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Green tea catechins inhibit pancreatic phospholipase A_2 and intestinal absorption of lipids in ovariectomized rats

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Abstract

This study was conducted to examine whether the inhibition of intestinal lipid absorption by green tea is associated with the inhibitory effect of its catechins on pancreatic phospholipase A_2 (PLA_2). PLA_2 activity was assayed by using 1,2-dioleoylphosphatidylcholine (DOPC), porcine pancreatic PLA_2 and catechins at varying concentrations (0.075–1.80 μmol/L). The amount of 1-oleoyl-2-hydroxyphosphatidylcholine liberated was determined by HPLC. The percentage of inhibition of PLA_2 by catechins at 0.6 μmol increased in the order of (−)-epicatechin (23.3%), (+)-catechin (CAT; 24.8%), (−)-epigallocatechin (25.7%), (−)-epicatechin gallate (39.7%) and (−)-epigallocatechin gallate (EGCG; 64.9%). In an in vivo study, ovariectomized rats with lymph cannula were infused intraduodenally for 8 h with a triolein emulsion containing [dioleoyl-1-^{14}C]-phosphatidylcholine, DOPC, α-tocopherol (αTOH) and retinol (ROH) without (CAT0) or with CAT or EGCG. The lymphatic total ^{14}C-radioactivity was significantly lowered by EGCG (45.5±4.9% dose) compared with CAT (56.2±5.2% dose) and CAT0 (64.7±2.0% dose). The ^{14}C-radioactivity remaining in the small intestinal lumen and cecum was higher in EGCG (24.1% dose) than in CAT (9.5% dose) and CAT0 rats (9.0% dose). Significantly less ^{14}C radioactivity was incorporated into lymph triacylglycerol and cholesteryl ester in EGCG rats. The absorption of αTOH, used as a marker of extremely hydrophobic lipids, was significantly lower in EGCG (7.8±1.7 μmol) than in CAT (14.4±2.8 μmol) and CAT0 rats (16.8±2.1 μmol). The absorption of ROH was unaffected, whereas oleic acid output was lower in EGCG rats. The results show that EGCG inhibits the intestinal absorption of lipids, which is in part associated with its inhibition of phosphatidylcholine hydrolysis. Data suggest that EGCG may inhibit the absorption of other highly lipophilic organic compounds.

Keywords: Lipids; Phosphatidylcholine; Green tea; (−)-Epigallocatechin gallate; Phospholipase A_2; α-Tocopherol; Rats

1. Introduction

Green tea (GT) is a beverage consumed worldwide and produced from the tea plant *Camellia sinensis* by treating fresh tea leaves with hot steam and air. The treatment inactivates polyphenol oxidase and preserves its peculiar green color, yielding polyphenol-rich GT. The major polyphenols in GT are catechins, constituting about one third of its total dry weight. The major catechins present in GT are (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-epicatechin (EC). In recent years, much attention has been directed to the potential health benefits of GT catechins. Evidence from cell and animal studies indicates that GT catechins have antioxidant, antiatherogenic, anticarcinogenic and anti-inflammatory properties as reviewed recently [1–3]. However, whether GT catechins prevent or reduce the risk of coronary heart disease, cancer and other inflammatory diseases in humans remains to be determined.

GT catechins are not well absorbed, and only a small percentage of orally ingested catechins appear in the blood in rats [4] and humans [5,6]. Particularly, EGCG, the principal GT catechin known to possess strong antioxidant and anti-inflammatory properties [7–9], is poorly absorbed, and its bioavailability is much lower than other GT catechins. In rats, about 0.1% of orally administered EGCG

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is shown to be bioavailable [4]. Because of their rather poor absorption, ingested catechins are present in the intestinal lumen in higher concentrations and affect the digestive and absorptive processes. Animal studies have shown that GT catechins increase the fecal excretion [10,11] and decrease the intestinal digestion and absorption [11–13] of lipids. The inhibitory effect of GT catechins on lipid absorption has been attributed to interference with the micellar solubilization of lipids [11,12] and inhibition of luminal lipolysis by pancreatic lipase [14].

Considerable evidence from cell and animal studies suggest the importance of pancreatic phospholipase A_2 (PLA_2) in facilitating the digestion and absorption of lipids. Numerous studies using in vitro systems and intestinal cells [15–19] demonstrated that phosphatidylcholine (PC) hydrolysis by pancreatic PLA_2 is required for the efficient absorption of fatty acids, cholesterol and other extremely hydrophobic lipids. The present study was conducted to investigate whether the inhibitory effect of GT catechins on lipid absorption is due to the inhibition of pancreatic PLA_2 in ovariectomized (OX) rats. In the present study, we used OX rats as a model to mimic the physiologic conditions of ovarian hormone deficiency or the postmenopausal state in which serum levels of lipids are elevated with increased risk of coronary heart disease [13]. Using an in vitro PLA_2 assay and an in vivo animal model with lymph cannula, the present study provides first evidence that GT catechins, particularly EGCG, inhibit pancreatic PLA_2 activity and markedly lower the lymphatic absorption of lipids.

2. Materials and methods

2.1. Assay for pancreatic PLA_2 activity in vitro

The activity of porcine pancreatic PLA_2 was assayed [20] in duplicate by using a substrate mixture consisting of 500 nmol 1,2-dioleoylphosphatidylcholine (DOPC; 99%; Avanti Polar Lipids, Alabaster, AL), 0.667 μmol $CaCl_2$, 0.134 μmol SDS and 0.674 μmol Triton X-100 in 200 μl of 100 mM Tris–HCl buffer (pH 7.9). The substrate mixture was incubated at 37°C for 30 min with 0.1 unit (0.0625 μg protein) of porcine pancreatic PLA_2 (1600 units/mg protein; Sigma Chemical, St. Louis, MO) in the absence (blank) or presence of a catechin. All catechins were of high purity (>98%) and purchased from Sigma Chemical. The catechins tested were (+)-catechin (CAT), EC, ECG, EGC or EGCG at varying concentrations (0.075–1.80 μmol/300 μl assay mixture). The range of concentrations approximates the amounts of total catechins in 0.4–10 cups of GT. The amount of 1-oleoyl-2-hydroxyphosphatidylcholine liberated was determined by HPLC as described previously [21]. The inhibition of PLA_2 activity by catechins was expressed as percentage of control (blank) activity. The percentage of inhibition was plotted against catechin concentrations, and the concentration of each catechin at 50% inhibition (IC_{50}) was determined.

2.2. Animals and diet

Eighteen female Sprague–Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) initially weighing 235±7 g were placed individually in plastic cages with stainless-steel wire bottoms in a windowless room maintained at 22°C to 24°C and a daily 12-h light/dark cycle with the light period from 0330 to 1530 h. The rats were fed a rodent diet ad libitum and allowed free access to deionized water delivered via an automatic watering system. The diet was formulated by Dyets Inc. (Bethlehem, PA) with modifications [21] of the AIN-93G diet [22]. All rats were cared for in an animal care facility in the Department of Human Nutrition, Kansas State University, in accordance with a protocol approved by the Institutional Animal Care and Use Committee.

2.3. Ovariectomy and cannulation of mesenteric lymph duct

At the end of 3 weeks, rats weighing 322–335 g were starved for 12 h and ovariectomized [23] under halothane anesthesia. Six weeks after ovariectomy, rats were starved for 16 h, and the major mesenteric lymph duct was cannulated as previously described [13].

2.4. Measurement of lymphatic lipid absorption

After overnight postoperative recovery, each rat was infused via the duodenal catheter with a lipid emulsion at 3.0 ml/h. The lipid emulsion contained 5 kBq [dioleoyl-1-^{14}C]-phosphatidylcholine (^{14}C-DOPC; specific activity, 3.8 GBq/mmol; Dupont-New England Nuclear, Boston, MA), 451.8 μmol triolein (95%; Sigma Chemical), 3.1 μmol α-tocopherol (αTOH; all-rac-α-tocopherol, 97%; Aldrich Chemical, Milwaukee, WI), 75.4 nmol retinol (ROH; all *trans*-retinol, 95%; Sigma Chemical), 396 μmol sodium taurocholate and 40 μmol DOPC (99%; Avanti Polar Lipids), with 199.3 μmol EGCG or with 199.3 μmol CAT (98%; Sigma Chemical) or with no catechin (CAT0) in 24 ml phosphate-buffered saline (6.75 mM Na_2HPO_4, 16.5 mM NaH_2PO_4, 115 mM NaCl and 5 mM KCl, pH 6.35). The choice of EGCG and CAT was based on the in vitro observation that EGCG was the most potent inhibitor of PLA_2, whereas CAT was the weakest. By including the two catechins, this experiment was performed to compare their effects on the luminal hydrolysis and absorption of labeled PC under in vivo conditions. The total amount of EGCG included in the emulsion (199.3 μmol/24 ml) is equivalent to that present in one tea bag (2 g GT leaves). Lymph was collected hourly for 8 h into preweighed conical centrifuge tubes containing 25 mM disodium EDTA cooled in ice-filled beakers under subdued light. From the hourly lymph samples, the ^{14}C-radioactivity was determined in 100-μl aliquots after mixing with scintillation liquid (Scintiverse; Fisher Scientific Co., Fair Lawn, NJ) by scintillation spectrometry (Beckman LS-8100; Beckman Instruments Inc., Fullerton, CA). The ^{14}C-radioactivities appearing in hourly lymph samples were expressed as percentage of the total ^{14}C-radioactivity infused.

Table 1
Inhibition (%) of PLA_2 activity by catechins at varying concentrations in vitro

μmol (per 300 μl assay mixture)	% Inhibition				
	EGCG	ECG	EC	EGC	CAT
0.075	2.2±2.1[a]	11.1±6.1[b]	18.8±2.6[c]	8.5±2.8[b]	12.9±5.2[b,c]
0.15	5.2±2.1[a]	15.3±2.3[b]	17.1±0.9[b]	17.2±0.9[b]	18.6±0.8[b]
0.30	30.1±0.5[a]	12.7±3.2[b]	21.3±0.5[c]	22.7±4.4[c]	21.4±0.9[c]
0.60	64.9±1.3[a]	39.7±0.3[b]	23.3±1.4[c]	25.7±3.2[c]	24.8±1.0[c]
1.20	77.9±2.9[a]	67.4±2.1[b]	28.8±1.9[c]	28.5±3.6[c]	28.4±1.0[c]
1.80	83.6±1.6[a]	73.1±9.8[a]	36.1±4.2[b]	32.0±3.6[b,c]	31.3±0.2[c]
IC_{50} (μmol)	0.48	0.84	>1.8	>1.8	>1.8

Values are means±S.D., $n=2$ (duplicates).
Values in the same row not sharing a common superscript ([a,b,c]) are significantly different ($P<.05$).

2.5. Measurement of ^{14}C-radioactivity in mucosal, luminal and cecal samples

At the end of lipid infusion, the rats were anesthetized with halothane and killed by cervical dislocation. The small intestine and the cecum were removed separately and chilled immediately on ice. The luminal content of the small intestine was emptied into a plastic tube and washed three times with 10 ml ice-cold phosphate-buffered saline (6.75 mM Na_2HPO_4, 16.5 mM NaH_2PO_4, 115 mM NaCl and 5 mM KCl, pH 6.35) containing 16.5 mM sodium taurocholate. After washing, the intestine was divided into four equal segments and cut opened longitudinally. The most proximal intestinal segment was designated as Segment 1, and the subsequent segments were designated as Segments 2, 3 and 4. Lipids from the intestinal segments were extracted according to the method of Folch et al. [24]. ^{14}C-radioactivity was determined from an aliquot (100 μl) of lipid extracts and expressed as percentage of the dose infused. The cecal content and intestinal luminal washings were separately homogenized. The ^{14}C-radioactivities were determined from aliquots (350 μl cecal content) and luminal washings (1.0 ml) after mixing with 10 ml scintillation liquid (Scintiverse; Fisher Scientific Co.) by scintillation spectrometry (Beckman LS-8100; Beckman Instruments Inc.).

2.6. Distribution of ^{14}C-radioactivities among lipids in lymph, mucosa and luminal washings

From the lipid extracts, the distribution of ^{14}C radioactivity among different lipids was determined by separating lipids by thin-layer chromatography on Silica Gel G (20×20 cm, 250 μm, Analtech, Newark, DE) with *n*-hexane–diethylether–glacial acetic acid (80:20:1, v/v/v). This solvent system gave an excellent separation of cholesterol ester, triglyceride (TG), free fatty acid (FFA) and phospholipid. After drying, the plate was placed into the second solvent system (chloroform–methanol–distilled water; 130:60:7, v/v/v) to separate lysophospholipid from intact phospholipid. Separated lipids were visualized with iodine vapor. The lipid spots were scraped into counting vials and eluted with 0.5 ml of chloroform–methanol (2:1, v/v) mixture for 10 min prior to mixing with scintillation liquid (Scintiverse, Fisher Scientific Co.). The distribution (%) of ^{14}C-radioactivity among lipid classes was calculated.

2.7. Determination of lymphatic phospholipid and oleic acid

Total lipids from 100 μl lymph were extracted by 2 ml of chloroform–methanol mixture (2:1, v/v) containing 10 mg of butylated hydroxytoluene/300 ml. An internal standard (19:0) was added prior to lipid extraction. Lymph phospholipid (PL) was measured colorimetrically (UV-1201 Spectrophotometer; Shimadzu Scientific Instruments Inc., Columbia, MD) using the method of Raheja et al. [25]. For oleic acid (OA) analysis, the lipids were hydrolyzed with methanolic NaOH, and fatty acids were saponified, methylated simultaneously with BF_3-methanol [26] and analyzed by gas chromatography [21] as previously described.

2.8. Determination of ROH and αTOH in lymph by HPLC

Total ROH (free and esterified ROH) and αTOH were extracted from lymph by a slight modification of the method of Bieri et al. [27]. A 100-ml lymph sample was saponified in 10 vol of 95% ethanol and 1% potassium hydroxide solution (Fisher Scientific, Pittsburgh, PA) containing 1% pyrogallol (99%, Acros Organics, Pittsburgh, PA) at 60°C for 10 min. After cooling, the contents were mixed vigorously with 20 vol of hexane for 7 min and then with

Table 2
Total lymphatic outputs of ^{14}C-radioactivity, PL, OA, αTOH, ROH and lymph volume in rats infused with a lipid emulsion containing ^{14}C-DOPC with EGCG, CAT or CAT0 (control)

Lymph	CAT0	CAT	EGCG
Total ^{14}C-radioactivity (% recovery)	64.7±2.0[a]	56.2±5.2[b]	45.5±4.9[c]
PL (μmol)	23.4±1.9[a]	20.7±3.2[b]	20.6±2.8[b]
OA (μmol)	772.6±89.5[a]	756.6±49.4[a,b]	705.6±63.4[b]
αTOH (nmol)	527.5±65.4[a]	450.4±86.9[b]	243.7±51.7[c]
ROH (nmol)	11.7±1.6[a]	11.8±1.4[a]	12.8±1.8[a]
Lymph volume (ml/8 h)	20.6±3.2[a]	21.3±2.7[a]	19.9±3.4[a]

Values are means±S.D., $n=5$.
Values in the same row not sharing a common superscript are significantly different ($P<.05$).

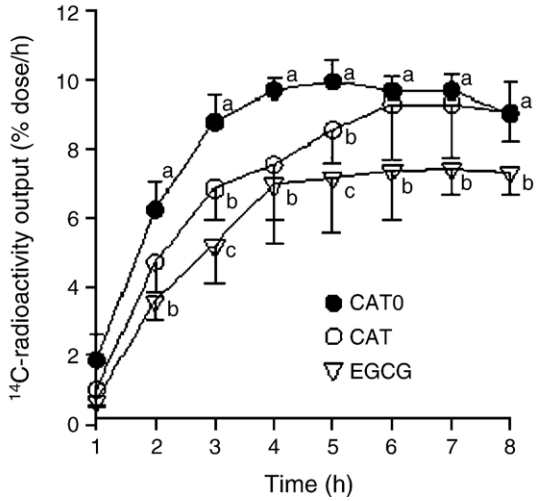

Fig. 1. Hourly rates of the lymphatic output of ^{14}C-radioactivity in rats infused with a lipid emulsion containing ^{14}C-DOPC with EGCG, CAT or CAT0 (no catechin). Values are means±S.D., $n=5$. Values at each hourly interval not sharing a common letter are significantly different ($P<.05$).

10 vol of water. After a brief centrifugation, the upper phase was transferred into a vial, dried under N_2 and resuspended in chloroform–methanol (1:3, v/v). Tocol (gift from Hoffmann-La Roche Ltd., Basel, Switzerland) was added as an internal standard to each sample to monitor extraction efficiency, which exceeded 95%. ROH and αTOH analysis was performed by using a Beckman HPLC as previously described [21].

2.9. Statistics

All statistical analyses were performed using PC SAS [28]. ANOVA and the least significant difference test were performed to compare multiple group means, and repeated-measures ANOVA was carried out to detect time-dependent changes within groups. Differences were considered

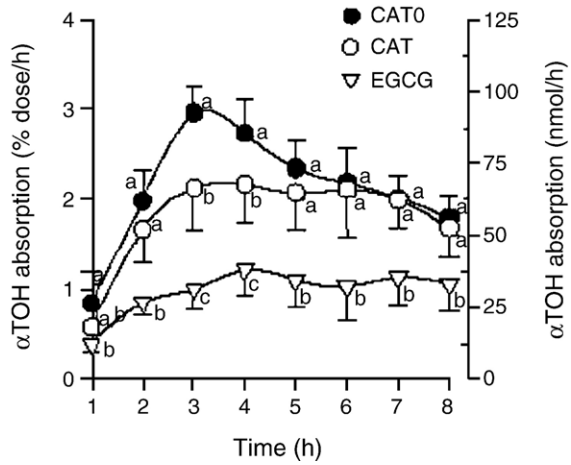

Fig. 2. Hourly rates of the lymphatic output of αTOH in rats infused with a lipid emulsion containing EGCG, CAT or CAT0 (no catechin). Values are means±S.D., $n=5$. Values at each hourly interval not sharing a common letter are significantly different ($P<.05$).

Table 3
Recovery (%) of ^{14}C-radioactivity in the intestine of rats after 8 h infusion of a lipid emulsion containing ^{14}C-DOPC with EGCG, CAT or CAT0 (control)

Fractions	% Recovery		
	CAT0	CAT	EGCG
Small intestinal lumen	8.8±2.9[a]	9.3±1.5[a]	22.2±4.0[b]
Small intestinal mucosa	12.9±1.0[a]	13.9±2.5[a]	12.5±0.4[a]
Cecal content	0.1±0.1[a]	0.2±0.1[a]	1.9±0.7[b]

Values are means±S.D., $n=5$.
Values in the same row not sharing a common superscript are significantly different ($P<.05$).

significant at $P<.05$. Values in tables and figures are expressed as means±S.D.

3. Results

3.1. Pancreatic PLA_2 activity with GT catechins in vitro

Among the tea catechins, EGCG was the most potent inhibitor of PLA_2 activity. The extent of inhibition increased in the order of CAT≅EC<EGC<ECG<EGCG. The concentrations at 50% inhibition (IC_{50}) for EGCG and ECG were 0.48 and 0.84 μmol, respectively. The IC_{50} values for EC and EGC were greater than 1.8 μmol. EC and EGC were much weaker inhibitors of PLA_2 relative to EGCG and ECG (Table 1).

3.2. Lymphatic output of ^{14}C-radioactivity, PL, OA, αTOH and ROH

The total ^{14}C-radioactivity appearing in mesenteric lymph after ^{14}C-DOPC infusion for 8 h was significantly lower in the rats infused with EGCG (45.5±4.9% dose) than those infused with CAT (56.2±5.2% dose) and CAT0 (control; 64.7±2.0% dose; Table 2). The hourly rate (% dose/h) of ^{14}C-radioactivity output was also markedly lower in the rats infused with EGCG than those infused with CAT0 at 2 h and thereafter and lower than in controls at 5 h and thereafter (Fig. 1). The cumulative output of total PL was moderately but significantly lower in EGCG (20.6±2.8 μmol) and CAT (20.7±3.2 μmol) than in CAT0 rats (23.4±1.9 μmol). The output of OA, as a measure of the efficiency of triolein digestion and absorption, was significantly lower in rats infused with EGCG (705.6±63.4 μmol) than those infused with CAT0 (772.6±89.5 μmol). No

Table 4
Distribution (%) of ^{14}C-radioactivity in PC, LPC and FFA in the small intestinal lumen of rats after 8 h infusion with a lipid emulsion containing ^{14}C-DOPC with EGCG, CAT or CAT0 (control)

Lipids	CAT0	CAT	EGCG
PC	11.9±2.9[a]	15.8±6.8[a]	22.8±7.4[b]
LPC	34.4±8.6	30.2±6.2	36.0±10.7
FFA	53.7±9.9	53.9±7.9	41.2±14.1

Values are means±S.D., $n=5$.
Values in the same row not sharing a common superscript are significantly different ($P<.05$).

significant difference was noted in OA output between EGCG and CAT (756.6±49.4 μmol). EGCG infusion also markedly lowered the hourly and cumulative output of αTOH (Table 2 and Fig. 2), whereas no significant differences were observed in hourly or cumulative lymphatic ROH outputs among groups (Table 2).

3.3. ^{14}C-radioactivity in the intestinal lumen, cecum and mucosa

The ^{14}C-radioactivity remaining in the small intestinal lumen was significantly higher in the rats infused with EGCG (22.2±4.0% dose) than those infused with CAT (9.3±1.5% dose) and CAT0 (8.8±2.9% dose; Table 3). The ^{14}C-radioactivity remaining in the cecal content was also significantly higher in the rats infused with EGCG than those infused with CAT and CAT0 (0.1±0.1% dose). The total ^{14}C-radioactivities remaining in the intestinal lumen and cecal content in rats infused with EGCG, CAT and CAT0 were 24.1%, 9.5% and 8.9%, respectively. No significant difference was noted in the ^{14}C-radioactivity remaining in the intestinal mucosa among groups.

3.4. Distribution of ^{14}C-radioactivity in luminal and mucosal lipids

A significantly higher percentage of the luminal ^{14}C-radioactivity was associated with intact PC in rats infused with EGCG (22.8±7.4%) than in those infused with CAT (15.8±6.8%) and CAT0 (11.9±2.9%). No differences were noted in ^{14}C-radioactivity associated with lysophosphatidylcholine (LPC) and FFA (Table 4). Also, a higher proportion of the mucosal ^{14}C-radioactivity was present in PC in the rats infused with EGCG (21.1±2.0%) than in those infused with CAT (13.7±1.9%) and CAT0 (7.2±0.9%; Table 5). In contrast, less ^{14}C-radioactivity was associated with mucosal TG in the rats infused with EGCG (31.9±4.8%) than in those infused with CAT0 (55.6±4.4%), with no significant difference between EGCG and CAT (41.3±5.4%) groups. A similar distribution of ^{14}C-radioactivity in mucosal PC and TG was noted in Segments 2 and 3, except that no difference was noted in ^{14}C-radioactivity associated with PC in Segment 4 (data not shown). The consistently greater incorporation of the labeled fatty acid into mucosal PC but less incorporation into TG may suggest a compensatory response to meet the PC demand for chylomicron formation in the rats treated with EGCG.

Table 5
Distribution (%) of ^{14}C-radioactivity in mucosal lipids in small intestinal Segment 1 of rats after 8 h infusion with a lipid emulsion containing ^{14}C-DOPC with EGCG, CAT or CAT0 (control)

Lipids	CAT0	CAT	EGCG
PC	7.2±0.9^{a}	13.7±1.9^{b}	21.1±2.0^{c}
LPC	1.6±0.4^{a}	3.2±0.8^{b}	4.4±0.7^{c}
FFA	26.6±3.4^{a}	26.2±3.3^{a}	29.3±2.2^{a}
TG	55.6±4.4^{a}	41.3±5.4^{b}	31.9±4.8^{b}
CE	4.3±0.7^{a}	2.8±0.7^{b}	2.8±0.6^{b}
Other lipids[1]	4.8±1.2^{a}	12.8±2.0^{b}	10.6±1.8^{b}

Values are means±S.D., $n=5$.
Values in the same row not sharing a common superscript are significantly different ($P<.05$).
[1] Lipids are detectable but not identified.

4. Discussion

The present study provides the following new evidence: (a) that EGCG and ECG, containing the gallate moiety, are potent inhibitors of pancreatic PLA_2 activity in vitro. Among the GT catechins, EGCG was most effective in inhibiting PLA_2 activity; (b) that EGCG interferes with PC hydrolysis and markedly lowers the lymphatic output of ^{14}C-radioactivity following the intraduodenal infusion of ^{14}C-DOPC under in vivo conditions; (c) that EGCG also significantly lowers the lymphatic absorption of fat and αTOH but with no effect on ROH absorption.

The lipid-lowering effect of GT has been reported in various animal models [10,29–32], although such an effect is yet to be conclusively demonstrated in humans [33–35]. The hypolipidemic effect may be partly associated with the inhibition of hepatic synthesis and release of lipids. An in vitro study with HepG2 cells [36] showed that EGCG was a potent inhibitor of the hepatic secretion of apoB-100, which is required for the formation and release of VLDL [36]. However, the study used a rather high level of EGCG (50 μM) in vitro, which may not be attainable by a dietary means. Thus, whether the hepatic secretion of apoB-100 is inhibited under physiological conditions remains to be determined. A study, using rats fed diets containing 1.0% cholesterol and 1–4% GT in drinking water, showed that GT did not significantly affect the liver activities of lipid-metabolizing enzymes, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, cholesterol 7α-hydroxylase and fatty acid synthase, whereas GT markedly increased the fecal excretion of cholesterol and bile acids [30]. This finding is consistent with the observations by others that GT catechins, especially EGCG, increase the fecal excretion of fat and cholesterol [10,11,37] and decrease the intestinal absorption of lipids [11–13,38]. Several studies [11,12,14] suggest that GT catechins interfere with the emulsification and micellar solubilization of lipids, which are critical steps for luminal lipolysis and uptake of hydrolyzed lipids by the enterocyte. Evidence also indicates that tea catechins inhibit pancreatic lipases interfering with luminal digestion of fat [14,38].

The present study provides new evidence that EGCG is a potent inhibitor of pancreatic PLA_2 interfering with the intestinal hydrolysis of PC, hence decreasing the absorption of lipids. Our findings here strongly suggest that the decreased absorption of lipids was partly attributable to the inhibition of PLA_2 activity by EGCG. The importance of PC hydrolysis in facilitating intestinal lipid digestion and absorption has been well demonstrated in studies using intestinal cells and enzymes in vitro [15–19] and in our study using a rat model in vivo with lymph cannula [39].

Since PC is present on the surface of lipid emulsions, the outer coat of PC hinders the hydrolysis of the core TG by pancreatic lipase, whereas even a limited hydrolysis of PC by pancreatic PLA_2 facilitates the binding of lipase/colipase to the substrate interface and results in a rapid hydrolysis of TG [15,40,41]. In studies using a rat intestinal cell line [42], pancreatic lipase/colipase was shown to be ineffective in hydrolyzing TG that was incorporated into PC-containing lipid emulsions, and the initial hydrolysis of the surface PC by pancreatic PLA_2 significantly increased the hydrolysis of TG by pancreatic lipase/colipase. In addition, the study of Young and Hui [42] demonstrated that minimal hydrolysis of TG was required for stimulation of the cell uptake of other extremely hydrophobic lipids such as cholesterol, suggesting that fatty acids and monoacylglycerol liberated from TG are key determinants in facilitating lipid transfer to the enterocyte. Thus, the initial action of pancreatic PLA_2 is critical to the hydrolysis of TG by lipase/colipase, formation of mixed micelles and subsequent transfer of micellar lipids to the enterocyte through the unstirred water layer [16,17].

In the present study, we included αTOH in the lipid emulsion as a marker of extremely hydrophobic lipids and ROH as a relatively less hydrophobic lipid to determine whether EGCG differentially inhibits the absorption of the two fat-soluble vitamins. In a study using Caco-2 cells in vitro, Homan and Hamelehle [19] demonstrated that the presence of intact PC in bile salt micelles markedly reduced the uptake of cholesterol, a 27-carbon molecule of extreme hydrophobicity, whereas it did not interfere with the cell uptake of less hydrophobic lipids such as ROH, OA and monoacylglycerol. The inhibition of luminal PC hydrolysis by EGCG may explain the rather marked inhibition of the lymphatic absorption of αTOH, another extremely hydrophobic 29-carbon lipid, while it had no effect on ROH absorption with a relatively moderate decrease in fat (OA) absorption. The potential impact of the decreased absorption of αTOH on the body status and availability of the vitamin in the lower intestinal tract (e.g., colon) remains to be determined. Also, it would be of interest to determine whether EGCG interferes with the absorption of nonnutrient lipophilic compounds including persistent organic pollutants [43].

At present, the mechanism underlying the inhibitory effect of EGCG is unknown. Flavonoids are known to readily form complexes with proteins through hydrophobic interactions and hydrogen bonding [44,45]. EGCG may bind to the active site of pancreatic PLA_2 or may change the protein conformation by nonspecific binding. There is also the possibility that EGCG may inhibit pancreatic PLA_2 activity by chelating Ca^{++}, which is required for its activity [20].

In summary, the present study provides first evidence that EGCG, the principal catechin from GT, markedly inhibits the luminal hydrolysis of PC by pancreatic PLA_2 and the intestinal absorption of lipids, particularly those of extreme hydrophobicity including αTOH. Further studies are warranted to evaluate the effect of chronic intake of GT on αTOH status and antioxidant defense, particularly in people at risk of vitamin E deficiency. Currently, attention is also being directed to assess the efficacy of GT or EGCG in reducing the intestinal absorption of other extremely lipophilic compounds.

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