Effects of Highly Purified Structured Lipids Containing Medium-chain Fatty Acids and Linoleic Acid on Lipid Profiles in Rats

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The purpose of this study is to examine the effects of highly purified structured lipids on serum and liver lipid profiles in rats. We also investigated in vitro hydrolysis of lipid emulsions by porcine pancreas. Hydrolysis rates of medium chain (M)-linoleic (L)-medium chain (M) types were 2 to 3 times higher than those of L-M-L types. The diet containing structured lipids or corn oil was administered to rats for 4 weeks. There were no significant differences in growth and food efficiency. Serum cholesterol levels were significantly lower (P < 0.05) in the 2-octanoyl-1,3-dilinoleoyl-glycerol, 2-linoleoyl-1,3-didecanoyl-glycerol, and 2-decanoyl-1,3-dilinoleoyl-glycerol groups than in the corn-oil group. Serum triglyceride levels were significantly lower (P < 0.05) in rats fed L-M-L types than those in the other groups. Serum non-esterified fatty acid (NEFA) and β-hydroxybutyrate levels were significantly higher (P < 0.01) in rats fed M-L-M types than those of the other groups. These results indicate that the feeding of highly purified L-M-L types could effectively improve serum and liver lipid profiles and that M-L-M types may be a preferable substrate for the pancreas and contribute to energy supply in rats.

Key words: structured lipids; lipid profiles; medium-chain fatty acid; rats

There has been much interest in structured lipids (SLs) in recent years because they can effectively supply functional fatty acids distributed in the glycerol structure. Therefore, a number of SLs have been synthesized by combination of a variety of fatty acids such as short-,1) and medium-chain fatty acids (MCFA),2) stearic,3) linoleic,4) α5) and γ-linolenic acid,5) and fish oils.6) In particular, SLs containing medium- and long-chain fatty acids that are esterified at the sn-1, 2 or 3 positions of the glycerol molecule, can be used for distinct nutritional and medical purposes utilizing their unique metabolism. Based on previous experimental evidence, it has been obvious that the SLs are far superior to conventional oils or their physical mixtures regarding physiological effects.7) Thus these unique lipids were considered as bioactive functional triglycerides to improve metabolic problems such as nitrogen balance8) and malabsorption.9)

In general it is well known that medium-chain triglyceride (MCT) are very different from long-chain triglyceride (LCT) with respect to their absorption and metabolism. They are absorbed via the portal vein to the liver and more readily oxidized than LCT, and used as an energy source without the carnitine transport system for mitochondrial entry. 10) Moreover, MCT may have advantages compared with LCT, such as a higher plasma clearance,10) with little tendency to deposit as body fat,11,12) decrease protein loss, and improved nitrogen-sparing effect.13) Although we now have available cooking oil consisting of MCT as a commercial product, a large intake of pure MCT may induce a deficiency of essential polyunsaturated fatty acid and create metabolic acidosis.10,14) The relative advantages and disadvantages of MCT remain controversial.

Although SLs have been exclusively applied in the clinical field as enteral and parenteral nutrition for the nourishment of infants or the treatment of patients with several specific conditions,15) such lipids are presently designed for uses in selected nutritional and food applications. However, it is rather difficult to obtain highly purified SLs as it entails an enormous cost of production. From these reasons, most studies have been confined to the effects of the...
unpurified materials. Accordingly, the relationship between the location of fatty acids in triglyceride molecules and the physiological functions of SLs have not been strictly elucidated. There is so far little information concerning digestion and the nutritional aspects of the highly purified SLs administered by enteral feeding in healthy models.

The SLs containing both medium- and long-chain fatty acids (LCFA) can retain most of the desired qualities of fatty acids while reducing the adverse effects of each. Therefore, it is important to evaluate whether the effective and safe administration of SLs containing MCFA might contribute to beneficial physiological functions in healthy models.

This study was designed to elucidate the nutritional and metabolic features of the highly purified SLs with a specific sn-positional distribution of fatty acids in healthy rats. The specific SLs were obtained by trans-esterification followed by high pressure liquid chromatography to obtain desired structural configurations. In addition, we examined the in vitro hydrolysis of lipid emulsions by porcine pancreas.

Materials and Methods

**Materials.** 2-linoleoyl-1,3-dioctanoyl-glycerol (C8:0-C18:2-C8:0; 8-L-8) and 2-linoleoyl-1,3-didecanoyl-glycerol (C10:0-C18:2-C10:0; 10-L-10) were prepared by transesterification of trilinolein (C18:2-C18:2-C18:2) and either caprilyl (C8:0) or caprin (C10:0) with 1,3-specific lipase (Lipozyme; Novozyme, Denmark). The enzyme reaction was done at 60°C for 5 hr. After completion of the reaction, the products were separated by HPLC with a reverse-phase column (Soken Chemical & Engineering Co., LTD., Tokyo), and then eluted at 1 ml/min with a mixture of ethanol:acetone (1:1, v/v).16)

2-octanoyl-1,3-dilinoleoyl-glycerol (C18:2-C8:0-C18:2; L-8-L) and 2-decanoyl-1,3-dilinoleoyl-glycerol (C18:2-C10:0-C18:2; L-10-L) were prepared by transesterification of trilinolein (C18:2-C18:2-C18:2) and either tricaprylin (C8:0-C8:0-C8:0) or tricaprin (C10:0-C10:0-C10:0) by the methods mentioned above. Purities of the triglycerides were as follows: L-10-L, 92.2%; L-10-10, 90.4%; L-8-L, 91.0%, and 8-L-8, 91.2%, respectively. These lipids were used in a series of experiments.

**Hydrolysis of lipid emulsions by porcine pancreas.** The comparison of the rates of hydrolysis of 8-8-8, 10-10-10, 8-10-8, 8-L-8, 10-L-10, L-8-L, L-10-L, olive oil, and trilinolein was made with a commercially available porcine pancreatic powder. The substrates were a lipid emulsion consisting of 8 ml of each lipid, 128 ml of gum arabic solution (10% wt/vol), and 64 ml of tris-HCl buffer (pH 9.0). These were homogenized by an ultrasonicator in ice bath for 10 min and the particle size were adjusted to the range of 2 to 10 μm in diameter. The assay solution, which contained 25 ml of substrate and 2 ml of sodium taurocholate (10% wt/vol), was adjusted to pH 9.2 with 0.1 N NaOH. With the mixture maintained at 37±0.1°C, 1 ml of porcine pancreatin was added, which produced a concentration of 8 to 16 USP units of lipase activity per ml. The pH was kept constant at pH 9.0 by titration with 0.1 N NaOH for 9 min. The data presented are the average of the titration volume of 0.1 N NaOH against time.

**Animals and diets.** Male Wistar rats, 4-weeks-old, were purchased from Japan SLC Inc. (Shizuoka, Japan) and were divided into 5 groups. Animals were housed individually under controlled room temperature (24±2°C) and lighting cycle (lighting 0800 to 2000 hours). Rats were fed the semipurified experimental diets ad libitum for 4 weeks. Experimental diets contained the following ingredients (g/kg diet): casein, 200; corn starch, 150; test oil, 100; AIN-76 mineral mixture,17) 35; AIN-76 vitamin mixture,17) 10; cellulose, 50; D,L-methionine, 3; choline bitartrate, 2 and ~1000 sucrose. The fatty acid compositions of dietary fats are shown in Table 1. At the end of the experiment period, after overnight fasting, they were anesthetized and killed for analysis. The care and use of animals were in accordance with the guidelines of the National Institute of Health and Nutrition.

**Chemical analysis.** Serum cholesterol and triglyceride concentrations were analyzed enzymatically using the Cholesterol E-test WAKO and Triglyceride E-test WAKO (Wako Pure Chemicals Co., LTD., Osaka, Japan). The concentrations of serum ketone bodies and non-esterified fatty acid (NEFA), and GOT and GPT activities were measured by a commercially available kit; Ketorex (Sanwa Chemical Co., Tokyo, Japan), NEFA-HA test Wako, GOT-UV test Wako, and GPT-UV test Wako (Wako Pure Chemicals Co., LTD.), respectively.

The liver lipids were extracted with chloroform-methanol (2:1, v/v). Cholesterol and triglyceride were assayed as described elsewhere.18)

The total lipids extracted from serum with chloroform-methanol (2:1, v/v) were directly trimethylated by the use of a boron trifluoride methanol

| Table 1. Fatty Acid Composition of Dietary Fats (Weight%) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Corn oil        | 8-L-8           | L-8-L           | 10-L-10         | L-10-L          |
| 8:0             | —               | 45.6            | 20.3            | —               | —               |
| 10:0            | —               | —               | 60.6            | 23.7            | —               |
| 16:0            | 10.9            | —               | —               | —               | —               |
| 18:0            | 1.6             | —               | —               | —               | —               |
| 18:1            | 25.8            | 2.6             | —               | 1.1             | —               |
| 18:2            | 60.4            | 50.7            | 78.3            | 37.7            | 75.4            |
| α-18:3          | 1.1             | —               | —               | —               | —               |
The degrees of hydrolysis by the porcine pancreas were estimated by the titrated volume of 0.1 N NaOH vs time to maintain the pH at 9.0 for 9 minutes. 8-8-8, tricaprylin; 10-10-10, tricaprin; 8-10-8, 2-decanoyl-1,3-dioctanoyl-glycerol; 10-L-10, 2-linoleoyl-1,3-didecanoyl-glycerol; 8-L-8, 2-linoleoyl-1,3-dioctanoyl-glycerol; L-8-L, 2-octanoyl-1,3-dilinoleoyl-glycerol; L-10-L, 2-decanoyl-1,3-dilinoleoyl-glycerol, olive oil and trilinolein were hydrolyzed in this study. Data were expressed as the means of duplication.

Fig. 2. Concentration of β-Hydroxybutylate in Serum of Rats Fed Structured Lipids-containing Diets.
Data are mean ± SEM of 9 or 10 rats. Values not sharing a common letter show a significant difference at P < 0.01.

No significant differences were observed in the body weight gain or the food efficiency. Relative liver weights of the rats varied within a small range, and the variation was negligible.

Serum concentrations of β-hydroxybutylate, which was measured as an index of energy production from fatty acids, were also significantly higher (P < 0.01) in the 8-L-8 and 10-L-10 groups than those of the other groups (Fig. 2).

Cholesterol and triglyceride concentrations of serum and liver are shown in Fig. 3. Serum cholesterol concentrations of rats fed the diets containing L-8-L, 10-L-10, and L-10-L were significantly lower (P < 0.05) than that of rats fed the diet containing corn oil. Serum cholesterol concentrations of rats fed SLs containing decanoic acid tended to be lower than those of rats fed the diets containing octanoic acid. The serum triglyceride concentration of rats fed the L-8-L was significantly lower (P < 0.05) than those of the corn oil and 10-L-10 groups. In the case of SLs containing LCFA at sn-1 and 3 positions, serum triglyceride concentrations tend to be lower than those of the other groups.

Although there was no significant difference in the liver cholesterol concentrations among any of the treatment groups, the liver triglyceride concentration was significantly lower (P < 0.05) in rats fed either the 10-L-10 or L-10-L diet than that of rats fed the corn oil diet. These were approximately 50% decrease compared with that of rats fed either the corn oil or 8-L-8 diets.

Concentrations of serum non-esterified fatty acid were significantly higher (P < 0.01) in rats fed the SLs containing MCFA at sn-1 and 3 positions than those of the other groups (Fig. 4). Those of rats fed the L-10-L diet were significantly lower than in the other groups.

Fatty acid compositions of the serum lipid are
Fig. 3. Cholesterol and Triglyceride Concentrations in Serum and Liver of Rats Fed Structured Lipids-containing Diet. Data are mean ± SEM of 9 or 10 rats. Values not sharing a common letter show a significant difference at $P<0.05$.

Fig. 4. Serum Non-esterified Fatty Acid (NEFA) Concentration of Rats Fed Structured Lipids-containing Diet. Data are mean ± SEM of 9 or 10 rats. Values not sharing a common letter show a significant difference at $P<0.01$.

listed in Table 2. Proportions of the linoleic acid in the serum of rats fed the SLs were significantly higher ($P<0.05$) than that of rats fed the corn oil-diet. Although the proportion of palmitic (16:0), oleic (18:1), and docosahexaenoic acid (22:6) showed statistical differences ($P<0.05$) between each group, these differences were likely to be a lower degree of magnitude.

The activities of serum GOT and GPT, which were used as indices of parenchymal cell injuries mainly in the liver, were not significantly different among any of the treatment groups fed the SLs (data not shown).

Discussion

This study demonstrates that lipid emulsions of the highly purified SLs, which containing MCFA at sn-1 and 3 positions and linoleic acid at sn-2 position, are more rapidly hydrolyzed, and that the feeding of these SLs provides a quick energy source and efficient absorption of essential fatty acid in the healthy rats as compared with those of either conventional oil or SLs composed of linoleic acid at sn-1 and 3 positions and MCFA at sn-2 position. In addition, significant decreases in serum cholesterol and liver triglyceride concentrations were observed by the feeding SLs (10-L-10 and L-10-L) with decanoic and linoleic acids. Serum triglyceride concentrations tended to decrease in rats fed diets containing L-M-L types such as L-8-L and L-10-L. In particular, statistical decrease in serum triglyceride was observed in rats
fed L-8-L diets as compared with those of rats fed corn oil, 8-L-8, and 10-L-10 diets. There were no significant differences in other biochemical parameters such as serum GOT and GPT activities and liver cholesterol level among any of the treatment groups.

As a result of in vitro hydrolysis of lipid emulsions, the hydrolysis of M-L-M types of SLs, such as 8-L-8 and 10-L-10, was greater than that of L-M-L types such as 8-L-8 and L-10-L. In addition, it seems that the hydrolysis rate of M-L-M type consisted of octanoic acid is little different from that of decanoic acid. Our results are not consistent with the report of Jandacek et al., which showed the in vitro hydrolysis rate of 1,3-dioctanoyl triglycerides with long-chain fatty acids at the sn-2 position was similar to that of medium-chain triglycerides with octanoic and decanoic acids in random positions.19) The reasons for such conflicting results may be in part attributed to the degree of free fatty acid released from the triglycerides by the action of pancreatic lipase or the degree of purification of SLs. Thus it is likely that the differences in hydrolysis rate between MCT and SLs may be due to the number of MCFA contained within the triglyceride molecule. In addition to the hydrolysis of SLs, we had also found that the SLs containing MCFA were absorbed more efficiently than a physical mixture of MCT and LCT through the lymph duct in restrained rats.20) On the basis of this evidence, we concluded that the SLs which contained MCFA at the sn-1 and 3 positions and linoleic acid at the sn-2 position might be preferable substrates for the pancreatic lipase, and that both MCFA and LCFA of the SLs were efficiently absorbed through the portal vein and the lymph duct, allowing the efficient supply of both energy source and essential fatty acid.

From these reasons, it is conceivable that the feeding of M-L-M types of SLs affects in vivo lipid metabolism and energy production. In this study, no difference was observed in the serum β-hydroxybutyrate and NEFA concentrations between octanoic and decanoic acid, which were located at sn-1 and 3 positions of the highly purified SLs, although the significant increase in serum ketone body concentration and retention of linoleic acid in rats fed M-L-M types reflect the features of both MCFA and LCFA. Therefore, SLs with either MCFAs served efficiently as an energy source even in the healthy model.

In this study, special attention has been paid to the significant decrease of serum cholesterol in the healthy rats fed the SLs with decanoic and linoleic acids. It is currently apparent that the feeding of highly purified SLs shows a tendency to decrease the serum cholesterol concentration, in particular in rats fed a diet containing either L-8-L, 10-L-10, or L-10-L compared with that of either the corn oil or 8-L-8 diet, suggesting that the SLs with decanoic and linoleic acids act advantageously on cholesterol metabolism. These results were also in agreement with the report by Nakagawa et al.21) Although these functions may be ascribed to the specific sn-1-position fatty acids, a closer mechanism of MCFA with SLs on cholesterol metabolism is not yet clear and deserves further study, because the action of MCFA with respect to the cholesterol metabolism also remains controversial.22,23)

Although it is plausible that the feeding of MCFA modulates to some extent the serum triglyceride concentration because these fatty acids were readily hydrolyzed and then used as an energy source, in the case of the feeding of SLs with decanoic and linoleic acids, our data are not necessarily applicable to the previous reports relating to MCT.24)

In this study, it is considered that the significant increases in serum β-hydroxybutyrate and NEFA concentrations are attributable to the MCFA located at the sn-1 and 3 positions of SLs. Our interpretation on the supply of SLs (8-L-8 and 10-L-10) as energy sources is consistent with the observations reported by Babayan.25) However, it is likely that much attention must be paid to the acidic stress of MCFA and metabolic disorders such as ketosis or acidosis, although the supplementation of LCFA is known to alleviate the impact of MCFA.26) Therefore it may be

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### Table 2. Serum Fatty Acids Compositions (wt%) in Rats Fed Diets Containing Structured Lipids

<table>
<thead>
<tr>
<th></th>
<th>Corn oil</th>
<th>8-L-8</th>
<th>L-8-L</th>
<th>10-L-10</th>
<th>L-10-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:0</td>
<td>nd</td>
<td>1.27±0.50</td>
<td>0.13±0.07</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>10:0</td>
<td>nd</td>
<td>0.19±0.10</td>
<td>0.90±0.19</td>
<td>0.12±0.08</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>0.72±0.27</td>
<td>0.49±0.05</td>
<td>0.51±0.09</td>
<td>0.48±0.02</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td>16:0</td>
<td>18.1±0.46ab</td>
<td>18.9±0.25a</td>
<td>17.2±0.48b</td>
<td>18.5±0.33ac</td>
<td>17.5±0.41bc</td>
</tr>
<tr>
<td>16:1</td>
<td>1.72±0.30</td>
<td>2.93±0.32</td>
<td>2.31±0.41</td>
<td>2.43±0.20</td>
<td>1.80±0.26</td>
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<td>18:0</td>
<td>10.5±0.40</td>
<td>10.7±0.55</td>
<td>10.3±0.27</td>
<td>9.99±0.57</td>
<td>10.4±0.25</td>
</tr>
<tr>
<td>18:1</td>
<td>12.9±0.91a</td>
<td>9.56±0.61bc</td>
<td>8.07±0.43b</td>
<td>10.2±0.72c</td>
<td>7.92±0.50b</td>
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<tr>
<td>18:2</td>
<td>21.0±1.26a</td>
<td>23.6±1.30b</td>
<td>26.8±0.70bc</td>
<td>26.8±1.81bc</td>
<td>30.2±1.00c</td>
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<tr>
<td>γ-18:3</td>
<td>0.55±0.19</td>
<td>0.19±0.09</td>
<td>0.68±0.11</td>
<td>0.62±0.14</td>
<td>0.19±0.13</td>
</tr>
<tr>
<td>20:4</td>
<td>26.5±1.79</td>
<td>28.9±1.86</td>
<td>29.0±1.22</td>
<td>24.6±1.75</td>
<td>29.3±1.15</td>
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<tr>
<td>22:6</td>
<td>2.29±0.24a</td>
<td>2.49±0.38a</td>
<td>2.31±0.45a</td>
<td>2.13±0.25a</td>
<td>0.66±0.34b</td>
</tr>
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</table>

Values are shown as mean±SEM of 10 rats.

Values in the same row not sharing a common letter show a significant difference at *P*<0.05.
necessary to distinguish the function and safety of SLs composed of MCFA and linoleic acid from those of triglyceride comprised of MCFA that have some deleterious impacts such as metabolic acidosis and the dysfunction of hepatocytes.

In conclusion, the feeding of highly purified SLs, which were composed of MCFA at the sn-1 and 3 positions and linoleic acid at the 2-position, resulted in more rapid hydrolysis, high concentration of NEFA, and remarkable production of ketone bodies in healthy rats. These SLs may be advantageous to effectively supply both an energy substrate and essential fatty acid even in the healthy model. Although the feeding of SLs combined both decanoic and linoleic acids may improve the serum cholesterol and triglyceride concentrations, these effects occurred independently of the sn-specific position of fatty acids. Contrary to the SLs containing decanoic and linoleic acids, it is likely that the feeding of SLs containing octanoic and linoleic acids exert stereospecific functions on the serum triglyceride concentration and energy production. Therefore, it seems to be of enormous importance to select SLs appropriately in accordance with the purpose of use. We expect that the production of triglycerides with specific fatty acids at specific sn-positions in the molecule may contribute to prevention of life-style-related diseases such as hypertriglyceremia or obesity, via the effective supply of physiologically functional fatty acids.

References

21) Nakagawa, M., Hiramatsu, Y., Mitsuyoshi, K., Yamamura, M., Hioki, K., and Yamamoto, M., Effect of various lipid emulsions on total parenteral


