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GREEN TEA AND ITS CATECHINS AS DIETARY AND PHARMACOLOGICAL MEANS OF LOWERING CHOLESTEROL ABSORPTION

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Abstract

Evidence shows that the serum level of cholesterol (CH) is decreased with increasing green tea (GT) consumption. This presentation summarizes our recent findings on the effect of GT extract on intestinal absorption of ^{14}C-labeled CH and phosphatidylcholine (PC). Ovariectomized (OX) adult rats were infused intraduodenally with lipid emulsions containing radiolabeled lipids [^{14}C-CH or ^{14}C-phosphatidylcholine (PC)] in the presence of GT extract or catechins to determine the rates and amounts of CH absorption and the intestinal hydrolysis and lymphatic output of PC. During lipid infusion, lymph was collected hourly for 8 h. The lymphatic absorption of ^{14}C-CH was drastically lowered by infusion of GT extract at two dosage levels (GT1 = 5.4 mg catechins/h and GT2 = 15.1 mg catechins/h). The cumulative lymphatic absorptions of ^{14}C-CH in rats infused with GT1 and GT2 were 20.7 ± 4.3 and 4.8 ± 4.1% dose, respectively, whereas the absorption of ^{14}C-CH in rats infused with no GT extract (GT0) was 36.3 ± 1.1% dose. GT extracts also significantly lowered the absorption of -tocopherol (TP) in a dose dependent manner (29.6 ± 4.9% dose in GT0, 20.8 ± 5.8% dose in GT1, and 7.9 ± 5.4% dose in GT2 groups). Both (+)-catechin and EGCG significantly lowered the lymphatic outputs of ^{14}C-radioactivity after intraduodenal ^{14}C-PC infusion. A significantly higher amount of ^{14}C-PC remained unhydrolyzed in the intestinal lumen of the EGCG rats (22.8%) compared with the (+)-catechin (15.8%) and control groups (11.9%). GT extracts, (+)-catechin, and EGCG significantly reduced the absorption of TP. The inhibitory effect of GT extract and catechins on lipid absorption may be mediated in part through the inhibition of pancreatic PLA_2. The findings provide the first direct evidence that green tea and catechins have a profound inhibitory effect on the intestinal absorption of CH in OX rats. Results suggest that green tea and catechins may be used as a dietary or pharmacological means of lowering cholesterol absorption.

Key words: green tea, catechins, cholesterol, -tocopherol, absorption, rats

INTRODUCTION

Epidemiological evidence suggests that dietary flavonoids, as naturally present in fruits, vegetables, and beverages including green tea and wine, may reduce the risk of coronary heart disease (CHD) in men and postmenopausal women (1-4). Evidence also shows that the serum level of cholesterol is lowered with increasing tea or green tea (GT) consumption in young and middle-aged men and women (5,6). At present, however, little is known about the mechanisms underlying the hypocholesterolemic effect of GT. Furthermore, no information exists on whether and how GT or its flavonoids (catechins) may influence cholesterol metabolism and serum cholesterol levels in postmenopausal women. GT is produced from freshly harvested leaves of the tea plant, *Camellia sinensis*. The fresh leaves are exposed to hot steam, cooled, and dried. The exposure of fresh tea leaves to hot steam and air inactivates polyphenol oxidase and assures peculiar green color, resulting in polyphenol-rich GT (7). The major catechins present in GT are (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epicatechin, and (+)-catechin (7,8).

Studies with various animal models have shown that green tea or its catechins lower the blood levels of cholesterol in cholesterol-fed male rats (9,10), mice (11), and hamsters (12), and retard the development or progression of atherosclerosis in apolipoprotein E-deficient mice (13) and hypercholesterolemic hamsters (14). Muramatsu et al. (9) first demonstrated that, when weanling rats were fed a high cholesterol diet containing a crude mixture of catechins, the fecal excretion of cholesterol was increased significantly. Similarly, in a study using hamsters (12), GT extract and catechins increased the fecal excretion of neutral and acidic sterols. However, a recent study (15) showed that the fecal excretion of bile acids remained unaffected in sucrose-fed male rats receiving GT in drinking water. Available information points to the possibility that tea catechins may inhibit pancreatic lipolytic enzymes interfering with lipid digestion and absorption. An in vitro study showed that gastric and pancreatic lipase activities were drastically inhibited by GT extract enriched in catechins (16), which is consistent with the observation that the apparent absorption of fat was decreased in rats fed GT extract under in vivo conditions (15). Evidence also shows that flavonoids such as hesperetin and myricetin are strong inhibitors of pancreatic phospholipase A_2 (PLA_2), which hydrolyzes PC to lysophosphatidylcholine (LPC) (17).

Women after natural or surgical menopause are substantially more susceptible to CHD than during their reproductive years (18-22). In view of the paucity of information on the potential cholesterol-lowering effect of GT in women, we used an ovariectomized rat model with lymph cannula to determine whether GT and catechins lower the intestinal absorption of cholesterol and fat. Our data presented here provide the first direct evidence that GT drastically lowers the intestinal absorption of

cholesterol and fat and that catechins significantly inhibit the activity of pancreatic PLA_2, which is a key determinant of the intestinal absorption of cholesterol and other lipids.

MATERIALS AND METHODS

Animals and diet

Adult female Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 210-240 g were individually housed in plastic cages with stainless steel wired floors in a windowless room. All rats were subjected to a daily 12-h light/dark cycle and had free access to deionized water (Millipore Corporation, Malboro, MA). The rats were fed ad libitum a nutritionally adequate diet containing soybean oil as the fat source and egg white as the protein source. The diet was formulated according to the AIN-93G recommendations (23,24). Animal care protocols were approved by the Kansas State University Institutional Animal Care and Use Committee. Animals were cared for in an animal facility accredited by the American Association for the Accreditation of Laboratory Animal Care. At 3-6 wk, rats were ovariectomized under halothane anesthesia (25).

Mesenteric lymph duct cannulation

At 5-9 wk following ovariectomy, rats were starved overnight (16 h) and anesthetized with halothane using a halothane vaporizer (2.0% halothane in 2.0 L O_2/min) prior to and during cannulation of the mesenteric lymph duct. Cannulation of the mesenteric lymph duct and insertion of an intraduodenal infusion catheter were performed as described in detail (26). Both the lymph cannula and the intraduodenal catheter were exteriorized through the right flank and the abdominal incision closed by suture. The rats were placed in restraining cages and housed in a postoperative recovery chamber at 30°C for 20 h. During the recovery period, a maintenance solution (277 mmol/L glucose, 6.75 mmol/L Na_2HPO_4, 16.5 mmol/L NaH_2PO_4, 5 mmol/L KCl and 115 mmol/L NaCl, pH 6.6) was infused at 3.0 mL/h through the duodenal cannula via an infusion pump (Harvard Apparatus, South Natick, MA).

Preparation of GT extract

GT extracts were prepared by soaking 4 g or 20 g of GT leaves (Salada Foods Division, Redco Foods Inc, Little Falls, New York) in 200 mL boiling deionized water for 5 min. Extracts were allowed to cool and filtered (0.45 μm HATF filters, Millipore Corp., Bedford, MA). Tea catechins were quantitated by HPLC (Beckman Instruments, Fullerton, CA; System Gold software) with a C_{18} reverse phase column (Alltima C_{18}, 5 μm, 4.6 × 150 mm; Alltech Associates). Water:acetonitrile: trifluoroacetic acid (83:17:0.05, v/v/v) was used as the mobile phase at 1.0 mL/min. Detection was monitored at 210 nm. Calibration standards were prepared using pure catechins (> 98%) and a linear

standard curve (r = 0.998) was generated for each catechin within the expected range of sample concentrations. The amounts of catechins extracted from the two preparations are shown by Table 1. The efficiency of catechin extraction was reduced with increasing the amount of tea leaves from 4 to 20 g.

Table 1. The amounts of green tea (GT) catechins extracted in 200 mL deionized water

Catechin	From 4 g GT leaves		From 20 g GT leaves	
	mg	%	mg	%
(-)-Epicatechin (EC)	34.2	9.6	86.7	8.6
(-)-Epicatechin gallate (ECG)	24.2	6.8	73.3	7.3
(-)-Epigallocatechin (EGC)	100.8	28.1	304.2	30.3
(-)-Epigallocatechin gallate (EGCG)	198.3	55.5	540.0	53.8
Total	357.5	100.0	1004.2	100.0

Preparation of lipid emulsions

For Experiment 1, lipid emulsions containing two different levels of tea catechins were prepared from the extracts prepared as above: one containing 112 μmol (42.9 mg) of total tea catechins (GT1) and the other containing 316 μmol (120.5 mg) of tea catechins (GT2), in 24 mL PBS buffer. A lipid emulsion containing no GT extract (GT0) was used for controls. The catechin levels in GT1 and GT2 were set to approximate a daily consumption of 7 and 20 cups of GT, respectively, in humans consuming 2,500 kcal/day. The lipid levels of the emulsion were based on the recommendations of the AIN-93G diet (23,24) and the daily food intake of 20 g/rat containing 0.12% cholesterol. Na^+-taurocholate (bile salt) was used at a physiological non-toxic level to emulsify the lipids (27).

For Experiment 2, the lipid emulsion contained 5 kBq ^{14}C-dioleoyl-phosphatidylcholine (^{14}C-DOPC; specific activity, 3.8 GBq/mmol; Dupont NEN, Boston, MA), 40.0 μmol DOPC (99%; Avanti Polar Lipids, Alabaster, AL) 451.8 μmol triolein (95%; Sigma Chemical, St. Louis, MO), 3.1 μmol -tocopherol (all-rac--tocopherol, 97%; Aldrich Chemical, Milwaukee, WI), 75.4 nmol retinol (all trans-retinol, 95%; Sigma Chemical, St. Louis, MO), and 396.0 μmol sodium taurocholate plus 199.3 μmol (-)-EGCG (98%; Sigma Chemical, St. Louis, MO), 199.3 μmol (+)-catechin (98%; Sigma Chemical, St. Louis, MO), or no catechin in 24 mL PBS buffer.

Measurement of ^{14}C-CH absorption (Experiment 1)

After postoperative recovery, each rat was infused via the duodenal catheter a lipid emulsion containing GT1 or GT2, or no GT extract (GT0) at 3.0 mL/h for 8 h (n = 5/group) under subdued light. The emulsions were stable and no phase separation was noted at room temperature. During infusion, mesenteric lymph was collected via the lymph cannula at hourly intervals into pre-weighed, ice chilled plastic tubes containing 4 mg Na_2-EDTA and 30 μg n-propyl gallate as antioxidant. Hourly lymph samples (100 μL) were used to determine the amount of ^{14}C-CH absorbed. The ^{14}C-radioactivity in total lymph was expressed as percent of the total radioactivity infused (% dose). The distribution of ^{14}C-radioactivity between free and esterified cholesterol fractions was determined by the method of Sperry and Webb (28) using digitonin.

Lipid analysis

Analyses for cholesterol (29), α-tocopherol (30), phospholipid (31), and fatty acids (32) were performed, as modified in our previous studies (25,26,33).

Determination of luminal hydrolysis and lymphatic output of PC (Experiment 2)

During lipid infusion, mesenteric lymph was collected via the lymph cannula at hourly intervals and the lymphatic output of ^{14}C-radioactivity was measured as above. At 8 h after lipid infusion, rats were anesthetized with halothane and killed by cervical dislocation. The intestine was removed and chilled immediately in ice. The luminal content was emptied into a plastic tube and washed three times with 10 mL ice-cold PBS containing 16.5 mM sodium taurocholate and weighed. Cecum with its content was removed separately. ^{14}C-radioactivities in lipids extracts from the small intestine and homogenates of the cecal contents and luminal washings were determined. Distributions of the ^{14}C radioactivity among different lipids in lymph, intestinal segments, luminal washings, and cecal contents were determined by thin layer chromatography.

Statistics

Statistical analysis was performed with PC SAS (34). Repeated measures ANOVA and the least significance difference test were used to compare multiple group means and time-dependent changes within groups. Differences were considered significant at $P < 0.05$.

RESULTS

Lymphatic absorption of ^{14}C-CH (Experiment 1)

3GT extracts markedly lowered the hourly rates and total amounts of ^{14}C-CH absorption in a dose-dependent manner (Fig. 1, Table 2). The average rates of ^{14}C-CH absorption in GT0, GT1, and GT2

were 4.5, 2.6, and 0.6% dose/h, respectively. The cumulative lymphatic absorptions of ^{14}C-CH over 8 h were 36.3 ± 1.1% dose in GT0, 20.7 ± 4.3% dose in GT1, and 4.8 ± 4.1% dose in GT2. Similarly, the average rates of lymphatic cholesterol output (exogenous and endogenous CH) also were lowered by GT extracts. The rates of CH output in GT0, GT1 and GT2 were 1.9 ± 0.4, 1.3±0.2, and 0.6 ± 0.1 μmol/h, respectively, with significant differences among all three groups. The cumulative lymphatic outputs of total CH were 15.0 ± 0.8 μmol/h in GT0, 10.0 ± 1.5 μmol/h in GT1 and 5.0 ± 1.4 μmol/h in GT2.

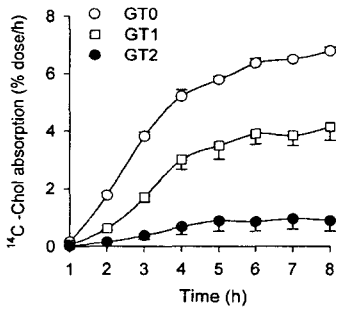

Fig. 1. Hourly rates of ^{14}C-CH absorption in OX rats infused with GT extracts.

Table 2. Cumulative lymphatic absorption of ^{14}C-cholesterol (^{14}C-CH), ^{14}C-radioactivity in esterified cholesterol (EC), -tocopherol (TP), and outputs of total cholesterol (CH), phospholipid (PL), oleic acid (OA), and lymph volume in rats infused with GT extracts for 8 h[1-3]

Lymph lipids	GT0	GT1	GT2
^{14}C-CH, % dose	36.3 ± 1.1^{a}	20.7 ± 4.3^{b}	4.8 ± 4.1^{c}
^{14}C-EC, % total	80.2 ± 2.3^{a}	79.0 ± 1.7^{a}	69.1 ± 6.8^{b}
CH, μmo	15.0 ± 0.8^{a}	10.0 ± 1.5^{b}	5.0 ± 1.4^{c}
EC, μmol	12.0 ± 0.8^{a}	8.0 ± 1.4^{b}	3.5 ± 1.1^{c}
PL, μmol	21.2 ± 1.5^{a}	20.6 ± 1.5^{a}	17.7 ± 5.0^{b}
TP, % dose	29.6 ± 4.9^{a}	20.8 ± 5.8^{b}	7.9 ± 5.4^{c}
nmol	1048.8 ± 174.9^{a}	736.5 ± 204.9^{b}	281.0 ± 190.8^{c}
OA, μmol	559.0 ± 25.0^{a}	704.0 ± 135^{b}	452.2 ± 210.4^{c}
Total fatty acid, μmol	717.7 ± 39.1^{b}	862.6 ± 151.1^{a}	557.9 ± 252.2^{c}
Lymph volume, mL	18.7 ± 3.1^{a}	20.8 ± 5.5^{a}	13.5 ± 3.8^{b}

[1]Mean ± SD; n = 5. [2]Values in a row not sharing a superscript letter are different (P < 0.05).
[3]GT0 = 0.0 mg GT catechins/h; GT1 = 5.36 mg GT catechins/h; GT2 = 15.6 mg GT catechins/h.

The lymphatic outputs of EC were significantly lower in rats infused with GT extracts (8.0 ± 1.4 μmol in GT1 and 3.5 ± 1.1 μmol in GT2) than in GT0 (12.0 ± 0.8 μmol). The average percent distribution of ^{14}C-radioactivity in the esterified cholesterol (EC) fraction did not differ between GT0 (80.2%) and GT1 (79.0%), but was significantly lower in GT2 (69.1%) than in GT0 and GT1. The lymphatic output of oleic acid, as infused in triolein, was significantly increased by GT1 but lowered by GT2 compared with GT0, indicating that GT differentially affect cholesterol and fat absorption. The lymphatic output of PL was unaffected by GT1, but was significantly lowered by GT2. GT extracts also markedly lowered the rates and amounts of TP absorption in a dose-dependent manner. The average rates of TP absorption in GT0, GT1 and GT2 were 131.1 ± 42.8, 92.1 ± 29.9, and 35.1 ± 10.8 nmol/h, respectively, with significant differences among all groups. The absorptions of TP in GT0, GT1, and GT2 were 1048.8 ± 174.9 nmol (29.6 ± 4.9% dose), 736.5 ± 204.9 nmol (20.8 ± 5.8% dose), and 281.0 ± 190.8 nmol (7.9 ± 5.4% dose), respectively.

Luminal hydrolysis and lymphatic output of ^{14}C-PC (Experiment 2)

Both (+)-catechin and EGCG significantly lowered the lymphatic output of ^{14}C-radioactivity after intraduodenal ^{14}C-DOPC infusion (Table 3). The total ^{14}C-radioactivity remaining in the luminal and cecal contents did not differ between the groups infused with no catechin (8.97% dose) and with (+)-catechin (9.45% dose). However, EGCG significantly increased the amount (24.1% dose) of ^{14}C-radioactivity in the intestinal contents. A significantly higher amount of ^{14}C-DOPC remained

Table 3. Recovery of ^{14}C-radioactivity in the intestine and lymph from ovariectomized rats at 8 h after intraduodenal infusion of ^{14}C-dioleoylphosphatidylcholine (^{14}C-DOPC) with (+)-catechin, or (-)-epigallocatechin gallate (EGCG), or without catechin[1,2]

Control	(+)-Catechin	EGCG	
Lymph, % dose	64.71 ± 2.00^a	56.15 ± 5.20^b	45.49 ± 4.90^c
Small intestine, % dose	12.87 ± 0.97^a	13.92 ± 2.46^a	12.49 ± 0.43^a
Cecum, % dose	0.14 ± 0.06^a	0.15 ± 0.07^a	1.92 ± 0.69^b
Lumen, % dose	8.83 ± 2.91^a	9.30 ± 1.47^a	22.18 ± 3.96^b
^{14}C-distribution, %			
PC	11.86 ± 2.93^a	15.84 ± 6.75^a	22.78 ± 7.39^b
LPC	34.42 ± 8.63^a	30.22 ± 6.15^a	36.03 ± 10.68^a
FFA	53.72 ± 9.91^a	53.94 ± 7.85^a	41.19 ± 14.12^a
Remainder	13.44 ± 1.09^a	20.42 ± 1.35^b	17.91 ± 1.28^c

[1]Means ± SD, n = 5. [2]Values in the same row not sharing a superscript are significantly different ($P < 0.05$).

unhydrolyzed in the intestinal lumen of the EGCG rats (22.8%) compared with the (+)-catechin (15.8%) and control groups (11.9%) as determined at 8 h postdosing. The total lymphatic output of phospholipid was moderately lowered in both (+)-catechin and EGCG groups, whereas the lymphatic output of oleic acid was lowered only in the EGCG groups, compared with controls (Table 4). Both (+)-catechin and EGCG significantly reduced the absorption of TP. However, EGCG showed a more pronounced inhibitory effect on TP absorption. The lymphatic total absorptions of TP in the rats infused with (+)-catechin and EGCG were 85.7 and 46.4%, respectively, of the control level. The absorption of retinol was not affected by either (+)-catechin or EGCG.

Table 4. Lymphatic outputs of phospholipid (PL), oleic acid (OA), -tocopherol (TP), and retinol (ROH) in rats infused for 8 h with ^{14}C-dioleoylphosphatidylcholine (^{14}C-DOPC) without catechin or with (+)-catechin or (-)-epigallocatechin gallate (EGCG)[1,2]

Lipid	Control	(+)-Catechin	EGCG
PL, μmol	23.4 ± 1.9[a]	20.7 ± 3.2[b]	20.6 ± 2.8[b]
OA, μmol	386.3 ± 44.8[a]	78.3 ± 24.7[a,b]	352.8 ± 31.7[b]
TP, % dose	16.8 ± 2.1[a]	14.4 ± 2.8[b]	7.8 ± 1.7[c]
ROH, % dose	15.5 ± 2.2[a]	15.6 ± 1.8[a]	17.0 ± 2.4[a]

[1]Means ± SD, n = 5.[2]Values in the same row not sharing a superscript are significantly different ($P<0.05$).

DISCUSSION

Our studies, as presented here, demonstrate that GT extract has a pronounced inhibitory effect on intestinal cholesterol absorption and that EGCG, a major catechin in GT, interferes with the luminal hydrolysis and lymphatic output of phosphatidylcholine (PC).

In the past, much attention has been directed to the antioxidant property of GT catechins, because it is thought to inhibit LDL oxidation and thus reduce the risk for coronary heart disease (CHD). Tea catechins are absorbed into the circulation in a dose-dependent manner and significantly contribute to the total antioxidant capacity of blood plasma (35,36). Numerous studies have shown that catechins bind to lipoproteins and possess antioxidant activities greater than -tocopherol, effectively inhibiting LDL oxidation and lipid peroxidation in vitro (37-41). Among the tea catechins, EGCG is most effective in inhibiting LDL oxidation (42). However, a recent study reported that, in human subjects drinking eight cups of green tea per day for three days, the amount of tea catechins associated with plasma LDL was less than 10% of the total amount in plasma, which was not sufficient to increase the

resistance to LDL oxidation ex vivo in adult humans (43). Much (60%) of green tea catechins were present mainly in the non-lipoprotein protein-rich fraction and 23% in high density lipoproteins (HDL) (43). Another study also showed that consumption of six cups of green tea for four weeks did not affect resistance to LDL oxidation or markers of oxidative damage to lipids in healthy adult humans (44). Thus, it still remains debatable whether the reduction in CHD risk associated with green tea consumption is attributable to the antioxidant potential of green tea catechins.

Our findings strongly suggest that in addition to its potential benefit as a source of polyphenolic antioxidants, GT may be used as dietary or pharmacological means of inhibiting the intestinal absorption of cholesterol and hence lowering the serum levels of cholesterol. At present, however, the precise mechanism underlying the effect of GT extract is far from clear. Previously, Ikeda et al. (45) reported that green tea catechins, particularly, their gallate esters, markedly reduced the absorption of cholesterol in male rats with thoracic lymph cannula. Under in vitro conditions, the investigators also observed that catechins were coprecipitated with cholesterol, when added directly to mixed micelles, and suggested that catechins might reduce the micellar solubility of cholesterol, thereby inhibiting its uptake by the intestinal absorptive cells. However, in this study (45), consistent relationships were shown between the catechin-induced inhibition of cholesterol absorption in vivo and the magnitude of micellar cholesterol precipitation measured in vitro. Furthermore, the absorption of cholesterol was measured after gastric intubation of a bolus (3.0 mL) of lipid emulsion containing excessive amounts of bile salt (200 mg/3.0 mL), cholesterol (25 mg/3 mL), and catechin preparations (100 mg/3.0 mL, 65-73% purity) containing oxidative and polymeric derivatives of catechins. Thus, it may be premature to conclude that the inhibition of cholesterol absorption by tea catechins is attributable to the formation of insoluble cholesterol-catechin precipitates. In the present study, we observed no precipitation of cholesterol in a lipid emulsion, when prepared with fresh GT extract containing 42.9 or 120.5 mg of catechins in 24 mL PBS buffer. The lipid emulsion contained 8.0 mg cholesterol and 213 mg Na^+-taurocholate per 24 mL. Under these conditions, the lipid emulsion remained stable over 32 h at room temperature.

Recently, we examined the inhibitory effects of tea catechins on porcine pancreatic phospholipase A_2 (PLA_2) activity in vitro (46). The percentages of inhibition of PLA_2 by EC, EGC, ECG, and EGCG were 23.3, 25.7, 39.7, and 64.9%, respectively, indicating that EGCG is the most potent inhibitor of the enzyme under the in vitro conditions used. Consistent with the in vitro findings, our data here show that when rats were infused intraduodenally with a lipid emulsion containing ^{14}C-labeled PC and EGCG, the total amount of the label appearing into the lymph were markedly reduced (45.5 ± 4.9% dose), compared with those (controls) infused with no EGCG (64.7 ± 2.0% dose). Also, significantly greater amounts of the unhydrolyzed PC were found in the intestinal lumen and cecum in EGCG-

infused rats. This observation is of significance in view of the emerging evidence that the initial hydrolysis of PC to lysophosphatidylcholine (LPC) by PLA_2 is a critical prerequisite step not only for efficient hydrolysis of triacylglycerol by pancreatic lipase/colipase, but also for stimulation of cholesterol absorption by the enterocyte (47-49).

An important new finding from our study is that GT extract and EGCG also lower the intestinal absorption of vitamin E (TP). Of particular interest is the observation that EGCG, a major GT catechin, inhibits the absorption of vitamin E, but does not interfere with vitamin A (retinol) absorption. The exact mechanism governing the differential effects of EGCG on the two lipid-soluble vitamins is unknown. In recent studies, we have provided evidence that luminal PC drastically inhibits the lymphatic absorption of both cholesterol and TP, and that LPC enhances the absorptions of both lipids in rats with lymph cannula (26). Similarly, Homan and Hamelehle (47), using Caco-2 cells, demonstrated that micellar PC does not interfere with the cell uptake of relatively less hydrophobic lipids such as retinol and fatty acids, but inhibits the uptake of cholesterol, an extremely hydrophobic lipid, whereas substitution of LPC for PC in mixed micelles or addition to PLA_2 increases the uptake of lipids regardless of their hydrophobicity. These findings suggest that intact PC may interfere with the micellar transfer of the lipids of extreme hydrophobicity to the enterocyte (47-49). Thus, based on the observations, it appears that the hydrolysis of PC by pancreatic PLA_2 is critical to the micellar incorporation and subsequent absorption of cholesterol and TP. Although the precise action of GT or catechins remains to be elucidated, it is probable that they may slow the rate of intestinal absorption of cholesterol and TP by inhibiting pancreatic PLA_2 activity, as we have demonstrated under both in vitro (46) and in vivo conditions in the present study.

In summary, our studies, as presented here, clearly show that GT extract, particularly at a higher dosage, is a potent inhibitor of the intestinal absorption of cholesterol and fat. Further studies are needed to answer the question of whether GT or catechins lowers the intestinal absorption of cholesterol under physiological conditions in humans, and to elucidate the precise mechanisms underlying the inhibitory effect of catechins on cholesterol absorption. Caution should be exercised regarding the potential adverse effect of chronic GT consumption on vitamin E status. The interactive roles of GT polyphenolic antioxidants and vitamin E in the antioxidant defense system should be further examined.

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