into 3 groups of 9–11 animals each. Mice were housed in suspended stainless-steel cages with wire mesh bottoms. The animal room was kept at a temperature of 24±0.5°C and a relative humidity of 65±5%. Room lighting consisted of 12-h periods of light and dark. Diets and water were provided ad libitum. The diets given to each group had similar energy content, and all mice were fed experi-

E-mail: higuchi@eiyo.ac.jp

Abbreviations: ANOVA, one-way analysis of variance; CHD, coronary heart disease; DHA, docosahexaenoic acid; ELISA, enzyme linked immunoassay; EPA, eicosapentaenoic acid; FO, fish oil; HDL, high-density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SEM, standard error of the mean; SFA, saturated fatty acid; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

Table 1. Proximate composition of Kazunoko (%).

| Substances | |
|---|---|
| Moisture | 80.3 |
| Protein | 16.1 |
| Lipid | 2.7 |
| Ash | 0.4 |
| Carbohydrate | 0.5 |

Table 2. Composition of experimental diets (%).

| Materials | Dietary group | | |
|---|---|---|---|
| | Control | 1% Kazunoko | 4% Kazunoko |
| Corn starch | 47.8 | 47.8 | 47.7 |
| Casein | 20 | 19.1 | 16.7 |
| Granulated sugar | 15 | 15 | 15 |
| Cellulose | 5 | 5 | 5 |
| Mineral mixture[a] | 4 | 4 | 3.9 |
| Vitamin mixture[b] | 2 | 2 | 2 |
| L-Methionine | 0.2 | 0.2 | 0.2 |
| Lard | 6 | 5.9 | 5.5 |
| Lyophilized Kazunoko | — | 1 | 4 |

Mineral and vitamin mixtures were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan).

[a] Mineral mixture contained (per kg): $CaHPO_4 \cdot 2H_2O$, 14.56 g; $KH_2PO_4$, 25.72 g; $NaH_2PO_4$, 9.35 g; NaCl, 4.66 g; Ca-lactate, 35.09 g; Fe-citrate, 3.18 g; $MgSO_4$, 7.17 g; $ZnCO_3$, 0.11 g; $MnSO_4 \cdot 4H_2O$, 0.12 g; $CuSO_4 \cdot 5H_2O$, 0.03 g; and KI, 0.01 g.

[b] Vitamin mixture contained (per kg): retinyl acetate, 0.1 g; cholecalciferol, 0.00025 g; $\alpha$-tocopheryl acetate, 0.5 g; menadione, 0.52 g; thiamin·HCl, 0.12 g; riboflavin, 0.4 g; pyridoxine·HCl, 0.08 g; cyanocobalamine, 0.00005 g; ascorbic acid, 3 g; biotin, 0.002 g; folic acid, 0.02 g; calcium pantothenate, 0.5 g; *p*-aminobenzoic acid, 0.5 g; niacin, 0.6 g; inositol, 0.6 g; choline chloride, 20 g; and cellulose powder, 73.1 g.

mental diets for 12 wk. Body weight was measured once every 2 wk. All mice were maintained according to the guidelines for experimental animals of the National Food Research Institute, Japan.

*Diets.* Lard was kindly supplied by NOF Co., Ltd., Tokyo, Japan. Salted Kazunoko was provided by a fisheries company in Japan and by the Canadian Pacific Kazunoko Association (Tokyo, Japan). Measurement of the proximate composition of Kazunoko was performed by Japan Food Research Laboratories (Tokyo, Japan). The composition is shown in Table 1. Contents of cholesterol, phospholipid and triacylglycerol in Kazunoko were 0.26±0.04%, 2.14±0.26% and 0.54±0.06%, respectively (*2*). Kazunoko was not bleached. Salted Kazunoko (20 kg) was immersed in distilled water overnight for demineralization, and was then lyophilized (FTS Systems, Stone Ridge, NY) for 3 d. Lyophilized Kazunoko (4.1 kg) was powdered and mixed with the experimental diets. Experimental diets were as follows:

Table 3. Main fatty acid contents of experimental diets (%).

| | Dietary group | | |
|---|---|---|---|
| | Lard | 1% Kazunoko | 4% Kazunoko |
| SFA | | | |
| 14:0 | 2.0 | 2.5 | 2.8 |
| 16:0 | 24.7 | 26.1 | 26.9 |
| 18:0 | 12.1 | 11.4 | 10.1 |
| Other | 0.2 | 0.2 | 0.0 |
| Total | 39.0 | 40.2 | 39.8 |
| MUFA | | | |
| 16:1*n*-9 | 0.3 | 0.3 | 0.4 |
| 16:1*n*-7 | 3.2 | 3.5 | 3.9 |
| 18:1*n*-9 | 42.3 | 40.1 | 37.7 |
| 18:1*n*-7 | 3.0 | 2.8 | 3.1 |
| 20:1*n*-9 | 0.6 | 0.5 | 0.5 |
| Other | 0.1 | 0.0 | 0.0 |
| Total | 49.4 | 47.3 | 45.5 |
| PUFA | | | |
| 18:2*n*-6 | 8.0 | 7.6 | 7.4 |
| 18:3*n*-3 | 0.4 | 0.4 | 0.5 |
| 20:4*n*-6 | — | 0.1 | 0.2 |
| 20:5*n*-3 | — | 0.2 | 1.4 |
| 22:6*n*-3 | — | 0.3 | 2.2 |
| Other | 0.2 | 0.3 | 0.2 |
| Total | 8.6 | 8.9 | 11.8 |
| *n*-3/*n*-6 | 0.1 | 0.1 | 0.5 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values are given as means.

without Kazunoko (control diet), with 1% (w/w) Kazunoko (1% Kazunoko diet), and with 4% (w/w) Kazunoko (4% Kazunoko diet). The composition of experimental diets is shown in Table 2. The composition of each experimental diet was adjusted to have the same lipid, protein and carbohydrate contents. To prevent oxidative loss of *n*-3 PUFAs during storage, experimental diets were stored below −40°C.

The fatty acid composition of each experimental diet is shown in Table 3. The predominant fatty acids in all experimental diets were 16:0, 18:0, 18:1*n*-9 and 18:2*n*-6. The percentage of 16:0 in the 1% Kazunoko and 4% Kazunoko diets was higher than that in the control diet. The percentages of 18:0, 18:1*n*-9 and 18:2*n*-6 in the control diet were higher than those in the 1% Kazunoko and 4% Kazunoko diets. The *n*-3/*n*-6 ratio of the 4% Kazunoko diets was higher than those of the control and 1% Kazunoko diets.

*Preparation of plasma and liver homogenates.* At the end of the feeding trials (Week 12), all mice were fasted for 24 h before being anesthetized with diethyl ether. Blood was then collected from the inferior vena cava with a heparinized syringe and was placed in ice-cold tubes. After collecting blood, the liver and visceral adi-

pose tissues were removed. The obtained liver was rapidly frozen using liquid nitrogen, and was homogenized in 0.15 M NaCl and 20 mM Tris-HCl (pH 7.5) using a Teflon-glass homogenizer. Plasma was separated by centrifugation at 900 $\times g$ for 20 min at 4°C. Plasma samples and liver homogenates were stored at −40°C until required for analysis.

*Analyses of plasma and hepatic lipids, and plasma glucose, insulin and adiponectin.* Total cholesterol, triacylglycerol and phospholipid concentrations in plasma samples and liver homogenates were assayed by the enzymatic methods of Allain et al. (*10*), Spayd et al. (*11*) and Takayama et al. (*12*) using Cholesterol E-test Wako, Triglyceride E-test Wako, and Phospholipid C-test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively. Plasma glucose concentrations were measured by the method of Trinder using an oxidase-peroxidase system (*13*) with Glucose CII-test Wako (Wako Pure Chemical Industries, Ltd.). Plasma insulin and adiponectin concentrations were measured with a mouse insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan) and a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), respectively.

*Analyses of fatty acid compositions.* Lipid extraction from the experimental diets was carried out by the method of Folch et al. (*14*). Lipid extraction and fatty acid derivatization from plasma and liver samples were performed by the method of Shirai et al. (*15*). Fatty acids were measured on a gas chromatograph (GC-18A, Shimadzu Co., Ltd., Kyoto, Japan) equipped with a fused silica capillary column, Supelcowax 10 (30 m× 0.25 mm i.d., Supelco Co., Ltd., Bellefonte, USA), fitted with a Class 10 integrator (Shimadzu Co., Ltd.). The carrier gas was helium (flow, 1 mL/min) with a split injection of 40 : 1. The temperature profiles were as follows: initial temperature, 170°C; heat rate, 1°C/min; final temperature, 220°C; injector temperature, 250°C; and detector temperature, 270°C. Total run time was 60 min. Fatty acids were identified by comparison of retention times with those of standard purified fatty acids and already identified marine fish oil (*16*, *17*).

*Statistical analyses.* Results are expressed as means±SE. The statistical significance of differences in lipid components, glucose, hormone concentrations and fatty acid composition between dietary groups was determined by one-way analysis of variance (ANOVA), and Spjotvoll and Stoline tests using the Statistica statistical program package (StatSoft, Tusla, OK, USA).

## RESULTS

*Body, liver and visceral adipose tissue weights, and food intake*

There were no significant differences in mean food consumption between dietary groups. Food intake (mean±SE) in each group was as follows: control diet group, 4.4±0.0 g/d; 1% Kazunoko diet group, 4.4±0.0 g/d; and 4% Kazunoko diet group, 4.3±0.0 g/d. Body, liver and visceral adipose tissue weights are shown in Table 4. There were no marked differences in the body or liver weights among the dietary groups, and although visceral adipose tissue weights tended to be lower in the 1% Kazunoko diet group than in the control and 4% Kazunoko diet groups, there were no significant differences in these weights between any of the dietary groups.

*Concentrations of plasma total cholesterol, triacylglycerol, phospholipid, glucose, insulin and adiponectin*

Plasma total cholesterol, triacylglycerol, phospholipid, glucose, insulin and adiponectin concentrations in mice are shown in Table 5. Plasma total cholesterol and phospholipid concentrations tended to be lower in the 1% and 4% Kazunoko diet groups than in the control diet group, while concentrations of plasma triacyl-

Table 4. Weights of body, liver and visceral adipose tissue in mice (g).

| | Dietary group | | |
|---|---|---|---|
| | Control | 1% Kazunoko | 4% Kazunoko |
| Body | 42.2±1.8 | 41.6±2.9 | 43.4±6.0 |
| Liver | 1.48±0.14 | 1.44±0.14 | 1.53±0.31 |
| Adipose tissue | 1.72±0.34 | 1.24±0.49 | 1.63±0.95 |

Table 5. Concentrations of plasma total cholesterol, triacylglycerol, phospholipid, glucose, insulin and adiponectin in mice fed experimental diets.

| | Dietary group | | |
|---|---|---|---|
| | Control | 1% Kazunoko | 4% Kazunoko |
| Total cholesterol (mg/dL) | 146.7±9.2 | 125.9±5.5 | 126.0±10.6 |
| Triacylglycerol (mg/dL) | 78.7±7.0 | 88.8±5.0 | 80.7±7.9 |
| Phospholipid (mg/dL) | 245.9±14.2 | 218.7±8.5 | 200.8±14.5 |
| Glucose (mg/dL) | 190.6±12.5 *a* | 163.8±8.5 *ab* | 142.4±15.4 *b* |
| Insulin (pg/mL) | 601.2±228.4 | 546.5±211.5 | 623.5±204.3 |
| Adiponectin ($\mu$g/mL) | 10.8±0.2 *a* | 11.4±1.2 *ab* | 12.9±0.6 *b* |

Levels of plasma glucose and plasma and hepatic lipids in mice fed experimental diets for 12 wk ($n$=9–11/group). Blood from the inferior vena cava and liver samples were obtained after fasting for 24 h. Values (means±SE) in the table labeled with different italic letters were significantly different in the Spjotvoll and Stoline test.

Table 6. Concentrations of hepatic total cholesterol, triacylglycerol and phospholipid in mice fed experimental diets (mg/g liver).

| | Dietary group | | |
|---|---|---|---|
| | Control | 1% Kazunoko | 4% Kazunoko |
| Total cholesterol | 4.4±0.3 | 3.7±0.3 | 3.8±0.1 |
| Triacylglycerol | 19.2±1.6 *a* | 22.9±2.1 *ab* | 25.8±2.1 *b* |
| Phospholipid | 20.1±0.4 *a* | 18.9±0.4 *ab* | 18.0±0.2 *b* |

Levels of plasma glucose and plasma and hepatic lipids in mice fed experimental diets for 12 wk ($n$=9–11/group). Blood from the inferior vena cava and liver samples were obtained after fasting for 24 h. Values (means±SE) in the table labeled with different italic letters were significantly different in the Spjotvoll and Stoline test.

glycerol in the 1% and 4% Kazunoko diet groups tended to be higher than in the control diet group. Plasma glucose concentrations in the 1% and 4% Kazunoko diet groups tended to be lower than in the control diet group and those in the 4% Kazunoko diet group were significantly lower than in the control diet group ($p<0.05$).

There were no differences in plasma insulin concentrations among the dietary groups. Plasma adiponectin concentrations were significantly higher in the 4% Kazunoko dietary group than in the control and 1% Kazunoko dietary groups ($p<0.05$). Plasma adiponectin concentrations thus tended increase with intake of Kazunoko.

*Hepatic total cholesterol, triacylglycerol and phospholipid contents*

Hepatic total cholesterol, triacylglycerol and phospholipid levels in mice are shown in Table 6. Total cholesterol content tended to be lower in the 1% and 4% Kazunoko diet groups than in the control diet group, but there were no marked differences in liver total cholesterol levels among the dietary groups. Hepatic triacylglycerol content tended to increase with Kazunoko, and the triacylglycerol content of the 4% Kazunoko diet group was significantly higher than that of the control and 1% Kazunoko diet groups. Hepatic phospholipid levels in mice fed the 4% Kazunoko diet were significantly lower than in those fed the control and 1% Kazunoko diets ($p<0.005$).

*Fatty acids in plasma and liver*

Plasma fatty acids in the mice fed each diet are shown in Table 7. Although the plasma saturated fatty acid and monounsaturated fatty acid percentages of mice fed Kazunoko were no different from those fed the control diet, plasma PUFA levels in mice fed Kazunoko were markedly different from those in animals fed the control diet. The percentages of 18:2$n$-6, 20:5$n$-3 and 22:6$n$-3 increased with intake of Kazunoko. In particular, 18:2$n$-6 and 22:6$n$-3 levels were significantly higher in the 1% Kazunoko and 4% Kazunoko diet groups than in the control diet group (18:2$n$-6, $p<0.0005$ and $p<0.0005$, respectively; 22:6$n$-3, $p<0.0005$ and $p<0.0005$, respectively). In contrast,

Table 7. Plasma fatty acid contents (%) and $n$-3/$n$-6 ratio in mice fed experimental diets for 12 wk.

| | Dietary group | | |
|---|---|---|---|
| | Control | 1% Kazunoko | 4% Kazunoko |
| SFA | | | |
| 14:0 | 0.4±0.0 | 0.5±0.0 | 0.4±0.0 |
| 16:0 | 21.6±0.2 *a* | 23.1±0.5 *b* | 21.7±0.3 *a* |
| 18:0 | 7.5±0.2 *a* | 7.0±0.2 *ab* | 6.9±0.2 *b* |
| Other | 0.2±0.0 *a* | 0.3±0.0 *ab* | 0.3±0.0 *b* |
| Total | 29.7±0.4 | 30.8±0.6 | 29.4±0.3 |
| MUFA | | | |
| 16:1$n$-9 | 0.5±0.0 *a* | 0.6±0.0 *b* | 0.6±0.0 *b* |
| 16:1$n$-7 | 4.4±0.2 | 3.9±0.2 | 4.2±0.2 |
| 18:1$n$-9 | 22.8±0.6 | 24.3±0.6 | 22.0±0.7 |
| 18:1$n$-7 | 2.8±0.1 | 2.3±0.2 | 2.3±0.2 |
| 20:1$n$-9 | 0.5±0.0 | 0.6±0.0 | 0.6±0.1 |
| Other | 0.3±0.0 | 0.2±0.0 | 0.3±0.0 |
| Total | 30.8±0.8 | 31.9±0.7 | 30.0±0.6 |
| PUFA | | | |
| 18:2$n$-6 | 12.3±0.4 *a* | 16.6±0.4 *b* | 19.2±0.7 *c* |
| 18:3$n$-6 | 0.3±0.0 | 0.5±0.1 | 0.4±0.0 |
| 20:3$n$-9 | 2.3±0.1 *a* | 0.9±0.1 *b* | 0.7±0.1 *b* |
| 20:3$n$-6 | 1.7±0.0 | 1.3±0.1 | 1.4±0.1 |
| 20:4$n$-6 | 18.6±0.8 *a* | 11.9±0.4 *b* | 8.9±0.6 *c* |
| 20:5$n$-3 | | 0.5±0.0 *a* | 2.1±0.1 *b* |
| 22:5$n$-6 | 0.7±0.1 | | |
| 22:5$n$-3 | — | 0.1±0.0 | 0.2±0.0 |
| 22:6$n$-3 | 2.9±0.1 *a* | 5.5±0.2 *b* | 7.7±0.3 *c* |
| Other | 0.0±0.0 *a* | 0.1±0.0 *ab* | 0.2±0.0 *b* |
| Total | 38.9±0.8 *ab* | 37.3±0.8 *a* | 40.6±0.7 *b* |
| $n$-3/$n$-6 | 0.1±0.0 *a* | 0.2±0.0 *b* | 0.3±0.0 *c* |

Values (means±SE) in the table labeled with different italic letters were significantly different in the Spjotvoll and Stoline test. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

20:4$n$-6 levels were lower with increasing intake of Kazunoko, and levels in the 1% Kazunoko and 4% Kazunoko diet groups were significantly lower when compared with the control diet group ($p<0.0005$ and $p<0.0005$, respectively). In addition, the $n$-3/$n$-6 ratio in the 1% Kazunoko diet and 4% Kazunoko diet groups was significantly higher than in the control diet group ($p<0.0005$ and $p<0.0005$, respectively).

The liver fatty acid composition of mice fed each diet is shown in Table 8. The percentages of 18:1$n$-9, 18:2$n$-6, 20:5$n$-3 and 22:6$n$-3 increased with intake of Kazunoko. In particular, the 18:2$n$-6 and 22:6$n$-3 percentages were significantly higher in the 1% Kazunoko and 4% Kazunoko diet groups than in the control diet group (18:2$n$-6, $p<0.001$ and $p<0.0005$, respectively; 22:6$n$-3, $p<0.05$ and $p<0.001$, respectively). Conversely, the 20:4$n$-6 percentage of the 1% Kazunoko and 4% Kazunoko diet groups was significantly lower than in the control diet group ($p<0.005$ and

Table 8. Hepatic fatty acid contents (%) and *n*-3/*n*-6 ratio in mice fed experimental diets for 12 wk.

| | Dietary group | | |
|---|---|---|---|
| | Control | 1% Kazunoko | 4% Kazunoko |
| SFA | | | |
| 14:0 | 0.5±0.0 | 0.6±0.0 | 0.6±0.0 |
| 16:0 | 24.1±0.5 *ab* | 25.3±0.6 *a* | 22.8±0.5 *b* |
| 18:0 | 7.5±0.5 *a* | 6.7±0.6 *ab* | 5.2±0.5 *b* |
| Other | 0.2±0.0 *a* | 0.3±0.1 *b* | 0.3±0.0 *b* |
| Total | 32.2±0.9 *a* | 32.8±1.0 *a* | 35.0±4.2 *b* |
| MUFA | | | |
| 16:1*n*-9 | 0.5±0.0 *a* | 0.7±0.1 *b* | 0.9±0.1 *b* |
| 16:1*n*-7 | 5.0±0.4 | 4.4±0.4 | 5.3±0.5 |
| 18:1*n*-9 | 34.2±1.5 | 35.2±1.5 | 37.6±1.4 |
| 18:1*n*-7 | 4.1±0.2 | 3.2±0.3 | 3.5±0.3 |
| 20:1*n*-9 | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 |
| Other | 0.2±0.0 *a* | 0.2±0.0 *a* | 0.4±0.0 *b* |
| Total | 44.7±2.1 | 44.4±2.1 | 48.3±2.1 |
| PUFA | | | |
| 18:2*n*-6 | 7.4±0.2 *a* | 9.6±0.3 *b* | 10.7±0.5 *b* |
| 20:3*n*-9 | 1.3±0.1 *a* | 0.6±0.1 *b* | 0.3±0.0 *c* |
| 20:3*n*-6 | 0.9±0.1 *a* | 0.6±0.0 *b* | 0.6±0.0 *b* |
| 20:4*n*-6 | 9.4±0.7 *a* | 6.2±0.5 *b* | 3.5±0.4 *c* |
| 20:5*n*-3 | — | 0.2±0.0 *a* | 0.8±0.1 *b* |
| 22:5*n*-6 | 0.8±0.1 | — | — |
| 22:5*n*-3 | — | 0.1±0.0 *a* | 0.4±0.0 *b* |
| 22:6*n*-3 | 3.2±0.3 *a* | 5.1±0.4 *b* | 6.2±0.7 *b* |
| Other | 0.2±0.0 | 0.3±0.0 | 0.4±0.0 |
| Total | 23.1±1.3 | 22.8±1.2 | 22.8±1.3 |
| *n*-3/*n*-6 | 0.2±0.0 *a* | 0.3±0.0 *b* | 0.5±0.0 *c* |

Values (means±SE) in the table labeled with different italic letters were significantly different in the Spjotvoll and Stoline test. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

$p<0.0005$, respectively). In addition, the *n*-3/*n*-6 ratio of the 1% Kazunoko diet and 4% Kazunoko diet groups was significantly higher when compared with the control diet group ($p<0.005$ and $p<0.0005$, respectively).

## DISCUSSION

In a previous study, we found that ingestion of extracted lipids from Kazunoko decrease plasma total cholesterol, triacylglycerol, phospholipid and glucose levels in mice (*9*). However, the lipids, protein and other nutrients in Kazunoko may have a synergistic effect on lipid and glucose metabolism. Lyophilized Kazunoko contains particularly large amounts of protein (approximately 82%) when compared with other fish roe products. The aim of this study was to clarify the effects of Kazunoko intake on plasma and hepatic lipids, and plasma glucose levels.

In the present study, plasma glucose concentrations in mice decreased with increasing Kazunoko intake, with plasma glucose concentrations in the 4% Kazunoko diet group being significantly lower than those in the control diet group (Table 5). However, there were no differences in plasma insulin concentration among the dietary groups, which suggests that Kazunoko has no effect on insulin secretion (Table 5). The lipid content in the 4% Kazunoko diet used in the present study was estimated at approximately 0.5%. In our previous study, we reported that an experimental diet containing 1% extracted lipids from Kazunoko did not reduce plasma glucose (*9*). Therefore, it is possible that the lipid content in the 4% Kazunoko diet (i.e., 0.5% Kazunoko lipids) was insufficient to reduce plasma glucose concentrations. Moriya et al. reported that concentrations of plasma glucose in C57bl/6J mice tended to decrease after ingestion of herring roe lipids and protein, while protein alone had no effect (*18*). In contrast, Lavigne et al. reported that cod protein improved fasting glucose tolerance and peripheral insulin sensitivity in rats and prevented obesity-induced muscle insulin resistance in high fat-fed obese rats (*19*, *20*). The combination of Kazunoko lipids and proteins may thus increase insulin sensitivity and reduce plasma glucose concentrations in mice.

Plasma adiponectin concentrations in the 4% Kazunoko diet group were significantly higher than those in the control diet group (Table 5). A previous study also showed that ingestion of an experimental diet containing extracted Kazunoko lipids increased plasma adiponectin concentrations in mice (*9*). These results suggest that the increases in plasma adiponectin concentration are caused by ingestion of Kazunoko lipids. It has been reported that adiponectin increases insulin sensitivity and/or glucose uptake via activation of adenosine monophosphate-activated protein kinase (*21*, *22*). It is thus possible that the increase in plasma adiponectin concentrations in mice fed the Kazunoko diet contributes to the reduction of plasma glucose concentration.

Plasma total cholesterol and phospholipid concentrations in mice fed 1% and 4% Kazunoko diets tended to be lower when compared to those in animals fed the control diet (Table 5). We previously reported that Kazunoko lipids have a lowering effect on plasma total cholesterol and phospholipid concentrations, despite containing large amounts of cholesterol (*9*). Moreover, saturated fatty acids other than stearic acid are a risk factor for elevated plasma cholesterol levels (*23*). The contents of saturated fatty acids in the 1% and 4% Kazunoko diets were slightly higher when compared to those in the control diet. Nevertheless, levels of plasma total cholesterol in the Kazunoko diet groups were lower than those in the control diet group. This suggests that the *n*-3 PUFAs in Kazunoko lipids reduce plasma total cholesterol and phospholipid concentrations, and overcome the effects of dietary cholesterol on plasma total cholesterol levels. In this study, the cholesterol contents of the control, 1% and 4% Kazunoko diets were estimated to be 9, 13 and 52 mg/100 g diet, respectively. Thus, ingestion of Kazunoko, as well as Kazunoko lipids, contributes to reductions in plasma

total cholesterol and phospholipid concentrations in mice.

Plasma triacylglycerol concentrations in mice fed Kazunoko diets did not differ from those in animals fed the control diet, and hepatic triacylglycerol contents in mice fed Kazunoko diets tended to increase with Kazunoko intake when compared to animals fed the control diet (Tables 5 and 6). We previously reported that the hepatic triacylglycerol contents in the 1% Kazunoko lipids diet group increased when compared to the lard diet group (*9*). Ueno et al. reported that fatty liver in rats fed a high-cholesterol diet was caused by decreased secretion of lipoproteins rather than increased lipogenesis (*24*). This suggests that the increases in hepatic triacylglycerol contents resulted from ingestion of dietary cholesterol in Kazunoko.

As shown in Tables 7 and 8, the percentages of plasma and hepatic 20:4*n*-6 in the 1% and 4% Kazunoko diet groups were significantly lower than those in the control diet group. The 18:2*n*-6 percentages in plasma and liver in mice fed the Kazunoko diet were significantly higher when compared to animals fed the control diet. These results indicate that 18:2*n*-6 is a precursor in the synthesis of 20:4*n*-6 in mammalian cells, and as synthesis of 20:4*n*-6 is suppressed by 22:6*n*-3 intake (*25*), ingestion of 22:6*n*-3 may inhibit the synthesis of 20:4*n*-6, resulting in accumulation of 18:2*n*-6. The 18:1 percentage in liver tended to increase with intake of Kazunoko (Table 8). The high content of 18:1 was probably due to the high triacylglycerol content in the liver in comparison to the control diet group, as 18:1 is the main fatty acid component of triacylglycerol.

In conclusion, we showed that Kazunoko intake decreased plasma total cholesterol, phospholipid and glucose concentrations, and increased plasma adiponectin concentration in mice. As the differences in the plasma glucose and adiponectin concentrations between the control diet and 4% Kazunoko diet groups were statistically significant, Kazunoko appears to reduce key risk factors for diabetes. Moreover, the results suggest that ingestion of Kazunoko influences lipid metabolism, as the profiles of plasma and hepatic fatty acids, particularly PUFAs, in mice fed the Kazunoko diets differed markedly from those in animals fed the control diet. However, it may be necessary to further investigate the effects of Kazunoko intake on hepatic triacylglycerol contents. Kazunoko and other fish roe contain large amounts of cholesterol and *n*-3 PUFAs; however, Kazunoko intake reduced plasma total cholesterol concentration. Kazunoko contains large amounts of proteins and slightly small amounts of cholesterol in comparison to other fish roe, while the fatty acids present in Kazunoko are not markedly distinct from those in other fish roe. Thus, it is possible that other types of fish roe will have similar effects on lipid and glucose metabolism.

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