Dietary Tea Catechins Increase Fecal Energy in Rats

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Summary  Much attention has been paid to the beneficial health effect of tea catechins as one of the effective strategies to prevent obesity. The current study was carried out to investigate the role of tea catechins on the utilization of dietary energy sources in rats. The addition of 1% (w/w) tea catechins, mostly in gallocatechin forms, to the diet brought about significant reductions in body weight gains and abdominal adipose tissue weights after 4-wk feeding periods compared to the control. A 2-d output of feces collected at the third week of feeding was significantly increased by a tea catechin diet (average dry weight±SD of 7.2±1.5 g) over that with a control diet (3.8±0.4 g). Only 0.1% of ingested starch appeared in the feces of rats fed the control diet, whereas 4.8% was excreted in the feces of the tea catechin group. Moreover, both apparent digestibility values for lipid and protein in the rats fed tea catechins were also lower than those of the control, suggesting that tea catechins increased the fecal excretion of these energy nutrients. Of the gross energy that the rats consumed from their respective diets during the fecal collection period, 1.6% (for control diet) and 5.8% (for tea catechin diet) were estimated to be excreted in feces. The energy loss originating from carbohydrate should contribute to the overall amount of energy in the feces, followed by protein. Intake of tea catechins suppressed the intestinal absorption of energy nutrients via the inhibition of digestive enzymes, which may at least partially influence the body fat reduction by tea catechins.

Key Words  tea catechins, body fat, fecal energy, apparent digestibility

The link between the consumption of green tea and its potential to reduce body fat has attracted much attention over the last decade. It is generally recognized that such a green tea effect is related to its catechins, particularly those having a galloyl moiety. Our previous study with moderately obese subjects provided direct evidence that daily consumption of a beverage containing 444 mg tea catechins (433 mg of which were tea catechins with a galloyl moiety) at mealtime for 12 wk suppressed body weight by 3.9% compared to the control beverage (1). Several intervention studies have so far been conducted to verify tea catechins as an aid in reducing body weight and fat in humans (2, 3).

The underlying mechanisms by which tea catechins are able to reduce body fat have been explored in animal models (4, 5). Approaches could be categorized by their actions of modulating the energy balance between the intake and expenditure of energy. Tea catechins may be viewed as a candidate for stimulating thermogenesis and fat oxidation. In particular, (−)-epigallocatechin gallate (EGCG) exerts synergistic effects in combination with caffeine (6). From another viewpoint, studies have focused on the intestinal absorption of energy nutrients in relation to the anti-obesity effects of tea catechins. Current investigations have suggested that tea catechins with a galloyl moiety function as modifiers of the intestinal absorption of dietary carbohydrate and lipids by inhibiting digestive enzymes (7, 8). The effects of those catechins on the inhibition of α-amylase and sucrase activities and on the reduction of glucose uptake from the rat intestine may lower the bioavailability of dietary carbohydrate (9, 10). Moreover, tea catechins also dose-dependently inhibited pancreatic lipase activities (11), leading to the suppression of fat digestion in the postprandial state (12). Treatment with EGCG increased fecal lipids in mice fed a high-fat diet compared with the control (13), supporting the hypothesis that EGCG modified lipid absorption. However, the contribution of increased fecal energy loss to the predicted change in body fat due to the consumption of tea catechins has yet to be systematically investigated. Fecal excretion of energy nutrients may provide a way to better evaluate the anti-obesity effect of tea catechins. Therefore, the present study aimed to verify the relative involvement of such an increased energy loss in the feces of rats fed a tea catechin diet.

Materials and Methods

Tea catechins. An extract rich in tea catechins was prepared according to the method of Kobayashi et al.

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(14). In brief, an aqueous solution of decaffeinated green tea containing a high level of the gallated type of tea catechins (THEA-FLAN 90S, ITO EN, Ltd., Tokyo, Japan) was autoclaved at 120°C for 5 min, and the resulting heat-treated sample was freeze-dried. The composition of tea catechins was as follows: EGCG, 22.2%; (+)-gallocatechin gallate, 27.5%; (+)-epicatechin gallate, 6.0%; (+)-catechin gallate, 5.0%; (+)-epigallocatechin, 4.4%; (+)-gallocatechin, 3.0%; (+)-epicatechin, 0.5% and (+)-catechin, 0.4% of dry matter. Other polyphenolic substances remaining in the green tea extract were not identified.

**Animals, diets, and experimental design.** Male Wistar rats, 4 wk old, were purchased from Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan), and were housed individually in stainless steel wire mesh cages at 23°C in a room with an automatically controlled 12-h lighting cycle. The rats were fed a commercial chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and acclimated to the facility for 1 wk before being fed the experimental diets. The rats were divided into two experimental groups that were assigned to either a control or a tea catechin diet. The control diet consisted of 20% casein, 10% corn oil, 50% corn starch, 10% sucrose, 5% cellulose powder, 3.5% AIN-76 mineral mixture, 1.0% AIN-76 vitamin mixture, 0.3% dl-methionine, and 0.2% choline bitartrate by weight. In the tea catechin group, a 1% tea catechin preparation was provided instead of cellulose. The rats had free access to tap water and the assigned diets for 4 wk.

Feces were collected in the metabolic cage (type KN-1002, Tokyo Kasei Gakuin University for the care and use of experimental animals). The plasma levels of glucose and albumin were also determined using assay kits of Glucose CII-test Wako and Cholesterol E-test Wako, respectively (Wako Pure Chemical Industries). The pH of this homogenate was measured using a pH electrode (model F-12; Horiba, Ltd., Kyoto, Japan). The organic acids (acetate, propionate, and butyrate) in the cecal homogenates were determined using a high-performance liquid chromatography system as described by Miwa et al. (18). After coupling with 2-nitrophenylhydrazine hydrochloride in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, the derivatives were separated with a 250×4.6 mm I.D. Cs reversed-phase column (YMC Co., Ltd., Kyoto, Japan) and detected with a variable-wavelength monitor (Model SPD-10AV PD, Shimadzu Co., Ltd., Kyoto, Japan) setting the absorbance at 400 nm.

**Statistical analysis.** Values are represented as mean±SD. The significance of differences between the groups was determined by Student’s t test using a commercial software package (GraphPad Prism 4 for Windows, GraphPad Software, Inc., San Diego, CA, USA). Differences were considered significant at p<0.05.

**Results and Discussion**

The addition of tea catechins (mostly those with a 6.25 to calculate the amount of protein in the feces. The carbohydrate content in the feces was determined after hydrolysis treatment with thermostable α-amylase and amyloglucosidase (Wako Pure Chemical Industries), followed by a determination of the released glucose using an enzymatic colorimetric assay kit of Glucose CII-test Wako (Wako Pure Chemical Industries). The results were corrected to starch basis by multiplying by 0.9 (17). Fecal ash was also measured by weighing after heating at 550°C for 5 h.

**Fecal analyses.** Before analysis, dried feces samples were ground through a 0.71-mm sieve. Fecal lipid was gravimetrically measured according to the method of Tsujita et al. (16). Total fecal nitrogen was determined by Kjeldahl’s method, and results were multiplied by 6.25 to calculate the amount of protein in the feces. The carbohydrate content in the feces was determined after hydrolysis treatment with thermostable α-amylase and amyloglucosidase (Wako Pure Chemical Industries), followed by a determination of the released glucose using an enzymatic colorimetric assay kit of Glucose CII-test Wako (Wako Pure Chemical Industries). The results were corrected to starch basis by multiplying by 0.9 (17). Fecal ash was also measured by weighing after heating at 550°C for 5 h.

**Cecum analysis.** Four volumes of distilled water were added to the weight of cecal content, and the mixture was sonicated for 1 min with a Branson Sonifier® (model 250; Branson Ultrasونics Co., CT, USA). The pH of this homogenate was measured using a pH electrode (model F-12; Horiba, Ltd., Kyoto, Japan). The organic acids (acetate, propionate, and butyrate) in the cecal homogenates were determined using a high-performance liquid chromatography system as described by Miwa et al. (18). After coupling with 2-nitrophenylhydrazine hydrochloride in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, the derivatives were separated with a 250×4.6 mm I.D. Cs reversed-phase column (YMC Co., Ltd., Kyoto, Japan) and detected with a variable-wavelength monitor (Model SPD-10AV PD, Shimadzu Co., Ltd., Kyoto, Japan) setting the absorbance at 400 nm.

**Table 1. Body weight, weight gain, food intake, food efficiency and organ weights in rats fed the experimental diet for 4 wk.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Tea catechin</th>
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<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>166±9</td>
<td>169±8</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>385±26</td>
<td>354±27</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>219±22</td>
<td>185±21*</td>
</tr>
<tr>
<td>Food intake (g/4 wk)</td>
<td>660±33</td>
<td>654±59</td>
</tr>
<tr>
<td>Food efficiency ratio</td>
<td>0.33±0.02</td>
<td>0.28±0.02**</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>12.6±1.1</td>
<td>10.5±0.8**</td>
</tr>
<tr>
<td>Cecal content (g)</td>
<td>1.9±0.5</td>
<td>4.4±1.9**</td>
</tr>
<tr>
<td>Abdominal adipose tissue (g)</td>
<td>22.6±3.9</td>
<td>17.2±2.6**</td>
</tr>
<tr>
<td>Epididymal</td>
<td>6.7±1.5</td>
<td>5.5±1.0</td>
</tr>
<tr>
<td>Perirenal</td>
<td>9.4±1.9</td>
<td>7.1±1.7*</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>6.5±1.7</td>
<td>4.5±1.2*</td>
</tr>
</tbody>
</table>

1Values are the means±SD for 7 rats per group. *p<0.05, **p<0.01.
2Calculated by dividing the weight gain of each rat over the total amount of food consumed per rat in 4 wk periods.
3Sum of the mass of epididymal, perirenal and mesenteric adipose tissues.
Tea Catechins Increase Fecal Energy

<table>
<thead>
<tr>
<th>Feces dry weight (g/2 d)</th>
<th>Control</th>
<th>3.8±0.4</th>
<th>Tea catechin</th>
<th>7.2±1.5**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal excretion</td>
<td>Carbohydrate (g/2 d)</td>
<td>0.04±0.00</td>
<td>1.45±0.06**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lipids (g/2 d)</td>
<td>0.16±0.02</td>
<td>0.21±0.04**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein (g/2 d)</td>
<td>0.41±0.07</td>
<td>1.05±0.15**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Energy (kcal/2 d)</td>
<td>3.2±0.4</td>
<td>11.9±3.4**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ash (g/2 d)</td>
<td>0.47±0.04</td>
<td>0.46±0.09</td>
<td></td>
</tr>
<tr>
<td>Apparent digestibility1</td>
<td>Carbohydrate (%)</td>
<td>99.9±0.0</td>
<td>95.2±1.6**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lipids (%)</td>
<td>96.8±0.8</td>
<td>95.7±0.4**</td>
<td></td>
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<tr>
<td></td>
<td>Protein (%)</td>
<td>95.8±0.9</td>
<td>89.3±0.8**</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are the means±SD for 7 rats per group. **p<0.01.

2 Calculated using Atwater’s general factors.

3 Expressed according to the formula: (ingested – excreted in feces)/ingested×100.

Table 3. Energy nutrients in rat feces collected for 2 d in the third week of feeding of experimental diets and their apparent digestibility.

Feces were subjected to a series of analyses of carbohydrate, lipids and protein. The results demonstrated that little carbohydrate was excreted in the feces of control rats, while that of tea catechin-treated rats contained significant amounts of carbohydrate (Table 3). From animals fed the control diet, only 0.1% of the ingested starch appeared in the feces, while 4.8% flowed through the large bowel and was excreted in the feces of rats in the tea catechin group. Provided that tea catechins do in fact inhibit pancreatic α-amylase activity (7, 9), it may be safely assumed that tea catechins may influence the hydrolysis of dietary starch in the small intestine, so that amounts of starch that cannot be digested move into the large intestine. The same result can be said of lipids. Tea catechins have an inhibitory effect against pancreatic lipase (11). Animal studies and clinical trials have actually revealed a significant reduction of the postprandial increase in plasma triacylglycerol by the ingestion of tea catechins via pancreatic lipase inhibition (23, 24). In this regard, limited but statistically significant amounts of lipids might be excreted in the feces of rats fed tea catechins. Our study also showed that the apparent digestibility of protein was 95.8% for the control group but 89.3% for the tea catechin group. This finding coincides with one in a previous paper by Ohnishi et al., who found that the result of fecal protein output significantly increased by addition to the diet of green tea extract at 0.4% (w/w) to the diet, without any influence on nitrogen balance (25). However, it remains to be determined whether tea catechins may enhance a secretion of endogenous proteins and/or a reduced degradation and reabsorption of endogenously secreted proteins. Provided that Atwater’s general factors were applied to calculate the fecal energy excreted, feces collected for 2 d during the third week of feeding contained a 3.2 kcal amount of energy amount in the control group, against 11.9 kcal in the tea catechin group. Of the total energy excreted in the feces, 49% originated from carbohydrate, followed by protein (35%). The energy value of these nutrients in the feces should contribute to a greater extent to the overall amount of energy loss in the feces. Since the rats consumed their respective diets of 205 kcal (for control diet) and 202 kcal (for tea catechin diet) of the gross energy during the fecal collection period, 1.6% and 5.8%, respectively, of the consumed energy amounts were estimated to be excreted in feces. Given this perspective, the difference of the fecal energy during the entire feeding period between the groups should amount to a simulated 139 kcal loss, thus greatly contributing to the body weight reduction in rats.

It is widely acknowledged that some of the starch that escapes digestion in the small bowel acts as a principal substrate for cecal bacteria. In the process of fermentation, short chain fatty acid (SCFA) is produced that will subsequently result in a decrease in pH. The present study showed that mean cecal pH values were 7.6±0.3 for the control and 7.4±0.1 for the tea catechin group. A comparison showed no significant statistical difference (p=0.059). Figure 1 displays the con-
centration of SCFA (acetate, propionate and butyrate) in the cecal content. Although acetate was the major SCFA in the cecum of the control group, supplementation with tea catechins resulted in a significant decrease of the acetate level in the cecum. Interestingly, the acetate concentration in the control group was almost equal to that of the control group. A previous study of chickens indicated that supplementation with 0.2% (w/w) tea polyphenols in the diet significantly decreased the number of total bacteria, but increased the number of lactobacilli (26). Tea catechins may be influential in the growth performance of a wide variety of bacteria and in the resulting bacterial fermentation of a dietary complex by large-bowel microflora, suggesting that such catechins may also have important implications for the cecal SCFA profiles due to the change in the microbial environment of the intestine. Further studies remain to be conducted to determine in detail the role and involvement of tea catechins in microbial fermentation in the intestine.

In conclusion, the results obtained here have demonstrated that a significant increase in energy excretion in rat feces is promoted by tea catechins, especially those with a galloyl moiety. Considering the presently available data indicating that tea catechins reduce the energy nutrient absorption by inhibiting digestive enzymes in the gastro-intestinal tract, the intestine may well be a target tissue to mediate the body fat-reducing effect of tea catechins. The relevance of these observations to humans still remains to be demonstrated.

REFERENCES


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