Larger Diet-Induced Thermogenesis and Less Body Fat Accumulation in Rats Fed Medium-Chain Triacylglycerols than in Those Fed Long-Chain Triacylglycerols

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(Received July 5, 2002)

Summary It has been previously shown that a diet containing medium-chain triacylglycerols (MCT) leads to less body fat accumulation as compared to a diet containing long-chain triacylglycerols (LCT). We investigated the involvement of diet-induced thermogenesis in the accumulation of body fat in rats fed a diet containing MCT. Twelve male Wistar rats were administered 1 g of MCT or LCT by gavage, and their oxygen consumption was measured for 6 h (experiment 1). Forty male Wistar rats were fed a diet containing 10% MCT or LCT for 6 wk, and their body composition was determined (experiment 2). In experiment 1, oxygen consumption increased to a greater extent after MCT administration than after LCT administration. Diet-induced thermogenesis was significantly (0.67 ± 0.14 kcal) larger after the administration of 1 g of MCT. In experiment 2, there were no differences in food intake or carcass protein content between the LCT group and MCT group. However, carcass fat and intra-abdominal fat content were significantly lower in rats fed MCT than in those fed LCT. We calculated that ingestion of 1 g of MCT decreased body fat by 0.94 ± 0.27 kcal relative to the ingestion of LCT. These results suggest that the larger diet-induced thermogenesis observed in rats fed MCT, compared to that of those fed LCT, is one of the main factors involved in the suppression of body fat accumulation in rats fed MCT.

Key Words medium-chain triacylglycerols, diet-induced thermogenesis, body fat, rats

Not only the amount, but also the type of dietary fat affects body fat accumulation in rats. Body fat accumulation is lower in rats fed vegetable oil than in those fed animal fat (1). A previous study from our laboratory indicated that the intake of unsaturated long-chain fatty acids, as compared to the intake of saturated long-chain fatty acids, promotes diet-induced thermogenesis (DIT) by increasing sympathetic activity in brown adipose tissue, resulting in the suppression of body fat accumulation (2). In addition, we demonstrated that sympathectomy by treatment with 6-hydroxydopamine hydrobromide eliminated the differences in body fat accumulation and DIT between rats fed diets rich in saturated or unsaturated long-chain fatty acids (3). These results suggest that the effects of fatty acids on DIT account for the difference in body fat accumulation when rats are fed different types of fat.

Medium-chain triacylglycerols (MCT) are edible oils that consist of C₈ and C₁₀ saturated fatty acids. Numerous animal studies have provided evidence that the intake of food containing MCT leads to less deposition of body fat as compared to diets containing long-chain triacylglycerols (LCT) (4–9). Recently, we reported that the daily ingestion of 10 g of MCT for 12 wk reduced body fat in healthy subjects to a greater extent than the ingestion of LCT (10). These results suggest that the substitution of MCT for LCT in dietary fat could reduce dietary obesity if energy intake remains constant (11). However, the mechanism of the greater body fat loss after ingestion of MCT is not clear (12). To test the hypothesis that the greater body fat loss after the ingestion of MCT results from increased DIT, we studied the effects of dietary MCT and LCT on DIT and body fat accumulation in rats.

MATERIALS AND METHODS

Animals. All animals were treated in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (13). Male Wistar rats (Japan SLC, Shizuoka, Japan) were individually housed in stainless-steel wire cages and allowed free access to filtered tap water. The temperature of the animal room was set at 22 ± 2°C, with a humidity of 50 ± 10% and lighting was provided from 08:00 to 20:00 h. Animals were also allowed free access to a commercial stock diet (Labo MR Stock; Nosan Corporation, Yokohama, Japan) before the experiment.

Test oils. MCT was purchased commercially (Nisshin OilliO, Tokyo, Japan). Soybean oil (Nisshin OilliO) was used as LCT. The fatty acid compositions of the test oils (Table 1) were determined by gas-liquid chromatography (6890 series; Agilent Technologies, CA, USA) with a capillary column (SP2340; Supelco, PA, USA).
Diet-Induced Thermogenesis and Body Fat with MCT

Measurements of DIT (experiment 1). Ten-week-old rats (196–210 g body weight) were used for this experiment. After 18 h of food deprivation, 1 g of LCT or MCT (n=6/group) was administered by gavage at 11:00 h. Rats were allowed to rest in a plastic chamber from 09:00 to 17:00 h. Oxygen consumption and carbon dioxide production before (30 min) and after (360 min) the administration were measured on a metabolism measuring system for small animals (MK-5000R; Muromachi Kikai, Tokyo, Japan). Oxygen consumption and carbon dioxide production were measured every 3 min, but the data from 0 to 30 min after administration were excluded since rats were taken out from the plastic chamber to administer the test oil at one time. DIT was evaluated by a method previously reported (14, 15).

Body fat accumulation (experiment 2). Six-week-old rats were randomized into two groups. Each group of rats (n=20/group) was allowed free access to the experimental diet containing 10% of one of the test oils for 6 wk. Experimental diets contained the following ingredients (g/kg feed): corn starch, 499; casein, 270; soybean oil, 30.0; test oil, 100; cellulose, 50; AIN-93G mineral mix (16), 35.0; AIN-93 vitamin mix (16), 10.0; L-cysteine, 3.00; choline bitartrate, 2.50; and tert-butyldihydroquinone, 0.026. At the end of the feeding period, rats were sacrificed by decapitation after 18 h food deprivation. Intra-abdominal fat (perirenal, mesenteric and epididymal adipose tissues) was removed carefully with scissors and weighed. Carcass samples, obtained by removing head, tail, digestive tract, heart, lungs, liver, kidneys and testes, were stored at −20°C until analysis. Carcass protein and fat content were analyzed as described by Takeuchi et al. (17). Analyses of serum triacylglycerol and cholesterol were carried out in a 7450 automated system (Hitachi, Tokyo, Japan) by enzymatic methods. Analyses of serum ketone bodies were conducted using a JCA-BM12 automated system (JEOL, Tokyo, Japan) by enzymatic methods. Serum aspartate aminotransferase (AST; UV method) and alanine aminotransferase (ALT; UV method) were assayed in a 7170 automated system (Hitachi).

Table 1. Fatty acid composition of test oils.

<table>
<thead>
<tr>
<th></th>
<th>LCT (g/100 g)</th>
<th>MCT (g/100 g)</th>
</tr>
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<tbody>
<tr>
<td>C8:0</td>
<td>—</td>
<td>74.9</td>
</tr>
<tr>
<td>C10:0</td>
<td>—</td>
<td>25.1</td>
</tr>
<tr>
<td>C16:0</td>
<td>10.4</td>
<td>—</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.1</td>
<td>—</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.0</td>
<td>—</td>
</tr>
<tr>
<td>C18:1</td>
<td>23.9</td>
<td>—</td>
</tr>
<tr>
<td>C18:2</td>
<td>52.9</td>
<td>—</td>
</tr>
<tr>
<td>C18:3</td>
<td>7.8</td>
<td>—</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.3</td>
<td>—</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.2</td>
<td>—</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.4</td>
<td>—</td>
</tr>
</tbody>
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1 Number of carbon atoms: number of double bonds.

LCT, long-chain triacylglycerols; MCT, medium-chain triacylglycerols.

RESULTS

Experiment 1

Oxygen consumption was measured for 6 h to assess the DIT caused by 1 g of LCT or MCT. There was no difference in body weight between the LCT and MCT administration groups (data not shown), and preprandial oxygen consumption did not differ between the two groups at the same time-points (Fig. 1). After administration of the test oils, oxygen consumption increased in
Table 2. Body weight, food intake, and body composition of rats fed a diet containing long-chain triacylglycerols (LCT) or medium-chain triacylglycerols (MCT) for 6 wk.

<table>
<thead>
<tr>
<th></th>
<th>LCT</th>
<th>MCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>177.3±1.2</td>
<td>177.3±1.1</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>282.0±2.9</td>
<td>272.3±2.6*</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>501.7±8.6</td>
<td>497.5±7.1</td>
</tr>
<tr>
<td>Test oil intake, g</td>
<td>50.2±0.9</td>
<td>49.8±0.7</td>
</tr>
<tr>
<td>Carcass protein, g</td>
<td>40.0±0.7</td>
<td>40.1±0.5</td>
</tr>
<tr>
<td>Carcass fat, g</td>
<td>26.0±0.6</td>
<td>22.2±0.7*</td>
</tr>
<tr>
<td>Liver, g</td>
<td>6.76±0.10</td>
<td>6.78±0.10</td>
</tr>
<tr>
<td>Intra-abdominal fat, g</td>
<td>17.7±0.4</td>
<td>16.0±0.5*</td>
</tr>
<tr>
<td>Difference of body fat energy, kcal/6 wk</td>
<td>49.3±14.0</td>
<td></td>
</tr>
</tbody>
</table>

Each value is expressed as mean±SE (n=20).
Results were tested by the Mann-Whitney U-test (LCT versus MCT).
* Significantly different from LCT at p<0.05.

Fig. 2. Diet-induced thermogenesis (DIT) during 6 h after administration of 1 g of long-chain triacylglycerols (LCT) or medium-chain triacylglycerols (MCT). Each value is the mean±SE (n=6). Results were tested by the Mann-Whitney U-test. *Significantly different from LCT at p<0.05.

Experiment 2
Growth, body composition, and body fat content are shown in Table 2. There were no differences in initial body weight, food intake, carcass protein content, and liver weight between the LCT and MCT groups. However, the final body weight was significantly lower in the MCT group than in the LCT group. Carcass fat and intra-abdominal fat content were also significantly lower in the MCT group than in the LCT group. Body fat energy was 49.0±10.2 kcal lower in rats fed the MCT-containing diet for 6 wk as compared to with the LCT group. The results of the blood analysis are shown in Table 3. There was no difference in serum total cholesterol concentration between the LCT and MCT groups. However, serum triacylglycerol concentration was significantly higher in the MCT group than in the LCT group. Serum ketone body concentrations were significantly lower in the MCT group than in the LCT group. On the other hand, AST and ALT did not differ between the two groups.

DISCUSSION
The objective of this study was to estimate the involvement of DIT in less body fat accumulation in rats fed a diet containing MCT as compared with those fed a diet containing LCT. The results from experiment 1 indicate that the intake of MCT is associated with an increase in DIT as compared with the intake of LCT. Other authors (8, 18, 19) have also reported that oxygen consumption is higher in rats ingesting MCT than in those ingesting LCT. DIT refers to the increase in metabolic rate that occurs for approximately 6 h after the ingestion of food (20). DIT plays an important role in the regulation of energy balance (21). DIT is increased after overfeeding.
Diet-Induced Thermogenesis and Body Fat with MCT

Fig. 3. Increase in diet-induced thermogenesis (A, experiment 1) and decrease in body fat energy (B, experiment 2) associated with the ingestion of 1 g of medium-chain triacylglycerols as compared to those associated with the ingestion of long-chain triacylglycerols. Each value is the mean ± SE (n=6).

and conversely, is decreased by starvation (22). DIT accounts for approximately 10% of daily energy expenditure (20). The capacity for DIT in genetically obese rodents is limited (23). These observations indicate that DIT plays a role in body fat accumulation. In experiment 2, the mean total body fat content (carcass fat and intra-abdominal fat) was 5.5 g (49 kcal) lower in rats fed the diet containing 10% MCT for 6 wk than in those fed the diet containing 10% LCT. The rats in the MCT group ingested 49.8 g of MCT during the 6 wk experimental period. Therefore, we calculated that ingestion of 1 g of MCT decreased total body fat by 0.94 kcal relative to the ingestion of LCT. On the other hand, the results of experiment 1 show that DIT after the administration of 1 g of MCT was 0.67 kcal higher than after the ingestion of 1 g of LCT. The increase in DIT after the administration of 1 g of MCT amounts to more than 70% of the reduction in total body fat caused by the ingestion of 1 g of MCT (Fig. 3). These results suggest that the increase in DIT after the ingestion of MCT is largely responsible for the lower accumulation of body fat in rats fed the MCT diet as compared to those fed the LCT diet.

In the present study, the difference in dietary fat did not affect the carcass protein content, but did affect total body fat content. Other researchers have reported similar observations (24). The increase in DIT observed after the ingestion of MCT cannot explain this phenomenon by itself, and other mechanisms are probably also involved in the differential effect on carcass protein and fat content. The rate of triacylglycerol uptake into adipose tissue is proportional to lipoprotein lipase activity (25). Turkenkopf and coworkers (26) reported that MCT-based diets lead to a lower adipose tissue lipoprotein lipase activity in obese Zucker rats as compared to the activity observed after the ingestion of LCT. Guo et al. (27) found that less octanoic acid than oleic acid was stored, and that more octanoic acid than oleic acid was oxidized in 3T3-L1 adipocytes. In addition, glycerol release from fat cells pretreated with octanoic acid was greater than from cells pretreated with oleic acid, indicating higher lipolysis after pretreatment with octanoic acid. A reduction of triacylglycerol uptake into adipose tissue and an increase in fatty acid oxidation and triacylglycerol degradation in adipose tissue may be involved in the differential effect of MCT on body fat and carcass protein content.

The respiratory quotient before administration of the test oil fell to about 0.7 because of 18 h of food deprivation. The cause of no difference in the respiratory quotient of both groups may be this low respiratory quotient. On the other hand, oxygen consumption after the administration of MCT was significantly higher than after the administration of LCT. The values of respiratory quotient and oxygen consumption indicate that, after the administration of MCT, the rats oxidized more fatty acids as compared to after the administration of LCT since the rats were administered only MCT or LCT, and respiratory quotients after the administration of test oil were approximately 0.7 in both groups.

The reasons for the increase in DIT after the administration of MCT compared with LCT are unknown, but may involve an increase of sympathetic activity in brown adipose tissue (BAT). In rats, DIT occurs mainly in BAT, in spite of the small total mass of such tissue. Because heat production by BAT is regulated by the sympathetic nervous system (28), an increase in sympathetic activity in BAT enhances DIT. Sakaguchi et al. (29) demonstrated that ketone bodies in the brain activate the sympathetic nervous system in interscapular BAT. Many studies have shown that the intake of MCT increases the ketone body concentration in blood (30). It is also possible that the increase in DIT observed after MCT administration as compared to LCT administration results from larger thermogenesis in the liver. Long-chain fatty acids absorbed at the small intestine flow into the venous system via lymphatic vessels and are transported to peripheral tissues. On the other hand, compared with LCT, ingested MCTs are more easily degraded to medium-chain fatty acids and glycerol by pancreatic lipase. Most medium-chain fatty acids absorbed at the small intestine are transferred to the liver via the portal vein. Medium-chain fatty acids can be transported through the mitochondrial membrane without binding to carnitine and easily move into the mitochondria, where β-oxidation occurs (31). Direct transportation of MCT from the small intestine to the liver may result in an increase in DIT (11, 32). The liver contributes about 30% to the basal metabolic rate (33).

Part of the acetyl-CoA massively produced during medium-chain fatty acids oxidation is directed towards the production of ketone bodies (30). In this study, however, the serum level of ketone bodies was lower in the MCT group than in the LCT group, but it was remarkably high in both groups. Fasting might have influenced the results. The serum concentration of triacylglycerol was higher in the MCT group than in the LCT group in this study. Other authors (9, 26) have also reported higher serum levels of triacylglycerol in rats fed MCT as compared to LCT. On the other hand, we have observed
that when 40 g/d of MCT or LCT was given to healthy men for 4 wk, their serum levels of triacylglycerol and ketone bodies did not differ significantly (34). The effect of dietary MCT on the serum levels of triacylglycerol may be different between rats and humans.

In conclusion, our study suggests that the increase in DIT observed in rats fed MCT is one of the main suppressors of body fat accumulation relative to animals fed LCT. DIT varies depending on the amount and composition of the diet and the manner of ingestion (20, 35). Further studies are required to clarify the mechanisms by which dietary MCT results in the suppressed accumulation of body fat as compared to dietary LCT.

REFERENCES
