Dietary Curcuminoids Prevent High-Fat Diet–Induced Lipid Accumulation in Rat Liver and Epididymal Adipose Tissue

Akira Asai and Teruo Miyazawa
Laboratory of Biodynamic Chemistry, Tohoku University Graduate School of Life Science and Agriculture, Sendai 981-8555, Japan

ABSTRACT Curcumin and its structurally related compounds (curcuminoids), the phenolic yellowish pigments of turmeric, display antioxidative, anticarcinogenic and hypocholesterolemic activities. In this study, we investigated the effects of dietary supplemented curcuminoids [commercial grade curcumin: a mixture of curcumin (73.4%), demethoxycurcumin (16.1%) and bisdemethoxycurcumin (10.5%)] on lipid metabolism in rats. Male Sprague-Dawley rats were assigned to three diet groups (n = 6) and fed a moderately high-fat diet (15 g soybean oil/100 g diet) for 2 wk. One diet group did not receive supplements (CONT), while the others were supplemented with 0.2 g curcuminoids/100 g diet (CUR0.2) or 1.0 g curcuminoids/100 g diet (CUR1.0). Liver triacylglycerol and cholesterol concentrations were significantly lower in CUR1.0 rats than in CONT rats. Plasma triacylglycerols in the VLDL fraction were also lower in CUR1.0 rats than in CONT rats (P < 0.05). Hepatic acyl-CoA oxidase activity of both the CUR0.2 and CUR1.0 rats was significantly higher than that of CONT rats. Furthermore, epididymal adipose tissue weight was significantly reduced with curcuminoid intake in a dose-dependent manner. These results indicate that dietary curcuminoids have lipid-lowering potency in vivo, probably due to alterations in fatty acid metabolism.


KEY WORDS: curcumin • triglyceride • liver • adipose tissue • rats

The rhizome of Curcuma longa (turmeric) has been widely used as a spice and coloring agent in many foods. Consumption of turmeric has been associated with various beneficial effects on human health (1). In tropical regions of Asia, turmeric has also been used as a traditional remedy for the treatment of inflammation and other diseases (1). Curcuminoids, curcumin and its structurally related compounds comprise the phenolic yellowish pigment of turmeric (Fig. 1). It has been reported that 2.71–5.18 g/100 g curcuminoids is present in commercially available turmeric powders (2) and that 0.34–0.47 g/100 g is present in curry powders (3). Dietary curcuminoids have been associated with antioxidative (4,5) and anticarcinogenic (6,7) activities. In recent years, much attention has been focused on the apoptotic action of curcumin (8–10).

With respect to lipid metabolism, several reports have shown that dietary curcuminoids reduce serum and liver cholesterol in cholesterol-fed rats (11,12). However, there have been few reports dealing with the effects of dietary curcuminoids on triacylglycerol (TG) (2) and fatty acid metabolism. Recently, we demonstrated that dietary turmeric extract prevents liver TG accumulation in mice (5). We speculated this was attributable to the intake of curcuminoids, the predominant constituents of the turmeric extract used in that study. Such a TG-lowering effect was of interest to us because TG accumulation in the liver is accompanied by biochemical modification of the mitochondrial and endoplasmic functions (13) and because fatty liver disease is associated with hyperlipidemia and obesity (14). To evaluate the TG-lowering effect of dietary curcuminoids, we fed rats a moderately high-fat diet (15% soybean oil) supplemented with 0.2 or 1.0% curcuminoids for 2 wk.

MATERIALS AND METHODS

Animals and diets. This study was conducted in conformity with the policies and procedures detailed in the Guide for the Care and Use of Laboratory Animals (15). Seven-week-old male Sprague-Dawley rats were obtained from Japan SLC (Hamamatsu, Japan) and housed individually in stainless steel wire-mesh cages in a room kept at 23 ± 1°C with a 12-h light:dark cycle (light period: 8:00–20:00) and every other day, respectively. Rat feces were collected in the final 3 d and stored at −30°C until analysis.

1 To whom correspondence should be addressed. E-mail: miyazawa@biochem.tohoku.ac.jp.
Sample collection. On completion of the experiment, all rats were weighed and blood was collected into EDTA-treated blood collection tubes after decapsulation. The blood collection was performed between 09:30 and 10:00 h. Plasma was prepared by centrifugation of blood at 1,000 g for 15 min at 4°C. From 3 mL of the prepared plasma, chylomicron and VLDL fractions were isolated by sequential ultracentrifugation (17). The isolated lipoproteins and residual plasma were stored at −80°C until analysis. Immediately after blood collection, livers were perfused in situ with ice-cold saline. Livers and epididymal adipose tissue were then removed and weighed. Two identical portions (~1 g) of each liver were divided and kept on ice for the enzyme preparations (described below), and the remainder was stored at −80°C until lipid analysis.

Liver enzyme preparations were performed on the day of dissection. For the fatty acid synthase (FAS) assay, a 105,000 g supernatant fraction was prepared by differential centrifugation (18). For the acyl-CoA oxidase (ACO) assay, a peroxisome-rich fraction was prepared as reported by Small et al. (19). Both enzyme preparations were stored at −30°C until assayed.

Analytical methods. The total fatty acid content of rat feces was measured by gas-liquid chromatography (GLC) using a 50-m CP-SIL 88 capillary column (Chrompack, Middelburg, the Netherlands). After adding pentadecanoic acid as an internal standard, fecal lipid was extracted with a mixture of chloroform/methanol (20). For GLC analysis, fatty acid methyl esters were prepared simultaneously from both free and esterified fatty acids with anhydrous HCl in methanol (21). The fatty acid content in the soybean oil used in the diet was also measured by GLC as described above, and the fatty acid content in the diet was calculated from that of the soybean oil. The apparent digestibility of dietary TGs was calculated from the difference between the fatty acid content of diet intake and that of feces.

Plasma phospholipids, TG and total cholesterol were measured using Phospholipid-test, Triglyceride-E-test, and Cholesterol-E-test kits (Wako Pure Chemical, Osaka, Japan), respectively. TG in chylo micron and VLDL fractions were also measured using the Triglyceride-E-test. For liver lipid analysis, total lipids were extracted with a mixture of chloroform/methanol (20). Liver TGs and total cholesterol were measured enzymatically as described above after total lipids were dissolved in Triton X-100 (22). Phospholipid phosphorus in total liver lipid was measured by the method of Bartlett (23). The fatty acid content in the soybean oil used in the diet was also measured by GLC as described above, and the apparent digestibility of dietary TGs was calculated from the difference between the fatty acid content of diet intake and that of feces.

RESULTS AND DISCUSSION

In a preliminary study, the liver TG concentration of the rats fed a 15 g/100 g fat (soybean oil) diet for 2 wk was approximately threefold greater than that of the rats fed a 5 g/100 g fat diet (unpublished observations). Therefore, for the present study, we designed the 15 g/100g fat diet used as a model of high-fat–diet–induced lipid accumulation in the liver. We then estimated the lipid-lowering efficiency of dietary curcuminoids.

The diets were well accepted over the feeding period, and neither the body weight gain nor the relative liver weight differed among the three diet groups (Table 1). Epididymal adipose tissue weight was reduced significantly by curcuminoid intake in a dose-dependent manner (Table 1). Hepatic TG concentration was also reduced with curcuminoid intake to 76% (CUR0.2, P = 0.06) and 64% (CUR1.0, P = 0.01) of CONT rats (Table 2). Because the intestinal digestibility of curcuminoids was estimated to be 88% (21), and these effects were similar to those reported previously (24), the apparent lipid-lowering efficiency of dietary curcuminoids was calculated to be approximately 70% (21).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>CONT</th>
<th>CUR0.2</th>
<th>CUR1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g/d</td>
<td>24.1 ± 1.0</td>
<td>22.1 ± 0.5</td>
<td>22.2 ± 0.8</td>
</tr>
<tr>
<td>Body weight gain, g/2 wk</td>
<td>93.5 ± 6.6</td>
<td>81.3 ± 5.3</td>
<td>83.6 ± 6.8</td>
</tr>
<tr>
<td>Liver weight, g/100 g body weight</td>
<td>4.66 ± 0.10</td>
<td>4.81 ± 0.09</td>
<td>4.94 ± 0.15</td>
</tr>
<tr>
<td>Epididymal adipose tissue weight, g/100 g body weight</td>
<td>2.26 ± 0.04</td>
<td>1.90 ± 0.08</td>
<td>1.59 ± 0.09</td>
</tr>
<tr>
<td>Digestibility of TGs, %</td>
<td>99.1 ± 0.1</td>
<td>99.1 ± 0.1</td>
<td>99.0 ± 0.3</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 6. Values in a row not sharing a superscript differ, P < 0.05.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>CONT</th>
<th>CUR0.2</th>
<th>CUR1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Phospholipids</td>
<td>32.0 ± 1.52</td>
<td>31.7 ± 1.80</td>
<td>31.7 ± 1.31</td>
</tr>
<tr>
<td>TG</td>
<td>47.8 ± 4.12b</td>
<td>36.2 ± 3.10ab</td>
<td>30.7 ± 4.56a</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>9.02 ± 0.58b</td>
<td>7.31 ± 0.45ab</td>
<td>6.39 ± 0.80a</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>CONT</th>
<th>CUR0.2</th>
<th>CUR1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Phospholipids</td>
<td>2.71 ± 0.15</td>
<td>2.42 ± 0.14</td>
<td>2.31 ± 0.18</td>
</tr>
<tr>
<td>TG</td>
<td>3.49 ± 0.50</td>
<td>3.18 ± 0.30</td>
<td>2.62 ± 0.39</td>
</tr>
<tr>
<td>Chylomicron-TG</td>
<td>2.05 ± 0.28</td>
<td>2.09 ± 0.26</td>
<td>1.57 ± 0.35</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>1.05 ± 0.19b</td>
<td>0.78 ± 0.07ab</td>
<td>0.56 ± 0.06a</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>2.23 ± 0.16</td>
<td>1.98 ± 0.10</td>
<td>2.02 ± 0.16</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 6. Values in a row not sharing a superscript differ, P < 0.05.
TGs was not affected by curcuminoid supplementation to the diet (Table 1), these effects apparently were not due to inhibition of TG digestion or absorption. As reported previously (5,11,12), liver cholesterol concentration was also reduced with curcuminoid intake (Table 2). Babu and Srinivasan (12) have suggested that such a cholesterol-lowering effect could be mediated by the stimulation of hepatic cholesterol-7α-hydroxylase activity. Further studies of sterol and bile acid balance in feces would be useful to examine the alteration in bile acid synthesis secondary to curcuminoid ingestion.

In curcuminoid-fed rats, TG concentration in the plasma VLDL fraction (VLDL-TG) was lower than that of CONT rats (74 and 53% of CONT for CUR0.2 (P = 0.23) and CUR1.0 (P = 0.02), respectively) (Table 2). In general, blood from animals with free access to food has high levels of chylomicrons and VLDL, both of which are rich in TGs. Therefore, no significant difference in TG concentration in whole plasma (Table 2) should be caused by the higher levels of chylomicrons, which are synthesized in intestinal epithelial cells and secreted into blood circulation via lymph ducts. On the other hand, VLDLs are synthesized in hepatic mesenchymal cells and secreted into the blood circulation. Thus, this hypotriglycerolemic action in VLDLs, but not in chylomicrons, also suggests that dietary curcuminoids alter TG metabolism in the liver and/or the VLDL clearance in the peripheral tissues without affecting intestinal absorption of TGs.

Curcumin (11,12) and turmeric extract (24,25) exhibit hypotriglycerolemic effects, particularly in cholesterol-fed animals. In contrast with these reports, in this study without cholesterol supplementation, the plasma cholesterol concentration was not affected by the curcuminoid supplementation (Table 2). Several previous reports (5,11,12) also indicated that the plasma cholesterol levels of animals fed a cholesterol-free diet were not affected by curcumin supplementation. Therefore, the hypocholesterolemic effect of curcuminoids seems to be limited in cholesterol-fed, hypercholesterolemic animals.

Hepatic ACO activity of curcuminoid-fed rats was significantly stimulated to 1.7-fold (CUR0.2) and 2.2-fold (CUR1.0) of that of CONT rats, whereas FAS activity was not affected (Fig. 2), suggesting that dietary curcuminoids affect fatty acid catabolism in the liver rather than de novo synthesis of fatty acids. ACO performs the first catalytic step enzyme of peroxisomal fatty acid β-oxidation, and its gene expression is regulated by the peroxisome proliferator-activated receptors (PPARs) (26). PPARs are ligand-activated transcriptional factors that regulate gene expression of a variety of lipid metabolizing proteins, such as ACO, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, acyl-CoA synthase, the cytochrome P-450 4A family, fatty acid binding proteins, lipoprotein lipase and apolipoproteins (26). Therefore, PPARs are proposed to play a central role in a signaling system that controls lipid homeostasis. Hypolipidemic fibrates (27), antidiabetic thiazolidinodiones (28,29), polyunsaturated fatty acids (PUFAs) (27,30) and some eicosanoids (27,30) are ligands for PPAR. Although we cannot explain entirely how curcuminoids lower the TG levels in rats, one possible mechanism may be through multiple inductions of intra- and extracellular fatty acid catabolism and utilization pathways (e.g., induction of fatty acid β-oxidation and TG hydrolysis), with metabolites of absorbed curcuminoids serving as ligands that can activate PPAR. Recent reports have indicated that some of the curcuminoids administered orally to rodents is absorbed and present in the blood circulation conjugated with glucuronic acid and sulfuric acid (31–33). The formation of further degradation products of curcumin has also been proposed (34). Thus, some of these degradation products may function as hypolipidemic drugs by serving as ligands for PPAR. However, until now, the distribution of the metabolites of orally administered curcuminoids in liver and other tissues, the interaction of the metabolites and PPAR, and the modulation of a variety of the PPAR-regulated lipid metabolizing proteins have not been revealed. To evaluate our hypothesis, further elucidation of these issues is necessary.

In conclusion, our results indicate that dietary curcuminoids prevent liver TG accumulation and epididymal adipose tissue weight gain and decrease plasma VLDL-TG in rats fed a high-fat diet. These TG-lowering effects are probably due to multiple inductions of fatty acid catabolism and utilization pathways by the metabolites of curcuminoids. Because turmeric has been used as a spice and coloring agent, curcuminoids are the minor constituents in turmeric-supplemented foodstuffs. Therefore, the doses of curcuminoids (0.2 and 1.0%) used in this study were not physiological. Further studies with lower doses will be helpful to clarify the nutritional and therapeutic importance of curcuminoids.

LITERATURE CITED

CURCUMINOIDS ALTER LIPID METABOLISM IN RATS


