Gallated Form of Tea Catechin, Not Nongallated Form, Increases Fecal Starch Excretion in Rats

Tomonori UNNO, Yoshimi MATSUMOTO and Yukari YAMAMOTO

Department of Health and Nutrition, Tokyo Kasei Gakuin University, Chiyoda-ku, Tokyo 102–8341, Japan

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Summary This study was carried out to elucidate the structural advantage of a gallated form of tea catechin on modulating bioavailability of dietary starch in rats. Animal studies demonstrated that the addition of 0.5% (w/w) (−)-epigallocatechin gallate (EGCG) to the diet brought about a significant increase in the starch content in the feces collected for 2 d at the fourth week of feeding over that with the control diet. Of the gross starch that the rats consumed from their respective diets during the fecal collection period, 0.1% (for control diet) and 1.9% (for EGCG diet) were estimated to be excreted in the feces. However, such a significant increase in the fecal excretion of starch by the EGCG diet was lost by undergoing hydrolysis of EGCG to (−)-epigallocatechin (EGC) and gallic acid (GA). In vitro investigation also showed that EGCG inhibited porcine pancreatic α-amylase activity in a concentration-dependent fashion, whereas the hydrolyzed preparation (the mixture of EGC and GA) exhibited a lack of the inhibitory activity for α-amylase. The modification of dietary starch digestion by inhibiting intestinal α-amylase activity with EGCG may be responsible at least in part for increasing fecal output of starch in rats. Thus, the attachment of a galloyl moiety to the tea flavan-3-ol skeleton may be of key importance for reducing intestinal digestion of dietary starch in rats.

Key Words (−)-epigallocatechin gallate, starch, apparent digestibility, (−)-epigallocatechin

The consumption of green tea is associated with a number of beneficial health effects. In the last decade, particular attention was paid to the effect of drinking green tea as an alternate strategy for weight management (1, 2). Intervenational studies have been conducted to verify green tea as an aid in reducing body weight and fat in humans (3–5). It is generally recognized that such a green tea effect is related to its catechins (flavan-3-ols). Four major flavan-3-ols occurring in green tea are (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epicatechin. Two of the green tea flavan-3-ols (EGCG, ECG) contain a gallic acid (GA) moiety at position 3 on the C ring. EGCG is commonly the most abundant catechin in green tea, accounting for up to 50% of total green tea catechin content (6). It is suggested that suppression of intestinal absorption of energy nutrients by tea catechins may be specific to a mechanism underlying the anti-obesity effect of green tea (7, 8). In vitro investigations have shown that EGCG functions as an inhibitor of digestive enzymes of α-amylase and sucrase (9, 10) and for glucose uptake from the intestine (11). Our previous animal study also demonstrated that addition of green tea extract to the diet increased fecal output of carbohydrate in rats compared with the control, supporting the hypothesis that tea catechins modified the bioavailability of carbohydrate (12). However, only a few attempts have so far been made to determine whether structural differences between gallated and nongallated forms of tea catechins may have importance for the excretion of carbohydrate in the feces. Therefore, the aim of the present study is to compare the critical involvement of EGCG itself and its hydrolyzing preparation (the mixture of EGCG and GA) in the increase of energy nutrients in the feces.

MATERIALS AND METHODS

Samples. Highly purified EGCG, the purity of which was >95%, was kindly donated by Ito En, Ltd. (Tokyo, Japan). An aqueous solution of EGCG (10 g/L) was treated with 50 mg of tannase from Aspergillus oryzae (SUMIZYME TAN, Shin Nihon Chemical Co., Ltd., Aichi, Japan). The reaction mixture was incubated at 37˚C for 60 min, then evaporated and freeze-dried to yield 10.2 g on a dry basis. High-performance liquid chromatography analysis confirmed the disappearance of the peak EGCG after treatment with tannase, and the peaks of EGC and GA were also generated. Except for EGC and GA, other newly-generated peaks were undetectable.

Animals and diets. Male Wistar rats, 4 wk old, were purchased from Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan), and were housed individually in stainless steel 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.,
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Ltd., Tokyo, Japan) and acclimated to the facility for 3 d before being fed the experimental diets. The rats were divided into three groups that were assigned to a control diet, an EGCG diet, or an EGC/GA diet (Table 1).

The rats had free access to tap water and the experimental diets for 5 wk. Food intake and body weight were determined tri-weekly. Feces were collected in the metabolic cage (type KN-647, Natsume Seisakusho, Co., Ltd., Tokyo, Japan) for 2 d of the fourth week (from day 24 to 25). At the end of the experimental period, the rats were sacrificed by drawing blood from the heart under diethyl ether anesthesia. Liver and abdominal adipose tissues (epididymis, perirenal, and mesenteric adipose) were immediately excised and weighed. All experimental procedures followed the guidelines of Tokyo Kasei Gakuin University for the care and use of experimental animals.

Table 1. Percentage composition of the experimental diets.

| Ingredients         | Control | EGCG   | EGC+GA |
|---------------------|---------|--------|--------|
| Casein              | 20      | 20     | 20     |
| DL-Methionine       | 0.3     | 0.3    | 0.3    |
| Cornstarch          | 55      | 55     | 55     |
| Sucrose             | 10      | 10     | 10     |
| Corn oil            | 5       | 5      | 5      |
| Cellulose powder    | 5       | 4.5    | 4.48   |
| Mineral mix         | 3.5     | 3.5    | 3.5    |
| Vitamin mix         | 1       | 1      | 1      |
| Choline bitartrate  | 0.2     | 0.2    | 0.2    |
| EGCG                | —       | 0.5    | —      |
| EGCG hydrolysate    | —       | 0.52   | 0.52   |

1 Source of ingredients: casein, cornstarch, sucrose, cellulose powder, mineral AIN-76 mix and vitamin AIN-76 mix (Oriental Yeast Co., Ltd., Tokyo); DL-methionine and choline bitartrate (Wako Pure Chemical Industries, Ltd., Osaka).

2 EGCG was enzymatically hydrolyzed to EGC and GA. Dried sample of EGCG hydrolysate (mixture of EGC and GA) was added equal to a molar basis of EGCG.

Fig. 1. Enzymatic hydrolysis of EGCG to EGC and GA. A: EGCG was hydrolyzed to EGC and GA by tannase from Aspergillus oryzae. B: The dotted line shows an HPLC chromatogram of EGCG, and the solid line shows a chromatogram of the sample after treatment with tannase. HPLC condition: column, Shim-pack CLC-ODS (6.0 mm×150 mm) at 40 °C; mobile phase, 15% (v/v) acetonitrile containing 0.1% phosphoric acid at flow rate of 1.0 mL/min; detection, UV detector at 230 nm.
Results

Characteristics of experimental animals

Food intake in each group showed no significant differences throughout the feeding period (Table 2). The average body weight of rats before and after the feeding experiment had no significant effect among the groups. The addition of EGCG to the diet had an impact on reducing the mass of abdominal adipose tissue weight compared to the control group. The EGCG+GA group showed no significant differences in the abdominal adipose tissue weights compared to other groups.

Energy nutrients in feces

The average dry weight of feces collected for 2 d in the EGCG group was significantly higher than in the control group and the EGCG+GA group (Table 3). Little starch appeared in the feces of rats fed the control diet, whereas the feces of rats fed the EGCG diet contained a significant amount of starch. Of the gross starch that the rats consumed from their respective diets during the fecal collection period, 0.1% (for the control diet) and 1.9% (for the EGCG diet) were estimated to be excreted.

Table 2. Food intake, body weight and organ weights in rats fed the experimental diet for 5 wk.1

|                          | Control  | EGCG     | EGCG+GA  |
|--------------------------|----------|----------|----------|
| Number of rats           | 8        | 8        | 7        |
| Food intake (g/35 d)     | 772 ± 22 | 756 ± 25 | 777 ± 38 |
| Initial body weight (g)  | 102 ± 7  | 103 ± 7  | 103 ± 3  |
| Final body weight (g)    | 381 ± 12 | 365 ± 16 | 374 ± 25 |
| Body weight gain (g)     | 279 ± 8  | 263 ± 12 | 270 ± 23 |
| Liver weight (g)         | 15.5 ± 1.6 | 14.0 ± 1.4 | 15.9 ± 2.0 |
| Abdominal adipose tissue (g)1 | 20.7 ± 3.1ab | 16.9 ± 2.1ab | 18.1 ± 2.9ab |
| Mesenteric               | 5.2 ± 1.2 | 4.1 ± 0.4 | 4.5 ± 0.8 |
| Perirenal                | 7.8 ± 1.0a | 6.1 ± 0.9b | 6.6 ± 1.2ab |
| Epididymal               | 7.6 ± 0.9 | 6.7 ± 0.8 | 7.0 ± 0.9 |

1 Values are means ± SD. A different superscript letter means significant difference at p < 0.05.
2 Sum of the mass of mesenteric, perirenal and epididymal adipose tissues.

Table 3. Energy nutrients in rat feces collected for 2 d in the fourth week of feeding of experimental diets and their apparent digestibility.1

|                         | Control  | EGCG     | EGCG+GA  |
|-------------------------|----------|----------|----------|
| Feces dry weight (g/2 d)| 3.34 ± 0.40b | 4.61 ± 1.02a | 3.26 ± 0.35b |
| Fecal excretion         |          |          |          |
| Carbohydrate (g/2 d)2   | 0.02 ± 0.00b | 0.48 ± 0.42a | 0.03 ± 0.01ab |
| Protein (g/2 d)         | 0.39 ± 0.06b | 0.68 ± 0.16a | 0.44 ± 0.08b |
| Lipids (g/2 d)          | 0.13 ± 0.03 | 0.14 ± 0.05 | 0.11 ± 0.01 |
| Energy (kcal/2 d)1      | 2.8 ± 0.3b  | 5.9 ± 2.2a  | 2.9 ± 0.4a |
| Apparent digestibility4 |          |          |          |
| Carbohydrate (%)        | 99.9 ± 0.0a | 98.1 ± 1.6b | 99.9 ± 0.1ab |
| Protein (%)             | 95.7 ± 0.6a | 92.3 ± 1.8b | 95.4 ± 0.8ab |
| Lipids (%)              | 94.3 ± 1.2 | 93.5 ± 2.0 | 95.1 ± 0.7 |

1 Values are means ± SD. A different superscript letter means significant difference at p < 0.05.
2 Measured as starch basis.
3 Calculated using Atwater’s general factors.
4 Expressed according to the formula: (ingested—excreted in feces)/ingested×100.
Inhibition of pancreatic $\alpha$-amylase by EGCG and its hydrolyzing preparation (mixture of EGC and GA). Data are expressed as mean±SD from triplicate measurements. ○, EGCG; □, hydrolyzed preparation of EGCG (mixture of EGC and GA).

Fig. 2. Inhibition of pancreatic $\alpha$-amylase by EGCG and its hydrolyzing preparation (mixture of EGC and GA). Data are expressed as mean±SD from triplicate measurements. ○, EGCG; □, hydrolyzed preparation of EGCG (mixture of EGC and GA).

In the feces, however, there was no marked effect on starch excretion in the EGC+GA group. The same observation can be made regarding protein. The EGC diet brought about more protein excretion in the feces than other groups. The lipid levels were unchanged among groups.

Provided that Atwater's general factors were applied to calculate the fecal energy excreted, the feces collected for 2 d during the fourth week of feeding contained 2.8±0.3 kcal of energy value in the control group, 5.9±2.2 kcal in the EGC group, and 2.9±0.4 kcal in the EGC+GA group. The rats consumed their respective diets of 178±26 kcal (for the control diet), 171±16 kcal (for the EGC diet) and 184±23 kcal (for the EGC+GA diet) of the gross energy during the fecal collection period; therefore 1.6, 3.5, and 1.6% of the consumed energy amounts were estimated to be excreted in the feces, respectively.

Inhibition of pancreatic $\alpha$-amylase

EGCG inhibited porcine pancreatic $\alpha$-amylase in a concentration-dependent manner (Fig. 2), with an IC$_{50}$ value of 2.2 mg/mL. However, the mixture of EGC and GA lacked the inhibitory activity for $\alpha$-amylase, the IC$_{50}$ value being much higher than 10 mg/mL.

DISCUSSION

The relevance of proposed mechanisms for the reduction of body fat by tea catechins has been discussed in the literature (7, 8). Mechanistic studies have suggested that tea catechins influence sympathetic nervous system activity, increasing energy expenditure and promoting fat oxidation. Other potential mechanisms include appetite regulation and decreased absorption of dietary nutrients. Our previous study indicated that a green tea extract diet supplement brought about a significant increase in fecal energy excretion in rats (12). In addition, the loss of fecal energy originated from carbohydrate substantially contributed to the total energy in the feces, suggesting that a modified bioavailability of dietary carbohydrate by tea catechins may influence the body fat reduction in rats. In order to understand the issue of decreasing the utilization of dietary carbohydrate by tea catechins, the present study focused on the structural importance of the gallated form of tea catechins, particularly making a comparison between EGCG and its hydrolyzed preparation (the mixture of EGC and GA).

Dietary supplementation with EGCG could reduce the mass of abdominal adipose tissue weight compared to the control group. This observation reflects a reproducible result of body fat reduction in animal models as previously reported (12, 14–17). The present study also revealed that the feces of rats fed the EGCG diet contained a significant amount of starch as compared with the control. Moreover, an in vitro study showed that EGCG inhibited porcine pancreatic $\alpha$-amylase in a concentration-dependent fashion. Given that EGCG has an impact on inhibiting pancreatic $\alpha$-amylase activity, it may be safely assumed that EGCG influences the hydrolysis of dietary starch in the small intestine, so that amounts of undigested starch flowed through the large intestine, and were excreted in the feces of rats in the EGCG group. Of course, having suggested that EGCG works as a potent inhibitor for $\alpha$-amylase in vitro, the assumption that EGCG may reflect the corresponding effect under in vivo conditions should be examined carefully. Meanwhile, such a significant suppression of abdominal adipose tissue was lost by undergoing hydrolysis of EGCG to EGC and GA. The hydrolyze preparation also lacked the fecal output of starch. Since the active property of EGCG should be lost by enzymatic hydrolysis to EGC and GA, the attachment of a galloyl moiety to the tea flavan-3-ol skeleton may be of key importance for reducing intestinal digestion of dietary starch in rats.

Table 3 also shows that the feces of rats fed the EGCG diet contained more protein than other groups, and as a consequence, the reduced apparent digestibility of protein by EGCG should be indicated. The reduced apparent digestibility of protein by tea catechins coincides with a previous paper by Ohnishi et al. (18), who reported that the fecal protein excretion was significantly increased by addition of green tea extract at 0.4% (w/w) to the diet. Bajerska et al. (19) also reported that supplementation with 1.1% green tea extract reduced the apparent digestibility of protein. Provided that EGCG interacts with proteins (20), one explanation for a reduction of apparent digestibility of protein may be that EGCG directly bound to dietary casein, or more specifically that EGCG bound to the protein-hydrolyzing enzymes in the intestinal tract. However, it remains unclear whether tea catechins influence a secretion of endogenous proteins and/or reabsorption of endogenously secreted proteins. As is the case in starch-based carbohydrate, the liberation of GA esters of EGCG canceled out the fecal output of protein. A 2-d output of feces collected at the fourth week of feeding contained a significant amount of energy in the EGCG group. The energy values originating from carbohydrate and protein were calculated at 0.1 kcal and 1.6 kcal for the control, and 1.9 kcal and 2.7 kcal for the EGCG group, respectively. Thus, compared to the control, the increase in energy value originating from carbohydrate ac-
counted for a substantial portion of the total amount of fecal energy in the EGCG group. Throughout a 5-wk feeding period, the differences in energy loss in the feces between the control and the EGCG group may contribute to a greater extent to the overall significant level of abdominal adipose tissue weights.

In conclusion, considering that the gallated form of tea catechins has a greater impact on the inhibition of α-amylase than the nongallated form, the results of this study may provide evidence of the in vivo effectiveness of the gallated form of tea catechins to influence the apparent digestibility of starch in rats, being responsible at least in part for the decrease in body fat observed in the EGCG-treated rats. The relevance of these observations to human subjects remains to be demonstrated, but the postprandial digestive and absorptive processes of energy nutrients reportedly were partially suppressed by a tea extract preparation in healthy humans (21). In recent studies, microbial degradation of tea catechins in the colon is largely explained. EGCG can be hydrolyzed by human intestinal bacteria, and subsequently EGC is anaerobically degraded to its ring-fission metabolites (22, 23). According to a paper by Schantz et al. (24), cleavage of the GA esters of EGCG by gut flora may occur not only in the colon, but also in small intestine. In order to better evaluate the role of dietary EGCG in the digestive enzymes of the intestine, further investigation is warranted into the rate and extent of the microbial catabolism for EGCG, that might finally affect the bioactivity of this compound.

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