Different Palatability of Various Fish Oils to Mice: Beta-endorphin May Not Be Involved in the Mechanism

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Abstract: Given the importance of fish oil in the development of functional food, we investigated the palatabilities of various fish oils (pollock, saury, sardine, and tuna) to BALB/c mice. In the food consumption test, mice showed a preference for the diet supplemented with 10% pollock oil over those supplemented with the other oils, though the calories did not differ among the diets. In the two-bottle selection test, mice preferred 5% pollock oil to saury or sardine oil. In the one-bottle test, concomitant with a noticeable increase in fluid intake, plasma β-endorphin levels increased significantly in the 5% pollock oil, saury oil and sardine group as compared with the vehicle group immediately after a 20-min fluid intake. On the other hand, plasma β-endorphin levels did not differ among the three fish oil groups, although the fluid intake in the 5% pollock oil group was significantly higher than that in the 5% saury oil group or 5% sardine oil group. Thus, endogenous opioid peptide levels may contribute to the preference for oily over non-oily food but may not be the cause of variation in the palatability of different fish oils to mice. In contrast, the different fatty acid compositions of these fish oils may be involved in the different feeding behaviors.

Key words: Fish oil · Palatability · Two-bottle choice · One-bottle test · β-endorphin · Fatty acid composition

INTRODUCTION

The intake of n-3 polyunsaturated fatty acids (n-3 PUFA) in the form of dietary fish or fish oil supplements has health benefits ranging from a reduced risk of cardiovascular disease (Saremi and Arora, 2009) to combating sepsis (Barton et al., 1991; Daly, Lieberman et al., 1992), cystic fibrosis (Lawrence and Sorrell, 1993), diabetes (Rudkowska, 2010), rheumatoid arthritis (Kremer, 1996), and depressive disorders (Liperoti et al., 2009). In contrast, the intake of n-3 PUFA in a typical western-style diet is far below the recommended consumption levels (Hibbeln et al., 2006). Furthermore, compared with fish, fish oils have a lower toxicity risk because of reduced organochlorines and mercury contamination (Melanson et al., 2005; Foran et al., 2003). Fish oils may therefore provide a better long-term source of n-3 PUFA. In addition to its use in supplements, fish oils can be added to food products as functional ingredients (Kolanowski, 1999; Kolanowski and Laufenberg, 2006). Therefore, a better understanding of the orosensory properties and palatability of fish oils is expected to aid the development of food products with added fish oil.

It is well established that both animals and humans have a preference for fat (Drewnowski, 1997; Imaizumi et al., 2001; Mela and Sacchetti, 1991), the mechanism for which may involve endogenous opioid peptides (Appleyard et al., 2003; Mizushige et al., 2009). Free fatty acids are recognized by CD36, a putative fatty acid receptor on the tongue, therefore, free fatty acids may be involved in fatty food recognition (Laugerette et al., 2005). Furthermore, long-chain fatty acids seem to be the chemical cue involved in this orosensory perception of lipids. Rats subjected to a two-bottle preference test display a lower appetite for triglycerides and medium-chain fatty acids than for long-chain fatty acids (Tsuruta et al., 1999). On the other hand, dietary lipids consist mainly of triglycerides, and it is still not known whether there is a difference in the palatability among different fish oils containing mainly long-chain fatty acids.

The importance of fish oils as functional ingredients in the food industry and the limited knowledge of the differences in the palatability of dietary oils, in particular different fish oils, led us to undertake this study. We investigated the differences in the palatability of pollock oil, saury oil, sardine oil and tuna oil, and
whether those differences were associated with fatty acid compositions of the fish oils and endogenous opioid peptide levels.

**Methods:**

**Fish Oils:**

Pollock oil, saury oil, sardine oil, and tuna oil were obtained from Nippon Suisan Kaisha, Ltd. (Tokyo, Japan). The fish oils were refined with silica gel and activated clays and then deodorized by steam distillation. All the oils were stored at -20 °C until use. The fatty acid composition of the fish oils was measured with gas chromatography with a 6890N Network GC System (Agilent Technologies Japan, Ltd., Japan). The acid value (AV) of the fish oil was expressed as the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of substance. The anisidine value (AnV) was determined as the absorbance of the solution resulting from the reaction of 1g oil in 100ml of isooctane solvent and reagent (0.25% anisidine and glacial acetic acid). The peroxide value (POV) was determined with the ferric thiocyanate method (Lips et al., 1943).

**Animals:**

All animal experiments were conducted in complete compliance with the National Institutes of Health: Guide for the Care and Use of Laboratory Animals. Male BALB/c mice at 5 weeks of age were obtained from CLEA Japan Inc. (Shizuoka, Japan). Mice were housed one per cage with paper bedding at 23 ± 1 °C on a 12-h light/dark cycle and given free access to water and powdered standard mouse chow MF (Oriental Yeast Co. Ltd., Tokyo, Japan) for an acclimation period of 1 week.

**Food Consumption Test:**

Mice were housed three per cage (40 × 40 × 18 cm) with plastic walls and stainless steel grid covers. One drinking bottle was mounted on the cover of each cage. Four feed trays were placed at four corners of each cage. Prior to the food consumption test, mice were fed the powdered MF diet containing a 10% mixture of fish oils comprised of equal amounts of pollock oil, saury oil, sardine oil, and tuna oil for 5 days to habituate them to the fish oil–supplemented food source. The food was placed in all four feeding trays in each cage during the habituation period, and food intake from each feed tray was measured every day for five consecutive days to investigate whether the mice had a position preference. After the pre-test habituation period, mice were deprived of food for 7 h from 9:00 to 16:00 and then exposed to powdered MF diet individually mixed with 10% pollock oil, saury oil, sardine oil, or tuna oil, in the four feed trays for 17 h daily for another 5 days. The placement of the four feed types was randomized with respect to the four trays in each cage. The mice were given free access to water throughout the test. The components of the experimental diets are given in Table 2. Moisture, crude protein, crude fat, crude fiber, and crude ash were determined according to the standard method of the Association of Official Analytical Chemists (AOAC). The amount of energy in the diet was determined by bomb calorimetry (McLean and Tobin, 1987). Fresh food was provided every day, and food intake was measured from each feed tray. Throughout the pre-test and experimentation period, the room was maintained on a 12:12-h light/dark cycle with lights off at 18:00, so that the food consumption of animals occurred mainly in the dark phase (from 16:00 to 9:00).

**Two-bottle Selection Test:**

We also assessed the palatability response to different fish oils using two-bottle selection test to reduce postigestive influences. Mice were housed three per cage and given five training days to habituate them to the two-bottle selection situation. During the training period, animals were exposed to 2% sucrose solution or water for 20 min daily. The solutions were contained in two separate bottles with bent ball-bearing sipper tubes to prevent leakage. After the training period, in experiment 1, mice were deprived of water and food for 2 h from 14:00 to 16:00 and then exposed to the 5% pollock oil solution suspended in 0.3% xanthan gum aqueous solution (vehicle) in one bottle and 5% saury oil solution (in vehicle) in the other bottle for 20 min daily for another five consecutive days. The vehicle solution masked the oil texture. The positions of the bottles were changed on alternate days to avoid potential position bias. Bottles were weighed immediately before and at the end of the 20-min drinking session. Differences in bottle weights (g) were converted to solution consumption. In another experiment, differences in fluid intake between 5% pollock oil and sardine oil were also investigated, and solution consumption was evaluated in the same way. Throughout the training and experimentation period, the room was maintained on a 12:12-h light/dark cycle with lights off at 15:00 so that the animals were exposed to the solution 1 h after the start of the dark phase (16:00).
One-bottle Test:

One-bottle test was performed to investigate the relationship between the palatability response to different fish oils and endogenous opioid peptide levels. Twenty-four mice were divided randomly into four groups (vehicle, pollock oil group, saury oil group, and sardine oil group) and housed one per cage during the experimentation period. After water and food deprivation for 2 h from 14:00 to 16:00, the control group was provided with the vehicle solution, the pollock oil group was provided with 5% pollock oil (in vehicle), the saury oil group was provided with 5% saury oil (in vehicle), and the sardine oil group was provided with 5% sardine oil (in vehicle) for 20 min daily for five consecutive days. It was reported that 5% was the best concentration for rat in one-bottle test to consume appropriate volume of the oil solution (Mizushige, Saitoh, et al., 2009). In the preliminary test, we found that 20 min was the most suitable time course to evaluate the palatability of the oil fluids to mice as compared to 10 min and 30 min course. The intake reached maximum value at 20 min when the mice were provided with the oil fluid (data not shown). The light/dark cycle used and the method of evaluating solution consumption during the experiment were the same as those in the two-bottle test. Mice were then used to examine β-endorphin levels.

β-endorphin Measurement

On the last day of the one-bottle test, the mice in the vehicle, pollock oil, saury oil, and sardine oil group were subjected to β-endorphin measurement. The animals were decapitated after the 20-min solution consumption, and plasma was collected from carotid arteries for β-endorphin measurement with an ELISA kit (Peninsula Laboratories, San Carlos, CA, USA). All measurements were performed in duplicate.

Statistical Analysis:

All data are expressed as the mean ± SE. Statistical differences between two groups were determined using the Student’s t-test, and those among three or four groups were evaluated by Tukey’s multiple comparison test. All differences with a P value <0.05 were considered to be significantly different.

RESULTS AND DISCUSSION

Characterization of Fish Oils:

As shown in Table 1, regarding the fatty acid composition of the fish oils, the amounts of monounsaturated fatty acids (MUFA) contained in pollock oil and saury oil were greater than those in sardine oil and tuna oil (P < 0.05). The n-3 polyunsaturated fatty acids (n-3 PUFA) contents in sardine oil and tuna oil were significantly higher than those in pollock oil and saury oil (P < 0.05). Moreover, regarding the MUFA components in pollock oil and saury oil, 16- and 18-carbon fatty acids combined were higher in pollock oil than in saury oil (P < 0.05). In contrast, 20- and 21-carbon MUFA combined were lower in pollock oil than in saury oil (P < 0.05). There were no noticeable differences in saturated fatty acids or n-6 PUFA among the four types of fish oil. As shown in Table 2, no significant differences in acid value (AV), anisidine value (AnV) and peroxide value (POV) were detected among all the tested fish oils.

Consumption of Fish Oil-supplemented Diets:

The consumption of diets supplemented with different fish oils is shown in Fig. 1. Consumption of food containing 10% pollock oil was significantly higher than the consumption of food containing 10% saury oil (P < 0.05), or 10% sardine oil (P < 0.01), or 10% tuna (P < 0.05). In the preliminary test, there were no considerable changes observed in feed tray compartment preference when measuring the consumption of the mixed fish oil-supplemented diet in each feed tray every day (data not shown). Furthermore, diet composition analysis revealed that there were no differences in calories among the four fish oil-supplemented diets. Thus, the mice used in this study did not make selections based on the position of the feed tray in the cage or the calories of the diets.

Two-bottle Selection Test:

 Compared with the intake of a 5% saury oil solution, intake of a 5% pollock oil solution was significantly increased (P < 0.001; Fig. 2A). The intake of a 5% pollock oil solution was noticeably higher than that of a 5% sardine oil solution (P < 0.01; Fig. 2B).

One-bottle Test:

As shown in Fig. 3, compared with the vehicle control group, the solution intake was increased in the 5%
pollock oil group \((P < 0.01)\), saury oil group \((P < 0.05)\), and sardine oil group \((P < 0.05)\). Furthermore, the intake in the pollock oil group was higher than that in the saury oil group \((P < 0.05)\) and sardine oil group \((P < 0.05)\).

**Plasma β-endorphin Levels:**

As shown in Fig. 4, compared with the control group, plasma β-endorphin levels increased significantly in the 5% pollock oil group \((P < 0.05)\), saury oil group \((P < 0.05)\) and sardine oil group \((P < 0.05)\) in the one-bottle test. On the other hand, β-endorphin plasma concentrations did not differ among the pollock oil, saury oil and sardine oil groups.

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**Fig. 1:** Comparison of the food consumption of powdered diet supplemented with 10% pollock oil, saury oil, sardine oil, or tuna oil. Mice were exposed to the diets for 17 h daily for five consecutive days, and food consumption was expressed as grams consumed per day per mouse \((n = 6; {\^*}P < 0.05, {\^*}P < 0.01\) as compared with the pollock oil-supplemented diet).

**Fig. 2:** Comparison of the intake of 5% pollock oil, saury oil, and sardine oil solutions in the two-bottle selection test. The oils were suspended in 0.3% xanthan gum. Mice were exposed to the solution for 20 min daily for five consecutive days, and the solution intake (in grams) was expressed as the daily intake per mouse \((n = 6)\). (A) Intake of pollock oil solution and saury oil solution \(({{^*}^*}P < 0.001)\). (B) Intake of pollock oil solution and sardine oil solution \(({{^*}^*}P < 0.01)\).
Fig. 3: Comparison of the intake of the vehicle solution, 5% pollock oil, saury oil and sardine oil solution in the one-bottle test. The oils were suspended in 0.3% xanthan gum (vehicle). Mice (n = 8) were exposed to the solution for 20 min daily for five consecutive days, and the 20-min intake shown was measured on the last day. *P < 0.05, **P < 0.01 versus the vehicle group, #P < 0.05 versus the pollock oil group.

Fig. 4: Comparison of the plasma β-endorphin levels in the vehicle, 5% pollock oil, saury oil and sardine oil groups in the one-bottle test. Mice (n = 8) were exposed to the solution for 20 min daily for five consecutive days, and the plasma β-endorphin levels were measured 20 min after exposure to the solution on the last day. *P < 0.05 versus the vehicle group.

Table 1: Fatty acid composition (%) of the fish oils

<table>
<thead>
<tr>
<th>Fatty acids (g/100 g fatty acids)</th>
<th>Vehicle</th>
<th>Pollock oil</th>
<th>Saury oil</th>
<th>Sardine oil</th>
<th>Tuna oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>4.4 ± 0.1</td>
<td>6.6 ± 0.2</td>
<td>5.8 ± 0.1</td>
<td>5.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>10.5 ± 0.2</td>
<td>10.4 ± 0.3</td>
<td>13.7 ± 0.1</td>
<td>11.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>2.6 ± 0.08</td>
<td>2.2 ± 0.06</td>
<td>2.4 ± 0.05</td>
<td>2.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>16:1</td>
<td>7.8 ± 0.09 a</td>
<td>3.0 ± 0.02 b</td>
<td>8.5 ± 0.06 a</td>
<td>5.3 ± 0.04 ab</td>
<td></td>
</tr>
<tr>
<td>18:1</td>
<td>16.0 ± 0.8*</td>
<td>5.8 ± 0.4 b</td>
<td>14.2 ± 0.5*</td>
<td>21.1 ± 1.1*</td>
<td></td>
</tr>
<tr>
<td>20:1</td>
<td>11.4 ± 0.2*</td>
<td>15.7 ± 0.4 b</td>
<td>3.4 ± 0.06 b</td>
<td>3.0 ± 0.04*</td>
<td></td>
</tr>
<tr>
<td>22:1</td>
<td>13.6 ± 1.2*</td>
<td>20.6 ± 1.4 b</td>
<td>3.0 ± 0.07*</td>
<td>1.9 ± 0.01*</td>
<td></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>1.0 ± 0.01</td>
<td>1.6 ± 0.01</td>
<td>1.2 ± 0.01</td>
<td>1.2 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>20:4n-6</td>
<td>1.5 ± 0.01</td>
<td>1.2 ± 0.01</td>
<td>1.5 ± 0.01</td>
<td>1.3 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.7 ± 0.02</td>
<td>1.1 ± 0.01</td>
<td>0.7 ± 0.01</td>
<td>0.5 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>20:4n-3</td>
<td>0.7 ± 0.01*</td>
<td>1.2 ± 0.02*</td>
<td>0.9 ± 0.03*</td>
<td>10.6 ± 0.7*</td>
<td></td>
</tr>
<tr>
<td>20:5n-3</td>
<td>10.3 ± 0.8*</td>
<td>6.4 ± 0.7*</td>
<td>19.2 ± 0.6*</td>
<td>7.0 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.1 ± 0.04</td>
<td>1.4 ± 0.01</td>
<td>1.6 ± 0.06</td>
<td>1.5 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>22:6n-3</td>
<td>7.4 ± 0.6*</td>
<td>11.8 ± 0.6*</td>
<td>17.6 ± 0.4*</td>
<td>23.3 ± 0.9*</td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>17.5 ± 0.5</td>
<td>19.2 ± 0.8</td>
<td>21.9 ± 0.9</td>
<td>18.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>MUFA</td>
<td>48.8 ± 1.3*</td>
<td>45.1 ± 1.2*</td>
<td>29.1 ± 1.2 b</td>
<td>31.3 ± 1.5 b</td>
<td></td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td>2.5 ± 0.8</td>
<td>2.8 ± 0.6</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td>20.2 ± 1.1*</td>
<td>21.9 ± 1.6*</td>
<td>40.0 ± 0.9 b</td>
<td>42.9 ± 1.5*</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE. Values are the means of three separate samples processed independently. Means in a row with superscripts without common letter are different, P < 0.05. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Table 2: Acid value, anisidine value, and peroxide value of the fish oils

<table>
<thead>
<tr>
<th>Component</th>
<th>Pollock oil</th>
<th>Saury oil</th>
<th>Sardine oil</th>
<th>Tuna oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid value (AV)</td>
<td>0.16 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Anisidine value (AnV)</td>
<td>4.43 ± 0.1</td>
<td>4.87 ± 0.22</td>
<td>4.16 ± 0.17</td>
<td>4.23 ± 0.09</td>
</tr>
<tr>
<td>Peroxide value (POV) (meq/kg)</td>
<td>0.85 ± 0.12</td>
<td>0.81 ± 0.12</td>
<td>0.93 ± 0.23</td>
<td>0.78 ± 0.18</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE. Values are the means of three separate samples processed independently. AV, acid value; AnV, anisidine value; POV, peroxide value.

Table 3: Composition of the 10% fish oil–supplemented diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Pollock oil (g/100g diet)</th>
<th>Saury oil (g/100g diet)</th>
<th>Sardine oil (g/100g diet)</th>
<th>Tuna oil (g/100g diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>7.3</td>
<td>7.4</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.7</td>
<td>20.8</td>
<td>20.7</td>
<td>20.9</td>
</tr>
<tr>
<td>Crude fat</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.6</td>
<td>2.7</td>
<td>2.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Crude ash</td>
<td>5.2</td>
<td>5.3</td>
<td>5.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Energy (MJ/100g)</td>
<td>1988.6</td>
<td>1987.9</td>
<td>1988.1</td>
<td>1988.4</td>
</tr>
</tbody>
</table>

Discussion:

Our current study compared the palatability of four fish oils (pollock, saury, sardine, and tuna) to BALB/c mice and showed that mice had different preference on various fish oils.

Oxidation of lipids is a common and frequently undesirable chemical change that may impact the flavor, aroma, nutritional quality, and, in some cases, even the texture of a product. Therefore, we first investigated the degree of rancidity of four types of refined fish oils by measuring acid value (AV), anisidine value (AnV) and peroxide value (POV). As lipid oxidation indicators, AV is a measure of fat acidity that reflects the amount of fatty acids hydrolyzed from triglycerides. AnV test is used to assess the secondary oxidation of lipid, which is mainly imputable to aldehydes and ketones, and POV is a measure of the formation of peroxide or hydroxyperoxide groups that are the initial products of lipid oxidation. Our results showed no significant differences in AV, AnV or POV among the tested fish oils. Thus, the difference in palatability of the different types of fish oils to BALB/c mice was not due to the quality of the oils.

The mice chose pollock oil over saury oil, sardine oil and tuna oil in 10% fish oil-supplemented diets consumption test. The long-term (17 h) session is considered to include postprandial feedback effects. It has been demonstrated that independent of the orosensory pleasure derived from eating calorie-dense foods, ingestion of nutrients positively reinforces feeding behavior (Sclafani, 2001). Postigestive fat metabolism, in particular fatty acid oxidation, influences the intake and palatability of fat (Leonhardt and Langhans, 2004; Suzuki et al., 2006). Fatty acid composition analysis showed that the content of n-3 polyunsaturated fatty acids (PUFA) in sardine oil and tuna oil was higher than that in pollock oil. n-3 PUFA including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are considered nutritional factors with the potential to modulate food intake. n-3 PUFA may directly increase postprandial satiety in humans immediately after and 2 h after a test dinner (Parra et al., 2008). n-3 PUFA may suppress the appetite because of an effect on appetite-related hormones such as leptin, glucagon-like peptide-1 (GLP-1), and cholecystokinin (CCK). Leptin, an adipocyte-derived hormone that acts on hypothalamic neurons to suppress the appetite and regulate energy, plays an important role in energy homeostasis in humans and rodents (McGarry 1995). When n-3 PUFA consumption increases, plasma leptin levels and mRNA levels for leptin-related neural peptides become optimized in C57BL/6J mice (Wang et al., 2002). In addition, the gut also participates in the hunger-satiety circuit by secreting satiety hormones such as GLP-1 and CCK (Little et al., 2005; Verdich et al., 2001). GLP-1 and CCK are key hormones in appetite control and satiety, and the G protein-coupled receptor GPR120 stimulates secretion of GLP-1 and CCK upon binding of free fatty acids, in particular, n-3 PUFA (Hirasawa et al., 2004; Oh et al., 2010). The low intake of sardine oil and tuna oil, which both have a high content of n-3 PUFA, may be partly due to the effect of these oils on the secretion of satiety hormones. Interestingly, mice showed a preference for pollock oil over saury oil, although both fish oils contain comparable amounts of saturated and unsaturated fatty acids. Regarding monounsaturated fatty acids (MUFA) components, the 16- and 18-carbon fatty acid content was higher and the 20- and 21-carbon fatty acid content was lower in pollock oil than in saury oil. How the altered MUFA compositions affects postigestive actions remains to be clarified.

In two-bottle selection test, the mice showed preference for pollock oil over saury oil and sardine oil. Since the session was carried out in a short term (20 min), the differences in ingestive behavior among different fish oil fluids were thus possibly relevant to the orosensory response to different oils. It has been demonstrated that the licking responses of BALB/c mice to 16- and 18-carbon unsaturated long-chain fatty acids are high, and those to palmitic acid (16:0) and arachidonic acid (20:4) are low, suggesting that the
saturated state of the fatty acid and the carbon chain length affect the sensitivity to fatty acids (Yoneda et al., 2009). In addition, significant amounts of triglycerides are immediately lipolyzed into fatty acids in the oral cavity by lingual lipase, which is usually secreted from von Ebner's glands (Kawai and Fushiki, 2003). It is thus suggested that the different palatability response to different fish oils may be partly attributed to the altered fatty acid compositions.

It is well established that palatable compounds including those that taste sweet or fatty activate the reward systems of the brain (Kelley et al., 2005; Neary and Batterham, 2010; Fulton, 2010), and extensive research indicates a strong relationship between endogenous opioid peptides and food intake (Mercer and Holder, 1997; Gosnell and Levine, 2009). β-endorphin is an endogenous opioid peptide neurotransmitter found in the neurons of both the central and peripheral nervous systems, and the levels of β-endorphin in serum and cerebrospinal fluid increase when rats are provided with sweet, but not bitter, solutions (Yamamoto et al., 2000). The one-bottle test in our current study showed that the plasma levels of β-endorphin were elevated in the pollock oil and saury oil group as compared with the vehicle group, concomitant with significantly increased solution intake. Mizushige et al. demonstrated that together with the significantly increased intake in the 5% corn oil group as compared with the vehicle group, β-endorphin levels in serum and cerebrospinal fluid of Wistar rats are significantly increased 15 min after the ingestion of corn oil, followed by a rapid decrease and maintenance at the basal level throughout the rest of the experimental period (Mizushige et al., 2009). Thus, a rapid release of β-endorphin after oil ingestion contributes to the hedonic preference and ingestive behavior for fat.

In contrast, our results showed that the plasma β-endorphin levels in the pollock oil and saury oil groups were comparable, although the amount of the solution consumed by the saury oil group was significantly lower than that consumed by the pollock oil group in the one-bottle test. Thus, the levels of endogenous opioid peptides may not be a crucial factor contributing to the variation in palatability of different fish oils to the mice. In other words, different feeding behaviors of BALB/c mice with respect to different fish oils provided in a short term may not be due to a difference in hedonic preference.

**Conclusion:**

This study showed different palatability of various fish oil to BALB/c mice using the food consumption test, the two-bottle selection test, and the one-bottle test. β-endorphin levels may have been involved in the preference for oily food over non-oily food, but a mechanism that does not involve β-endorphin levels, such as fatty acid compositions, may be related to differences in the palatability of various types of oils to mice.

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