A Comparative Study of Lipids Extracted from Herring Roe Products and Fish Oil on Plasma Glucose and Adipocytokine Levels in ICR Aged Mice

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Male mice (11 months old) were fed 5% lard, fish oil, or kazunoko (salted fish roe product) lipids for 4 months to investigate the effects of diet on plasma glucose and adipocytokines. Plasma glucose levels were significantly lower in the fish oil group than in the lard group and there was a non-significant tendency towards lower levels in the kazunoko lipid group. Plasma leptin and adiponectin levels were also significantly higher in fish oil group than in lard group and resistin levels were significantly lower in the kazunoko lipid group than the lard group, but not the fish oil group. The results suggest that kazunoko lipids might be effective as a dietary component in the prevention of coronary heart disease and diabetes even though the metabolic effects of kazunoko lipids were different from those of fish oil.

Keywords: kazunoko, lipid, glucose, leptin, resistin, adiponectin

Introduction

It is well established that the prevention of metabolic syndrome is important in the maintenance of human health, as this syndrome substantially increases the risk of coronary heart disease (CHD). Numerous studies and clinical investigations indicate that the onset of metabolic syndrome is associated with lifestyle disorders, such as insufficient exercise, smoking, unbalanced diet, and excess drinking and eating (Firdaus, 2005; Gill and Malkova, 2006; Morriss and Mohammed, 2005; Azadbakht et al., 2005; Riccardi et al., 2004). A variety of foods, and their components, have been proposed as useful agents in the prevention of the onset of metabolic syndrome (Shirai et al., 2006c; Shirai and Suzuki, 2003; Kao et al., 2006; Borek, 2006; Murakami et al., 2005). In particular, there is evidence that intake of fish oil containing eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) may help prevent CHD and diabetes (Ebbesson et al., 2005; Lombardo and Chicco, 2006; Shirai and Suzuki, 2004; Carpentier et al., 2006).

Fish roe, such as caviar, are consumed throughout the world. In Japan, the most frequently consumed roe are salted roe products from salmon, pollock, flyingfish, and herring, usually known as ikura, tarako, tobiko, and kazunoko, respectively. Studies on the detailed lipid composition of fish roe have shown that, in general, they contain large amounts of eicosapentaenoic acid (20:5n-3), docosahexaenoic acid (22:6n-3, DHA), phospholipids (PL), and cholesterol (Shirai et al., 2006a; Kaitaranta, 1980; Tocher and Sargent, 1984; Bledsoe et al., 2003). PLs are effective in lowering plasma lipids (Brook et al., 1986; Iwata et al., 1992) and the majority of 22:6 in fish roe lipids is associated with PL; it is a characteristic of fish roe lipids that differs from fish oil. We have previously reported that intake of lipids extracted from salted herring roe products reduces plasma lipid and glucose concentrations in mice (Higuchi et al., 2006a). This evidence suggests that intake of fish roe lipids may be beneficial to human health.

Recent studies indicate that the adipocytokines, such as leptin and adiponectin, may also be involved in the prevention of metabolic syndrome (Kobayashi, 2005; Matsuzawa et al., 2004) and there have been reports that levels of these adipocytokines are influenced by fish oil intake (Shirai et al., 2006c; Flachs et al., 2006; Peyron-Caso et al., 2002; Perez-Matute et al., 2005; Neschen et al., 2006). In our previous study of the effect of intake of lipids of the herring roe prod-

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uct, kazunoko, in mice, plasma glucose levels declined and insulin and adiponectin levels were modulated (Higuchi et al., 2006a). However, the difference, if any, between the effects of kazunoko lipids and fish oil on plasma glucose (Glu) and adipocytokines has not been established. Furthermore, the prevalence of diabetes mellitus increases with the aging process (Nakano and Ito, 2007). The purpose of this study was to compare the effects of kazunoko lipids and fish oil on plasma lipids, glucose, insulin, and adipocytokines in aged mice.

Materials and Methods

Diet  Lard was purchased from NOF Co., Ltd., Tokyo, Japan. Kazunoko (salted herring roe) processed in Canada was supplied by Kato Suisan CO., LTD (Rumoi, Hokkaido, Japan). After removal of the salts, lipids were extracted from kazunoko with a hexane/ethanol (1:1) solvent, as described previously (Higuchi et al., 2006a). Total lipid content of kazunoko was 3.0% (Shirai et al., 2006a). Fish oil containing the same percentages of 20:5n-3 and 22:6n-3 fatty acids, in the lipid component, as the kazunoko lipids, were produced by mixing sardine oil, tuna oil (NOF Co., Ltd., Tokyo, Japan), and fish oil refined oil (Nippon Chemical Feed Co., Ltd., Hakodate, Japan). The experimental diets contained 5% fat (lard), 5% fish oil, or 5% extracted kazunoko lipids and other components as follows: corn starch, 48.8%; casein, 20.0%; granulated sugar, 15.0%; cellulose powder, 5.0%; salt mixture, 4.0%; vitamin mixture, 2.0% and L-methionine, 0.2%. Salt and vitamin mixtures were purchased from Oriental Yeast Co., Ltd., Tokyo, Japan. In order to prevent oxidative changes to the fatty acids during storage, experimental diets were stored below –30°C. The composition of the main fatty acids and lipid components of each experimental diet are shown in Table 1.

Animals  Male mice of Crlj:CD-1(ICR) strain (5 weeks old) were obtained from Charles River Japan Inc. (Atsugi, Kanagawa, Japan). All animals were switched from laboratory chow, MF (Oriental Yeast Co., Ltd., Tokyo, Japan), and fish oil refined oil (Nippon Chemical Feed Co., Ltd., Hakodate, Japan). The experimental diets contained 5% fat (lard), 5% fish oil, or 5% extracted kazunoko lipids and other components as follows: corn starch, 48.8%; casein, 20.0%; granulated sugar, 15.0%; cellulose powder, 5.0%; salt mixture, 4.0%; vitamin mixture, 2.0% and L-methionine, 0.2%. Salt and vitamin mixtures were purchased from Oriental Yeast Co., Ltd., Tokyo, Japan. In order to prevent oxidative changes to the fatty acids during storage, experimental diets were stored below –30°C. The composition of the main fatty acids and lipid components of each experimental diet are shown in Table 1.

Preparation of plasma samples and liver homogenates  At the end of the feeding trials, all mice were fasted for 24 hours before being anesthetized with diethyl ether. Blood was then collected from the inferior vena cava with a heparinized syringe and placed into ice-cold tubes. The plasma was separated from the remaining blood sample by centrifugation at 900g for 20 min at 4°C. After collecting the blood, the livers were removed, and homogenized with 0.05 mol/L phosphate buffered saline (pH=7.4) using a Teflon-glass homogenizer. Plasma samples and liver homogenates were stored at –30°C until lipid, glucose and hormone analyses.

Fatty acid analyses of plasma and liver lipids  The fatty acid components of the total lipids from plasma and liver were derivatized with 0.5 mol/L NaOH methanol solution and 14% boron trifluoride methanol solution (Shirai et al., 2005) and then 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, PA, USA) and manipulated with a Class 10 work station (Shimadzu Co., Ltd., Kyoto, Japan). The carrier gas was helium (flow 1 ml/min) with a split injection of 40:1. The temperature profile was as follows:

| Table 1. Fatty acid composition of experimental diets. |
|-----------------|------------|-----------|
|                 | Lard       | Fish      | Kazunoko  |
| 14:0            | 1.8        | 4.7       | 3.0       |
| 16:0            | 24.2       | 16.3      | 24.8      |
| 18:0            | 13.4       | 3.7       | 2.6       |
| Others          | 1.1        | 2.2       | 0.9       |
| Total           | 40.5       | 26.9      | 31.2      |
| MUFA            |            |           |           |
| 16:1n-9         | 0.2        | 0.3       | 0.7       |
| 16:1n-7         | 2.8        | 6.3       | 5.0       |
| 18:1n-9         | 42.2       | 11.1      | 13.9      |
| 18:1n-7         | 3.6        | 2.5       | 5.1       |
| 20:1            | 0.7        | 2.3       | 1.1       |
| Others          | 1.0        | 2.2       | 1.1       |
| Total           | 50.5       | 24.7      | 26.8      |
| PUFA            |            |           |           |
| 18:2n-6         | 8.3        | 1.5       | 1.1       |
| 18:3n-3         | 0.4        | 0.7       | 0.6       |
| 18:4n-3         | -          | 2.2       | 0.5       |
| 20:4n-6         | -          | 1.9       | 1.2       |
| 20:5n-3         | -          | 14.2      | 13.9      |
| 22:5n-3         | -          | 1.9       | 1.2       |
| 22:6n-3         | -          | 22.7      | 22.7      |
| Others          | 0.3        | 3.3       | 0.7       |
| Total           | 9.0        | 48.4      | 41.9      |
| n-3/n-6         | 0.05       | 8.23      | 17.33     |

| Cholesterol (mg/diet/g) | - | - | 4.4 |
| Phospholipids (mg/diet/g) | - | - | 35.7 |
| Fatty acid content (mg/100g diet) | - | - | - |
| 20:4n-6 | - | 72 | 24 |
| 20:5n-3 | - | 514 | 283 |
| 22:6n-3 | - | 862 | 476 |

The fatty acid content of the total lipids from plasma and liver were derivatized with 0.5 mol/L NaOH methanol solution and 14% boron trifluoride methanol solution (Shirai et al., 2005) and then 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, PA, USA) and manipulated with a Class 10 work station (Shimadzu Co., Ltd., Kyoto, Japan). The carrier gas was helium (flow 1 ml/min) with a split injection of 40:1. The temperature profile was as follows:
initial temperature, 175°C; heating rate, 1°C/min; final temperature, 220°C (15 min isolate); injector temperature, 250°C; and detector temperature, 270°C. The fatty acids were identified by comparison of the retention times with those of standard purified fatty acids.

**Measurements of plasma and liver lipids, plasma glucose, insulin, leptin, adiponectin, resistin, and C-peptide** Total cholesterol (T-chol), triacylglycerol (TG), and PLs in plasma and liver, and non-esterified fatty acids (NEFA) and glucose (Glu) in plasma were determined by Wako commercial analytical kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Plasma insulin, leptin, resistin, and C-peptide were measured by mouse insulin (Morinaga Institute of Biological Science, Yokohama, Japan), leptin (Morinaga Institute of Biological Science), resistin (LINCO Research, St. Charles, MO, USA), and C-peptide (Yanaihara Institute Inc., Shizuka, Japan) ELISA kits, respectively. Plasma adiponectin was determined by a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan).

**Statistical analyses** All results were expressed as mean ±SE. The statistical analyses were performed with the STATISTICA statistical program package (StatSoft Inc., Tulsa, OK, USA). The significance of differences between dietary groups was determined by one-way analysis of variance with the Spjotroll/Stoline test at P<0.05.

**Results**

**Food consumption and final body weight** The mean food consumption of mice in the lard, fish oil, and kazunoko lipid diet groups was 4.3 g/d/mouse, 4.2 g/d/mouse, and 4.4 g/d/mouse, respectively. The final mean body weights were as follows: lard group, 46.3±1.4 g; fish oil group, 51.4±1.3 g; and kazunoko lipid group, 48.8±1.3 g. There were no marked differences in food consumption between these experimental diet groups. There were no significant differences in final body weights between kazunoko lipid and lard diet groups, but mice in the fish oil group had a significantly greater body weight than those in the lard group.

**Plasma and liver lipids** The plasma and liver lipid concentrations of mice fed lard, fish oil, or kazunoko lipid diets are shown in Fig. 1. The plasma T-chol and PL concentrations were significantly lower in the fish oil and kazunoko lipid groups than in lard diet group. There were no significant differences in plasma TG or NEFA concentrations among the three experimental diet groups. In liver, although the T-chol levels were significantly lower in mice fed a fish oil diet than those fed a lard diet, this difference was not significant between the kazunoko lipid and lard diet group. However, the lower T-chol level in the fish oil group compared with the kazunoko diet group was not statistically significant. There were no significant differences in liver TG and PL contents among the experimental diet groups.

**Plasma glucose, insulin, leptin, adiponectin, resistin, and C-peptide** The plasma Glu, insulin, leptin, adiponectin, resistin, and C-peptide content of mice fed lard, fish oil, or kazunoko lipid diets are shown in Fig. 2. Mice on the fish oil diet had a significant reduction of plasma Glu content compared with the lard group, and although levels tended to be lower in the kazunoko lipid group compared with the lard group, this difference was not statistically significant. Further, the difference between plasma Glu in the kazunoko lipid and fish oil diet groups was not significant.

Levels of plasma insulin, leptin, and adiponectin in the kazunoko lipid group were not significantly different from those in either the lard or fish oil diet groups. In contrast, the fish oil group had a significantly higher plasma insulin, leptin, and adiponectin content than the lard group. Levels of plasma resistin were significantly lower in the kazunoko lipid diet group than the lard diet group, but this difference was not evident in the fish oil group. However, there was no statistically significant difference in plasma resistin levels between the kazunoko and fish oil diet groups.

The levels of plasma C-peptide in the kazunoko lipid
group was significantly lower than the fish oil group, but was similar to the lard group, although the difference between the fish oil diet group and the lard group was not statistically significant.

**Fatty acid composition of plasma and liver** The plasma and liver fatty acid compositions of mice fed the lard, fish oil, or **kazunoko** lipid diets are shown in Table 2. The percentage of plasma 16:0 was significantly higher in both the fish oil and **kazunoko** lipid diets than in the lard diet group. The 18:1n-9 and MUFA percentages were significantly lower in order of fish oil < **kazunoko** lipid < lard diet groups. The **kazunoko** lipid group had a significantly lower 18:2n-6 percentage than the lard group. The percentage of plasma 18:2n-6 in the fish oil diet group did not significantly differ from that in the lard diet group. The 20:4n-6 percentage was significantly lower in the order of **kazunoko** lipid.
< fish oil < lard diet groups. Although the 20:5n-3 percentage and the ratio of n-3 to n-6 were significantly higher in the order of kazunoko lipid > fish oil > lard diet groups, the 22:6n-3 percentage was significantly higher in the order of fish oil > kazunoko lipid > lard diet groups.

In the liver, the main differences in fatty acids between the diet groups were in 16:0, 18:1n-9, 20:4n-6, 20:5n-3, and 22:6n-3. The trends in these fatty acids of each diet group were similar to those observed in plasma. However, in the liver, the lower percentage of 18:2n-6 in the kazunoko lipid group was significant compared with both the lard and fish oil diet groups where the percentage of this fatty acid was similar. The ratios of n-3 to n-6 in both the liver and plasma were significantly higher in the order of kazunoko lipid > fish oil > lard diet groups.

**Discussion**

In our previous study, plasma T-chol and PL levels were significantly reduced in mice following an intake of 3% or more kazunoko lipid (Higuchi et al., 2006a). It has been reported that a fish oil intake also significantly lowers the plasma T-chol and PL levels (Higuchi et al., 2006b; le Morvan et al., 2002; Huang et al., 1986; Kuda et al., 2000). This is confirmed in the present study, where plasma T-chol and PL levels in mice on the kazunoko lipid diet were significantly lower than those in animals in the lard group and this significant decrease was also seen in the fish oil diet group. In contrast, the T-chol content of liver was not significantly reduced in the kazunoko lipid group, whereas it was significantly reduced in the fish oil diet group. This is consistent with our previous study on kazunoko lipids where they did not appear to influence levels of liver T-chol (Higuchi et al., 2006a).

An intake of n-3 PUFA inhibits the absorption and synthesis of, and enhances the degradation of cholesterol (Choi et al., 1989; Chen et al., 1987; Smit et al., 1994). The 20:5n-3 and 22:6n-3 content of the kazunoko diet was lower compared with the fish oil diet, even though fish oil was produced in order to have the same fatty acid percentages as in the kazunoko lipids (Table 1). The low 20:5n-3 and 22:6n-3 content of the kazunoko diet compared with fish oil diet was due to cholesterol and PLs, including kazunoko lipid. Perhaps, this low n-3 PUFA content reflects the lower percentage of liver and plasma 22:6n-3 in the kazunoko lipid group than those in the fish oil group. However, the lower percentage of liver and plasma 22:6n-3 seen in mice of the kazunoko lipid group compared with the fish oil group might not contribute to the difference in cholesterol levels seen in plasma and livers of animals on these two dietary regimens. The kazunoko lipid diet contained a large amount of cholesterol and phospholipids (Shirai et al., 2006a) compared with the fish oil diet and it is likely that these contributed to the liver T-chol content. However, lowering plasma T-chol level with kazunoko lipid intake was suggested as the amount of cholesterol included in kazunoko lipid hardly influenced the plasma T-chol level. The large amount of phospholipids could contribute the suppression of plasma cholesterol level. These results indicate that kazunoko lipids could have a similar lowering effect on plasma lipids as does fish oil.

Reports from a number of studies have indicated that an intake of fish oil or 22:6n-3 suppresses plasma Glu content (Shirai and Suzuki, 2004; Kuda et al., 2000; Miura et al., 1997). In our previous study, a significant reduction in plasma Glu content was also seen following an intake of diets containing 3% or more of kazunoko lipids (Higuchi et al., 2006a). In the present study, mice in the fish oil diet group had significantly reduced plasma Glu concentrations compared with mice fed the lard diet, but the tendency for a reduction in plasma Glu in mice fed the kazunoko lipid diet compared with the lard diet was not significant. The fact that the plasma Glu reduction in the kazunoko lipid diet group was not significant compared with the lard diet group may be related to the low dietary 20:5n-3 and 22:6n-3 content. Thus, it would appear that a kazunoko lipid intake can lower plasma Glu content in mice, but its effect is less marked than that of a fish oil diet.

A dietary intake of fish oil or 22:6n-3 has been shown to influence plasma insulin and adipocytokine levels (Flachs et al., 2006; Peyron-Caso et al., 2002; Perez-Matute et al., 2005; Neschen et al., 2006; Miura et al., 1997; Steerenberg et al., 2002). In the present study, an intake of fish oil also significantly increased insulin, leptin, and adiponectin levels but not resistin when compared with the lard diet. However, these increases were not observed in mice fed the kazunoko oil diet even though this oil has a similar 22:6n-3 percentage as fish oil. On the other hand, resistin levels were significantly decreased in mice on the kazunoko lipid diet. It is thought that leptin and adiponectin improve insulin sensitivity and, thus, the significant reduction of plasma Glu following intake of a fish oil diet could be related to changes in these hormone levels. The smaller reductions in plasma Glu levels in the kazunoko diet group are consistent with the more limited changes in these hormones in this group. Furthermore, the C-peptide level, which indicates the insulin secretion from the pancreas, of the lard diet group did not show the significant differences between the fish oil and the kazunoko lipid diet groups. On the contrary, the insulin level of lard lipid diet group was significantly lower than that of fish oil diet group, but there was no significant difference with the kazunoko lipid diet. These differences imply that the insulin metabolism of fish oil and kazunoko lipid diet groups may
differ from those of the lard diet group.

Following both the kazunoko lipid and fish oil diets, there was a marked increase in plasma and liver n-3 PUFA levels. The significantly lower plasma and liver 20:4n-6 levels in mice on the kazunoko lipid diet compared with those in the fish oil diet group were consistent with our previous observations of an intake of kazunoko lipid reducing plasma, liver, and brain 20:4n-6 levels (Higuchi et al., 2006a; Shirai et al., 2006b). The extent of PC intake does not appear to influence plasma fatty acid composition (Lim and Suzuki, 2002), but a large cholesterol intake, as would occur with the kazunoko lipid diet, has been shown to reduce the percentage of 20:4n-6 in plasma and liver (Shimada et al., 2003). Therefore, the mechanism of lowering plasma and liver 20:4n-6 levels by intake of kazunoko lipids may be also associated with dietary cholesterol. At any rate, these changes of fatty acid composition may influence the secretion of insulin and adipokines. Some reports have suggested that an intake of 20:5n-3 and 22:6n-3 activates the expression of leptin and adiponectin mRNAs (Flachs et al., 2006; Peyron-Caso et al., 2002; Perez-Matute et al., 2005; Neschen et al., 2006) and that 20:4n-6 enhances the expression of resistin mRNA (Haugen et al., 2005). Thus, in this study, it is conceivable that plasma adipokine concentration is related to the differences in the levels of 20:5n-3, 22:6n-3, and 20:4n-6 in plasma and liver from animals on the kazunoko lipid and fish oil diets.

In conclusion, we have shown that kazunoko lipids decrease plasma lipids and glucose to a similar or slightly lesser extent than that observed with fish oil. However, there were marked differences in the effects on levels of plasma insulin, C-peptide, and adipokines between these two oils as dietary components. These results suggest that the mechanism by which levels of plasma Glu are modulated may be different in animals fed a kazunoko lipid diet than for those fed a fish oil diet. Further, the difference between the levels of adipokines observed in animals fed either the kazunoko lipid diet or the fish oil diet may be influenced by the overall fatty acid composition of tissues. Based on these results, we suggest that a diet containing either kazunoko lipids or fish oil may help prevent CHD and/or diabetes.

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References


Effect of Kazunoko Lipids on Adipocytokines

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