Effects of Different Triglyceride Saturated Fatty Acids on Tissue Lipid Level, Fatty Acid Composition, Fecal Steroid Excretion, Prostacyclin Production, and Platelet Aggregation in Rats

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Received August 20, 1992

Male Sprague-Dawley rats were fed for 3 weeks cholesterol-enriched diets containing 7% interesterified fats in which saturated fatty acids, laurie, myristic, palmitic, and stearic acids, were the sole variable. The dietary fat was composed of 50% saturated fatty acid, 30% oleic acid, 14% linoleic acid, and 6% a-linolenic acid (P/S = 0.4, n-6/n-3 = 2.3). There was no difference in food intake, body weight gain, or liver and epididymal adipose tissue weight among the groups, although the apparent fatty acid absorption decreased with increasing chain-length of dietary saturated fatty acids. Plasma lipid levels were comparable among the groups, but the concentration of liver cholesterol was significantly lower, and fecal excretion of neutral but not acidic steroids was significantly higher in the stearic acid group even compared with the palmitic acid group. The liver triglyceride decreased upon feeding palmitic or stearic acid fats. The fatty acid composition of adipose tissue but not liver triglyceride apparently reflected the source of dietary fats, but a considerable portion of stearic acid appeared to be desaturated to oleic acid. The proportion of arachidonic acid in plasma cholesterol ester and that of linoleic acid in liver phosphatidylethanolamine decreased with the chain-length of saturated fatty acids. The stearic acid-fat significantly reduced platelet aggregation by ADP and aortic production of prostacyclin, and there was a similar but to a less marked response pattern in the palmitic acid-fat. These observations suggest that the different saturated fatty acids exert differential effects on various lipid parameters.

The type of dietary fats is a crucial determinant for the plasma cholesterol level. The classical studies by Keys et al.1) and Hegasted et al.2) clarified the contrasting effects of saturated (S) and polyunsaturated (P) fatty acids on the level of plasma cholesterol. Although these pioneer studies pointed out the importance of individual fatty acids, recent information is indicative that saturated fatty acids can not be regarded as as a single entity, and different saturated fatty acids appear to differently influence plasma cholesterol levels more than previously indicated.

Grundy and his colleagues3,4) showed that stearic acid is not hypercholesterolemic but it rather reduces plasma cholesterol in hamsters. They suggested palmitic acid may be responsible for an elevating effect of the saturated fatty acids. In contrast, Hayes et al.5,6) presented evidence that supports a antihypercholesterolemic propensity of palmitic acid in hamsters and in monkeys. The latter authors pointed to a possible hypercholesterolemic tendency of myristic acid. In accordance with these observations, palm oil has been shown to be less hypercholesterolemic than predicted from its high content of palmitic acid.8,9) In these contexts, the P/S ratio should be evaluated by considering the effects of individual fatty acids on the plasma cholesterol level. It is interesting that, insofar as the supply of linoleic acid is above the threshold level, approximately 6 energy %, no fatty acids may increase the plasma cholesterol level.8,9)

To understand the effects of different fatty acids on hypercholesterolemia, the use of dietary fats with a defined fatty acid composition is prerequisite. This may be achieved by mixing different fats appropriately, but it is virtually impossible to prepare fats with a reasonably comparable composition in respect to the individual fatty acids. The difference in the structure of dietary fat10,11) and/or the content of the non-glyceride components11) should also be taken into account.

Hence, we prepared dietary fats, in which the saturated fatty acids was the sole variable, by the interesterification technique,12) and fed these to rats to examine the effects of different saturated fatty acids, laurie, myristic, palmitic, and stearic acids, on plasma and liver lipid levels and fatty acid compositions in rats. In addition, aortic production of prostacyclin and platelet aggregation were examined since these parameters are implicated in atherosclerotic diseases.

Materials and Methods

Preparation of dietary fats. All the natural and synthetic triglycerides used in these studies were kindly donated by Fuji Oil Company (Osaka). The company also permitted us to use their facilities for preparing rearrangements of fats. The triglycerides having saturated: oleic:linoleic: a-linolenic acid ratios of 5:3:1.4:0.6 (P/S = 0.4, n-6/n-3 = 2.3) were prepared by the method of Kukla12) and modified as follows. The mixture of saturated triglyceride, either trilaurin, trimyristin, tripalmitin, or tristearin (the purity as estimated by the content of the respective fatty acids was above 97%), rapeseed oil and soybean oil were dehydrated at 110°C in vacuo. After cooling to 80°C, 0.3% (w/w) of Na-methoxide was added and the mixture was kept in vacuo for 1 h to produce interesterification. The reaction was stopped by 1% (w/w) water, and the product became dark immediately. Subsequently, 5% (w/w) of 10% phosphoric acid solution was added to neutralize the contents. The water layer was separated by centrifugation at 300 x g for 10 min and discarded. The oil layer was washed with water (20%, w/w) and dehydrated at 110°C in vacuo. The product was decolorized by white clay and charcoal and deodorized by vapor distillation at reduced pressure as is done for commercial edible oils. To the refined fats were added 100 ppm a-tocopherol and 25 ppm citric acid. The fatty acid composition of the purified interesterified fats was measured by gas-liquid chromatography (GLC) using a 10% Silar 10C column.12) The melting point and chemical
Table I. Chemical and Physical Characteristics and Fatty Acid Composition of Interesterified Fats

<table>
<thead>
<tr>
<th></th>
<th>Lauric fat</th>
<th>Myristic fat</th>
<th>Palmitic fat</th>
<th>Stearic fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>15.6</td>
<td>28.4</td>
<td>38.6</td>
<td>44.5</td>
</tr>
<tr>
<td></td>
<td>17.5</td>
<td>32.7</td>
<td>45.7</td>
<td>54.5</td>
</tr>
<tr>
<td>Chemical characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid value</td>
<td>0.18</td>
<td>0.11</td>
<td>0.38</td>
<td>0.67</td>
</tr>
<tr>
<td>Saponification value</td>
<td>219</td>
<td>206</td>
<td>190</td>
<td>185</td>
</tr>
<tr>
<td>Iodine value</td>
<td>60.1</td>
<td>60.0</td>
<td>60.5</td>
<td>61.3</td>
</tr>
<tr>
<td>Peroxide value</td>
<td>1.10</td>
<td>1.30</td>
<td>1.29</td>
<td>2.28</td>
</tr>
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<td>Fatty acid composition (wt%)</td>
<td></td>
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<td></td>
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<tr>
<td>12:0</td>
<td>43.6</td>
<td>0.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>14:0</td>
<td>0.2</td>
<td>44.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>16:0</td>
<td>3.0</td>
<td>2.6</td>
<td>48.0</td>
<td>3.9</td>
</tr>
<tr>
<td>18:0</td>
<td>1.1</td>
<td>1.0</td>
<td>1.8</td>
<td>43.3</td>
</tr>
<tr>
<td>18:1</td>
<td>30.9</td>
<td>30.8</td>
<td>29.8</td>
<td>31.3</td>
</tr>
<tr>
<td>18:2n-6</td>
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<td>13.6</td>
<td>14.1</td>
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<tr>
<td>18:3n-3</td>
<td>6.2</td>
<td>6.3</td>
<td>6.2</td>
<td>6.3</td>
</tr>
<tr>
<td>SFA</td>
<td>48.3</td>
<td>49.0</td>
<td>50.0</td>
<td>47.9</td>
</tr>
<tr>
<td>MUFA</td>
<td>30.9</td>
<td>30.8</td>
<td>30.1</td>
<td>31.8</td>
</tr>
<tr>
<td>PUFA</td>
<td>20.8</td>
<td>20.2</td>
<td>19.9</td>
<td>20.3</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Values were measured by the conventional methods. These characteristics are summarized in Table I.

Animal experiment. Male Sprague-Dawley rats, 4 weeks old (Seiwa Experimental Animals, Fukuoka), were given commercial rat chow (NMF, Oriental Yeast Co., Tokyo) and acclimated for 3 days in an air-conditioned room (20–23 °C, lights on 0800–2000 h). Then, the rats were divided into 4 groups of 6 rats each. The experimental diet was prepared according to the recommendation of the American Institute of Nutrition, and contained by wt%; casein 20, interesterified fat 7, vitamin mixture 1, mineral mixture 3.5, choline bitartrate 0.2, pt-methionine 0.3, cellulose 5, choleesterol 0.2, Na-cholate 0.05, corn starch 15, and sucrose 47.75. Vitamin and mineral mixtures AIN-TM were purchased from Oriental Yeast Co., Tokyo. The animals were fed diets ad libitum for 3 weeks. At the end of feeding, the animals were deprived of food for 7 h (0600–1300 h), and blood (10 ml) was withdrawn from the abdominal aorta in a syringe containing 1 ml of 3.8% NaCl-citrate under light diethyl ether anesthesia. The thoracic aorta, liver, and epididymal adipose tissue were immediately excised.

Lipid analyses. Plasma, liver, and adipose tissue lipids were extracted and analyzed for cholesterol, triglyceride, and phospholipid. Tissue lipids were separated into triglyceride, cholesterol ester, and phospholipids by thin-layer chromatography and their fatty acid compositions were measured by GLC. Apparent fatty acid absorption rate was calculated on the basis of fecal fatty acid analysis.

Platelet aggregation. After harvesting platelet-rich plasma (PRP) by centrifugation of blood at 160 × g for 10 min, platelet-poor plasma (PPP) was obtained by centrifugation at 1700 × g. To PRP was added either ADP (final concentration 5 µM) or collagen (10 µg) to induce platelet aggregation. In these inducer concentrations, platelets aggregated to an appropriate extent. The aggregation was measured by the platelet aggregation analyzer (Aggrecorder II, Kyoto Daischi Kagaku Co., Kyoto) according to the instructions of the manufacturer. PPP served as a blank reference. After measuring aggregation, platelets were harvested by centrifugation at 1500 × g, washed and analyzed for their phospholipid fatty acid composition as above.

Measurement of aortic production of prostacyclin (PG12). The thoracic aorta, approximately 25 mg, was incubated in Krebs-Henseleit bicarbonate buffer (pH 7.4) at 25 °C for 30 min, and the concentration of 6-keto-PGF1α in the medium was measured by a radioimmunoassay using a commercial kit (NEK-008, New England Nuclear, Boston, MA). The concentration of 6-keto-PGF1α was measured in a range of around 50% of the normalized percentage bound (% B/Bo) for the standard according to the instruction manual provided by the supplier. A portion of the aorta was used for the fatty acid analysis.

Statistical analysis. Data were analyzed by one-way analysis of variance, followed by inspection of all differences by Duncan’s multiple-range test.

Results
As shown in Table II, all groups or rats consumed the same amount of food, and there was no difference in weight gain among the groups. The relative weight of liver and epididymal adipose tissue was also comparable in all groups.

The fecal excretion of fatty acid increased with an increase in the chain-length of the saturated fatty acids in the dietary fats. Consequently, the apparent absorption rate of dietary fat was lowest in the tristearin group and highest in the lauric and myristic acid groups, the tripalmitin group being intermediate (Fig. 1). The changes in fecal fatty acid excretion and the apparent absorption rate were more marked after 3 weeks than after 1 week of feeding. In fecal fatty acids of the stearic and palmitic acid groups, stearic and palmitic acids occupied approximately 80% of total

Table II. Effects of Different Saturated Fatty Acid Fats on Growth Parameters and Tissue Weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Food intake (d/day)</th>
<th>Relative weight (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Gain</td>
<td>Liver</td>
</tr>
<tr>
<td>Lauric fat</td>
<td>96 ± 3</td>
<td>147 ± 6</td>
<td>17.7 ± 0.9</td>
</tr>
<tr>
<td>Myristic fat</td>
<td>96 ± 3</td>
<td>142 ± 7</td>
<td>16.8 ± 0.6</td>
</tr>
<tr>
<td>Palmitic fat</td>
<td>96 ± 3</td>
<td>147 ± 11</td>
<td>17.3 ± 0.8</td>
</tr>
<tr>
<td>Stearic fat</td>
<td>96 ± 2</td>
<td>143 ± 4</td>
<td>17.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats. * Epididymal adipose tissue.

Fig. 1. The apparent Absorption Rates of Different Saturated Fatty Acids.

Values are means ± SE of 6 rats. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid.

* Values not bearing a common letter in each feeding period are significantly different at p < 0.05.

of 6-keto-PGF1α was measured in a range of around 50% of the normalized percentage bound (% B/Bo) for the standard according to the instruction manual provided by the supplier. A portion of the aorta was used for the fatty acid analysis.
Fig. 2. Effects of Different Saturated Fatty Acid Fats on Plasma (A–C) and Liver (D–F) Lipid Levels.

Values are means ± SE of 6 rats. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid.
*Values not bearing a common letter are significantly different at p < 0.05.

Fig. 3. Effects of Different Saturated Fatty Acid Fats on Fecal Weight (A), and Fecal Excretion of Neutral (B) and Acidic (C) Steroids.

Values are means ± SE of 6 rats. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid.
*Values not bearing a common letter in each feeding period are significantly different at p < 0.05.

acids excreted, whereas the proportion of myristic and lauric acids was approximately 40 and 8% in rats fed myristic and lauric acid fats, respectively.

Dietary fats did not significantly influence concentrations of plasma cholesterol, triglyceride, or phospholipid, although the triglyceride concentration tended to be lower in rats fed lauric or myristic acid fats (Fig. 2). The concentration of liver triglyceride decreased with an increasing chain-length of dietary saturated fatty acids, and it was highest in the lauric acid group and the lowest in the stearic acid group. The concentration of liver cholesterol also was lowest in the stearic acid group. There was no difference in the concentration of adipose tissue cholesterol among the group (data not shown).

Figure 3 shows the effects of dietary fats on fecal excretion of steroids. Although no difference was observed in fecal
Table III. Effects of Different Saturated Fats on Fatty Acid Composition of Epididymal Adipose Tissue Triglyceride

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fatty acids (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12:0</td>
</tr>
<tr>
<td>Lauric fat</td>
<td>12.6±0.6a</td>
</tr>
<tr>
<td>Myristic fat</td>
<td>0.2±0.1b</td>
</tr>
<tr>
<td>Palmitic fat</td>
<td>—</td>
</tr>
<tr>
<td>Stearic fat</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means±SE of 6 rats.

*ab Values not sharing a common superscript letter are significantly different at p<0.05.

Table IV. Effects of Different Saturated Fats on Fatty Acid Composition of Plasma Cholesterol Ester

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fatty acids (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
</tr>
<tr>
<td>Lauric fat</td>
<td>0.3±0.0a</td>
</tr>
<tr>
<td>Myristic fat</td>
<td>1.4±0.1b</td>
</tr>
<tr>
<td>Palmitic fat</td>
<td>0.2±0.0a</td>
</tr>
<tr>
<td>Stearic fat</td>
<td>0.2±0.0a</td>
</tr>
</tbody>
</table>

Values are means±SE of 6 rats.

*ab Values not sharing a common superscript letter are significantly different at p<0.05.

Table V. Effects of Different Saturated Fats on Fatty Acid Composition of Liver Phosphatidylcholine and Phosphatidylethanolamine

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fatty acids (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td></td>
</tr>
<tr>
<td>Lauric fat</td>
<td>0.4±0.0a</td>
</tr>
<tr>
<td>Myristic fat</td>
<td>0.7±0.1b</td>
</tr>
<tr>
<td>Palmitic fat</td>
<td>0.2±0.0a</td>
</tr>
<tr>
<td>Stearic fat</td>
<td>0.2±0.0a</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td></td>
</tr>
<tr>
<td>Lauric fat</td>
<td>0.2±0.0</td>
</tr>
<tr>
<td>Myristic fat</td>
<td>0.2±0.0</td>
</tr>
<tr>
<td>Palmitic fat</td>
<td>0.2±0.0</td>
</tr>
<tr>
<td>Stearic fat</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

Values are means±SE of 6 rats.

*ab Values not sharing a common superscript letter are significantly different at p<0.05.

weight on the dry basis (Fig. 3A), there was a significant increase in fecal neutral but not acidic steroids in rats fed stearic acid fat as compared with those fed other triglycerides (Fig. 3B and C). This increase could be observed even after 1 week of the experimental diets.

The fatty acid composition of adipose tissue triglyceride in general reflected that of dietary fat, and there was a predictable increase in respective fatty acids in the lauric, myristic, and palmitic acid-fat groups (Table III). In the stearic acid-fat group the extent of increase in the proportion of stearic acid was less marked, and instead that of oleic acid was increased to a similar extent as in the case of an increase in saturated fatty acids. However, dietary fats did not modify the composition of polyunsaturated fatty acids. These dietary fat-dependent changes were not observed in liver triglyceride (data not shown).

In plasma cholesterol ester, there was a predictable increase in respective fatty acids in the lauric, myristic, palmitic, and stearic acid-fat groups (Table IV). In the palmitic and stearic acid-fat groups, oleic acid increased at the expense of arachidonic acid. Docosahexaenoic acid was also lower in the palmitic and stearic acid-fat groups than in lauric and myristic acid-fat groups.

In liver phosphatidylcholine, the effects of dietary fats were limited only to the composition of saturated fatty acids (Table V). Palmitic acid fat reduced the proportion of stearic acid and stearic acid fat reduced that of palmitic acid. The same results were demonstrated in plasma phosphatidylcholine (data not shown). However, in liver phosphatidylethanolamine, there was an increasing trend of stearic acid and a decreasing trend of linoleic acid when the chain-length of saturated fatty acids increased. There was no difference in the proportions of other major polyunsaturated fatty acids. In addition, in the plasma cholesterol ester fraction, the arachidonic acid percentage decreased accompanying an increase in the chain-length (38.4±6.0, 39.0±3.5, 26.6±3.9, and 25±3.3% for lauric, myristic, palmitic, and stearic acid fats, respectively), and the oleic acid percentage...
increased \( (21.8 \pm 3.1, 21.8 \pm 2.0, 20.4 \pm 2.4, \text{ and } 36.7 \pm 2.5\% \).

The fatty acid compositions of platelet phospholipids and aortic phosphatidylcholine were not largely influenced by the dietary fat sources, and no fatty acid-dependent difference could be seen (data not shown).

The maximum platelet aggregation by ADP but not by collagen tended to decrease with an increase in the chain-length of saturated fatty acids, and the difference between both extremes was significant (Fig. 4A). The aortic production of prostacyclin also tended to decrease with an increase in the chain-length, and again the difference between the lauric acid and stearic acid groups was significant (Fig. 4B).

**Discussion**

In this study, we prepared interestrified fats with P/S ratio of 0.4 referring to the profile of dietary fats commonly consumed in the Western countries, since the hypercholesterolemic effect of saturated fatty acid seems to be more marked when the P/S ratio is low.\(^5\) However, we have included a fairly high proportion of \( \alpha \)-linolenic acids as a source of polyunsaturated fatty acids in order to discover any preferable effect of this type of polyunsaturated fatty acids in the regulation of lipid metabolism.\(^1\) Under these situations, there was still a detectable difference in the effects of different saturated fatty acids on various lipid parameters examined herein.

There was no dietary fat-dependent difference in the plasma cholesterol level in this study. However, the concentration of liver cholesterol was significantly lower in rats fed stearic acid fat than in those fed other saturated fatty acid fats. This can at least in part be attributable to an increase in fecal excretion of neutral steroids on feeding stearic acid fat. Fat absorption also tended to decrease with an increase in the chain-length of saturated fatty acids in dietary fats. Although these intestinal events may be ascribed to the high melting point of stearic acid fat, there was also an decreasing trend of apparent absorption rate in palmitic acid fat, which contrarily did not decrease liver as well as plasma cholesterol. Since weight gain was not influenced by the type of dietary fats, it seems therefore likely that stearic acid has an effect on cholesterol metabolism which is distinguishable from that of palmitic acid.\(^3\)\(-7\) Thus, the different effects of different saturated fatty acids cannot totally be explained by the difference in the absorption rate.

There was apparently a contrasting effect of dietary fats on liver and plasma triglyceride levels, although the change in the latter was not statistically significant. This observation suggests that the longer chain saturated fatty acids promote hepatic secretion of VLDL.\(^20\)

The effects of different saturated fatty acids on tissue fatty acid composition were also marked. An increasing trend of stearic acid at the expense of linoleic acid was observed in liver phosphatidylethanolamine. Although the physiological significance of this observation is not clear, the desaturation index for linoleic acid, \( (20:3 + 20:4)/18:2 \), increased with an increase in the chain-length of saturated fatty acids, indicating a possible interaction of dietary saturated fatty acid and linoleic acid metabolism.\(^21,22\) However, these changes were not observed in liver phosphatidylcholine. The fatty acid composition of plasma cholesterol ester also was modified by the dietary fats in which the proportion of oleic acid increased at the expense of arachidonic acid. The fatty acids of plasma cholesterol ester are set by the hepatic acyl CoA cholesterol acyltransferase and the lecithin : cholesterol acyltransferase reaction; the fatty acids of the 2-position of plasma phosphatidylcholine are transferred to cholesterol ester.\(^23\) Since the fatty acid composition of plasma phosphatidylcholine was not influenced, this observation at least suggest a saturated fatty acid-dependent change in the fatty acid specificity of the acyltransferase enzyme\(^24\) and/or in the metabolic fate of individual cholesterol esters.\(^25\)

Thus, different saturated fatty acids differently influenced cholesterol ester metabolism.
In addition, stearic acid reduced maximum platelet aggregation when it was induced by ADP. The physiological significance of this observation may be limited, since this was not the case when collagen was added as an inducer, which represents a net effect on platelet aggregation.\textsuperscript{26} The reduction of the aortic production of prostacyclin by stearic acid fat rather points to a possible untoward effect of this saturated fatty acid in relation to other saturated fatty acids.\textsuperscript{8,9}

The results of this study did not reflect all the parameters in respect to the effects of different saturated fatty acids. Our dietary fats were low in the P/S ratio but contained a high proportion of n-3 polyunsaturated fatty acid relative to the n-6 counterpart. A more significant effect of n-3 polyunsaturated fatty acids on plasma cholesterol and triglyceride in the rat model in relation to humans\textsuperscript{17,27} may conceal the different effects of different saturated fatty acids. The study with dietary fats having the same P/S ratio but containing linoleic acid as the sole source of the polyunsaturated fatty acids will provide more conclusive information, and this line of study is in progress in our laboratory. In this context, the effects of fatty acid distribution in the triglyceride should be taken into consideration.\textsuperscript{10}

Acknowledgment. This paper was supported in part by a Grant-in-Aid for the Scientific Research B from the Ministry of Education, Science, and Culture of Japan.

References