Note

Heat-Epimerized Tea Catechins Have the Same Cholesterol-Lowering Activity as Green Tea Catechins in Cholesterol-Fed Rats

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Received July 15, 2005; Accepted September 7, 2005

Tea catechins are known to be epimerized by heat treatment. The effect of heat-epimerized tea catechins on serum cholesterol concentration was compared with that of green tea catechins. Our observations strongly suggest that both tea catechins and heat-epimerized tea catechins lower serum cholesterol concentration by inhibiting cholesterol absorption in the intestine. There was no differential effect between the two catechin preparations.

Key words: tea catechins; heat-epimerized tea catechins; cholesterol; rats

Green tea catechins mainly include (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG). About a half of these catechins are known to be epimerized during heat pasteurization to (−)-catechin (C), (−)-gallocatechin (GC), (−)-catechin gallate (CG), and (−)-gallocatechin gallate (GCG).1,2) Since consumption of canned and bottled tea drinks is increasing in Asian countries, in particular in Japan, and they are autoclaved for pasteurization, intake of heat-epimerized tea catechins cannot be ignored. Although several physiological functions have been reported in green tea catechins,3–6) data on heat-epimerized tea catechins are scarce.7,8) Several observations have shown that green tea catechins have hypocholesterolemic activity in experimental animals.9–11) Some studies have found that feeding of green tea catechins significantly increases fecal excretion of cholesterol in rats.9,10) These observations suggest that green tea catechins reduce plasma cholesterol concentrations by inhibiting cholesterol absorption. Our previous study showed that ECG and

![Chemical Structure of Various Catechins and Heat-Epimerized Catechins](image)

Fig. 1. Chemical Structure of Various Catechins and Heat-Epimerized Catechins.

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Abbreviations: EC, (−)-epicatechin; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EGCG, (−)-epigallocatechin gallate; C, (−)-catechin; GC, (−)-gallocatechin; CG, (−)-catechin gallate; GCG, (−)-gallocatechin gallate
EGCG effectively coprecipitated cholesterol from bile salt micelles and reduced intestinal absorption of cholesterol in lymph-cannulated rats. This might be a cause of increased fecal excretion of cholesterol. Our recent study showed that CG and GCG were more effective in eliminating cholesterol from bile salt micelles than ECG and EGCG, but whether heat-epimerized tea catechins are more hypcholesterolemic than green tea catechins has not yet been investigated. In the present study, the hypcholesterolemic effect of heat-epimerized tea catechins was compared with that of green tea catechins in rats fed a cholesterol-supplemented diet.

The mixture of green tea catechins used was THEA-FLAN 90S (Ito En, Tokyo), a decaffeinated green tea extract rich in polyphenols (about 90%) that includes tea catechins with a galloyl moiety at about 60–70%. Beyond tea catechins, the remaining polyphenols have not been identified. Heat-epimerized 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, 40.5%; GCG, 4.7%; ECG, 18.3%; and CG, 1.3%. The composition of heat-epimerized tea catechins was as follows: EGCG, 20.1%; GCG, 21.2%; ECG, 11.0%; and CG, 7.9%. Heat-epimerized tea catechins contained increased amounts of GCG and CG.

Five-week-old Male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) were acclimatized in a room maintained at 20–23°C with a 12-h light-dark cycle. Before the experiments, all rats were allowed free access to commercial rat feed (Type CE-2, CLEA Japan, SLC, Shizuoka, Japan) for 3 d. Experimental diets were prepared according to the recommendations of the American Institutes of Nutrition and contained (g/kg diet) casein, 200; high oleic safflower oil, 100; a vitamin mixture (AIN-93), 10; a mineral mixture (AIN-93), 35; choline bitartrate, 2.5; l-cystine, 3; cellulose, 50; α-cornstarch, 132; sucrose, 100; β-butyldihydroquinone, 0.014; cholesterol, 5; and cornstarch to 1,000 g. Vitamins and mineral mixtures were purchased from Oriental Yeast (Tokyo). In the green tea catechin (C + T) and heat-epimerized tea catechin (C + H) groups, 10 g/kg diet of catechin preparations were added at the expense of cornstarch. The amounts of these catechins added to the diets and the catechin preparation: cholesterol ratio were taken from Muramatsu et al. and Raederstorff et al. respectively. Since food intake in rats fed catechins tended to be lower compared with that in rats fed the control diet, the rats were pair-fed for 21 d. On the final day, blood was collected via the abdominal aorta under anesthesia after 7 h of food deprivation, and the liver was immediately excised. Feces were collected at 18–19 d of feeding for analysis of neutral and acidic steroids. This animal study was carried out under the guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animals.

Serum HDL-cholesterol and total cholesterol concentrations were determined using enzyme assay kits: the HDL-C test Wako and the cholesterol C test Wako (Wako Pure Chemicals, Osaka, Japan). Liver lipids were extracted by the method of Folch et al. and the concentration of liver lipids was measured as described previously. Fecal steroids were extracted with ethanol and were subjected to analysis by GC using a SPB-1 column (Supelco, Bellefonte, PA) and a Silicone XE-60, 3% Chromosorb WAW DMCS packed column (Supelco), respectively.

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.

Body weight and food intake are shown in Table 1. There were no significant differences in final body weight or food intake among the three groups. The concentrations of serum lipids are shown in Table 1. The concentration of cholesterol was significantly lower in the C + T and C + H groups than in the control group. The concentration of HDL-cholesterol was significantly higher in the C + H group than in the control group.

Table 1. Body Weight, Food Intake and Concentrations of Lipid Parameters in Serum and Liver of Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C + T</th>
<th>C + H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>160 ± 3</td>
<td>160 ± 3</td>
<td>160 ± 2</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>274 ± 4</td>
<td>287 ± 6</td>
<td>294 ± 7</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>17.1 ± 0.2</td>
<td>16.8 ± 0.4</td>
<td>17.5 ± 0.8</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>48.0 ± 3.6a</td>
<td>51.7 ± 1.09b</td>
<td>57.9 ± 2.9b</td>
</tr>
<tr>
<td>Total-Cholesterol (mg/dl)</td>
<td>125 ± 8a</td>
<td>96.2 ± 7.7b</td>
<td>98.7 ± 3.6b</td>
</tr>
<tr>
<td>Atherogenic index*</td>
<td>1.68 ± 0.24a</td>
<td>0.856 ± 0.135b</td>
<td>0.698 ± 0.096b</td>
</tr>
<tr>
<td>Liver lipid concentration</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total-Cholesterol (mg/g liver)</td>
<td>37.4 ± 2.4a</td>
<td>14.1 ± 1.9b</td>
<td>12.3 ± 0.6b</td>
</tr>
</tbody>
</table>

1 10 g/kg diet of green tea catechin preparations was added.
2 10 g/kg diet of heat-epimerized tea catechin preparations was added.
3 (Total-Cholesterol – HDL-Cholesterol)/HDL-Cholesterol
4 Results are means ± SE (n = 5 or 6).

Different superscript letters show significant difference at p < 0.05.
control group. The concentration of liver cholesterol was significantly lower in the C + T and C + H groups than in the control group (Table 1), but there was no difference between the two catechin groups in these parameters.

Fecal excretion of neutral and acidic steroids are shown in Table 2. Excretion of fecal neutral steroids (cholesterol and coprostanol) in the C + T and C + H groups was significantly higher than in the control group. Excretion of fecal acidic steroids in the C + T and C + H groups was significantly lower than in the control group. Total excretion of neutral and acidic steroids was significantly higher in the two catechin groups than in the control group.

Although several studies have shown that dietary tea and catechins lower plasma cholesterol concentration in experimental animals fed a high-cholesterol diet, the effect of heat-epimerized tea catechins on serum cholesterol concentration has never been studied. Our result clearly shows that green tea catechins and heat-epimerized tea catechins lower serum cholesterol concentrations as green tea catechins. Differential effects between these two catechin preparations might be observed if prolonged feeding were done.

In the present study, both catechin preparations significantly decreased fecal excretion of acidic steroids (Table 2). Nakamura et al. reported that oral administration of 0.01 to 1.0 g/kg green tea polyphenol, which contained catechins at about 80%, did not affect bile acid excretion in feces in rats. In contrast, Yang and Ko(18) reported that Lung Chen tea, a Chinese green tea, the major ingredient of which is EGCG, increased fecal excretion of both cholesterol and bile acids in rats. Since our previous study showed that catechins added to bile salt micelles did not affect the concentration of bile acid in micellar solutions, it is not thought that catechins influence reabsorption of bile acids. The reasons for the discrepancies among these studies are not clear at present. Hence, more detailed studies are necessary in this regard.

The present study indicates the possibility that heat-epimerized catechins might be as effective in increasing fecal excretion of cholesterol, and consequently in lowering serum cholesterol concentrations as green tea catechins. Recently we reported that 394 mg/d of heat-epimerized tea catechins for 12 weeks was an effective dose to lower serum cholesterol concentration in mildly hypercholesterolemic subjects. It has been established that an increase in plasma cholesterol concentration is an independent risk factor for atherosclerosis. Therefore, both green tea catechins and heat-epimerized tea catechins can be effective in preventing atherosclerosis through hypocholesterolemic activities.

### Table 2. Fecal Excretion of Neutral and Acidic Steroids in Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C + T</th>
<th>C + H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal weight (g/d)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Neutral steroids*</td>
<td>1.33 ± 0.06(^a)</td>
<td>1.79 ± 0.10(^b)</td>
<td>1.89 ± 0.18(^b)</td>
</tr>
<tr>
<td>(mg/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent cholesterol absorption** (%)</td>
<td>54.8 ± 1.8(^b)</td>
<td>30.2 ± 4.7(^b)</td>
<td>25.8 ± 7.2(^b)</td>
</tr>
<tr>
<td>Acidic steroids (mg/d)</td>
<td>15.7 ± 2.2(^b)</td>
<td>9.27 ± 0.95(^b)</td>
<td>8.78 ± 1.78(^b)</td>
</tr>
<tr>
<td>Total steroids*** (mg/d)</td>
<td>55.0 ± 2.4(^b)</td>
<td>70.5 ± 3.3(^b)</td>
<td>76.6 ± 7.9(^b)</td>
</tr>
</tbody>
</table>

1\(^a\) 10 g/kg diet of green tea catechin preparations was added.
2\(^b\) 10 g/kg diet of heat-epimerized tea catechin preparations was added.
3\(^c\) Cholesterol + coprostanol
4\(^d\) Ingested cholesterol (mg/2 d) / excreted neutral steroids (mg/2 d)
5\(^e\) Apparent cholesterol absorption
6\(^f\) (mg/2 d) ingested cholesterol (mg/2 d) × 100

Different superscript letters show significant difference at \(p < 0.05\).

References

3) Okuda, T., Kimura, Y., Yoshida, T., Hatono, T., Okuda,


