LONG-TERM EFFECT OF MEDIUM-CHAIN TRIGLYCERIDE ON HEPATIC ENZYMES CATALYZING LIPOGENESIS AND CHOLESTEROGENESIS IN RATS

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Summary This study was conducted to investigate the long-term effect of dietary medium-chain triglyceride (MCT) as compared with that of corn oil feeding on lipid metabolism in rats. Both serum cholesterol and triglyceride levels in MCT-fed rats showed significant decrease during the experimental period of eight weeks, although liver cholesterol and triglyceride contents were not distinguishable between the two groups. Significant elevation of the activity of lipogenic enzymes, such as fatty acid synthetase (FAS) and malic enzyme (ME) of the liver, was observed in MCT-fed rats without any fat accumulation of the liver (fatty liver). The increase of lipogenic enzyme activity was accompanied by a significant reduction of essential fatty acids (EFA) such as 18:2 (ω6) and 20:4 (ω6) in total liver lipid. In contrast, hepatic β-hydroxy-β-methylglutaryl CoA (HMG-CoA) reductase activity was significantly decreased in MCT-fed rats, that would play an important role in achieving hypocholesterolemia. From these results obtained in a long-term experiment, it is concluded that exogenous MCT depresses the key enzyme catalyzing cholesterol synthesis with a concomitant elevation of lipogenic enzyme activity in the rat liver.

Hyperlipidemia, mainly hyper-cholesterolemia and/or -triglyceridemia, is considered to be one of the major risk factors responsible for the development of atherosclerosis, although more precise information on its pathogenesis is not yet

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Abbreviations: MCT, medium-chain triglyceride; FAS, fatty acid synthetase; ME, malic enzyme; EFA, essential fatty acid; HMG-CoA, β-hydroxy-β-methylglutaryl CoA; GLC, gas-liquid chromatography.

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available. Since certain types of dietary fats may induce hyperlipidemia, it is of practical importance to have a listing of those dietary fats that may be responsible in order to prevent premature atherosclerosis associated with obesity or not with it.

It has been widely accepted that a diet containing an EFA such as linoleic acid is useful for achieving a lowering of serum cholesterol and triglyceride in humans (1). However, it can not be overlooked that a tendency to overweight is often complicated because of the inevitable availability of energy supplying linoleate-containing fats.

During the past ten years, considerable attention has been received regarding physiological and nutritional aspects of MCT containing fatty acids of 8 and 10 carbons in chain length (2). The metabolism of MCT by the liver is different from that of long-chain triglyceride in that most of which is ingested is rapidly catabolized into CO₂ and ketone bodies (2, 3). Therefore, the clinical application of MCT will be of considerable importance in the prevention of the hyperlipidemia (2, 4, 5) as well as in the avoidance of the undesirable consequences of overweight (6).

The present study was undertaken to investigate the nutritional influence of MCT on lipid metabolism, focusing particularly on the key enzymes catalyzing cholesterogenesis and lipogenesis in the rat liver.

METHODS

Animals and diets. Male Wistar-strain rats (Shiihashi Co., Tokyo), weighing about 60 g, were housed in wire cages in a temperature- and humidity-controlled room (23°C and 50% relative humidity). Six rats were used in all experimental subgroups. Food and water were freely available, and food consumption and body weight were measured at 3 or 4 days intervals.

Synthetic diets containing by weight corn oil, 5% (CI) or 15% (CII), and MCT, 5% (MI) or 15% (MIT) were prepared as shown in Table 1, unless indicated otherwise. Each diet contained an identical amount of carbohydrate (57% sucrose) in order to obtain a fixed level of lipogenic enzyme activity. A MCT preparation, kindly supplied by Ono Pharmaceutical Co., Osaka, contained only trioctanolate by GLC analysis.

Animals received either MCT (experimental) or corn oil (positive control) for an observation period of 8 weeks. Then, they were sacrificed successively at 3 min intervals by decapitation, starting at 9:00 am after 4 hr of starvation. The blood was collected immediately, and the serum obtained by centrifugation after clotting. Livers were also removed immediately after decapitation and rinsed with a cold saline solution. Half of the liver was used for determination of lipogenic and cholesterogenic enzyme activity, and the remainder for other chemical analysis.
Enzyme and lipid analysis. The liver was homogenized in two volumes of buffer containing 0.25 M sucrose, 0.1 M potassium phosphate buffer (pH 7.4), 0.07 M KHCO₃, 1 mM EDTA, and 1 mM dithiothreitol. The homogenate was centrifuged at 105,000×g for 60 min and the soluble liver supernatant ("clear" sup) was used for determining the activity of FAS and ME spectrophotometrically as previously described (7).

The homogenate was centrifuged at 8,000×g for 20 min and the resulting postmitochondrial supernatant at 105,000×g for 60 min. The microsomal fraction thus obtained was used for the assay of HMG-CoA reductase activity. The enzyme activity was determined by modification of the method of Goldfarb and Pitot (8) as described by Iijima and Maruyama (9). The amount of mevalonate-3-¹⁴C formed from DL-HMG-3-¹⁴C-CoA as a substrate was determined with a dioxane-scintillation counting solution in a Packard Liquid Scintillation Spectrometer model 3380. The DL-HMG-3-¹⁴C-CoA preparation was kindly supplied by Drs. Y. Iijima and M. Maruyama (Sankyo Pharmaceutical Co., Tokyo).

Protein was assayed by the biuret method of Gornall et al. (10) and the method of Lowry et al. (11). Cholesterol concentrations in the serum and liver were determined according to Zlatkis et al. (12), and the triglyceride contents of the two tissues by the method of Van Handel and Zilversmit (13). Liver lipid was extracted by the method of Folch et al. (14), and contained hydroquinone (10 mg per g liver) and arachidic acid (Applied Science Laboratories, Inc.) as an internal standard for GLC analysis (5 mg per g liver). After washing the crude lipid extract by adding one-fifth volume of 0.2% HCl solution, methylation was carried out with 2 ml of redistilled methanol, containing 2% H₂SO₄, under N₂ gas overnight at 60°C (15). The methyl esters were separated by GLC (10% EGSS-X
coated on Gaschrom P, column temperature 180°C) using a Shimadzu GC-6A. The data were analyzed statistically by means of t-test.

RESULTS

The animals fed corn oil showed a normal growth rate, and those of group CII were the biggest among the four groups during the experimental period of 8 weeks. No difference between groups MI and MII was observed: the final body weight of group MI was as low as 92% of that of group CI, and that of group MII 75.3% of group CII. Total food intake and carbohydrate consumption (see Table 2) for the entire observation period of 8 weeks were not reduced in MCT-fed rats. Moreover, neither enlargement nor reduction of organ weight per 100 g body weight, such as of the liver, kidneys and heart, was observed in the MCT group.

Table 2. Effects of corn oil and MCT on lipogenic enzyme and HMG-CoA reductase activities.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipogenic enzyme activities</th>
<th></th>
<th>HMG-CoA reductase activity</th>
<th>Carbohydrate intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mµmoles/min/mg protein</td>
<td></td>
<td>pmoles/min/mg protein</td>
<td>(8 weeks) g</td>
</tr>
<tr>
<td>CI</td>
<td>FAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CII</td>
<td>32.5±3.1</td>
<td></td>
<td>105±6</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>33.5±2.9</td>
<td>89±7</td>
<td>296±9</td>
<td></td>
</tr>
<tr>
<td>MII</td>
<td>42.7±4.4</td>
<td>265±45</td>
<td>297±14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58.5±14.0</td>
<td>251±7</td>
<td>289±15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CII</td>
<td>58±11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCT 10% + corn oil 5%</td>
<td></td>
<td>16±3</td>
<td></td>
</tr>
</tbody>
</table>

1) In Experiment I, CI, corn oil 5% diet; CII, corn oil 15% diet; MI, MCT 5% diet and MII, MCT 15% diet were further designated a) to d), respectively, in order to represent each matched pair with statistical significance. In Experiment II, group corn oil 15%, and MCT 10%+corn oil 5% were also designated e) and f), respectively.

2) Mean±SEM

3) T-test analysis; * P<0.05; ** P<0.01; *** P<0.001.

Serum cholesterol concentrations in groups MI and MII were significantly lower than that in groups CI and CII (Fig. 1). The serum triglyceride level in group MII was the lowest among the experimental groups (Fig. 1). However, none of the changes in cholesterol and triglyceride levels of the liver were observed among the four experimental groups.

More significant elevation of the activity of lipogenic enzymes such as FAS and ME in the liver was observed in the MCT group compared with that in the corn oil group as shown in Table 2. In contrast, hepatic HMG-CoA reductase
activity in rats fed MCT-containing fats (10% MCT and 5% corn oil) was found to be significantly reduced compared with that in animals fed only 15% corn oil (Table 2).

No difference in total fatty acid content in the liver was observed among the four experimental groups. However, a striking difference in the fatty acid composition between the two oil feedings was evident as indicated in Table 3. Marked increase of monoenoic acid, such as 16:1 (ω7) and 18:1 (ω9), with concomitant decrease of polyunsaturated fatty acid, such as 18:2 (ω6) and 20:4 (ω6), in the liver lipid was induced by MCT feeding. In addition, a significant increase in trienoic acid, such as 20:3 (ω9), was revealed in MCT-fed rats. Therefore, the EFA index: ω7+ω9/ω6+ω3 was significantly higher in groups MI and MII than in groups CI and CII as indicated in Table 3.

DISCUSSION

The present study clearly demonstrates that the marked reduction in serum cholesterol and triglyceride levels is achieved in rats fed 5 or 15% MCT (exclusively trioctanoate) over the entire experimental period of 8 weeks with corn oil feeding provided as a positive control. The finding is generally in agreement with previous reports (4, 5), in which rats were maintained on diets containing 12 or 20% MCT with or without 2% corn oil for an observation period of 2 to 3 weeks. However, the mechanism of hypolipidemic effect by MCT ingestion still remains to be elucidated.
Table 3. Effects of corn oil and MCT on fatty acid composition in total liver lipid.

<table>
<thead>
<tr>
<th>Group</th>
<th>CI (a)</th>
<th>CII (b)</th>
<th>MI (c)</th>
<th>MII (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fatty acid content (mg/g liver)</td>
<td>29.6±2.1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>30.8±2.5</td>
<td>25.3±0.4</td>
<td>33.1±6.3</td>
</tr>
<tr>
<td>Fatty acid composition (%)&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 : 0</td>
<td>0.3±0.02</td>
<td>0.2±0.03</td>
<td>0.3±0.04</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>16 : 0</td>
<td>18.2±0.6</td>
<td>19.4±0.7</td>
<td>18.7±0.7</td>
<td>17.9±1.9</td>
</tr>
<tr>
<td>16 : 1 (ω7)</td>
<td>2.6±0.2</td>
<td>1.5±0.1</td>
<td>5.9±0.6&lt;sup&gt;3&lt;/sup&gt;</td>
<td>5.1±0.4&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>18 : 0</td>
<td>20.8±0.7</td>
<td>20.2±0.6</td>
<td>20.2±1.5</td>
<td>22.8±0.3</td>
</tr>
<tr>
<td>18 : 1 (ω9)</td>
<td>16.4±1.0</td>
<td>12.9±1.2</td>
<td>27.8±1.5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>26.5±1.8&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>18 : 2 (ω6)</td>
<td>11.4±0.8</td>
<td>18.0±0.7&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.9±0.3&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.7±0.5&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 : 3 (ω9)</td>
<td>—</td>
<td>0.3±0.09</td>
<td>16.9±1.4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>14.7±0.4&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 : 4 (ω6)</td>
<td>28.4±0.5</td>
<td>25.8±0.8</td>
<td>7.7±1.0&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10.2±0.3&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 : 5 (ω3)</td>
<td>0.4±0.04</td>
<td>0.4±0.04</td>
<td>0.2±0.03&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.2±0.03&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>22 : 5 (ω6)</td>
<td>0.6±0.06</td>
<td>0.6±0.09</td>
<td>0.5±0.07&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.4±0.07&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>22 : 6 (ω3)</td>
<td>0.8±0.05</td>
<td>0.7±0.1</td>
<td>0.9±0.1</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>EFA index (ω7+ω9)/(ω3+ω6)</td>
<td>0.48±0.04</td>
<td>0.43±0.11</td>
<td>3.50±0.52</td>
<td>2.24±0.13</td>
</tr>
</tbody>
</table>

<sup>1</sup> CI, corn oil 5% diet; CII, corn oil 15%; MI, MCT 5% diet and MII, MCT 15% diet were designated a) to d), respectively, in a similar way as indicated in Table 2.

<sup>2</sup> Means±SEM.

<sup>3</sup> Fatty acid compositions are indicated as percentage of total fatty acid in liver.

<sup>4</sup> T-test analysis; * P<0.05; ** P<0.01.
Marked accumulation of fat in the liver is usually associated with severe EFA deficiency within several days, when animals are refed a high carbohydrate, fat-free diet following two days of starvation: a striking reduction in linoleic and arachidonic acids with a consistent appearance of 5,8,11-eicosatrienoic acid occurs (20: 3, ω9) (16, 17). The present experiment, however, was aimed initially at examining the long-term effect by ingestion of MCT itself, in order to evaluate its clinical application, and then to establish the optimum requirement for EFA when MCT is ingested as a major source of dietary fats. Of interest is the fact that no fatty liver was induced in rats fed MCT without the addition of EFA, in sharp contrast to the animals fed a fat-free diet even for a longer period of 20 weeks (18).

It has been reported (4, 5, 19) that hepatic hyperlipogenesis by MCT ingestion is mainly due to accelerated incorporation of acetate-1-14C into the liver fatty acid fraction. However, no direct determination of lipogenic enzyme activity itself has been examined: the significant enhancement of FAS and ME activities (Table 2) was associated with the striking reduction of EFA in such proportions as 18: 2 and 20: 4 (ω6) in the liver lipid (Table 3). Therefore, the present finding strongly suggests that the increased incorporation of acetate-1-14C into the liver fatty acid is due mainly to the elevation of lipogenic enzyme activity when MCT ingested as a dietary source of fats. Since there is no difference in carbohydrate consumption between the MCT and corn oil groups, the finding is compatible with the contention that exogenous polyunsaturated fatty acids in a short-term experiment repress lipogenic enzyme activity specifically and directly (7, 15, 20).

The discrepancy between the increased lipogenic enzyme activity and the decreased level of serum triglyceride remains to be elucidated. Further detailed studies of the synthesis and secretion of triglyceride-rich lipoprotein (very low density lipoprotein; VLDL) from the liver as well as degradation of the VLDL by the peripheral tissue are required.

Of considerable importance is the fact that present study revealed a marked depression of HMG-CoA reductase activity in the MCT-fed rats, in sharp contrast to the elevated activity of lipogenic enzyme (Table 2); i.e., an inverse relationship between cholesterogenesis and lipogenesis in the liver. This finding strongly supports the previous observation reported by KRITCHEVSKY and TEPPER (4) that exogenous MCT represses the incorporation to cholesterol of the liver not from mevalonate-2-14C, but from acetate-1-14C as precursors. Hence, it can be concluded that a reduction in the activity of the key enzyme catalyzing cholesterol synthesis plays one of the major roles in achieving hypocholesterolemia by MCT ingestion, although study of cholesterol degradation by the liver is still necessary. Moreover, the nature of the common signal(s) for control and regulation of the key enzyme catalyzing both lipogenesis and cholesterogenesis remains to be elucidated.

In conclusion, the experimental study on MCT administration provides a good
model for exploration of the mechanism of controlling and regulating lipid metabolism. Clinical application of MCT-containing diets may be of importance in controlling hypertriglyceridemia (i.e., exogenous chylomicronemia and alcoholic fatty liver etc.) as well as hypercholesterolemia, particularly when hepatic cholesterol synthesis is actually increased.

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REFERENCES

