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Effects of Tea Polyphenols on Emulsification of Olive Oil in a Small Intestine Model System

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Tea catechins have been shown to reduce plasma cholesterol and suppress hypertriacylglycerolemia by reducing triglyceride absorption. However, the mechanism is not yet clear. One of the possible mechanisms is that tea polyphenols may modify dietary fat emulsification in the gastrointestinal tract. The digestive enzyme (lipase) acts on specific emulsion interface properties (droplet size and surface area). Therefore, changes in these properties may modify emulsification and lead to changes in dietary fat digestion and absorption. In this study, the effect of both green and black tea on the changes of emulsification was examined by measuring the droplet size and the surface area. A model emulsion system containing olive oil, phosphatidylcholine (PC), and bile salt was developed to simulate small intestinal conditions. Initial changes in droplet size (from 1.4 to 52.8 μm and from 1.4 to 25.9 μm) of the emulsion were observed in the presence of 1.04 mg/mL and 0.10 mg/mL of total catechins prepared from green and black tea, respectively. Both teas caused similar changes on the emulsion properties; however, black tea was more effective than green tea. The underlying mechanisms of actions of tea polyphenols are discussed.

KEYWORDS: Olive oil; emulsion; tea; droplet size; surface area; catechin; caffeine; small intestine

INTRODUCTION

The protective effect of tea against cancers (*1*−*3*), cardiovascular disease (*4*, *5*), and other chronic diseases (*6*, *7*) has been studied extensively by many researchers. Mainly catechins and other polyphenols are suggested to be involved in these protective effects. In addition to those health benefits, tea has been reported to possess hypochlesterolemic effect (*8*, *9*), reduce triglyceride (TG) level, and prevent oxidation of low-density lipoprotein (*10*). Reduction in TG and cholesterol levels in blood plasma is an important effect as high TG and cholesterol levels are considered as the risk factors of cardiovascular disease. Several animal studies (*11*, *12*) have reported that green tea intake decreased the absorption of TG and cholesterol. In rats fed green tea catechins, a marked increase in fecal total lipids and cholesterol was observed as compared with the control group (*11*). Hamsters drinking green tea or green tea polyphenols also had higher fecal fat excretion than the control group (*12*). One study (*13*) reported that regular ingestion of green tea catechins decreased plasma total cholesterol and blood TG levels in human, and other animal studies (*14*, *15*) showed reduction in TG by regular ingestion of both green and black tea. Some studies have shown effects of tea on plasma, but the effects differ among studies (*16*, *17*). Nonetheless an increasing number of studies showed the effect of tea on reducing fat absorption and lowering TG level. Most of these findings are in accordance with the fact that fat excretion increases and TG level decreases in vivo or animal studies. There is still limited information on the mechanism of how tea may reduce fat absorption, and the mechanism remains yet to be determined.

One of the possible mechanisms is that tea polyphenols may modify emulsion properties of dietary fat in the gastrointestinal tract, consequently leading to changes in fat digestion and absorption. The characteristic of fat digestive enzyme (lipase) is its specificity to act on a specific emulsion interface. The emulsion interface properties, namely, droplet size and specific surface area, govern the activity of lipase on dietary fat emulsions (*18*). From detailed studies of lipid digestion and absorption (*19*, *20*), it is described that the droplets in the human small intestine are fine and very small in the range of 0.5−1.2 μm. Other recent study suggested (*18*) that the droplet size in the small intestine could be in the range of 0.5−12 μm depending on the initial emulsion droplet size formed in the stomach prior to entering the small intestine. Therefore, changes in the emulsion droplet size and surface area might have an important role in modifying fat digestion and absorption.

It is known that dietary fat digestion undergoes several complicated processes prior to absorption by the mucosa of the small intestine. Following ingestion of lipids, a crude emulsion is formed by mastication and subsequently travels to the stomach. After being held within the stomach for a few hours, the emulsion enters the duodenum where it is exposed to the surface-active components of bile, and further emulsification occurs (*21*). The majority of lipolysis is carried out in the duodenum by pancreatic lipase. The lipolysis products are then transferred into mixed micelles and are absorbed by the

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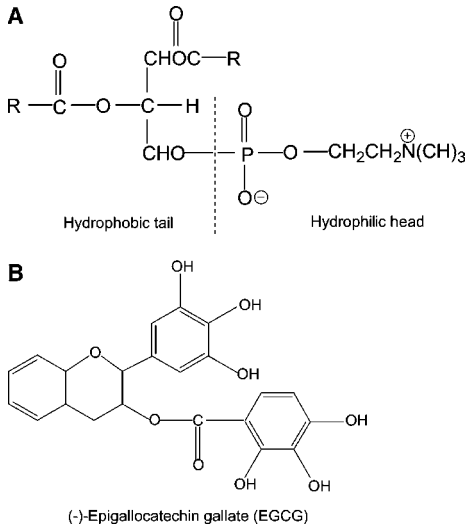

Figure 1. (**A**) Chemical structure of phosphatidylcholine (PC), where R = linoleic acid, linolenic acid, monounsaturated fatty acids, and saturated fatty acids. (**B**) Chemical structure of EGCG.

enterocytes. Any modulation of this complicated digestion steps may change fat digestion or absorption. The focus of the present study was on the effect of tea on the changes of dietary fat emulsification since emulsification is one of the first steps in digestion and is also a fundamental step creating a lipid−water interface essential for lipolysis.

In this study, to gain understanding of the mechanism, a simple oil in water emulsion was prepared mimicking the physiological conditions in the small intestine in the human body. The effect of tea, individual pure catechins, and caffeine on olive oil emulsion was examined by measuring the physiochemical properties (droplet size and specific surface area) of the emulsions.

MATERIALS AND METHODS

Chemicals. Olive oil (highly refined, low acidity, 77.3% oleic acid, 9.8% palmitic acid, 7.1% linoleic acid) was bought from Sigma-Aldrich, Poole, U.K. and used without further purification. Black teas (leaf teas; PG-Tips and English Breakfast) were bought from a local supermarket in Leeds, U.K., and green teas (leaf teas; Sayama-cha and Yabukita-cha) were bought from a local supermarket in Tokyo, Japan.

The bile salt sodium taurocholate (NaT) was also obtained from Sigma-Aldrich, Poole, U.K. at 98% purity and was used without further purification. Pure catechins (above 95% purity) (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (+)-catechin, (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), and caffeine (CF) were bought from the same source. Phosphatidylcholine (EPIKURON 200, 92.7% purity) from soya bean was obtained from Degussa Texturant Systems U.K. Ltd. All chemicals were of analytical grade. The chemical structures of EGCG and phosphatidylcholine are shown in **Figure 1**.

Preparation of Green and Black Tea Freeze-Dried Powder (GFD and BFD). Both green and black tea extracts (5% w/v) were prepared in boiling water by immersing in water for 10 min with constant stirring. The extracts were strained using a strainer and freeze-dried (500 mm of Hg, −20 °C). The freeze-dried powders were reconstituted in water and analyzed using RP-HPLC for catechins and caffeine composition by the method of Khokhar and Magnusdottir (*22*).

Preparation of Olive Oil Emulsion. For all studies, a standard olive oil emulsion preparation was used: 20% v/v olive oil, 4% phosphatidylcholine (PC) in 2 mM Tris/HCl buffer (pH 7.5). The PC was dispersed in the buffer by constant agitation before adding olive oil. PC was chosen in this study as one of the emulsifiers because the principle emulsifiers in the digestive system are thought to be phospholipids either intrinsic to the diet or supplied by digestive juice (*21*). In addition, PC is one of the most frequently used natural emulsifiers in the food industry. The 20% v/v standard emulsion was achieved by using high-pressure jet homogenizer at 300 bar. The stability of the emulsion to coalescence was monitored by Malvern Mastersizer 2000 (Malvern Instruments, Malvern, U.K.). To prepare the final emulsion, 20% v/v standard olive oil emulsion prepared as above was diluted with NaT stock solution containing sodium chloride and calcium chloride to give the final composition: 8 mM NaT, 1% olive oil, 2.3 mM PC, 10 mM $CaCl_2$, 150 mM NaCl, and 2 mM Tris/HCl buffer (pH 7.5).

A known amount of GFD, BFD (prepared as above), individual pure catechins, and caffeine was dissolved in 3 mL water and added to 20 mL of the final emulsion prepared as above. After addition of the tea components, the pH of the sample mixture was readjusted to 7.5 with $NaHCO_3$, if necessary. The pH was adjusted to 7.5 because in a healthy human body, pancreatic juice containing alkaline solution (sodium bicarbonate ($NaHCO_3$)) flows into the small intestine in order to maintain neutral pH. Distilled water (3 mL) was added to the emulsion and was considered as a control. The mean droplet size, size distribution, and specific surface area were measured immediately after preparing final samples and also after incubating at 37 °C with constant shaking (100 strokes/min) for 3 and 6 h.

Measurement of Distribution of Catechins in the Water and the Lipid Phase. The distribution of catechins and caffeine between the emulsified droplets and the water phase was determined by the depletion method. A single centrifugation step to separate the dispersed and oil phase of emulsions which is frequently used to determine the level of adsorbed protein to the emulsion droplets was applied (*23*).

Five milliliters of the final sample mixture was centrifuged in a Beckman centrifuge (JA-21 rotor, 36 300*g*, 30 min, 4 °C). The resulting water phase was analyzed for free catechins by HPLC to obtain the amount of free catechins present in the water phase. The catechin content in the lipid phase was calculated by subtracting the amount of free catechins in the water phase from the total amount of catechins added to the emulsion. Since catechins are known to be unstable at alkaline condition, the degradation of catechins at pH 7.5 was taken into account when calculation was carried out. The recovery rate of catechins was determined by incubating tea solutions at pH 7.5, 37 °C for 3 h. The catechin content in the lipid phase was calculated as follows; Catechin content in lipid phase = (initial amount) × (recovery rate at pH 7.5) − (free catechin in the water phase after centrifugation).

Five milliliters of emulsion (containing no tea) was centrifuged, the oil phase was carefully collected, and then resuspended in buffer to make up the final volume to 5 mL. The droplet size of this solution was measured using a Mastersizer. The results showed that there was no change in the droplet size distribution due to centrifugation. It was clear that the centrifuge condition used in this study did not induce breaking of the emulsions.

Particle Size Analysis. The droplet size distribution and specific surface area of the emulsions were determined using a Malvern Mastersizer 2000 (the refractive indices for the droplets and dispersant are 1.46 and 1.33, respectively). The particle size was expressed as the volume weighed mean d_{43} diameter, $d_{43} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3$, where n_i is the number of droplets of diameter d_i.

Viscosity Measurements. Rheological testing was carried out after 3 h of incubation of the emulsions with and without the presence of tea extracts, individual pure catechins, caffeine, and a mixture of pure tea compounds. Large-deformation steady-state shear rheology was performed at 37 °C with a Bohlin CS50 rheometer, using a C14 concentric cylinder measuring cell (inner diameter 14 mm, sample vol 2 mL). The sample was covered with a thin layer of silicone oil to prevent evaporation, and then allowed to equilibrate at 37 °C for 10 min, prior to viscosity measurement at a shear rate of 5 per s.

Confocal Micrograph Images. Confocal laser scanning micrographs were obtained using a Leica TCS SP2 confocal microscope.

Statistics. All experiments were performed 4−6 times, and the results are expressed as means ± standard deviation (SD). Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Level of Catechins and Caffeine in Freeze-Dried Powder. As expected, GFD (freeze-dried powder of green tea) contained

Table 1. Levels of Catechins and Caffeine in Freeze-Dried Powders (mg/g Powder)

	CF	EGC	C	EC	EGCG	ECG	total catechins
GFD	43	132	4	19	85	14	257
BFD	39	28	5	4	15	9	61

Table 2. Average Droplet Size and Specific Surface Area of Olive Oil Emulsion with and without GTEF[a]

level of total catechins (mg/mL)	incubation time (h) 0	3	6	viscosity (mPa)
0 (control)	1.36 ± 0.10 8.58 ± 0.66	1.44 ± 0.15 8.12 ± 0.74	1.39 ± 0.12 8.33 ± 0.75	3.1 ± 0.1
0.22	1.38 ± 0.24 8.42 ± 0.34	1.43 ± 0.33 8.36 ± 0.34	1.42 ± 0.12 8.37 ± 0.24	3.2 ± 0.2
0.52	1.61 ± 0.08 8.05 ± 0.48	1.45 ± 0.09 7.52 ± 0.75[b]	1.41 ± 0.20 6.62 ± 1.69[b]	3.1 ± 0.1
0.65	1.54 ± 0.10 5.90 ± 0.77[b]	1.48 ± 0.12 5.50 ± 0.36[b]	1.63 ± 0.05 4.50 ± 0.05[b]	3.0 ± 0.1
0.78[b]	2.44 ± 0.64 3.94 ± 0.32	2.48 ± 0.53 3.79 ± 0.17	2.81 ± 0.65 3.38 ± 0.19	3.2 ± 0.1
0.91[b]	2.52 ± 0.79 4.04 ± 0.72	4.49 ± 2.75 3.16 ± 0.44	22.43 ± 9.21 2.48 ± 0.41	3.1 ± 0.2
1.04[b]	50.04 ± 2.22 0.85 ± 0.09	46.30 ± 11.82 0.69 ± 0.07	52.79 ± 2.80 0.62 ± 0.12	3.1 ± 0.1

[a] The upper and lower values in the columns are droplet size (μm) and specific surface area (m^2/g oil), respectively. The values are expressed as the mean ± SD of 4–6 determinations. [b] Significantly different from the control. Both droplet size and specific surface area were significantly different from the control for 0.78, 0.91, and 1.04 mg/mL at all incubation times.

significantly ($p < 0.01$) higher levels of catechins (257 mg total catechins/g powder) than that of BFD (61 mg total catechins/g powder) but similar levels of caffeine (43 and 39 mg/g for green and black tea, respectively). The details of individual catechins and caffeine content are shown in **Table 1**. Freeze-dried powder (0.5 g) was reconstituted in 10 mL of distilled water. This solution was further diluted to obtain various concentrations of total catechins. These tea extracts prepared from freeze-dried powders were abbreviated as GTEF and BTEF for green and black tea, respectively.

Effect of Tea on Physicochemical Properties of the Emulsion. *1. Green Tea Extract (GTEF).* The average droplet size and specific surface area of the emulsion in the absence of tea components (control) were 1.36 ± 0.10 μm and 8.58 ± 0.66 m^2/g oil, respectively. There were no changes in the droplet size and specific surface area up to 12 h incubation period (measurements were carried out at every 3 h). Thus it was concluded that the control emulsion was stable at least for 12 h. In the presence of the GTEF, droplet size increased from 1.36 ± 0.10 to 52.79 ± 2.80 μm and specific surface area decreased from 8.58 ± 0.66 to 0.62 ± 0.12 m^2/g oil with increasing concentration of GTEF (0.00 to 1.04 mg total catechins/mL) (**Table 2**). It is, of course, natural that the specific surface area decreases with increase in droplet size because part of the surface area of the droplets is lost when two or more droplets coalescence together to form a larger droplet. A slightly wide range of SD values was observed when high concentrations of tea components were present. This may be due to droplets forming aggregates or coalescencing to create a less-uniform system (a mixture of coalescencing and noncoalescencing droplets in the presence of a high level of catechins). At concentrations below 0.65 mg/mL, there was a trend that specific surface area decreased slightly with increase in catechin concentration, even though there was no significant difference in the droplet size among the concentrations. This was due to the fact that although the average droplet size exhibited no significant change, a slight shift in droplet distribution occurred (**Figure 2**) resulting in a decrease in specific surface area. There was a significant ($p < 0.05$) increase in droplet size (2.44−52.79 μm), decrease in specific surface area (3.94−0.62 m^2/g oil), and change in droplet size distribution when the concentration of total catechins was increased from 0.78 to 1.04 mg/mL (**Table 2** and **Figure 2**). In addition to changes in droplet size, size distribution, and specific surface area, visual changes (creaming and phase separation) were observed at a concentration of 0.91 mg/mL and above. Further, from confocal micrographs (**Figure 3A**,**B**), it was clear that emulsion had undergone coalescence at a concentration of 0.91 mg/mL total catechins.

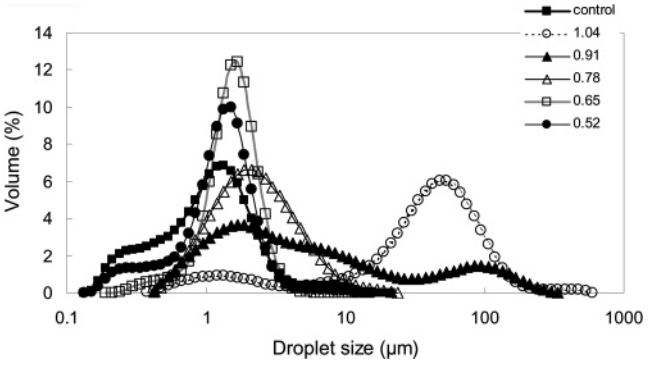

Figure 2. Changes in emulsion droplet size distribution in the presence of different levels of catechins from GTEF. The distributions are the average of 4–6 determinations, after incubation for 6 h. The symbols refer to different levels of catechins: ■, control; ●, 0.52 mg/mL; □, 0.65 mg/mL; △, 0.78 mg/mL; ▲, 0.91 mg/mL; ○, 1.04 mg/mL.

2. Black Tea Extract (BTEF). In the presence of BTEF, similar results were obtained as with GTEF, but the effects were much greater. The droplet size increased considerably to 25.89 ± 1.91 μm and the specific surface area was decreased to 1.85 ± 0.14 m^2/g oil at a concentration of 0.1 mg/mL (**Table 3**). This concentration of catechins was nearly 10 times lower than that required to produce a similar effect from GTEF (1.04 mg/mL). A significant ($p < 0.01$) decrease in specific surface area (4.50 m^2/g oil) was observed at the lowest concentration tested (0.033 mg/mL) from BTEF.

Because there were no significant differences in droplet size and specific surface area and did not show any particular trend for the longer incubation times for both GTEF and BTEF, it is suggested that the interaction between tea catechins and the emulsion was relatively quick and that further incubation had only a small effect.

Black tea is known to contain lower amounts of catechins than green tea due to its fermentation process. The total content of catechins in BFD (61 mg/g FD) used in this experiment was one-quarter of that found in GFD (257 mg/g FD) (**Table 1**). Taking this into account, it is clear that the black tea had a greater effect than green tea on the emulsion. This suggests that catechins may not be the only compounds affecting the marked changes in the droplet size and specific surface area of the olive oil emulsion.

3. Individual Tea Components (EGC, EC, EGCG, ECG, and Caffeine). Individual pure catechins, caffeine, and a mixture of four catechins and caffeine were added in a solution to the

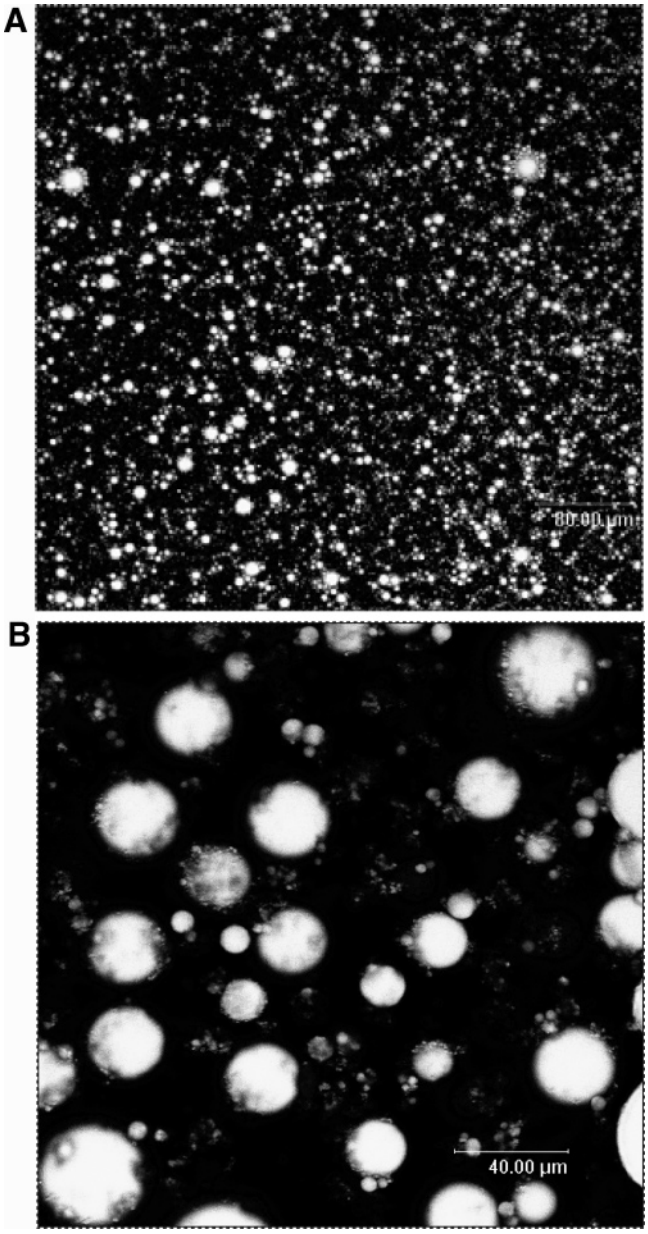

Figure 3. (**A**) Control emulsion (in the absence of tea components). (**B**) Coalesced emulsion (in the presence of 0.9 mg/mL total catechins from GTEF).

emulsion in the same manner as BTEF and GTEF to investigate the contribution of each compound independently and in a mixture. The highest concentration of individual catechins (EGC 0.33 mg/mL, EC 0.09 mg/mL, EGCG 0.35 mg/mL, ECG 0.1 mg/mL) and caffeine (0.35 mg/mL) in the emulsion were equivalent to GTEF (0.91 mg/mL of total catechins) which showed a significant change in the emulsion properties. The composition of the mixture of pure catechins and caffeine is shown in **Table 4**.

The interaction of pure compounds with the emulsion was also relatively quick, and longer incubation times had a small effect. In the presence of 0.35 mg/mL EGCG, the droplet size was 12.47 ± 4.23, 19.94 ± 3.42, 20.03 ± 5.40 μm at 0, 3, and 6 h of incubation, respectively. No significant difference ($p = 0.08$) in droplet size was observed between incubation times of 0 and 6 h. Thus, measurements of droplet size, distribution, and surface area were taken immediately after adding the catechin solutions to the emulsions and after 3 h of incubation. A significant decrease in specific surface area (from 7.92 to 3.04 m^2/g oil) and increase in droplet size (from 1.52 to 16.20 μm) were observed with increasing concentrations (0.13−0.35 mg/mL) of EGCG. When individual catechins were compared, EGCG caused changes in the droplet size and specific surface area, while EC, EGC, ECG, and CF showed no effect. ECG may also have a potential to affect the emulsion properties as there was a slight decrease in specific surface area in the presence of 0.1 mg/mL ECG (**Figure 4**). Because of the low solubility of ECG in water, it was not possible to examine the effects of higher concentrations. However, this concentration was higher than the levels contained in GTEF used in this study. Taking into account the low solubility of ECG in water and

Table 3. Average Droplet Size and Specific Surface Area of Olive Oil Emulsion with and without BTEF[a]

level of total catechins (mg/mL)	incubation time (h) 0	3	6	viscosity (mPa)
0 (control)	1.36 ± 0.10 8.58 ± 0.66	1.44 ± 0.15 8.12 ± 0.74	1.39 ± 0.12 8.33 ± 0.75	3.1 ± 0.1
0.033	1.62 ± 0.03 4.50 ± 0.01[b]	1.71 ± 0.15 4.65 ± 0.30[b]	1.66 ± 0.01 4.33 ± 0.04[b]	3.2 ± 0.1
0.07[b]	7.78 ± 3.41 3.21 ± 0.38	8.32 ± 2.91 3.11 ± 0.01	7.13 ± 1.19 3.32 ± 0.13	3.0 ± 0.0
0.10[b]	13.93 ± 2.20 2.83 ± 0.05	25.48 ± 0.44 1.89 ± 0.01	25.89 ± 1.91 1.85 ± 0.14	3.1 ± 0.1

[a] The upper and lower values in the columns are droplet size (μm) and specific surface area (m^2/g oil), respectively. The values are expressed as the mean ± SD of 4–6 determinations. [b] Significantly different from the control. Both droplet size and specific surface area were significantly different from the control for 0.07 and 0.10 mg/mL at all incubation times.

Table 4. Composition of the Mixtures Containing Pure Catechins and Caffeine (mg/mL)

	mixture 1 (0.16 mg/mL)	mixture 2 (0.33 mg/mL)	mixture 3 (0.65 mg/mL)
CF	0.035	0.07	0.13
EGC	0.08	0.15	0.3
EC	0.02	0.03	0.05
EGCG	0.07	0.13	0.26
ECG	0.01	0.02	0.04

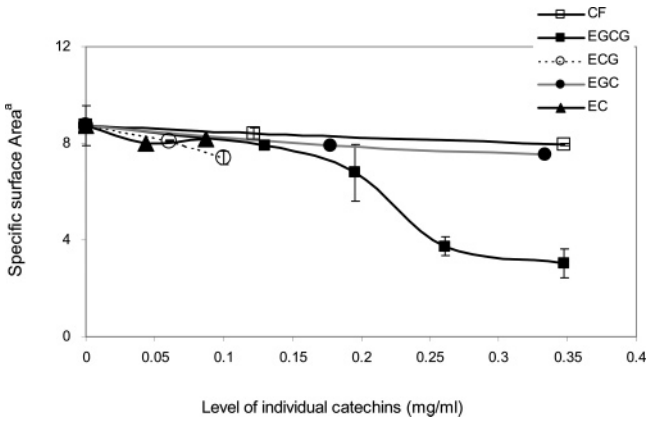

Figure 4. Effects of individual catechins and caffeine on specific surface area. The measurements are the average of three determinations. The symbols refer to individual tea compounds: □, CF; ■, EGCG; ○, ECG; ●, EGC; ▲, EC. The error bars represent the SD from three different determinations; "a" denotes that the specific surface area is expressed as (m^2/g oil).

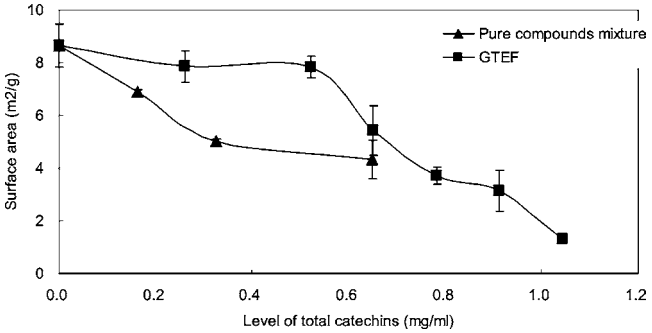

Figure 5. Effects on specific surface area; a comparison between a mixture of pure compounds and GTEF. Data are given on the basis of an average of 4–6 determinations for GTEF and 3 determinations for pure compounds mixture.

estimated daily consumption of tea, the possible concentration of ECG in the small intestine from tea beverage will be lower than 0.1 mg/mL. Higher concentrations of ECG will, therefore, not be relevant to any in vivo effects.

When three mixtures 1−3 consisting of four catechins and caffeine (representing the composition of GTEF) were added to the emulsion, similar but greater changes were observed as compared to those of GTEF (**Figure 5**). This might be due to slightly higher level of individual EGCG contained in the mixtures compared to the levels in GTEF. Another possibility is that there might be synergistic effects between catechins. When mixture 3 containing similar levels of EGCG was compared with individual pure EGCG (0.26 mg/mL), changes in specific surface area (4.33 m^2/g oil) and droplet size (2.28 μm) were, however, smaller than that of pure EGCG (droplet size, 5.00 μm and specific surface area, 3.74 m^2/g oil).

Overall, the results suggested that the main compounds responsible for affecting physicochemical properties of the emulsions are EGCG, abundant in green tea, and/or polymeric forms of catechins present in black tea.

Incorporation of Tea Catechins in the Lipid Phase. After incubating the emulsion mixture for 3 h, the levels of free catechins in the water phase were measured by HPLC as described in Materials and Methods. The difference between the initial amount of catechins present in the emulsion and the amount in the water phase after incubation was considered as the amount of catechin incorporated into the lipid phase. The recovery rates of individual catechins at pH 7.5 (37 °C) from tea extracts were 12.2%, 81.8%, 30.5%, and 69.2% for EGC, EC, EGCG, and ECG, respectively. The losses at pH 7.5 were taken into account for calculations. The calculation method is described under Materials and Methods. The level of catechins in the lipid phase increased significantly ($p < 0.01$) from 0.4 to 1.0 mg with increase in catechins added to the emulsion (from 0.22 to 0.78 mg/mL). There was no further increase in catechins in the lipid phase at further high concentration of catechins (**Figure 6A**). In the presence of 0.78 and 0.91 mg/mL total catechins from GTEF, similar amounts of total catechins (0.97 and 1.03 mg, respectively) were found in the lipid phase. Among the individual tea compounds, the recovery of caffeine and EC in water phase was 100% regardless of the concentrations. The major compound found in the lipid phase was EGCG, while a small amount of EGC and ECG was also present in the lipid phase (**Figure 6B**). These results were consistent with the results of the effects of the individual tea compounds on physicochemical properties of the emulsion except EGC. EGCG significantly affected the emulsion droplet size and specific surface area, while ECG showed a trend of a decrease in specific surface

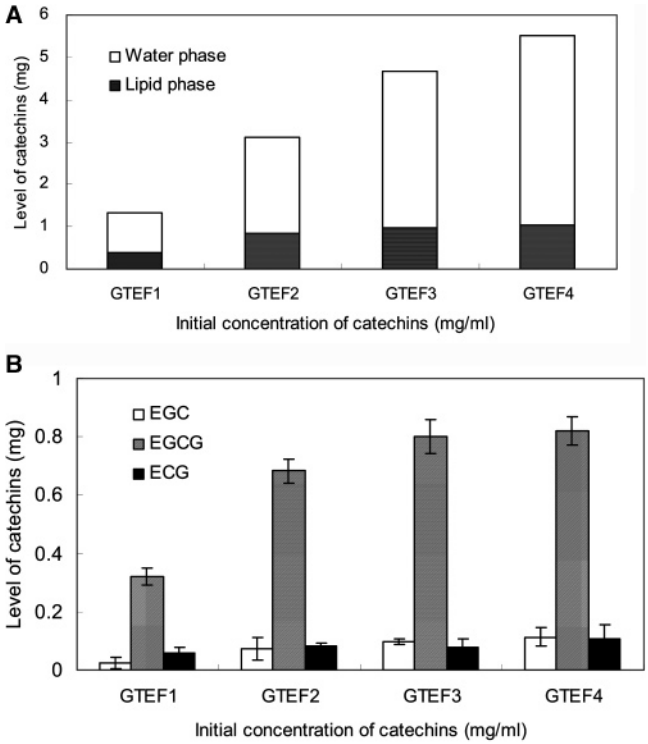

Figure 6. (**A**) Distribution of catechins between the emulsion water phase and the lipid phase: GTEF1, 0.22 mg/mL; GTEF2, 0.52 mg/mL; GTEF3, 0.78 mg/mL; GTEF4, 0.91 mg/mL. (**B**) Profile of individual catechins and levels in the lipid phase: GTEF1, 0.22 mg/mL; GTEF2, 0.52 mg/mL; GTEF3, 0.78 mg/mL; GTEF4, 0.91 mg/mL.

area. It is not clear, however, what the effect of EGC is on the emulsion. There was a possibility that the level of catechins in the lipid phase could be over- or underestimated as catechins stability in the emulsion could be different from that in tea extracts. Nonetheless, the results obtained in this study suggested that some catechins may be incorporated into the lipid phase of the emulsion.

DISCUSSION

One of the purposes of this study was to examine whether tea can affect the physicochemical properties (droplet size and specific surface area) of the dietary fat emulsion and to what extent. Identifying the main factors affecting these properties was another objective of this study. The present study clearly showed that both green and black teas had an effect on the physicochemical properties of olive oil emulsions (emulsified with PC and NaT) in the small intestine model system. Addition of tea solutions to the emulsions increased the mean droplet size and changed the droplet size distribution of the emulsions and, consequently, decreased the specific surface area and led to coalescence in the presence of high levels of catechins. Juhel et al. (*24*) also found that in the presence of green tea extract AR25, only 3% oil was emulsified in gastric and duodenal media in vitro. Our results suggested that EGCG and black tea polyphenols may be mainly responsible for the changes in emulsion properties. The possible mechanisms of actions of tea polyphenols on lipid emulsification are discussed below.

Possible Mechanisms Involved in Modification of Lipid Emulsification. Several factors could lead to changes in lipid emulsification. The possible mechanisms involved are likely to be one of the following mechanisms or the combination of the two: (1) interaction between tea polyphenols (catechins and/or

their polymeric forms) and PC; (2) incorporation of tea polyphenols (EGCG and the polymeric forms) within the lipid (emulsifier) layer.

1. Interaction between Tea Polyphenols (Catechins and/or Their Polymeric Forms) and PC. Catechins and some of their polymer forms present in black tea may interact with PC and form PC−catechin/PC−polymer complexes. Because PC possesses a hydrophilic head (see **Figure 1A**) at the exterior of the emulsion droplets, this may complex with the hydroxyl groups of catechins and some of their polymeric forms. It was proposed that the flavonoid's hydroxyl group may interact with the polar headgroup of a biological membrane which is composed of phospholipids and thus increase their local concentration at the water−lipid interface (*25*, *26*). The formation of hydrogen bonds between hydroxyl groups in the flavonoids and the polar headgroups of lipids can be particularly relevant for the interaction with the water−lipid interface (*25*). It is possible that these complexes form cross-links between neighboring droplets. This linkage may be followed by coalescence to larger droplets at high concentrations of tea. It has been reported that tea polyphenols can interact with some digestive enzymes (proteins) and form a complex (*24*). When enough polyphenols interact with protein, polyphenol can, then, act as a linker between other polyphenol−protein complexes to form further complex aggregates (*27*). This may occur between tea polyphenols and PC. Larger molecules with multiple binding sites would be expected to be more effective at cross-linking than small ones, overcoming any electrostatic repulsion between droplets, which tends to stabilize the emulsions. This might explain why black tea was more effective than green tea, despite the lower amount of catechins in black tea. Black tea contains higher amount of thearubigins and theaflavins (30−59%), which are oxidized polymeric forms of catechins, typically with a molecular weight in a range of 700−10 000.

Although the emulsion interface is stabilized by two emulsifiers, PC and bile, it is unlikely that tea catechins bind or interact with bile salts, because there is no possibility of electrostatic attraction between same charges. Ikeda et al. (*28*) has also reported that the bile salts concentration in the micelles remained unchanged on the addition of either catechin mixtures or purified catechins and suggested no interaction between catechins and bile salts occurred when bile salts were present within the physiological range.

2. Incorporation of Tea Polyphenols (EGCG and/or the Polymeric Forms) within the Lipid Layer. It is possible that tea catechins/polymeric forms may be incorporated into the PC lipid (emulsifier) layer. Incorporation in the lipid phase may lead to change in the physicochemical properties of the emulsion. Previous studies (*28*, *29*) showed that catechins were incorporated into the lipid bilayer of liposome and altered the membrane structure. It has also been reported that the affinity of each catechin for the lipid bilayer is mainly reflected by its hydrophobicity; for instance, EC and EGC have lower affinities than the corresponding gallic acid esters, namely EGCG and ECG (*30*). Similar incorporation of EGCG and other polymeric forms of catechins into PC-stabilized dietary lipid emulsion might also occur in the small intestine. The results from the present study (**Figure 6B**) showed that the main compound found in the lipid phase of the emulsion was EGCG with a small amount of ECG thus incorporating these catechins within the lipid phase.

Changes in the viscosity of the medium are a well-known factor that could affect the emulsion properties. Viscous soluble fibers, especially medium- to high-viscous fibers from guar gum, for example, were reported (*31*) to reduce emulsification of dietary lipids in the stomach and duodenum and subsequently lipolysis via a viscosity-mediated mechanism. It is reasonable to suppose that addition of tea may change the viscosity and lead to coalescence. This is, however, unlikely to be the mechanism in the presence of tea as there was no change in the apparent viscosity when tea or individual catechins were added (**Tables 2**, **3**, and **5**). The lack of any change in the viscosity also suggests that there was little extensive flocculation of droplets on addition of tea components. Rather, if instability occurred, this was manifested as coalescence (larger droplet size).

Table 5. Effects of Individual Tea Compounds on Physicochemical Properties of Olive Oil Emulsion[a]

	level of compounds (mg/mL)	droplet size (μm)	specific surface area (m^2/g oil)	viscosity (mPa)
CF	0.12	1.48 ± 0.08	8.42 ± 0.22	3.1 ± 0.1
	0.35	1.42 ± 0.01	7.94 ± 0.05	3.1 ± 0.2
EGC	0.18	1.52 ± 0.02	7.92 ± 0.09	2.9 ± 0.1
	0.33	1.48 ± 0.02	7.54 ± 0.15	3.1 ± 0.1
EC	0.04	1.41 ± 0.01	8.00 ± 0.01	3.0 ± 0.0
	0.09	1.49 ± 0.11	8.19 ± 0.04	3.1 ± 0.2
EGCG	0.13	1.52 ± 0.10	7.92 ± 0.03	3.0 ± 0.2
	0.20	1.55 ± 0.10	6.79 ± 0.17	3.2 ± 0.1
	0.26	5.00 ± 1.07[b]	3.74 ± 0.38[b]	3.0 ± 0.1
	0.35	16.20 ± 7.15[b]	3.04 ± 0.58[b]	3.1 ± 0.2
ECG	0.06	1.39 ± 0.01	8.10 ± 0.01	3.1 ± 0.2
	0.10	1.49 ± 0.05	7.61 ± 0.05	3.0 ± 0.1
mixture[c]	0.16	1.62 ± 0.02	6.89 ± 0.20	3.0 ± 0.1
	0.33	1.52 ± 0.01	5.02 ± 0.10[b]	3.1 ± 0.2
	0.65	2.28 ± 0.84[b]	4.33 ± 0.72[b]	3.1 ± 0.1

[a] The values are expressed as the mean ± SD of three determinations. [b] Significantly different from the control. [c] Mixture is composed of pure catechins and caffeine compounds mixture which represent the composition of tea extract. (The level of compounds is expressed as the total sum of pure catechins.)

Possible in Vivo Effect on Lipid Emulsification by Tea Consumption. One key issue is how far these results may be extrapolated to alter lipid emulsification in vivo. Fat emulsification is one important step among several stages in fat digestion. Several studies (*22*, *32*) have reported the levels of total catechins in a cup of tea. The level is reported to vary substantially depending on the type of tea, the agronomic condition of the tea leaf, individual preference (strength), and so on (*33*). It is estimated that the range of total catechins contained could be between 160−300 and 20−80 mg per cup (200 mL) for green tea and black tea, respectively. Imai et al. (*34*) reported from their epidemiology study in Japan that 50% of the population consumes 4−9 cups (180 mL) per day and 15% consumed more than 10 cups per day. Ten cups of green tea was estimated to be equivalent to 300−400 mg of EGCG and more than 1000 mg of total catechins per day. The latter level of consumption was found to give a negative association between tea consumption and cancer incidence. Therefore, it seems reasonable to assume that the daily intake of catechin could be as high as 1200−1500 mg per day from green tea in some Asian countries, especially Japan and China. For black tea, 100−200 mg per day of total catechins, from approximately 3−5 cups, may be estimated in some European countries, for instance The Netherlands and Britain (*35*, *36*).

Considering the amount of fluid entering (secretions from salivary glands, stomach, pancreas, liver, and the small intestine itself) the small intestine and from the estimated daily intake of tea, the concentration of catechins in the small intestine could

be in the range of 0.3−0.4 and 0.03−0.07 mg/mL for green and black tea, respectively. Comparison of these levels to those in the present study suggests that a significant increase in droplet size and decrease in surface area might be observed in vivo from daily black tea consumption and a slight decrease in surface area due to typical green tea consumption might be observed. It is not possible to conclude whether these changes can modulate subsequent fat digestion as emulsions undergo further enzyme hydrolysis by pancreatic lipase before absorption. However, it is likely that decrease in lipase activity occurs with an increase in emulsion droplet size. It has been reported (*18*) that fine dietary fat emulsions (<1 μm) behaved differently from coarse emulsions (10 μm) in the digestive tract of healthy humans and were digested and metabolized differently. Another study (*37*) has reported that lipase activity decreases when the mean droplet size increases, particularly obvious for the droplet size above 0.6 μm. These results may suggest that lipase activity could be affected by varying the emulsion droplet size within the physiological range. If lipase activity decreases with increase in droplet size within the normal physiological range, tea may have a much stronger impact on modifying fat absorption. Thus, a relatively low concentration of catechins from GTEF (0.22−0.65 mg/mL total catechins) that slightly increased the droplet size within the physiological range (1.43−1.63 μm) may be very efficient in reducing the lipase activity. However, the relationship between the emulsion droplet size, specific surface area, and enzyme activity is not yet clearly understood. Further investigation on the lipase activity is in progress in our laboratory.

The result from this study that black tea polyphenols may have a strong effect on dietary fat emulsion is also supported by an animal study (*15*) that showed a decrease in triglyceride (TG) level in blood plasma after regular ingestion of black tea. It has been also reported (*38*) that theaflavins-enriched green tea showed cholesterol- and TG-lowering effects in humans and concluded that tea extract containing both black tea theaflavins and green tea catechins might have a stronger impact on plasma lipid profile compared to a single dose of either tea. Most previous studies concluded that green tea is more effective in delivering beneficial health effects such as prevention of chronic diseases due to higher levels of catechins compared to those of black tea. Interestingly, our results indicate that black tea may be equally effective in reducing fat absorption as green tea.

In conclusion, the results obtained in this study have clearly demonstrated that both green and black teas have effects on physicochemical properties of an olive oil emulsion by increasing droplet size and decreasing specific surface area. Black tea had greater effects at lower catechin concentration compared to those of green tea. The levels of catechins (0.3−0.4 and 0.03−0.07 mg/mL for green and black tea, respectively) that showed effects on those properties are achievable by typical daily tea consumption. Therefore, reduction in dietary fat emulsification, consequently modification in fat digestion and absorption, may be observed in vivo. The possible mechanisms are likely to be (1) interactions between tea polyphenols and PC and (2) incorporation of tea polyphenols into the interface of an emulsion droplet. Among the individual tea compounds, EGCG and polymeric forms of catechins such as theaflavins and thearubigins may be mainly responsible for the changes in the physicochemical properties of the olive oil emulsion.

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