# Jasmine Green Tea Epicatechins Are Hypolipidemic in Hamsters (*Mesocricetus auratus*) Fed a High Fat Diet[1]

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ABSTRACT These studies were designed to test the hypolipidemic activity of green tea epicatechins (GTE) isolated from jasmine green tea. In Experiment 1, three groups of hamsters were given a semisynthetic diet containing 200 g lard/kg and 1 g cholesterol/kg for 4 wk. The control group received distilled water, and the other two groups received either 15 g/L green tea water extract (GTWE) or 5.0 g/L GTE solution. Both the GTWE and GTE groups had lower concentrations of serum total cholesterol (TC) and triacylglycerols (TG) than the controls ($P < 0.05$). In Experiment 2, four groups of hamsters received tap water as the drinking fluid, but they were given the same high fat and cholesterol diet supplemented with 0 (control), 1.1, 3.4 or 5.7 g GTE/kg diet. The hypolipidemic effect of jasmine GTE was dose dependent. In Experiment 3, the time-course of changes in serum TC and TG was monitored in hamsters given the high fat diet supplemented with 5.7 g GTE/kg in comparison with that of controls. The hypolipidemic effects of dietary GTE were evident after feeding for 2 wk. Dietary supplementation of GTE did not affect liver fatty acid synthase. However, GTE-supplemented hamsters had higher fecal excretions of total fatty acids, neutral sterols and acidic sterols compared with the control group. In Experiment 4, hamsters were fed nonpurified diet; the control group drank distilled water, and the GTE group drank distilled water containing 5.0 g GTE/L. No differences in activities of 3-hydroxy-3-methyl glutaryl coenzyme A reductase and intestinal acyl CoA:cholesterol acyltransferase were observed. This study suggests that the hypolipidemic activity of GTE is not due to inhibition of synthesis of cholesterol or fatty acid but is most likely mediated by its influence on absorption of dietary fat and cholesterol. J. Nutr. 129: 1094–1101, 1999.

KEY WORDS: • *cholesterol* • *Camellia sinensis* • *epicatechins* • *Mesocricetus auratus* • *triacylglycerols*

High plasma cholesterol has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease (Grundy 1986, Neaton et al. 1984). Tea is one of the most popular beverages in Chinese society. There is an increasing interest in green tea as a protective agent against cardiovascular disease (Chisaka et al. 1988, Imai and Nakachi 1995, Kono et al. 1992). In this connection, several epidemiologic studies have shown an inverse association between tea consumption and coronary heart diseases (Hertog et al. 1993, Stensvold et al. 1992). In fact, increased consumption of green tea has been associated with decreased serum triacylglycerols (TG)[3] and cholesterol (Kono et al. 1992). The effect of drinking green tea but not black tea on plasma lipoproteins appears to be characterized by decreasing LDL cholesterol and increasing HDL cholesterol (Green and Harari 1992, Imai and Nakachi 1995). This suggests that the beneficial effect of drinking green tea over black tea is attributed to the content of green tea epicatechins (GTE). In green tea, GTE remain relatively unchanged compared with the fresh tea leaves, whereas in black tea, they are degraded by the fermentation process during manufacture.

The GTE consists mainly of four derivatives; these include (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG) (**Fig. 1**). This study was designed to test the hypothesis that the GTE derivatives are the major active compounds responsible for this hypolipidemic activity; hamsters were used as an animal model. This was done by comparing the hypolipidemic activity of green tea water extract with that of isolated GTE from green tea. We also sought to determine whether supplementation of GTE would lead to any changes in liver fatty acid synthase (FAS), 3-hydroxy-3-methyl glutaryl coenzyme A redutase (HMG-CoA-R), a key enzyme in cholesterol synthesis, and intestinal acyl CoA:cholesterol acyltransferase (ACAT), which is believed to play an important role in intestinal cholesteryl esterification before cholesterol is absorbed and assembled in the chylomicrons.

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[3] Abbreviations used: ACAT, acyl CoA:cholesterol acyltransferase; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; FAS, fatty acid synthase; FFA, free fatty acids; GTE, green tea epicatechins; GTWE, green tea water extract; HDL-C, high density lipoprotein cholesterol; HMG-CoA-R, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; PL, phospholipids; TC, total cholesterol; TG, triacylglycerols; TMS, trimethylsilyl.



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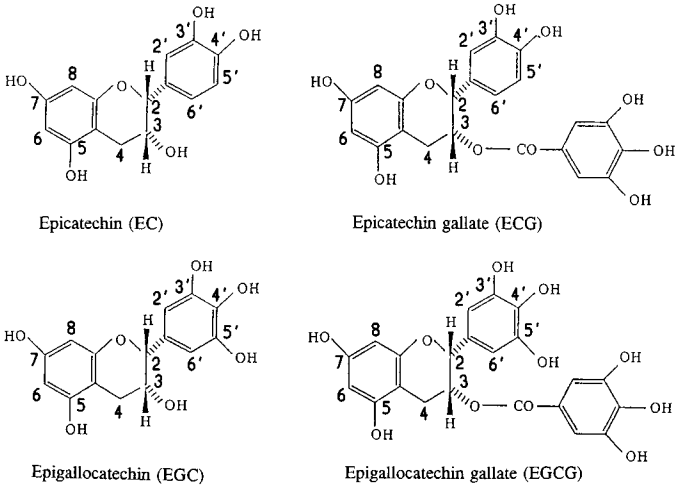


**FIGURE 1** Chemical structures of (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG).

## MATERIALS AND METHODS

***Preparation of jasmine green tea water extract (GTWE).*** The GTWE was prepared by adding 7.5 g jasmine tea (*Camellia sinensis*) leaves to 500 mL of freshly boiled water (80°C). After 15 min, the infusion was filtered and saved at 4°C until used the next day. It was found that GTE in water was stable at least for 2 d (Zhu et al. 1997).

***Preparation of GTE extract.*** Jasmine GTE was extracted and its individual derivatives were analyzed by HPLC as previously described (Zhang et al. 1997a). In brief, 10 g of dry jasmine tea leaves were extracted three times with 140 mL of hot distilled water (80°C). The infusion was then cooled to room temperature, filtered and extracted with an equal volume of chloroform to remove caffeine and pigments. The remaining aqueous layer was then extracted twice with an equal volume of ethyl acetate. The ethyl acetate containing GTE was then pooled and evaporated using a vacuum rotary evaporator. The resulting GTE was then dissolved in 10 mL of distilled water and freeze-dried overnight. Afterwards, a portion of the extract was used to make a 5.0 g GTE/L solution. The remaining dry GTE extract was then saved for supplementing the diet.

The total GTE and individual derivatives in jasmine GTE extract were analyzed as previously described (Zhang et al. 1997a and 1997b). The total amount of GTE extracted varies with variety of teas, methods used and different laboratories (Graham 1992). The extraction method used in this study yielded 75 g GTE/kg jasmine green tea leaves with a purity of 95% in which EGCG, EGC, ECG and EC accounted for 62.3, 19.2, 8.3 and 4.6%, respectively.

***Animals.*** Male Syrian golden hamsters (*Mesocricetus auratus*, The Chinese University of Hong Kong, Shatin, Hong Kong) were housed (2 hamsters/cage) in an animal room at 23°C with a 12-h light:dark cycle. Fresh semisynthetic diets were given to the animals daily, and uneaten food was discarded. Food intake was measured daily and body weight was recorded twice a week. The hamsters were given free access to food and fluid. The protocol was reviewed and approved by the Committee of Animal Ethics, The Chinese University of Hong Kong.

***Diet.*** The hypercholesterolemic diet described by Sanders and Sandaradura (1992) was modified and used in this study. The diet was high in fat and cholesterol and was prepared by mixing the following ingredients: casein, 200 g; lard, 200 g; cornstarch, 418 g; sucrose, 100 g; AIN-76 mineral mix, 40 g; AIN-76A vitamin mix, 20 g; DL-methionine, 1 g; and cholesterol, 1 g. The ingredients were purchased from Harlan Teklad (Madison, WI) except for lard, which was obtained from the local market, and DL-methionine and cholesterol, which were purchased from Sigma Chemical (St. Louis, MO). For Experiment 1, the hamsters were fed this hyperlipidemic diet with GTE supplemented in the distilled water. For Experiments 2 and 3, the GTE-supplemented diet was prepared by adding jasmine GTE extract to this hyperlipidemic diet (powder form), which was then mixed with 1 L gelatin solution (200 g/L). Once the gelatin had set, the food was cut into ~20-g cubed portions and stored frozen at −20°C. For Experiment 4, the hamsters were fed a nonpurified diet (Rodent Chow, Purina Mills, St. Louis, MO); the GTE was added to distilled water.

***Experiment 1.*** The objective was to test the hypolipidemic effect of GTWE solution and the same amount of GTE extract dissolved in drinking water. Male hamsters (2 mo; 95–110 g,) were randomly divided into three groups ($n$ = 12). They were fed the control diet containing no GTE as described above. One group of hamsters received the distilled water, whereas the other two groups received either 5.0 g GTE/L solution or 15 g GTWE/L solution as the only source of drinking water. For all three groups, sucrose was added to the fluid at the concentration of 15 g/L to overcome the bitterness of the green tea solutions. Freshly prepared solution was given and the intake was measured daily. At the end of 4 wk, all fluids were withdrawn and the distilled water was given instead. After food was withheld for 14 h, the hamsters were killed and blood was collected via the abdominal aorta. After clotting, the blood was centrifuged at 1300 × $g$ for 10 min, and serum was then collected.

***Experiment 2.*** The objective was to examine the dose-dependent hypolipidemic activity of jasmine GTE extract supplemented in the diet. Male hamsters (2.5 mo, 125–140 g) were randomly divided into four groups ($n$ = 9). All animals received the tap water as the only drinking fluid. One group of hamsters was fed the control diet, whereas the other three groups were given one of the three GTE-supplemented diets (1.1, 3.4 and 5.7 g GTE/kg). At the end of 4 wk, all of the hamsters were killed after food deprivation for 14 h. The blood was collected via the abdominal aorta and the serum was obtained as above.

***Experiment 3.*** The objective was to monitor the time-course changes in serum total cholesterol (TC) and TG in hamsters given a GTE-supplemented diet. In brief, male hamsters (2.5 mo, 125–140 g) were randomly divided into two groups ($n$ = 7). One group was fed the control diet containing no GTE, whereas the other group was fed the same diet supplemented with 5.7 g GTE/kg diet, a level previously shown to significantly reduce serum TG and TC concentrations. All hamsters were allowed free access to tap water. On d 2, 14 and 28, after food deprivation for 14 h, the hamsters were bled from the retro-orbital sinus into a heparinized capillary tube under light ether anesthesia (Silverman 1987). The total fecal output of each cage was combined into two periods, d 0–20 and d 21–34, to determine total fecal fatty acids, neutral and acidic sterols. At the end of 5 wk, after food deprivation for 14 h, the hamsters were killed and blood was collected via the abdominal aorta. The serum was collected and stored in aliquots at −76°C. The liver, feces and carcass (whole body-perirenal adipose tissue-liver-blood) was also retained to measure TG, phospholipids (PL) and free fatty acids (FFA) according to the methods previously described (Chen et al. 1997).

***Experiment 4.*** The objective was to measure the activity of HMG-CoA-R in the liver of hamsters fed low cholesterol nonpurified diet because high dietary cholesterol inhibits this enzyme. Male hamsters (2.5 mo, 125–140 g) were divided into two groups ($n$ = 9) and fed the nonpurified diet. The control group was supplied distilled water, whereas the GTE group was given water containing 5.0 g GTE/L. For both the control and GTE-supplemented group, sucrose was added to the fluid at a concentration of 15g/L to overcome the bitterness of GTE. Hamsters were then killed by anesthesia under carbon dioxide and the liver was saved for the HMG-CoA-R assay. The intestine was also saved; the first 10 cm from the stomach was discarded, and the next 30 cm taken for the intestinal ACAT assay. Tissues were stored at −78°C for 6 wk.

***Determination of blood cholesterol and apolipoproteins.*** Several enzymatic kits were purchased from Sigma Chemical to measure serum TG (#336–20), TC (#352–20), and HDL-cholesterol (HDL-C; #352–4). Serum apolipoprotein A-1 (Apo A-1) and apolipoprotein B (Apo B) were measured using Sigma commercial immunoassay kits (Apo B, #357-A; Apo A-1, #356-A) as previously described by Rifai and King (1986).

***Determination of liver cholesterol.*** Total lipids were extracted from 300 mg of liver with the addition of stigmastanol (Sigma

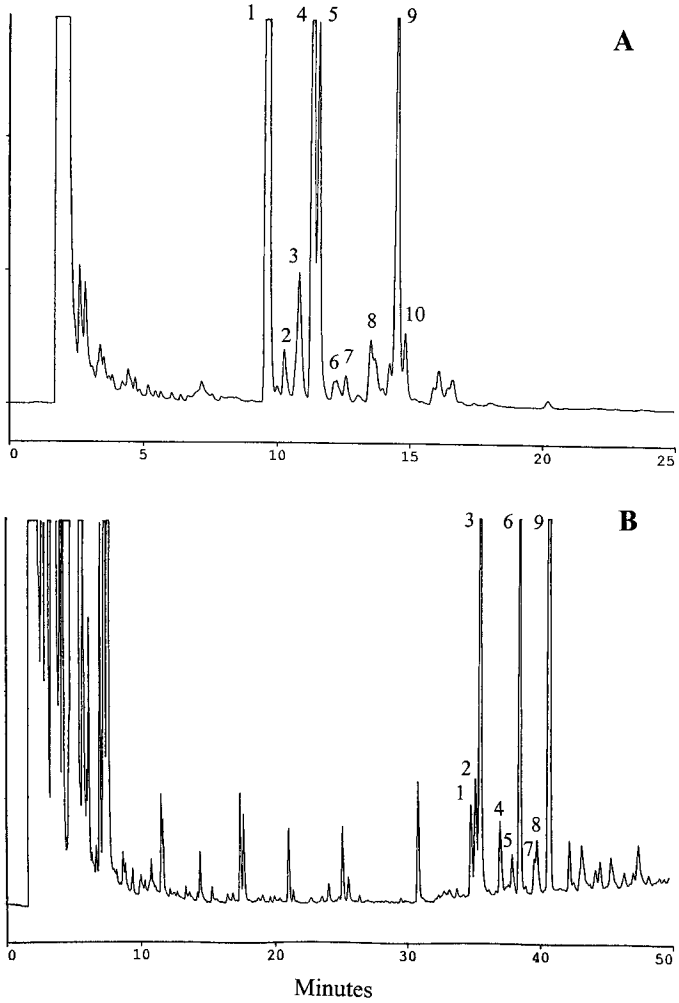


**FIGURE 2** Gas-liquid chromatographic trace of fecal neutral sterols (*A*) and acidic sterols (*B*). *Panel A*: 1, coprostanol; 2, unknown; 3, coprostanone; 4, cholesterol; 5, dihydrocholesterol; 6,7,8, unknown; 9, stigmasterol (internal standard); 10, unknown. *Panel B*: 1,2, unknown; 3, lithocholic acid; 4,5, unknown; 6, deoxycholic acid; 7,8, chenodeoxycholic acid + cholic acid; 9, hyodeoxycholic acid (internal standard).

Chemical) as an internal standard using chloroform/methanol (2:1, v/v). The lipid extracts were then saponified with 6 mL of 1 mol/L NaOH in 90% ethanol at 90°C for 1 h, and the nonsaponified substances including cholesterol were converted to their trimethylsilyl (TMS)-ether derivatives by a commercial TMS reagent (Sigma). Analysis of the cholesterol TMS-ether derivative was performed in a fused silica capillary column (SAC-5, 30 m × 0.25 mm, i.d.; Supelco, Bellefonte, PA) using a Shimadzu GC-14 B gas-liquid chromatograph equipped with a flame ionization detector (Kyoto, Japan).

***Determination of fecal neutral and acidic sterols.*** Stigmasterol (0.3 mg) as an internal standard for neutral sterols was added to a fecal sample (300 mg). The sample was then saponified using 9 mL of 1 mol/L NaOH in 90% ethanol containing 0.3 mg hyodeoxycholic acid as an internal standard for acidic sterols (Sigma). The total neutral sterols were extracted using 8 mL of cyclohexane and were then converted to their corresponding TMS-ether derivatives for GLC analysis. A typical chromatogram of the fecal neutral sterol profile is illustrated in **Figure 2**A.

After cyclohexane extraction, 1 mL of 10 mol/L NaOH was added to the remaining aqueous layer and heated at 120°C for 3 h. After a cooling down period, 1 mL of distilled water and 3 mL of 3 N HCl were added, followed by extraction with 7 mL of diethyl ether twice. The diethyl ether layers were then pooled, followed by the addition of 2 mL of methanol, 2 mL of dimethoxypropane and 40 μL of concentrated HCl (12 mol/L). After standing overnight at room temperature, the solvents were dried down and the acidic sterols were similarly converted to their TMS-ether derivatives at 60°C for 1 h. After the evaporation of TMS reagent under nitrogen, the TMS-ether derivatives of acidic sterols were dissolved in 300 mL of hexane and subject to GLC analysis as shown in Figure 2*B*.

***Assays of HMG-CoA-R, ACAT and FAS.*** Liver micosomes were isolated according to Erickson et al. (1977). The activity of liver HMG-CoA-R was measured as previously described by Shapiro et al. (1969) and modified by Heller and Strewsbury (1976). The activity of liver FAS was measured using the methods described by Nepokroeff et al. (1975). Mucosa microsomes were prepared according to the method described by Murakami et al. (1995). The activity of intestinal ACAT was determined using the method of Helgerud et al. (1981).

***Statistics.*** Data are expressed as means ± SD. For Experiments 1 and 4, both ANOVA and Student's *t* test (two-tailed) were used where applicable for statistical evaluation of significant differences between the control and the GTE-supplemented groups. For Experiments 2 and 3, data were analyzed using a multivariate procedure for repeated measurements as described previously (Cole and Grizzle 1966, LaTour and Miniard 1983) using Sigmastat (Jandel Scientific Software, San Rafael, CA). Differences were considered significant when $P < 0.05$.

## RESULTS

***Experiment 1.*** No significant differences in body weight gain and food intake were observed among the control, GTE and GTWE groups (**Table 1**). However, fluid consumption by the GTWE group was significantly higher than that in the control and GTE groups ($P < 0.01$). The GTE and GTWE solutions used had similar concentrations of total tea epicatechin derivatives (data not shown). Compared with the control, serum TG, TC and Apo B were significantly lower in both the GTE and GTWE groups ($P < 0.05$, **Table 2**). In contrast, the concentration of serum HDL-C and Apo A-1 did not differ among the three groups.

***Experiment 2.*** No differences in body weight or food intake were found between the control and the three groups supplemented with various levels of GTE in the diet (Table 1). Significantly lower levels of serum TG and TC were observed in all GTE-supplemented groups compared with those of the control group ($P < 0.05$, **Table 3**). The hypolipidemic effects of dietary GTE were dose dependent among the control, 3.4 and 5.7 g GTE/kg diet groups ($P < 0.05$), although serum TG and TC did not differ in the 1.1 and 3.4 g GTE/kg diet groups. As in Experiment 1, dietary GTE did not affect serum HDL-C.

***Experiment 3.*** After 14 d of consumption of the high fat and cholesterol diet, serum TG and TC levels were elevated in the controls and in hamsters fed the 5.7 g GTE/kg diet (**Fig. 3**). After 28 d, the differences between the control and 5.7 g GTE/kg diet groups were significant ($P < 0.05$) with the latter having lower serum TG and TC concentrations. At the end of the experiment, serum TG was 35% ($P < 0.05$; **Table 4**) lower, whereas serum TC was 31% lower ($P < 0.05$; Table 4) in the GTE-supplemented group compared with that of the control group. After 5 wk, hamsters fed the 5.7 g GTE/kg diet had significantly lower serum Apo B than controls, whereas Apo A-1 levels did not differ (Table 4). Thus, the ratio of Apo A-1 to Apo B was greater in the GTE-supplemented group.

Hamsters fed GTE had lower liver total cholesterol concentrations and lower carcass and liver TG concentrations than controls after 5 wk ($P < 0.05$, **Table 5**). In contrast, carcass and liver PL did not differ in the two groups (data not shown). The carcass but not the livers of the GTE-supplemented hamsters were characterized by a lower TG/FFA ratio compared with that of the control group. Carcass fatty acid

**TABLE 1**

*Changes in body weight, food intake and fluid intake in hamsters supplemented with green tea epicatechins (GTE) in either food or drinking water[1]*

| | Experiment 1[2] | | |
|---|---|---|---|
| | Control ($n$ = 12) | 5 g GTE/L ($n$ = 12) | 15 g GTWE/L ($n$ = 12) |
| Initial body weight, *g* | 105.0 ± 8.2 | 102.7 ± 7.5 | 104.2 ± 10.2 |
| Final body weight, *g* | 121.0 ± 12.1 | 120.6 ± 9.4 | 123.5 ± 11.5 |
| Food intake, *g/d* | 7.8 ± 0.5 | 7.8 ± 0.9 | 8.0 ± 0.4 |
| Fluid intake, *mL/d* | 10.1 ± 3.4[a] | 8.4 ± 1.7[a] | 15.7 ± 2.3[b] |

| | Experiment 2[3] | | | |
|---|---|---|---|---|
| | Control ($n$ = 9) | 1.1 g GTE/kg ($n$ = 9) | 3.4 g GTE/kg ($n$ = 9) | 5.7 g GTE/kg ($n$ = 9) |
| Initial body weight, *g* | 138.3 ± 13.9 | 137.8 ± 18.7 | 136.1 ± 10.2 | 137.2 ± 7. |
| Final body weight, *g* | 168.9 ± 14.4 | 166.7 ± 17.0 | 169.4 ± 8.8 | 171.3 ± 16.0 |
| Food intake, *g/d* | 19.3 ± 1.8 | 19.0 ± 1.4 | 19.5 ± 1.6 | 18.7 ± 1.7 |

| | Experiment 3[4] | |
|---|---|---|
| | Control ($n$ = 7) | 5.7 g GTE/kg ($n$ = 7) |
| Initial body weight, *g* | 126.4 ± 2.4 | 126.9 ± 2.6 |
| Final body weight, *g* | 172.9 ± 11.9 | 171.3 ± 6.4 |
| Food intake, *g/d* | 20.2 ± 1.4 | 20.4 ± 1.6 |

[1] Values are means ± SD; means in a row with different letters differ significantly, $P < 0.01$; numbers in parentheses indicate the number of hamsters used.
[2] Green tea epicatechins (GTE) were supplemented in the drinking water in Experiment 1 in comparison with green tea water extract (GTWE).
[3] GTE was supplemented in the diet set in gelatin solution in Experiment 2.
[4] GTE was supplemented in the diet set in gelatin solution in Experiment 3.

composition did not differ in the control and the GTE-supplemented groups except for oleic acid, which was lower in the latter (45.8 vs. 46.6% total fatty acids; $P < 0.05$). The activity of liver FAS did not differ in the control and GTE-fed groups [8.2 ± 1.6 vs. 8.3 ± 0.7 nmol/(min · mg protein)].

GTE-supplemented hamsters had higher fecal excretion of total neutral sterols and cholesterol per se during the first 20 d of the experiment compared with those fed the control diet ($P < 0.05$, **Table 6**, Experiment 3). The output of total fecal bile acids in the GTE-supplemented group was not different from that in the control group during the first 20 d but it was greater ($P < 0.05$) during d 21–34 (**Table 7**). Among the bile acids, deoxycholic acid and chenodeoxycholic acid plus cholic acid were significantly higher in the feces of hamsters fed the diet supplemented with GTE, whereas the concentration of lithocholic acid did not differ between the groups.

The GTE-supplemented group had a higher concentration of total fecal fatty acids (240 ± 9.5 μmol/g feces) than the control group (203 ± 9.0 μmol/g feces) during the first 20 d of the experiment ($P < 0.01$). Afterwards, there was no difference in the fecal total fatty acids between the control (203 ± 27 μmol/g feces) and GTE-supplemented groups (201 ± 16 μmol/g feces).

***Experiment 4.*** The activity of liver HMG-CoA-R in the hamsters drinking the fluid containing 5.0 g GTE/L [26.4 ± 2.8 pmol/(min · mg protein)] was not different from that of controls [27.3 ± 2.0 pmol/(min · mg protein)]. Similarly, no difference in the activity of intestinal ACAT existed between the control [171.9 ± 118.9 pmol/(min·mg protein)] and GTE groups [142.8 ± 69.6 pmol/(min · mg protein)].

**TABLE 2**

*Effects of consuming green tea water extract (GTWE) and green tea epicatechins (GTE) in distilled water for 4 wk on serum lipid and apolipoprotein profiles in hamsters (Experiment 1)[1]*

| | Control | 5.0 g GTE/L | 15 g GTWE/L |
|---|---|---|---|
| TG,[2] *mmol/L* | 3.39 ± 1.17[a] | 1.94 ± 0.65[b] | 2.36 ± 0.75[b] |
| TC, *mmol/L* | 5.38 ± 0.59[a] | 4.35 ± 0.67[b] | 4.81 ± 0.36[b] |
| HDL-C, *mmol/L* | 1.94 ± 0.25 | 1.94 ± 0.23 | 2.04 ± 0.26 |
| Apo A-1, *g/L* | 0.80 ± 0.04 | 0.80 ± 0.02 | 0.81 ± 0.03 |
| Apo B, *g/L* | 0.52 ± 0.01[a] | 0.37 ± 0.04[b] | 0.39 ± 0.07[b] |
| Apo A-1/Apo B | 1.6 ± 0.3[b] | 2.3 ± 0.5[a] | 2.1 ± 0.4[a] |

[1] Values are means ± SD, $n$ = 12; means in a row with different letters differ significantly, $P < 0.05$.
[2] TG, triacylglycerols; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; Apo B, apolipoprotein B; Apo A-1, apolipoprotein A-1.

## DISCUSSION

Green tea possesses hypolipidemic activity in hamsters fed a high fat diet in the form of either GTWE or GTE solutions. This observation is in agreement with two recent epidemiologic studies that showed an inverse correlation between tea consumption and the concentration of serum TC and TG in Japanese (Imai and Nakachi 1995, Kono et al. 1992). However, this is in contrast to two other studies in which no correlation between tea consumption and plasma cholesterol was found (Brown et al. 1993, Klatsky et al. 1985). Japanese consume mainly green tea, whereas Caucasians drink mainly

**TABLE 3**

*Effects of consuming varying levels of green tea epicatechins (GTE) in food for 4 wk on serum lipids in hamsters (Experiment 2)*[1]

| | Control | 1.1 g GTE/kg | 3.4 g GTE/kg | 5.7 g GTE/kg |
|---|---|---|---|---|
| | | *mmol/L* | | |
| TG[2] | 8.25 ± 1.79[a] | 5.87 ± 1.97[b] | 6.02 ± 1.97[b] | 4.56 ± 1.65[c] |
| TC | 5.04 ± 0.54[a] | 4.55 ± 1.16[b] | 4.53 ± 1.01[b] | 4.27 ± 0.62[c] |
| Serum HDL-C | 1.42 ± 0.15 | 1.40 ± 0.23 | 1.40 ± 0.15 | 1.50 ± 0.15 |

[1] Values are means ± SD, $n = 9$; means in a row with different letters differ significantly, $P < 0.05$.
[2] TG, triacylglycerols; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol.

black tea. The beneficial effect of drinking green tea over black tea may be attributed to its higher content of GTE for the following reason. In green tea, GTE remain relatively unchanged compared with the fresh tea leaves, whereas in black tea, they are mostly oxidized during the fermentation process (Graham 1992). This study demonstrated that jasmine GTWE solution and the isolated GTE possessed similar hypolipidemic activities (Table 2). This finding indicates that GTE are the major active components or contribute at least in part to the hypolipidemic activity of green tea.

A plausible mechanism for the hypocholesterolemic activity of GTE may be its inhibition of cholesterol and bile acid absorption. This study is the first report to examine the influence of dietary GTE on the profile of both fecal neutral and acidic sterols. The excretion of fecal neutral sterols (cholesterol and coprostanone) during d 0–20 and acidic sterols (deoxycholic acid, chenodeoxycholic acid and cholic acid) during d 21–34 were significantly greater in the GTE-supplemented hamsters than in controls (Tables 2 and 3). The observation that the excretion of bile acids was greater during d 21–34 but not during d 0–20 is unexplained. Perhaps the effect of the diet change from the nonpurified diet to the hyperlipidemic diet on fecal excretion of bile acids had not taken place during the first week of the experiment. It also is unknown why fecal cholesterol in the GTE-supplemented group was higher than that in the control group only during d 0–20 but not during d 21–34. Perhaps the hamsters adapted to the GTE, somehow compensating for its interference with cholesterol absorption. Further study is therefore needed to examine the effect of dietary GTE on both fecal neutral and acidic sterols on a daily basis. Nevertheless, the reduced absorption of dietary cholesterol during d 0–20 was directly associated with a lower serum cholesterol concentration in the GTE-supplemented group. In addition, the greater synthesis and excretion of acidic sterols (the major end products of cholesterol catabolism) in hamsters fed GTE during d 21–34 would also serve to lower the level of serum cholesterol.

Cholesterol homeostasis is a delicate balance among dietary intake, synthesis and catabolism. Serum total cholesterol can be lowered if cholesterogenesis is inhibited. HMG-CoA-R mediates the first committed step in the de novo synthesis of cholesterol from its precursor, acetate. The partial inhibition of this rate-limiting enzyme by dietary plant sterols and hypocholesterolemic drugs such as lovastin and simvastatin is a

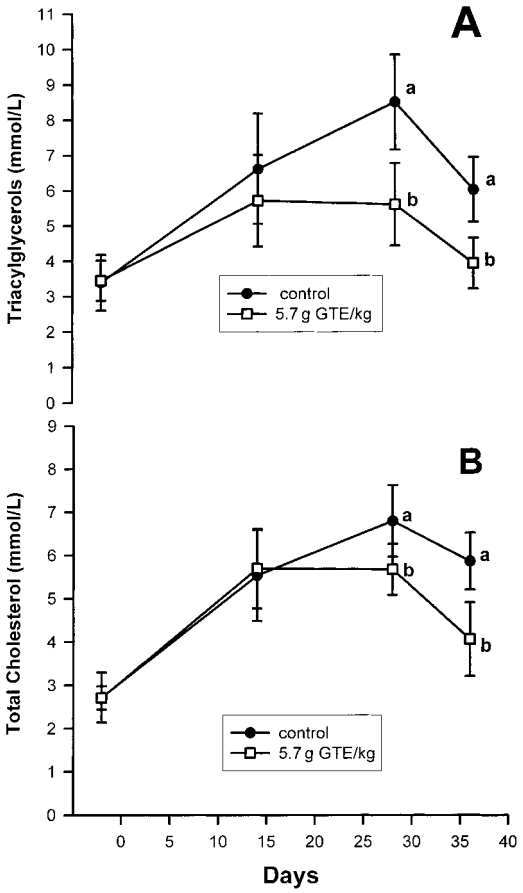


**FIGURE 3** Effect of dietary green tea epicatechins (GTE) on serum total triacylglycerols (TG, *panel A*) and total cholesterol (TC, *panel B*) in hamsters (Experiment 3). Values are means ± SD, $n = 7$. Means with different letters at a given time point differ significantly, $P < 0.05$.

**TABLE 4**

*Effects of consuming green tea epicatechin (GTE) in food for 5 wk on serum lipid and apolipoprotein profiles in hamsters (Experiment 3)*[1]

| | Control | 5.7 g GTE/kg |
|---|---|---|
| TG,[2] *mmol/L* | 6.03 ± 0.92[a] | 3.93 ± 0.71[b] |
| TC, *mmol/L* | 5.87 ± 0.67[a] | 4.06 ± 0.85[b] |
| HDL-C, *mmol/L* | 1.91 ± 0.26 | 1.99 ± 0.16 |
| Serum Apo A-1, *g/L* | 0.71 ± 0.04 | 0.67 ± 0.02 |
| Serum Apo B, *g/L* | 0.84 ± 0.13[a] | 0.48 ± 0.18[b] |
| Apo A-1/Apo B | 0.9 ± 0.2[b] | 1.6 ± 0.5[a] |

[1] Values are means ± SD, $n = 7$; means in a row with different letters differ significantly, $P < 0.05$.
[2] TG, tricylglycerols; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; Apo B, apolipoprotein B; Apo A-1, apolipoprotein A-1.

**TABLE 5**

*Effects of consuming dietary green tea epicatechins (GTE) for 5 wk on carcass and liver lipid concentration in hamsters (Experiment 3)*[1]

| | Control | 5.7 g GTE/kg |
|---|---|---|
| Carcass | | |
| TG, *μmol/g* | 296.0 ± 63.8[a] | 209.8 ± 19.9[b] |
| FFA, *μmol/g* | 207.0 ± 35.5[b] | 262.2 ± 15.6[a] |
| TG/FFA | 1.4 ± 0.3[a] | 0.8 ± 0.1[b] |
| Liver | | |
| TG, *μmol/g* | 16.8 ± 2.1[a] | 9.4 ± 3.0[b] |
| FFA, *μmol/g* | 10.9 ± 1.2[a] | 7.0 ± 1.2[b] |
| TG/FFA | 1.5 ± 0.2 | 1.3 ± 0.4 |
| Cholesterol, *μmol/g* | 30.5 ± 5.2[a] | 13.5 ± 5.4[b] |

[1] Values are means ± SD, $n = 7$; means in a row with different letters differ significantly, $P < 0.05$.
[2] TG, triacylglycerols; FFA, free fatty acids.

typical example of reducing plasma cholesterol (Brown and Goldstein 1986). In this study, the activity of HMG-CoA-R did not differ in the control and GTE-supplemented hamsters, suggesting that inhibition of this enzyme is not part of the hypocholesterolemic mechanism of GTE.

Intestinal ACAT plays a key role in the intestinal absorption of cholesterol by esterifying cholesterol before its absorption (Largis et al. 1989, Wrenn et al. 1995). It has been shown that tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats (Stensvold et al. 1992). We hypothesized that GTE may interfere with the absorption of cholesterol by inhibiting the ACAT activity. However, dietary GTE had no influence on the intestinal ACAT activity, suggesting that GTE increases the fecal output of cholesterol mainly as a result of its binding capacity and acceleration of cholesterol excretion. In fact, GTE has been shown to form insoluble coprecipitates with cholesterol and thus decrease cholesterol absorption (Muramatsu et al. 1986).

This study also demonstrated that dietary GTE had TG-lowering activity in hamsters. It is unlikely related to the inhibitory effect on liver fatty acid synthesis because there was no difference in hepatic FAS activity between the control and GTE-supplemented groups. Most likely, the higher output of fecal fatty acids in the GTE-supplemented group would be one of the possible mechanisms. It should be pointed out that the concentrations of serum TG in the control group of Experiments 1, 2 and 3 were inconsistent. We have no clear explanation for this discrepancy, but the following factors my be involved. First, the body weight gain and the food intake in Experiments 2 and 3 were greater than those in Experiment 1. Thus, the fat intake in Experiments 2 and 3 was greater than that in Experiment 1, resulting in a higher level of serum TG (Tables 2, 3 and 4). Second, the diet in Experiment 1 was in the form of powder, whereas in Experiments 2 and 3, the diet was set in gelatin. Third, the enzymatic kit measuring serum TG in Experiment 1 was not from the same batch as that used in Experiments 2 and 3, although both batches were purchased from Sigma Chemical.

Jasmine GTE not only reduced serum TG and TC but also Apo B with no effect on Apo A-1. Apo B is the principal protein in LDL, comprising ~90% of total LDL protein mass (Rifai 1986). It plays a major role in the recognition of cellular receptors for the catabolism of LDL (Naito 1986). Numerous studies have indicated that Apo A-1 and Apo B measurements are useful in assessing the risk of cardiovascular disease (Kottke et al. 1986, Maciejko et al. 1983, Naito 1986). It has been reported that they are more specific and sensitive biochemical markers of cardiovascular disease risk than HDL-C and LDL-C. People with a low Apo A-1/Apo B ratio might have a higher risk of cardiovascular disease (Kukita et al. 1984). The present results clearly demonstrated that supplementation of GTE in either diet or drinking water modified favorably the balance of these two apolipoproteins (Tables 2 and 4). If drinking green tea is associated with a significantly lower risk of cardiovascular disease in humans, part of the mechanism may involve an increase in Apo A-1/Apo B ratio.

Suppression of body TG accumulation in the GTE-supplemented group was another major finding in this study (Table 5). First, greater excretion in fecal total fatty acids (only during d 0–20 in Experiment 3) may contribute in part to a lower body TG accumulation in hamsters supplemented with GTE. Second, dietary GTE may enhance the hydrolysis of TG to FFA for oxidation. This was supported by the observation that carcass FFA concentration was significantly higher in hamsters supplemented with GTE (Table 5). This was also reflected in the significantly lower TG/FFA ratio of GTE-fed hamsters. In fact, a previous study by Sano et al. (1986) demonstrated that adrenaline-induced lipolytic activity in abdominal adipose tissue was significantly elevated in rats given green tea for 8 or 16 wk. It is unlikely that lower body TG accumulation in the GTE-supplemented group was associated with suppression in fat synthesis because no difference in the activity of liver FAS was observed between the control and GTE-supplemented groups.



**TABLE 6**

*Effect of consuming green tea epicatechins (GTE) on fecal output of neutral sterols in hamsters (Experiment 3)*[1,2]

| | d 0–20 | | d 21–34 | |
|---|---|---|---|---|
| | Control | 5.7 g GTE/kg | Control | 5.7 g GTE/kg |
| | *mg/g feces* | | | |
| Cholesterol | 3.90 ± 0.88[a] | 4.86 ± 0.99[b] | 1.87 ± 0.99 | 2.15 ± 0.62 |
| Coprostanol | 0.53 ± 0.47 | 0.49 ± 0.45 | 3.87 ± 1.45 | 3.94 ± 2.08 |
| Coprostanone | 0.35 ± 0.08[b] | 0.46 ± 0.10[a] | 0.34 ± 0.17 | 0.40 ± 0.12 |
| Dihydrocholesterol | 0.46 ± 0.11 | 0.50 ± 0.13 | 0.78 ± 0.21 | 0.82 ± 0.25 |
| Total | 5.24 ± 0.88[c] | 6.31 ± 0.55[d] | 6.86 ± 1.93 | 7.31 ± 2.22 |

[1] Values are means ± SD, $n = 4$ cages with 2 hamsters per cage except in 1 cage containing 1 hamster.
[2] Means in a row with different letters differ significantly; [ab]$P < 0.05$; [cd]$P < 0.01$.

**TABLE 7**

*Effect of consuming green tea epicatechins (GTE) on fecal output of acidic sterols in hamsters (Experiment 3)*[1,2]

| | d 0–20 | | d 21–34 | |
|---|---|---|---|---|
| | Control | 5.7 g GTE/kg | Control | 5.7 g GTE/kg |
| | *mg/g feces* | | | |
| Lithocholic acid | 1.03 ± 0.34 | 1.12 ± 0.40 | 1.33 ± 0.46 | 1.37 ± 0.36 |
| Deoxycholic acid | 1.21 ± 0.44 | 1.33 ± 0.60 | 1.20 ± 0.13[b] | 2.07 ± 0.41[a] |
| Chenodeoxycholic acid + cholic acid | 0.32 ± 0.20 | 0.21 ± 0.10 | 0.43 ± 0.17[d] | 0.68 ± 0.22[c] |
| Total | 2.56 ± 0.83 | 2.66 ± 0.40 | 2.96 ± 0.66[d] | 4.12 ± 0.73[c] |

[1] Values are means ± SD, $n = 4$ cages with 2 hamsters per cage except 1 cage containing 1 hamster.
[2] Means in a row with different letters differ significantly; [ab]$P < 0.05$, [cd]$P < 0.01$.

In this study, the amount of GTE supplemented in food or drinking water was similar to that commonly consumed by tea drinkers. Supplementation of 1.1, 3.4 and 5.7 g GTE/kg diet was equivalent to 0.06, 0.16 and 0.28 mg/kJ consumed by hamsters. If humans consumed 8360 kJ/d (2000 kcal/d), the three levels of dietary GTE supplementation in hamsters would be approximately equivalent to an intake of 0.45, 1.34 and 2.33 g GTE/d, respectively. The extraction method used in this study could yield 74 g GTE per kilogram jasmine tea leaves (Chen and Chan 1996). The intake of 0.45, 1.34 and 2.33 g GTE/d would therefore correspond to drinking 3, 9 and 15 cups (100 mL/cup) of jasmine tea beverage daily, provided that each cup of tea beverage contained 2 g of dry tea leaves. In fact, most Chinese and Japanese tea drinkers usually consume >3 cups of tea per day and some heavy drinkers may have >10 cups (1 L) each day (Imai and Nakachi 1995).

In addition to their hypolipidemic activities, GTE and its four major epicatechin derivatives, EC, ECG, EGC and EGCG (Lunder 1992), are believed to have a wide range of other pharmaceutical properties including being antioxidative (Ding et al. 1992, Miura et al. 1994), anticarcinogenic (Shi et al. 1994) and antihypertensive (Henry and Stephens-Larson 1984). We have previously shown that these GTE derivatives exhibited stronger antioxidative effects than the commercially available antioxidant, butylated hydroxytoluene, in foods (Chen and Chan 1996). It also has been demonstrated that these compounds are more protective than ascorbate against LDL-oxidation (Zhang et al. 1997a). These GTE derivatives protect not only $\alpha$-tocopherol but also docosahexaenoic acid and arachidonic acid in LDL and red blood cell membranes from oxidation (Zhang et al. 1997a and 1997b ). It has been suggested that oxidative modification of LDL may play a role in the development of atherosclerosis (Jialal and Devaraj 1996). If tea consumption in humans is associated with a significant decrease in cardiovascular disease, part of the mechanism may also involve protection of LDL against oxidative modification in addition to tea's hypolipidemic activity.

The high fat and cholesterol diet was used in this study to elevate serum TG and cholesterol to a level similar to that in humans. The present results in hamsters, although not directly applicable to humans, may have some implications for individuals who often consume a high fat and cholesterol diet. The reduction in serum TG and cholesterol by dietary GTE is not associated with inhibition of liver FAS, HMG-CoA-R and intestinal ACAT, but it is most likely mediated by its inhibition of absorption of dietary fat, cholesterol and reabsorption of bile acids.

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