Dietary Gallate Esters of Tea Catechins Reduce Deposition of Visceral Fat, Hepatic Triacylglycerol, and Activities of Hepatic Enzymes Related to Fatty Acid Synthesis in Rats


1Laboratory of Nutrition Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka 812-8581, Japan
2Laboratory of Nutrition Biochemistry, Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Saga 840-8502, Japan
3Central Research Institute, Ito En, Ltd., Shizuoka 421-0516, Japan

Received January 24, 2005; Accepted April 13, 2005

Tea catechins, rich in (+)-epigallocatechin gallate and (+)-epicatechin gallate, or heat-treated tea catechins in which about 50% of the (+)-epigallocatechin gallate and (+)-epicatechin gallate in tea catechins was epimerized to (−)-gallocatechin gallate and (−)-catechin gallate, were fed to rats at 1% level for 23 d. Visceral fat deposition and the concentration of hepatic triacylglycerol were significantly lower in the tea catechin and heat-treated tea catechin groups than in the control group. The activities of fatty acid synthase and the malic enzyme in the liver cytosol were significantly lower in the two catechin groups than in the control group. In contrast, the activities of carnitine palmitoyltransferase and acyl-CoA oxidase in the liver homogenate were not significantly different among the three groups. These results suggest that the reduction in activities of enzymes related to hepatic fatty acid synthesis by the feeding of tea catechins or heat-treated tea catechins can cause reductions of hepatic triacylglycerol and possibly of visceral fat deposition.

Key words: fatty acid synthase; malic enzyme; rat; tea catechins; visceral fat

Several physiological functions of tea catechins, such as antiobesity, hypocholesterolemic, antiatherogenic, antioxidantive, and anticarcinogenic activities, have been reported in experimental animals and humans. A tea drink supplemented with tea catechins is now utilized as a functional food having antiobesity activity in Japan. Murase et al. showed that dietary tea catechins rich in (+)-epigallocatechin gallate (EGCG) and (+)-epicatechin gallate (ECC) reduced the deposition of visceral fat in mice fed a high-fat diet for 11 months. Since mitochondrial β-oxidation in the liver was stimulated in mice fed a diet containing tea catechins for 1 month, they ascribed the reduction of visceral fat deposition to an increase in fatty acid oxidation. They did not observe any change in expression of hepatic fatty acid synthase mRNA. In contrast to their observation, Wang and Tian showed that EGCG was an inhibitor of fatty acid synthase from chicken liver in vitro. Although the inhibition of fatty acid synthesis in the body can be a cause of reduction in visceral fat deposition, no evidence is available as to whether tea catechins inhibit fatty acid synthase activity in vivo. Previously we showed that dietary tea catechins suppressed postprandial hypertriacylglycerolemia in rats. We suggested the possibility that suppression of postprandial hypertriacylglycerolemia might be a cause of reduction in body fat accumulation. Therefore, although antiobesity activity of tea catechins has been reported, the precise mechanisms of reduction in visceral fat deposition by the feeding of tea catechins have not been clearly elucidated.

Since tea catechins extracted from tea leaves are rich in EGCG and ECG, (+)-epigallocatechin (EGC) and (+)-epicatechin (EC), many studies of the physiological functions of catechins have been conducted using these catechins. It has been found that almost a half of EGCG, ECG, and EC are epimerized to (−)-gallocatechin gallate (GCG), (−)-catechin gallate (CG), (−)-galloca-
techin (GC), and (−)-catechin (C) during heat pasteurization. Because the consumption of canned and bottled tea beverages is increasing in Asian countries, in particular in Japan, the intake of heat-treated tea catechins cannot be ignored. But, research on the physiological functions of GCG, CG, GC, and C is scarce. In the present study, to reveal the precise mechanisms of the reduction of visceral fat deposition by tea catechins, we studied the effects of dietary tea catechins on visceral fat accumulation and triacylglycerol metabolism in rats. The effect of heat-treated tea catechins on these parameters was also compared with that of tea catechins.

THEA-FLAN 90S, a decaffeinated green tea powder provided by Ito En, Ltd. of Tokyo, Japan, was used as a mixture of tea catechins. This product is rich in EGCG and EGC. Heat-treated tea catechins were prepared by autoclaving the catechin mixture at 120°C for 5 min. The composition of tea catechins (weight %) was as follows: EGCG, 44.1%; GCG, 4.9%; EGC, 20.6%; CG, 1.6%; EC, 0.8%; and (−)C, 0.8%. The composition of heat-treated tea catechins was as follows: EGCG, 23.1%; GCG, 24.2%; EGC, 11.1%; CG, 8.6%; EGC, 0.6%; GC, 0.7%; EC, 0.6%; and (−)C, 0.6%. The heat-treated tea catechins contained increased amounts of GCG and CG.

Six-week-old male Sprague-Dawley rats (Seac Yoshihito, Fukuoka, Japan) were divided into three groups. The animals were housed individually in an air-conditioned room (21–24°C, lights on 08:00–20:00). Before the experiment, all rats were allowed free access to commercial chow (type NMF, Oriental Yeast, Tokyo) for 1 week. The experimental diets were prepared according to the recommendation of the American Institute of Nutrition and contained (g/kg diet) casein, 200; high oleic safflower oil, 100; vitamin mixture (AIN-93), 10; mineral mixture (AIN-93), 35; choline bitartrate, 2.5; l-cystine, 3; cellulose, 50; α-cornstarch, 132; sucrose, 100; r-butyldihydroquinone, 0.014; and cornstarch to 1,000 g. Vitamin and mineral mixtures were purchased from Nihon Nosan Kogyo, Tokyo. In the tea catechin and heat-treated catechin groups, 10 g/kg diet of catechin preparations was added at the expense of cornstarch. The experimental diets were given to the rats for 23 d and the rats were killed without starvation by withdrawing blood from the abdominal aorta under diethyl ether anesthesia. Liver and epididymal, mesenteric and perirenal + retroperitoneal adipose tissues were excised and weighed. An aliquot of liver was homogenized in 6 volumes of an ice-cold 0.25 mol/l sucrose solution containing 1 mmol/l EDTA in a 10 mmol/l Tris–HCl buffer (pH 7.4), and the cytosol was separated by ultracentrifugation at 125,000 × g for 1 h. The animal study was carried out under the guidelines for animal experiments of the Faculty of Agriculture of the Graduate School of Kyushu University and Law 105 and Notification 6 of the Government of Japan.

The concentrations of serum total cholesterol, triglycerides, and phospholipids were determined using enzyme assay kits, Cholesterol C test, Triglyceride E test, and Phospholipid C test (Wako Pure Chemicals, Osaka, Japan). Liver lipids were extracted by the method of Folch et al. The concentration of liver lipids being chemically measured as described previously. Protein in the cytosol and total homogenate of rat liver was measured by the method of Lowry et al. using bovine serum albumin as a standard. The enzyme activities of fatty acid synthase, the malic enzyme and glucose-6-phosphate dehydrogenase in the cytosol fraction, and carnitine palmitoyl transferase in total homogenate were measured as described previously. Acyl CoA oxidase in total homogenate were measured as described.

Values are represented as mean ± SEM. Statistical analysis of data was performed by one-way ANOVA followed by Fisher’s PLSD test to establish differences among groups. Differences were considered significant at P < 0.05.

Since some rats fed heat-treated catechins had unusually high food intake, average food intake tended to be higher and body weight was significantly higher in the heat-treated tea catechin group than in the control group (Table 1). Therefore, visceral fat weight was

<table>
<thead>
<tr>
<th>Table 1. Body Weight, Food Intake, and Visceral Fat Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
</tr>
<tr>
<td>Final body weight (g)</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
</tr>
<tr>
<td>Liver weight (g/100 g body weight)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adipose tissue weight (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal</td>
</tr>
<tr>
<td>Mesenteric</td>
</tr>
<tr>
<td>Perirenal + retroperitoneal</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Rats were given a diet containing tea catechins or heat-treated tea catechins at 1% for 23 d. A diet without the addition of catechin preparations was the control.

Heat-treated, heat-treated tea catechin group.

Data are means ± SE of 7 rats.

aDifferent letters show significant difference at P < 0.05.
expressed as g/100 g body weight. In separate studies, dietary heat-treated tea catechins did not increase food intake in rats (data not shown). Therefore, the higher food intake observed in this study appears to be accidental. The results showed that total visceral fat weight was significantly lower in the two catechin groups than in the control group (Table 1). Hepatic triacylglycerol concentration was also significantly lower in the two catechin groups than in the control group (Fig. 1). Tea catechins and heat-treated tea catechins had almost the same effect on these parameters. There were no significant differences in the concentrations of hepatic and serum cholesterol and phospholipids among the groups (data not shown). The concentration of serum triacylglycerol in the liver cytosol were significantly lower in the two catechin groups than in the control group (Table 1).

The activities of fatty acid synthase and the malic enzyme in the liver cytosol were significantly lower in the two catechin groups than in the control group (Table 2). There was no difference between the two catechin groups in the effects of these parameters. There were no significant differences among the three groups in the activities of cytosolic glucose-6-phosphate dehydrogenase, mitochondrial carnitine palmitoyl transferase, or peroxisomal acyl-CoA oxidase in the liver.

These observations suggest that the reduction in hepatic triglyceride concentration in rats fed a catechin preparation can be due to a reduction in fatty acid synthesis, because fatty acid synthesis is a determinant of triacylglycerol synthesis in the liver. Wang and Tian reported that EGCG is a direct inhibitor of fatty acid synthase from chicken liver in vitro. Yeh et al. also showed that the expression of fatty acid synthase mRNA in malignant human breast carcinoma MCF-7 cells was suppressed by the addition of the tea extract and EGCG. However, the concentration of EGCG added to the incubation medium and the culture medium in these studies appears to be considerably higher than the physiological concentration of EGCG in human plasma after ingestion of EGCG. Therefore, the effect of tea catechins on fatty acid synthase activity was never proved in vivo. The present study shows for the first time that dietary tea catechins and heat-treated tea catechins suppress the activity of fatty acid synthase in vivo.

Table 2. Activities of Enzymes Related to Fatty Acid Synthesis and Oxidation in the Liver

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Tea catechin</th>
<th>Heat-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS (nmol/min/mg protein)</td>
<td>23.2 ± 0.6a</td>
<td>17.3 ± 1.1b</td>
<td>17.5 ± 1.4b</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>82.1 ± 0.6a</td>
<td>62.4 ± 6.5b</td>
<td>62.1 ± 4.7b</td>
</tr>
<tr>
<td>G6PDH</td>
<td>88.6 ± 5.7</td>
<td>79.0 ± 5.1</td>
<td>79.2 ± 7.8</td>
</tr>
<tr>
<td>CPT</td>
<td>1.72 ± 0.21</td>
<td>1.58 ± 0.20</td>
<td>1.51 ± 0.21</td>
</tr>
<tr>
<td>ACO</td>
<td>0.79 ± 0.06</td>
<td>0.65 ± 0.03</td>
<td>0.73 ± 0.07</td>
</tr>
</tbody>
</table>

Rats were given a diet containing tea catechins or heat-treated tea catechins at 1% for 23 d. A diet without the addition of catechin preparations was the control. Heat-treated, heat-treated tea catechin group. Data are means ± SE of 6–7 rats. Different letters show significant difference at P < 0.05.

Sterol-responsive element-binding protein (SREBP)-1c, a transcription factor, is known to regulate fatty acid biosynthesis. SREBP-1c regulates transcription through binding to sterol regulatory elements (SREs) located in the promoters of its target genes, such as acetyl-CoA carboxylase and fatty acid synthase. Promoter-specific transcription factor (Sp-1), a coactivating factor for the activation of promoters by transcription factors, is thought to play a crucial role in the stimulation of target gene expressions by SREBP-1c. Yeh et al. showed that EGCG inhibits the epidermal growth factor (EGF)-mediated signal transduction pathway and reduces the DNA-binding capacity of Sp-1 in MCF-7 breast cancer cells. Therefore, there is a possibility that tea catechins weaken the activation of fatty acid synthase mRNA expression by SREBP-1c by reducing the DNA-binding capacity of Sp-1. It is not clear whether this mechanism works in hepatocytes. However, our results, in which both catechin preparations suppressed the activities of fatty acid synthase and the malic enzyme, but not that of glucose-6-phosphate dehydrogenase (Table 2), appears to support the above hypothesis. SREs are not known in the promoters of the latter two enzymes, but there are some SRE half sites in the promoters.
binds to the SRE half sites and activates target gene transcription.\textsuperscript{21} The promoters of fatty acid synthase and the malic enzyme have Sp-1 binding sites, but there is no Sp-1 binding site in the promoter of glucose-6-phosphate dehydrogenase.\textsuperscript{21} If catechins disturb the binding of Sp-1 to the binding site, mRNA expressions of fatty acid synthase and the malic enzyme might be suppressed and that of glucose-6-phosphate dehydrogenase might not be changed. Since the activities of these enzymes are thought to reflect their mRNA expressions, our results suggest that the activities of fatty acid synthase and the malic enzyme are suppressed through disturbance of the Sp-1 binding to its target genes by catechins. Our results also suggest that tea catechins and heat-treated tea catechins have similar effects on the suppression of fatty acid synthase synthesis in the liver.

In contrast to our results, Murase \textit{et al.} did not observe any change in mRNA expression of fatty acid synthase in the livers of mice fed a 30\% fat diet containing tea catechins,\textsuperscript{3} suggesting that fatty acid synthesis did not change in the feeding of tea catechins. Because sufficient amounts of fatty acids were supplied from dietary sources in their study, fatty acid synthesis had to be suppressed in the liver. Since the dietary fat content was 10\% in our experimental condition, fatty acid synthesis can be an important source of body fat. This might be a reason for the inconsistent observations between the two studies.

The feeding of catechin preparations did not influence the enzyme activities of hepatic carnitine palmitoyl transferase or acyl CoA oxidase in the present study, suggesting that mitochondrial and peroxisomal β-oxidation in the liver are not stimulated by catechin feeding. In contrast, Murase \textit{et al.} observed stimulation of mitochondrial β-oxidation and an increase in mRNA expression of acyl CoA oxidase in the livers of mice fed a high-fat diet containing tea catechins.\textsuperscript{3} The discrepancy between these studies cannot be explained at present. More studies are necessary to resolve this issue.

The suppression of fatty acid synthesis in the liver observed in this study might be a cause of the reduction in visceral fat deposition, because fatty acids synthesized in the liver are important sources of visceral fat. On the other hand, Murase \textit{et al.}\textsuperscript{3} and Osaki \textit{et al.}\textsuperscript{22} suggested that stimulation of β-oxidation in the liver by tea catechins might be a cause of the increase in energy expenditure and hence of the reduction in visceral fat deposition. Previously we observed that tea catechins and heat-treated tea catechins reduce postprandial hypertriglyceridemia by delaying lymphatic transport of triacylglycerol.\textsuperscript{39} We proposed that the suppression might be a cause of the reduction in visceral fat deposition, because several studies have suggested the possibility that delayed absorption of triacylglycerol prevents body fat accumulation.\textsuperscript{23,24} Liu \textit{et al.} also reported that a fatty acid synthase inhibitor prevented adipocyte differentiation of mouse 3T3-L1 cells.\textsuperscript{25} Furuyashiki \textit{et al.} reported that tea catechins suppressed adipocyte differentiation through the down-regulation of peroxisome proliferator-activated receptor (PPAR) γ2, CCAAT/enhancer-binding protein (C/EBP), and glucose transporter (GLUT) 4 in 3T3-L1 cells.\textsuperscript{26} Suppression of adipocyte differentiation might be another candidate for the action of catechins in the reduction in visceral fat deposition. All of these observations suggest the possibility that tea catechins and heat-treated tea catechins multi-functionally influence lipid metabolism and reduce visceral fat deposition. More detailed studies appear to be necessary to discover what is most potent to reduce fat deposition by catechins.

References


11) Seto, R., Nakamura, H., Nanjo, F., and Hara, Y., Preparation of epimers of tea catechins by heat treat-


