

# Combination Effect of Herring Roe Lipids and Proteins on Plasma Lipids and Abdominal Fat Weight of Mouse

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**ABSTRACT:** Dietary effects of herring roe lipids (HR-L) and proteins (HR-P) on plasma lipids and abdominal fat pad weight were determined. The main lipid class of HR-L was phospholipids (74%) and the main fatty acids were palmitic acid (16:0, 25.8%), DHA (22:6n-3, 21.6%), EPA (20:5n-3, 14.4%), and oleic acid (18:1n-9, 13.2%). A little increase in total cholesterol level was observed in plasma lipids of mouse fed with HR-L, although HR-L contained 9% cholesterol. This would be due to the lowering effect of EPA and DHA contained in HR-L on plasma cholesterol. Replacement of a part of dietary protein (5%) to HR-P reduced abdominal fat pad weight, but not significantly. On the other hand, combination of HR-P and HR-L significantly reduced the fat pad weight of the mouse as compared with the control. A significant effect of HR-P + HR-L was also observed in the reducing plasma lipid levels.

**Keywords:** anti-obesity, antihyperlipidemia, DHA, EPA, fish protein, herring roe

## Introduction

The potent beneficial health effects of n-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) have been reported extensively. EPA and DHA are the predominant fatty acids found in fish oils. Both n-3 PUFA are effective in lowering blood pressure, reducing hyperlipidemia and arrhythmias, and preventing arterial thrombosis and cardiovascular disease, and are anti-inflammatory, anti-cancerous, and anti-obesity (Li and others 2003; Narayan and others 2006; Shahidi and Miraliakbari 2006). However, the intakes of EPA and DHA in Western countries such as the U.S.A. and the U.K. are below the lowest of the recommended intakes. Therefore, there is a demand for fish oil as a functional food or nutraceutical.

Fish roes contain significant amount of lipids with high levels of EPA and DHA. Total contents of long chain n-3 PUFA (mainly EPA and DHA) in fish roe lipids vary from 30% to 50% of total fatty acids (Tocher and Sargent 1984; Cowey and others 1985). This value is the highest one among fish oils. EPA and DHA in fish roe lipids are mainly contained as a part of phospholipids (PL). Biological effects of EPA and DHA as triacylglycerol (TG) form are well known, while there has been relatively a little information on those as PL form. Herring roe lipid consists of PL (> 70%) and TG (< 30%) (Kaitaranta and Ackman 1981; Miyashita and others 1999), indicating that they would be useful models for the biological study of EPA and DHA as PL form.

Although herring roe products are favorite marine foods to most of Japanese people, the high cholesterol level in herring roe lipids has reduced their consumption in Japan due to the possibility of increase in plasma cholesterol level by herring roe intake. However, herring roe lipids contain a large amount of EPA and DHA, which are well known to exhibit a lowering effect on plasma cholesterol. The main nutrient components of herring roe are protein (> 80%)

and lipids (> 15%), whereas other fish roe such as salmon roe shows higher lipid contents (> 30%). Fish proteins have been reported to reduce cholesterol levels in plasma and liver of rats (Ait-Yahia and others 2003a; Wergedahl and others 2004). To better understand the nutritional value of herring roe, the determination of combined effects of the lipids and the proteins would be needed. Therefore, we analyzed the combination effects of herring roe lipids and proteins on lipid metabolism in mouse.

## Materials and Methods

### Samples and extraction of lipids

Herring roe was kindly donated from Canadian Pacific Kazunoko Assn., Tokyo, Japan. The raw material was washed with water, and homogenized in chloroform/methanol/water (10:5:3, v/v/v). The homogenate was separated into 2 layers in a separating funnel and the lower layer was collected. Organic solvents were removed from the lower layer under vacuum using a rotary evaporator. The last traces of the solvents and water remained were removed under high vacuum. The herring roe lipids (HR-L) obtained was then weighed.

### Preparation of herring roe proteins

The lipid in the raw material was removed by 2 times extraction with ethanol (2:1, v/v) by homogenizing and filtering. The residue after the filtration of the homogenate was dried and the dried matter was used as herring roe protein (HR-P). Protein content of HR-P was more than 95% (w/w) as measured by the Kjeldahl method.

### Lipid analysis

Lipid profile of HR-L was checked by thin-layer chromatography (TLC) (Christie 1982). TLC was carried out on a 0.25-mm silica gel plate (Merck, Darmstadt, Germany) developed with chloroform/methanol/water (25:10:1, v/v) or with *n*-hexane/diethyl ether (70:30, v/v). The lipid composition was determined quantitatively with a TLC-flame ionization detector (FID) on Chromarod S-III using an Iatroscan TH-10 (Iatron, Tokyo, Japan) equipped with a Shimadzu C-R6A integrator (Shimadzu Seisakusho Co., Kyoto, Japan).

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Chromarods (Iatron, Tokyo, Japan) were activated by passing them through the FID scanner and 1  $\mu$ L of the sample solution was applied on each rod. The rods were developed 10 cm from the origin with benzene/diethyl ether/acetic acid (50:20:0.2, v/v). After developing, the rods were dried in a desiccator for a few minutes and then scanned by the Iatrosan. A part of the HR-L was separated into neutral lipids and phospholipids by silicic acid column chromatography eluting with chloroform and methanol, respectively. Column chromatographic separation was monitored with TLC using authentic standards. The methanol fraction was further analyzed by TLC-FID to determine lipid class of PL. The experimental procedure was the same as described above except for the developing solvent (chloroform/methanol/acetic acid [65:25:10, v/v]).

### Fatty acid composition

The fatty acid composition of sample lipid was determined by gas chromatography (GC) after conversion of fatty acyl groups in the lipid to their methyl esters by heating in a sealed tube at 90 to 100 °C for 1 h with 7% boron fluoride methanol under nitrogen. The methyl ester obtained by the transesterification was purified on a silicic acid column (Silica gel 60; Merck, Darmstadt, Germany) eluting with *n*-hexane and a mixture of *n*-hexane/diethyl ether (95:5, v/v). The methyl ester eluted with *n*-hexane/diethyl ether (95:5, v/v) was subjected to GC. The GC analysis was performed on a Shimadzu GC-14B (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (Omegawax 320 [30 m  $\times$  0.32 mm i.d.]; Supelco, Bellefonte, Pa., U.S.A.).

### Animals and diets

ICR mouse (11 wk of age) and C57bl/6J mouse (7 wk of age) were purchased from Japan CREA Co., Tokyo and from Japan Liver Co., Tokyo, respectively. They were housed at a constant humidity (55%) and temperature (20  $\pm$  1 °C) with a 12-h light/dark cycle throughout the experiment. After the ICR mouse was acclimated for 1 wk by feeding control diets, animals were randomly divided into 2 groups of 6 mice and given free access to the water and the experimental diet. In the case of C57bl/6J mouse, after acclimation for 15 d feeding commercial chow diet, the animals were fed a high fat diet containing 3% soybean oils and 17% lard for 19 d. The fatty C57bl/6J mice were randomly divided into 3 groups of 6 mice and given free access to the water and the experimental diet. The diet was prepared according to the recommendation of the American Inst. of Nutrition (AIN-93G) (Reeves and others 1993). The dietary components are shown in Table 1 (ICR) and 2 (C57bl/6J). The study protocol was approved by the committee of Hokkaido Univ.

### Sample collections

After 3 wk of feeding, the animals were starved for 1 night and anesthetized with diethyl ether. The mice were killed by exsanguination, and their blood was withdrawn at the abdominal artery. The liver and the brain were then excised and weighed. Both organs were perfused with 0.9% NaCl and lipids were extracted with chloroform/methanol (2:1, v/v) as described previously by Folch and others (1957). The abdominal fat pads composed of epidermal adipose tissues were also excised and weighed. Plasma lipid levels were measured with analytical kits (Wako Pure Chemicals, Osaka, Japan).

### Statistical analysis

The results were expressed as means  $\pm$  SE. Analysis of variance (ANOVA) was used to test for significant difference between control and experimental diets administered rats. Statistical comparisons were made by Scheffe's *F*-test. Differences with *P* < 0.05 were considered significant.

## Results and Discussion

The TLC-FID analysis showed that HR-L mainly consisted of phospholipids (74%) with triacylglycerol (9%), free fatty acids (4%), and other neutral lipids (12%). Cholesterol (9%/total lipids) was contained in the neutral lipids. The TLC-FID analysis also indicated that the main phospholipids in herring roe lipids were phosphatidylcholine (72%), followed by lisophosphatidylcholine (11.8%), phosphatidylserine (8.7%), and phosphatidylethanolamine (6.6%). The main fatty acids of herring roe lipids were palmitic acid (16:0, 25.8%), DHA (22:6n-3, 21.6%), EPA (20:5n-3, 14.4%), and oleic acid (18:1n-9, 13.2%). DHA and EPA contents in PL were 29.2% and 16.1%, respectively, while those in neutral lipids were 5.8% and 6.7%, respectively.

Table 3 shows the final body weight, liver weight, and fat pad weight of the ICR mouse fed control and HR-L diets. All animals

**Table 1 – Percentage composition of experimental diets for ICR mouse**

Diet ingredients	Group	
	Control	HR-L
Casein	23.0	23.0
L-cystein	0.3	0.3
Corn starch	29.7	29.7
Dextrinized corn starch	9.9	9.9
Cellulose	5.0	5.0
Sucrose	7.5	7.5
Oil		
Soybean oil	3.0	3.0
Lard	17.0	12.0
HR-L	–	5.0
Mineral mix	3.5	3.5
Vitamin mix	1.0	1.0
Choline bitartrate	0.2	0.2

**Table 2 – Percentage composition of experimental diets for C57bl/6J mouse**

Diet ingredients	High fat diet	Group		
		Control	HR-P	HR-L + HR-P
Protein				
Casein	23.0	20	15	15
HR-P	–	–	5	5
L-cystein	0.3	0.3	0.3	0.3
Corn starch	29.7	39.8	39.8	39.8
Dextrinized corn starch	9.9	13.2	13.2	13.2
Cellulose	5.0	5.0	5.0	5.0
Sucrose	7.5	10.0	10.0	10.0
Oil				
Soybean oil	3.0	7.0	7.0	–
Lard	17.0	–	–	–
HR-L	–	–	–	7.0
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2

**Table 3 – Final body weight, liver weight, fat pad weight of ICR mouse fed control and HR-L<sup>a</sup>**

	Group	
	Control	HR-L
Initial body weight (g)	36.9 $\pm$ 2.2	36.7 $\pm$ 1.8
Final body weight (g)	39.9 $\pm$ 4.6	37.8 $\pm$ 2.3
Body weight gain (g)	3.0 $\pm$ 3.6	1.1 $\pm$ 3.2
Food intake (g/d)	5.2 $\pm$ 0.7	4.2 $\pm$ 0.3
Liver weight (g/100 g of BW)	4.3 $\pm$ 0.4	4.9 $\pm$ 0.5
Abdominal fat pad weight (g/100 g of BW) <sup>b</sup>	6.1 $\pm$ 2.8	4.6 $\pm$ 1.6

<sup>a</sup>The values are mean  $\pm$  SE for 6 mice.

<sup>b</sup>Abdominal fat pads are made up of epididymal and perirenal adipose tissues.

remained healthy through the experimental period. There was no significant difference in the body weight, food intake, liver weight, abdominal fat pad weight, and fat pad/body weight. Parrish and others (1990) reported that lard-fed rats had 77% more fat in perirenal fat pads and 51% more fat in epididymal fat pads compared with fish oil-fed rats. Kawada and others (1998) also reported an anti-obesity effect of fish oil. They also found that the expression of uncoupling protein 1 (UCP1) in interscapular brown adipose tissue (BAT) was significantly higher in the fish oil diet-fed rats compared to that in the lard-fed group. In BAT mitochondria, substrate oxidation is poorly coupled to ATP synthesis because of the presence of UCP, thereby leading to energy dissipation, that is, heat production. Kawada and others (1998) suggested that the intake of PUFA found in fish oil such as EPA and DHA causes UCP induction and enhancement of thermogenesis, resulting in suppression of the excessive growth of abdominal fat pads. PUFA from vegetable oils also suppressed the excessive accumulation of adipose tissue, as compared to animal fats (Shimomura and others 1990; Okuno and others 1997). However, the activity of PUFA from vegetable oils was less than EPA and DHA from fish oil (Kawada and others 1998). The present study showed the lower abdominal fat pad weight of the HR-L-fed mouse than that of control fed mouse, however, without significant difference (Table 3). In the anti-obesity study of fish oil, 10% fish oil (Kawada and others 1998) or 20% fish oil (Parrish and others 1990) was fed to animals, whereas only 5% HR-L was replaced in total 20% dietary lipid in the present study. Significant difference in the reduction of adipose tissue weight and plasma lipid levels might be found by higher content of HR-L in the dietary fat.

The herring roe lipids (HR-L) used in this experiment contained 9% cholesterol, which can be calculated to be 0.45% of the diet. This might increase plasma cholesterol level. However, a little increase in total cholesterol level was observed in plasma lipids in ICR mouse (Table 4). This would be due to the lowering effect of EPA and DHA in HR-L on the cholesterol. Both PUFA are known to decrease TG levels in plasma. The plasma TG content in ICR mouse fed HR-L was lower than that in control (Table 4), although there was no significant difference.

**Table 4—Effects of experimental diets on the plasma lipids of ICR mouse<sup>a</sup>**

	Control	HR-L
Total cholesterol (mg/dL)	148.7 ± 18.6	155.2 ± 13.1
HDL cholesterol (mg/dL)	89.5 ± 15.9	89.7 ± 8.7
LDL cholesterol (mg/dL)	44.7 ± 8.6	53.7 ± 8.2
Triacylglycerol (mg/dL)	73.7 ± 27.7	59.7 ± 21.2
Phospholipids (mg/dL)	258.8 ± 20.1	225.8 ± 14.0
Glucose (mg/dL)	145.7 ± 48.8	126.0 ± 65.9

<sup>a</sup>The values are mean ± SE for 7 mice.

**Table 5—Fatty acid composition of liver lipids from ICR mouse fed control (lard + soybean oil) and HR-L (lard + soybean oil + HR-L) diets<sup>a</sup>**

Fatty acid (wt%)	Control	HR-L
16:0	22.7 ± 1.3	23.4 ± 1.5
18:0	8.0 ± 2.3	8.8 ± 1.9
16:1n-7	1.3 ± 0.4	1.1 ± 0.3
18:1n-7	2.4 ± 0.5	1.7 ± 0.1 <sup>b</sup>
18:1n-9	26.0 ± 4.5	21.9 ± 3.7
18:2n-6	18.0 ± 2.2	18.7 ± 1.4
20:4n-6	8.9 ± 2.9	3.6 ± 0.8 <sup>b</sup>
20:5n-3	0.1 ± 0.1	3.0 ± 0.4 <sup>b</sup>
22:6n-3	5.4 ± 1.5	12.3 ± 1.4 <sup>b</sup>

<sup>a</sup>The values are mean ± SE for 6 mice.

<sup>b</sup>Significantly different from control ( $P < 0.05$ ).

The fatty acid profiles of lipids from liver of ICR mouse are shown in Table 5. Significant increases in EPA (20:5n-3) and DHA (22:6n-3) and significant decrease in arachidonic acid (20:4n-6) were found in the liver lipids from the mice fed HR-L diet as compared with control. These changes in the fatty acid composition have been reported in other studies on fish oil administrations to rodents. Significant increase in DHA and significant decrease in arachidonic acid by feeding HR-L diet were also found in brain lipids (Table 6).

Herring roe contains relatively high amount of protein as compared with other fish roes. Fish proteins improved blood pressure of spontaneously hypertensive rats (Ait-Yahia and others 2003b, 2005). Furthermore, hypocholesterolemic effects of fish proteins have been reported (Ait-Yahia and others 2003a, 2005; Wergedahl and others 2004). The main active components of fish proteins in its hypocholesterolemic effect were considered to be fish peptides formed by digestion (Wergedahl and others 2004); however, the mechanism has not been elucidated. To better understand the nutritional value of whole herring roe, the dietary effect of herring roe protein (HR-P) would be very important. The effects of fish proteins on blood pressure and plasma cholesterol levels indicate that they may have a cardioprotective activity, as fish oil does. This suggests that the combined effects of herring roe lipids and proteins may be higher than that of individual. Therefore, in the next experiment, adult C57bl/6J mouse were fed high fat diet for 19 d, and then the dietary effect of HR-P or HR-P + HR-L was examined.

Table 7 shows body weight, liver weight, and abdominal fat pad weight fed HR-P and HR-P + HR-L. There was no significant difference in the body weight, food intake, and liver weight. Replacement of a part of dietary protein (5%) to HR-P reduced abdominal fat pad weight, but not significantly. On the other hand, significant decrease was observed by combination of HR-P and HR-L. When 5% HR-L was fed to ICR mouse (Table 5), abdominal fat pad weight reduced as compared with control, but was not

**Table 6—Fatty acid composition of brain lipids from ICR mouse fed control (lard + soybean oil) and HR-L (lard + soybean oil + HR-L) diets<sup>a</sup>**

Fatty acid (wt%)	Control	HR-L
16:0	22.7 ± 0.9	21.5 ± 0.8
18:0	19.3 ± 0.5	19.5 ± 0.7
18:1n-7	4.4 ± 0.3	4.2 ± 0.1
18:1n-9	17.7 ± 0.9	18.6 ± 0.9
20:1n-9	2.2 ± 0.5	2.7 ± 0.4
18:2n-6	0.7 ± 0.1	0.7 ± 0.1
20:4n-6	8.4 ± 0.8	7.2 ± 0.4 <sup>b</sup>
22:6n-3	13.7 ± 1.3	15.8 ± 1.0 <sup>b</sup>

<sup>a</sup>The values are mean ± SE for 6 mice.

<sup>b</sup>Significantly different from control ( $P < 0.05$ ).

**Table 7—Final body weight, liver weight, fat pad weight of C57bl/6J mouse fed HR-P and FR-P + HR-L<sup>a</sup>**

	Group		
	Control	HR-P	HR-P + HR-L
Initial body weight (g)	27.3 ± 1.4	27.3 ± 1.5	27.3 ± 1.7
Final body weight (g)	30.2 ± 1.4	29.5 ± 1.3	29.0 ± 1.6
Body weight gain (g)	2.9 ± 1.1	2.2 ± 0.3	1.7 ± 0.8
Food intake (g/d)	4.1 ± 0.4	3.8 ± 0.3	3.8 ± 0.4
Liver weight (g/100 g of BW)	3.7 ± 0.3	3.8 ± 0.3	3.9 ± 0.1
Abdominal fat pad weight (g/100 g of BW) <sup>b</sup>	5.6 ± 0.6	4.9 ± 1.2	3.2 ± 0.3 <sup>c</sup>

<sup>a</sup>The values are mean ± SE for 6 mice.

<sup>b</sup>Abdominal fat pads are made up of epididymal and perirenal adipose tissues.

<sup>c</sup>Significantly different from control ( $P < 0.05$ ).

significant. In the case of C57bl/6J mouse, 7% HR-L was mixed with HR-P and then fed to animals after high fat treatment. Higher concentration of HR-L than that in ICR mouse might be the reason for the significant reduction of abdominal fat pad weight found in C57bl/6J mouse. In addition, combination with HR-P might have effects on the abdominal fat pad reduction. Further study is required for the combined anti-obesity effect of fish oil with fish protein.

Akit-Yahia and others (2003a, 2005) reported the significant decrease in cholesterol levels in blood and in liver of rats when dietary protein (20% w/w casein) was replaced with 20% w/w fish proteins. Blood total cholesterol, LDL cholesterol, and triacylglycerol levels reduced by feeding 5% HR-P, but the difference was not significant (Table 8). Significant decrease in blood cholesterol, triacylglycerol, and phospholipid levels was observed by feeding HR-P + HR-L. These effects of HR-P + HR-L would be due to the combined effect of HR-P and EPA and DHA in HR-L. High dose of fish oil or fish protein may be beneficial for the significant lowering blood lipid levels or abdominal fat pad weight. On the other hand, the present study indicates that combination of fish lipids and proteins would be expected to obtain significant effects, although the content of each component is not so high. Fish roe mainly consists of protein and lipids containing EPA and DHA. Furthermore, fish roe lipids contain bioactive minor compounds such as carotenoids and coenzyme Q10. The HPLC analysis showed that the herring roe lipids used in this study contained lutein (6.4 µg/g lipid) and coenzyme Q10 (100 µg/g lipid). Therefore, the whole fish roe intake may be beneficial to human health.

**Table 8 – Effects of experimental diets on the concentration of plasma lipids<sup>a</sup>**

	Control	HR-P	HR-P + HR-L
Total cholesterol (mg/dL)	134.3 ± 5.7	126.8 ± 16.5	103.4 ± 5.5 <sup>b</sup>
HDL cholesterol (mg/dL)	72.3 ± 2.6	74.4 ± 3.8	61.6 ± 3.4 <sup>b</sup>
LDL cholesterol (mg/dL)	46.7 ± 5.7	41.6 ± 14.1	35.9 ± 3.3 <sup>b</sup>
Triacylglycerol (mg/dL)	77.7 ± 23.9	53.6 ± 11.8	30.4 ± 12.0 <sup>b</sup>
Phospholipids (mg/dL)	256.0 ± 7.5	255.6 ± 19.9	168.0 ± 20.4 <sup>b</sup>
Glucose (mg/dL)	87.6 ± 22.5	94.3 ± 31.1	76.9 ± 16.7

<sup>a</sup>The values are mean ± SE for 6 mice.

<sup>b</sup>Significantly different from control ( $P < 0.05$ ).

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