Review

Mechanisms of Inhibition of Cholesterol Absorption by Green Tea Catechins

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Several studies have shown that green tea catechins reduce serum and liver cholesterol concentrations and increase the fecal excretion of neutral steroids originating from cholesterol in experimental animals. Furthermore, we have found that green tea catechins, particularly, their galloyl esters, could reduce the lymphatic absorption of cholesterol in rats. This finding suggests that green tea catechins having a galloyl moiety can inhibit cholesterol absorption in the intestine, thus reducing serum cholesterol concentration. The solubilization of cholesterol in bile salt micelles is essential for its absorption. Catechins having a galloyl moiety can decrease the micellar solubility of cholesterol; however, it is not well understood how these catechins decrease micellar solubility. This review presents novel mechanisms underlying the decrease in cholesterol micellar solubility mediated by catechins having a galloyl moiety.

Keywords: green tea catechins, cholesterol, absorption, bile salt micelle, micellar solubility of cholesterol, phosphatidylcholine

Introduction

Green tea is produced from the leaves of *Camellia sinensis*, and is one of the most widely consumed beverages worldwide. Catechins are the most commonly found polyphenols in green tea. Green tea catechins constitute about 10 – 18% of the total dry weight of green tea leaves. Green tea catechins extracted from tea leaves mainly include four types of catechins: (\(-\))-epicatechin (EC), (\(-\))-epigallocatechin (EGC), (\(-\))-epicatechin gallate (ECG), and (\(-\))-epigallocatechin gallate (EGCG) (Fig. 1). Catechins can be divided into two classes: free catechins (such as EC and EGC) and catechins having a galloyl moiety (such as ECG and EGCG). The consumption of canned and bottled tea beverages is increasing in industrialized countries, particularly in Japan. The heat-sterilization of these products epimerizes approximately 50% of catechins at the C2-position, forming (\(-\))-catechin (C), (\(-\))-gallocatechin (GC), (\(-\))-catechin gallate (CG), and (\(-\))-gallocatechin gallate (GCG) (Chen et al., 2001; Seto et al., 1997). Therefore, canned and bottled tea beverages typically contain eight types of catechin.

Green tea catechins have been reported to have various health benefits, including antiviral (Miura et al., 2001), antioxidative (Okuda et al., 1983; Yoshino et al., 1994), anticarcinogenic (Fujiki et al., 1998; Kada et al., 1985) and antiobesity activities (Ikeda et al., 2005; Kajimoto et al., 2005; Kobayashi et al., 2016; Murase et al., 2000). In addition, catechins have hypocholesterolemic activities. The relationship between green tea consumption and serum cholesterol concentration has been investigated in several epidemiological studies. Four cohort studies found that green tea consumption is associated with a reduction in serum cholesterol concentration (Imai and Nakachi., 1995; Kono et al., 1992, 1996; Tokunaga et al., 2002). Several interventional studies also revealed that the consumption of green tea or green tea catechins decreases serum cholesterol concentration (Kajimoto et al., 2003, 2006; Nantz et al., 2009; Samavat et al., 2016). Moreover, three meta-analyses and a systematic review reported that the intake of green
tea beverages or green tea extracts containing catechins results in significant reductions in serum total and LDL cholesterol levels (Khalesi et al., 2014; Kim et al., 2011; Momose et al., 2016; Zheng et al., 2011). Furthermore, Kuriyama et al. (2006) reported the association of green tea consumption with a reduction in mortality from cardiovascular disease in a large population-based prospective cohort study of 40,530 individuals in Japan (Ohsaki Study).

As hypercholesterolemia is an important risk factor of coronary heart disease (Martin et al., 1986; Okamura et al., 2007), studies have suggested that green tea or green tea catechins can prevent atherosclerosis and coronary heart disease through their LDL-cholesterol-lowering activity. A green tea beverage supplemented with tea catechins having a galloyl moiety, which is rich in EGCG, ECG, GCG and CG, is currently promoted in Japan as a functional food that has hypocholesterolemic activity.

![Chemical structures of various green tea catechins and heat-epimerized tea catechins](image)

Fig. 1. Chemical structures of various green tea catechins and heat-epimerized tea catechins
Left: green tea catechins, Right: heat-epimerized tea catechins
Hypcholesterolemic activity of green tea catechins

Muramatsu’s group (Fukuyo et al. (1986); Muramatsu et al. (1986)) was the first to report that green tea catechins could reduce serum cholesterol concentration in experimental animals. They found that when a crude catechin mixture containing EGCG, EGC, ECG and EC was added at 1% and 2% concentration to diets containing 1% cholesterol and fed to rats for 28 days, plasma total cholesterol and liver cholesterol levels were significantly reduced. Consumption of the catechin mixture increased the fecal cholesterol excretion of rats in the study. Fukuyo et al. (1986) also found that the cholesterol-lowering effects of EGCG were similar to those of a crude catechin mixture in rats. Matsuda et al. (1986) showed that mixtures containing ECG or EGCG, but not those containing EC or EGC, caused the cholesterol-lowering effects in mice. Moreover, Raederstorff et al. (2003) demonstrated that purified EGCG from green tea leaves added at 1% to diets containing 0.5% cholesterol reduced plasma and liver cholesterol concentrations in rats. Chan et al (1999) studied the hypolipidemic activity of green tea extracts in hamsters fed a high-fat diet; the extracts were isolated from jasmine green tea leaves with a purity of 95%, in which the percentages of EGCG, EGC, ECG and EC were 62.3%, 19.2%, 8.3% and 4.6%, respectively. In comparison with the control group, hamsters given the jasmine green tea extracts showed a higher fecal excretion of neutral steroids originating from cholesterol. The increase in the fecal excretion of neutral steroids originating from cholesterol in experimental animals fed green tea catechins was also reported by Nakamura et al. (2001) and Kobayashi et al. (2005). Nakamura et al. (2001) showed that oral administration of green tea extract high in EGCG at a dose of 1.0 g/kg for 23 days significantly increased the fecal excretion of neutral steroids including cholesterol, coprostanol and coprostanone in rats fed a commercial non-purified diet. The effect of green tea extracts high in heat-epimerized catechins having a galloyl moiety, in which CG and GCG levels were increased during pasteurization, on serum cholesterol concentration was compared with that of green tea extracts high in catechins having a galloyl moiety, in which the major components were ECG and EGCG (Kobayashi et al. 2005). In the study, both green tea catechin preparations, added at 1% to diets containing 0.5% cholesterol, significantly reduced serum and liver cholesterol concentrations and apparent cholesterol absorption, and they increased the fecal excretion of neutral steroids including cholesterol and coprostanol in rats. Yang and Koo (2000) showed that Lung Chen Tea, a Chinese green tea rich in EGCG (prepared by soaking 5 g, 10 g, and 20 g of tea leaves in 500 mL of boiled water for 30 minutes) reduced serum and liver cholesterol in diet-induced hypercholesterolemic rats after 8 weeks in a dose-dependent manner. The liver activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme for cholesterol synthesis, was not affected by Lung Chen Tea supplementation. Furthermore, Lung Chen Tea significantly increased fecal cholesterol excretion. These observations strongly suggest that green tea catechins including their C2-epimer, especially those having a galloyl moiety, exert hypcholesterolemic activity by increasing fecal cholesterol excretion.

Studies on the effects of green tea catechins on the intestinal absorption of cholesterol have been conducted. Chisaka et al. (1988) showed that the in situ uptake of 14C-cholesterol in the lumen of the intestine was suppressed by EGCG. They also demonstrated that orally administered EGCG decreased cholesterol absorption in the rat intestine using the dual isotope ratio method. We have used thoracic duct lymph-cannulated rats to investigate the effect of catechin mixtures on the intestinal absorption of cholesterol (Ikeda et al., 1992). In this study, a green tea extract high in ECG and EGCG was more effective in reducing the lymphatic absorption of cholesterol than a green tea extract high in EC and EGC. Löest et al. (2002) also reported that intraduodenal infusion of green tea extract high in EGCG and EGC significantly lowered the lymphatic absorption of cholesterol and α-tocopherol dose-dependently in ovariectomized rats cannulated into the mesenteric lymph duct. Furthermore, we have compared green tea extract high in heat-epimerized CG and GCG with green tea extracts high in ECG and EGCG and evaluated their effect on cholesterol absorption in rats cannulated in the thoracic duct (Ikeda et al., 2003). Green tea extract high in CG and GCG was more effective than green tea extract high in ECG and EGCG in reducing cholesterol absorption. These results strongly suggest that green tea catechins including their C2 epimers, especially green tea catechins having a galloyl moiety, inhibit cholesterol absorption in the intestine.

Mechanisms of the inhibitory effect of green tea catechins on cholesterol absorption

Cholesterol absorption takes place mainly in the upper portion of the small intestine. Approximately 50% of cholesterol in the intestine is absorbed, and the remainder is excreted in feces (Idkbal and Hussain., 2009). Woollett et al. (2006) reported that the solubilization of cholesterol in bile salt micelles was essential for absorption in humans. Therefore, cholesterol must be incorporated into bile salt micelles prior to absorption.

We have examined the effects of green tea catechins on the micellar solubility of cholesterol (Ikeda et al., 1992). Two green tea extracts, one high in catechins with a galloyl moiety (ECG and EGCG) and the other high in free catechins (EC and EGC), were added to a bile salt micellar solution containing sodium taurocholate, egg yolk phosphatidylcholine (PC), and cholesterol. When green tea extracts were added to the bile salt micellar solution, the solution immediately turned turbid, and precipitates were observed (Fig. 2). The addition of green tea extracts to the bile salt micellar solution decreased the micellar solubility of cholesterol in a dose-dependent manner. Green tea extracts high in catechins having a galloyl moiety were more effective than those
high in free catechins in precipitating micellar cholesterol. When purified EC, EGC, ECG, and EGCG were used, catechins with a galloyl moiety (ECG and EGCG) were more effective than free catechins (EC and EGC) in precipitating micellar cholesterol. Furthermore, the amount of EGCG precipitated from the micellar solution was increased linearly with increasing EGCG levels, and it showed a near linear correlation with precipitated cholesterol (correlation coefficient = 0.99). Moreover, the concentration of bile acids in micelles was not affected by the addition of green tea extracts or EGCG. We believed that EGCG eliminated cholesterol from the micelles and was coprecipitated with cholesterol following its addition to the micelle solution. Raederstorff et al. (2003) also reported that purified EGCG decreased the micellar solubility of cholesterol in vitro. Furthermore, they used light scattering to show that the addition of EGCG to micelles altered the size of the micelles. We have investigated the effects of eight different types of purified catechins on the micellar solubility of cholesterol in vitro (Ikeda et al., 2003). In this study, CG and GCG were more effective than ECG and EGCG (parent catechins of CG and GCG, respectively) in precipitating micellar cholesterol. These observations indicated that heat-epimerized tea catechins might be more effective than green tea catechins in reducing cholesterol absorption.

Several studies have been conducted to understand the association between green tea catechins and cholesterol. Kajiya et al. (2001) reported the presence of a hydrophobic domain in catechins having a galloyl moiety, which is not present in free catechins. They also showed that the affinity of green tea catechins with a galloyl moiety is higher than that of free catechins for hydrophobic lipid bilayers. Therefore, green tea catechins having a galloyl moiety are thought to interact directly with cholesterol through their hydrophobic domain since cholesterol is also a hydrophobic molecule. However, no evidence is available on the direct interaction between catechins having a galloyl moiety and cholesterol. Kumazawa et al. (2004) provided experimental evidence by solid-state $^{31}$P and $^2$H nuclear magnetic resonance (NMR) spectroscopy that EGCG interacts with dimyristoylphosphatidylcholine in the lipid bilayer. Uekusa et al. (2007) also showed that ECG and EGCG interact with the surface of lipid membranes through the trimethylammonium group in PC using solution NMR techniques. PC is known as an important substance for the solubilization of cholesterol in bile salt micelles (Lichtenberg et al., 1990). These observations suggest that the micellar solubility of cholesterol may be affected by the interaction of catechins having a galloyl moiety with PC.

We have investigated the involvement of PC in the EGCG-mediated decrease in cholesterol micellar solubility. When EGCG, GCG, and EGC were added to a bile salt micellar solution containing sodium taurocholate, egg yolk PC, and cholesterol, EGCG and GCG eliminated not only cholesterol but also PC from bile salt micelles in a dose-dependent manner in vitro (Fig. 3) (Kobayashi et al., 2014). The concentrations of micellar cholesterol and PC following the addition of GCG were significantly lower than those following the addition of EGCG at concentrations of 2 and 3 mmol/L micelles. EGC slightly decreased the micellar solubility of cholesterol and PC in the study. Five types of bile salt micellar solutions containing 0.6 mmol/L PC, phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, or phosphatidylyserine were prepared, and the effects of EGCG on the micellar solubility of both cholesterol and a phospholipid were examined. When the bile salt micelles contained a phospholipid other than PC, both cholesterol and phospholipid were not eliminated following the addition of EGCG (Fig. 4). Moreover, when five vesicular solutions containing 0.6 mmol/L PC, phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, or phosphatidylserine were prepared followed by the addition of EGCG, similar results were observed as in the case of bile salt micelles. When EGCG was added to these vesicular solutions, only the PC vesicular solution immediately turned turbid. Moreover, EGCG effectively and exclusively eliminated PC from the vesicles. We also found that EGCG and GCG, but not EGC, effectively decreased the vesicular solubility of PC in a dose-dependent manner. These observations suggest that green tea catechins having a galloyl moiety can interact with PC, and the binding of EGCG to PC decreases the solubility of PC and cholesterol.

We have performed binding analyses using a surface plasmon resonance (SPR)-based biosensor assay (Kobayashi et al., 2014). We prepared homogeneous liposomal solutions containing PC (the ligand) or phosphatidic acid (the control ligand); green tea catechins would not interact with phosphatidic acid based on the
results shown in Fig. 4. The liposome was immobilized on a sensor chip surface, and the SPR-based biosensor assay was performed using the single-cycle kinetics method (Karlsson et al., 2006). This method involves the sequential injection of various concentrations of an analyte without any regeneration steps. Eight green tea catechin solutions of catechins having a galloyl moiety (5–80 mmol/L) and catechins without a galloyl moiety (50–800 mmol/L) were prepared in running buffer. To examine the association of the green tea catechins with PC or phosphatidic acid, the green tea catechin solutions were injected over the lipid surface. To dissociate the PC- or phosphatidic acid-green tea catechin complexes, green tea catechin solutions were replaced with running buffer. Then, the apparent equilibrium dissociation constants (K_D) of the interaction between the eight green tea catechins and PC were calculated. The apparent K_D values of the interaction between green tea catechins having a galloyl moiety and PC were lower than those of the interaction between green tea catechins without a galloyl moiety and PC (Table 1). These results indicated that the affinities of green tea catechins having a galloyl moiety were higher than those of free catechins for PC. The different affinities of green tea catechins having a galloyl moiety and PC were lower than those of the interaction between green tea catechins without a galloyl moiety and PC (Table 1). These results indicated that the affinities of green tea catechins having a galloyl moiety were higher than those of free catechins for PC. The different affinities of green tea catechins having a galloyl moiety and PC were lower than those of the interaction between green tea catechins without a galloyl moiety and PC (Table 1). These results indicated that the affinities of green tea catechins having a galloyl moiety were higher than those of free catechins for PC.
for PC between green tea catechins having a galloyl moiety and free catechins may be a reason why EGCG and GCG, but not EGC,
effectively decreased the micellar and vesicular solubility of PC.

These findings suggest that green tea catechins having a galloyl
moiety can interact with PC, and the binding of EGCG to PC
decreases PC solubility. In our preliminary study, when a bile salt
micellar solution containing 0.5 mmol/L cholesterol was prepared
by sonication in the absence of phospholipids, the amount of
cholesterol solubilized in the bile salt micelles was extremely low;
no more than 2.47% was solubilized, and the rest was precipitated.
These results suggest that PC is eliminated from bile salt micelles
through its interaction with green tea catechins having a galloyl
moiety, thus reducing the micellar solubility of cholesterol and,
precipitating cholesterol.

In order to investigate the interaction of EGCG with PC in bile
salt micelles, we performed \(^1\)H NMR measurements of EGCG in
the absence and presence of bile salt micelles (Kobayashi et al.,
2014). A bile salt micellar solution (in D\(_2\)O) containing 6.6 mmol/L
sodium taurocholate, 0.6 mmol/L PC, 0.5 mmol/L cholesterol,
132 mmol/L NaCl, and 15 mmol/L NaD\(_2\)PO\(_4\) (pD 6.8) was prepared
by sonication and stored at room temperature overnight. EGCG
was then added to the micellar solution. The interaction of EGCG
with the bile salt micelles resulted in distinct shifts in the A-ring
(6-H and 8-H), B-ring (2'-H and 6'-H), C-ring (2-H and 3-H), and
galloyl moiety (2''-H and 6''-H) signals of EGCG (data not shown).
These results indicated that the magnetic environment of
these protons in EGCG was altered by interactions with some
molecules in the bile salt micelles or a conformational change in

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**Table 1.** Apparent equilibrium dissociation constants of the binding
of eight green tea catechins to phosphatidylcholine.

<table>
<thead>
<tr>
<th>Catechin</th>
<th>Apparent K(_D) (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>1.535 × 10(^{-5})</td>
</tr>
<tr>
<td>GCG</td>
<td>2.485 × 10(^{-5})</td>
</tr>
<tr>
<td>ECG</td>
<td>2.888 × 10(^{-5})</td>
</tr>
<tr>
<td>CG</td>
<td>3.662 × 10(^{-5})</td>
</tr>
<tr>
<td>EGC</td>
<td>6.685 × 10(^{-4})</td>
</tr>
<tr>
<td>GC</td>
<td>9.488 × 10(^{-4})</td>
</tr>
<tr>
<td>EC</td>
<td>1.036 × 10(^{-4})</td>
</tr>
<tr>
<td>C</td>
<td>4.316 × 10(^{-4})</td>
</tr>
</tbody>
</table>

Eight green tea catechin solutions of catechins having a galloyl
moiety (5 – 80 mmol/L) and catechins without a galloyl moiety
(50 – 800 mmol/L) were prepared in running buffer. To associate
the green tea catechins with phosphatidylcholine (PC) or phosphatidic
acid, the green tea catechin solutions were injected over the lipid
surface. To dissociate the PC- or phosphatidic acid-green tea catechin
complexes, green tea catechin solutions were replaced with running
buffer. The sensorograms obtained from the injection of running buffer
were subtracted from the sensorograms obtained from the injection
of green tea catechin solutions. Then, the sensorogram obtained from
the reference flow cell, in which phosphatidic acid was immobilized, was
subtracted from the sensorogram obtained from the measurement flow
cell, in which PC was immobilized, to determine the apparent binding
responses. Then, the apparent equilibrium dissociation constants (K\(_D\))
of the binding of the eight green tea catechins to PC were calculated.

Reproduction of Table 1 in Kobayashi et al. 2014.
Green Tea Catechins and Cholesterol Absorption

EGCG. 4α-H and 4β-H signals were not observed because they overlapped large signals derived from several molecules in the bile salt micelles. These changes in the chemical shift following the addition of EGCG to the bile salt micelles were similar to those observed in a previous study involving isotropic bicelles, which are composed of PC and frequently used as a lipid bilayer model (Uekusa et al., 2007). The findings suggest that EGCG can interact with PC in bile salt micelles and lipid bilayers.

It remained unclear how EGCG interacts with PC in the bile salt micelles in the 1H NMR experiment because chemical shift changes were observed for the all of the assigned protons of EGCG. Therefore, we conducted nuclear Overhauser effect-correlated spectroscopy (NOESY) experiments on bile salt micelles containing cholesterol and PC/EGCG samples (Kobayashi et al., 2014). NOESY experiments are suitable for estimating both intermolecular and intramolecular proton distances of up to 5 Å (Lee et al., 1999). The preparation of bile salt micellar solutions (in D2O) and the addition of EGCG were performed as previously described. By increasing the mixing time, an NOE was observed. We found that a mixing time of 800 ms was optimal for observing the cross-peak in the bile salt micelle/EGCG sample. An intermolecular NOE was observed between the γ-H of PC and 2''-H and 6''-H on the galloyl moiety of EGCG when EGCG was added to the bile salt micelles containing cholesterol and PC (Fig. 5). The cation-π interaction between the trimethylammonium group of PC and the galloyl moiety of EGCG was inferred from this observation. Furthermore, several intramolecular cross-peaks in the EGCG molecule were observed between the protons on the B-ring and the protons on the galloyl moiety (data not shown). Uekusa et al. (2011) suggested that the B-ring and the plane of the ring in the galloyl moiety of EGCG or ECG may be stacked via the π-π interaction in lipid bilayers containing PC and that the cation-π interaction between the trimethylammonium group of PC and the galloyl moiety of EGCG or ECG may be enhanced by the π-π interaction between the B-ring and the plane of the ring in the galloyl moiety of EGCG or ECG. An intramolecular NOE between the protons on the B-ring and the protons on the galloyl moiety was also observed for bicelle/EGCG and ECG samples (Uekusa et al., 2011) as well as the micelle/EGCG samples in our study (Kobayashi et al., 2014). Therefore, there is a strong possibility that the intramolecular interactions in the EGCG molecule enhance the cation-π interaction between the trimethylammonium group of PC and the galloyl moiety of EGCG in bile salt micelles and lipid bilayers. Furthermore, we believe that the cation-π interaction between the trimethylammonium group of PC and the galloyl moiety of EGCG may have led to distinct shifts in not only the galloyl moiety (2''-H and 6''-H) signals, but also the A-ring (6-H and 8-H), B-ring (2’-H and 6’-H), and C-ring (2-H and 3-H) signals of EGCG in the 1H-NMR study. In contrast, an intramolecular NOE between cholesterol and EGCG was not observed. These observations support our hypothesis that EGCG specifically interacts with PC, but not cholesterol, in bile salt micelles.

We have investigated the localization of EGCG in bile salt micelles containing cholesterol and PC by the fluorescence quenching of fluorescent substances with EGCG (Kobayashi et al., 2014). A bile salt micellar solution containing 6.6 mmol/L sodium taurocholate, 0.6 mmol/L PC from egg yolk, 0.5 mmol/L cholesterol, 132 mmol/L NaCl, and 15 mmol/L NaH2PO4 (pH 6.8) with 1 mmol/L 2-(9-anthroyloxy) stearic acid (2-AS) or 12-(9-anthroyloxy) stearic acid (12-AS) was prepared. The micellar solutions were incubated with PBS containing various amounts of EGCG for 20 min at 20℃. The fluorescence of 2-AS or 12-AS in

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**Fig. 5.** NOESY spectra of epigallocatechin gallate and bile salt micelles containing cholesterol and phosphatidylcholine (800 ms mixing time at 300 K).

Overall view of the NOESY spectra. (B) Enlarged view of the NOESY spectra.

Reproduction of Fig. 8 in Kobayashi et al. 2014.
the bile salt micellar solution was measured. The anthroyl group of 2-AS should be located on the surface of the bile salt micelles, whereas that of 12-AS should be located in the hydrophobic core of the bile salt micelles. EGCG effectively quenched the fluorescence of 2-AS in a dose-dependent manner (data not shown). This observation suggests that EGCG is localized on the surface of bile salt micelles via its interaction with the trimethylammonium group of PC, which is a hydrophilic group.

The results from our previous study suggested that cholesterol is directly precipitated with EGCG (Ikeda et al., 1992). To investigate whether EGCG interacts with cholesterol, we have performed binding analyses using an SPR-based biosensor assay (Kobayashi et al., 2014). A phospholipid bilayer with or without cholesterol was immobilized on a sensor chip surface, and SPR-based binding analyses were performed. We found that EGCG did not bind to cholesterol. This result strongly suggests that EGCG does not directly interact with cholesterol in bile salt micelles.

The results from our study (Kobayashi et al., 2014) indicated that EGCG formed a complex with PC. Hénin et al. (2006) showed that cholesterol and dimyristoylphosphatidylcholine formed a complex with a 1:1 stoichiometry in a fully hydrated cholesterol-dimyristoylphosphatidylcholine bilayer through molecular dynamics simulations. Furthermore, in preliminary studies, we found that cholesterol and PC formed a complex in bile salt micelles and that the interaction between EGCG and PC leads to the formation of a complex containing EGCG, PC, and cholesterol. However, at present, we do not have direct experimental evidence to confirm that the interaction between EGCG and PC leads to the formation of a complex containing EGCG, PC and cholesterol.

In humans, the amount of phospholipids delivered via the biliary pathway to the intestinal lumen is higher than that of phospholipids of dietary origin (Cohn et al., 2010). The predominant phospholipid in the intestinal lumen is PC because almost all biliary phospholipids are PC. Therefore, the intestinal lumen would be a suitable environment for the inhibition of cholesterol absorption by EGCG because the interaction between green tea catechins having a galloyl moiety and PC is the key to eliminating cholesterol from bile salt micelles.

In another study, Ogawa et al. (2015) suggested that the interaction between EGCG and bile acids may decrease micellar cholesterol and PC solubility. They showed that the magnitude of the chemical shift changes of EGCG following the addition of EGCG to bile salt micelles containing cholesterol and PC was similar to the magnitude following the addition of EGCG to sodium taurocholate solution in $^1$H-NMR experiments. Moreover, the seven characteristic signals (3-H, 7-H, 12-H, 18-H, 19-H, 25-H, and 26-H) of sodium taurocholate were also shifted in the study. These observations suggest that EGCG may interact with sodium taurocholate. Ogawa et al. (2015) also conducted rotating-frame NOE spectroscopy (ROESY) experiments on sodium taurocholate/EGCG samples. ROESY is similar to NOESY and can detect signals if the proton distance is around 4.5 Å. Intermolecular correlations were found between the protons of the galloyl moiety of EGCG and the methyl groups (at positions 18 and 19) of sodium taurocholate when EGCG was added to sodium taurocholate solution. Therefore, Ogawa et al. (2015) proposed that EGCG interacted with sodium taurocholate rather than PC or cholesterol, and the elimination of PC and cholesterol from bile salt micelles was caused by the interaction between EGCG and sodium taurocholate. However, we found that when bile salt micelles contained a phospholipid other than PC, neither cholesterol nor the phospholipid was eliminated following the addition of EGCG (Fig. 4). If the interaction between EGCG and sodium taurocholate leads to the elimination of PC and cholesterol from bile salt micelles, phospholipids other than PC and cholesterol should also be precipitated. The cause of the discrepancies between the results of Kobayashi et al. (2014) and Ogawa et al. (2015) remains unclear. Hence, additional in-depth studies are necessary.

**Conclusion**

Green tea catechins, particularly those having a galloyl moiety, can decrease the micellar solubility of cholesterol (Ikeda et al., 1992, 2003; Kobayashi et al., 2014, Ogawa et al., 2015; Raederstorff et al., 2003). The limited solubility of cholesterol induced by green tea catechins with a galloyl moiety can be a major cause of the inhibition of cholesterol absorption (Ikeda et al., 1992, 2003). However, the mechanism of the associations between catechins having a galloyl moiety and cholesterol, PC, and bile acids is not completely understood. Hence, additional in-depth studies are necessary to elucidate how green tea catechins having a galloyl moiety cause the precipitation of cholesterol from bile salt micelles.

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**References**


