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Inhibition of Gastrointestinal Lipolysis by Green Tea, Coffee, and Gomchui (*Ligularia fischeri*) Tea Polyphenols during Simulated Digestion

Kwang Hyun Cha,[‖] Dae-Geun Song,[‖] Sang Min Kim, and Cheol-Ho Pan*

Functional Food Center, Korea Institute of Science and Technology (KIST), Gangneung 210-340, Republic of Korea

ABSTRACT: Green tea, coffee, and gomchui (*Ligularia fischeri*) tea, which are rich in polyphenols, may exhibit antiobesity effects by inhibiting pancreatic lipase. However, the bioavailability of some polyphenols is poor due to either degradation or absorption difficulties in the gastrointestinal tract, thus making their beneficial effects doubtful. This study was conducted to evaluate the inhibitory effect of three beverages on lipolysis and the contribution of their major polyphenols during simulated digestion. During simulated digestion, gomchui tea was the most potent at inhibiting gastrointestinal lipolysis, whereas green tea was the least potent. The strongest lipase inhibitor among purified major polyphenols was a green tea polyphenol, (−)-epigallocatechin gallate (EGCG, IC_{50} = 1.8 ± 0.57 μM), followed by di-*O*-caffeoylquinic acid isomers (DCQA, IC_{50} from 12.7 ± 4.5 to 40.4 ± 2.3 μM), which are gomchui tea polyphenols. However, the stability of DCQA was greater than that of EGCG when subjected to simulated digestion. Taken together, gomchui tea, which has DCQA as the major polyphenol, showed stronger lipolysis inhibitory activity during simulated digestion compared to both green tea and coffee.

KEYWORDS: *EGCG, DCQA, lipase inhibition, green tea, coffee, Ligularia fischeri, simulated digestion*

INTRODUCTION

Obesity is a common chronic disorder of carbohydrate and fat metabolism characterized by excess fat deposition.[1] Obesity has become a critical public health problem in developed nations because of its increasing prevalence in all ages, ethnicities, and races.[2] Obesity alone can induce all symptoms of metabolic syndrome, which is associated with many additional health problems, including an increased risk of insulin resistance, nonalcoholic fatty liver disease, atherosclerosis, some immune-mediated disorders such as asthma, and certain cancers.[3,4]

Pancreatic lipase, a key triglyceride absorption enzyme in the small intestine, is secreted from the pancreas and hydrolyzes triglycerides into glycerol and fatty acids.[5] Suppressing triglyceride absorption by inhibiting lipase is a major approach to prevent obesity. Orlistat, which is an agent used clinically to manage obesity, hinders triglyceride absorption by inhibiting digestive lipase, and long-term administration results in weight loss.[6] Several studies have revealed various health benefits of natural products as potential natural inhibitors of pancreatic lipase.[7−9] Some natural product extracts show a major inhibitory effect on pancreatic lipase, and the bioactive compounds of the extracts are mostly polyphenols or saponins, such as catechins,[10] tannins,[11] isoflavonoids,[12] triterpene saponins,[13,14] or theasaponins.[15]

Tea and coffee, the most popular beverages in the world, contain large quantities of polyphenols. Both coffee and green tea are rich sources of phenolic compounds, including caffeoylquinic acids (CQAs) in coffee[16,17] and catechins in green tea.[18] Gomchui (*Ligularia fischeri*), which has been used traditionally as an herbal medicine in China, Korea, and Japan, has also been consumed as a healthy tea, and it is rich in di-*O*-caffeoylquinic acids (DCQAs).[19] Tea polyphenols have various biological and pharmacological functions, such as anti-human immunodeficiency virus,[20] antioxidant,[21] antimutagenic,[22] and anticarcinogenic activities.[23] Additionally, habitual tea consumption is generally assumed to suppress lipid catabolism by inhibiting pancreatic lipase activity due to the tea polyphenols,[10,24−27] which may lead to an antiobesity effect.[25,28]

Recent studies have demonstrated that tea polyphenols have poor bioavailability resulting from instability under digestive conditions, poor transcellular transport, and rapid metabolism followed by excretion.[29−31] In vitro studies show that about 80% of catechins in green tea are degraded during simulated digestion, including almost total degradation of (−)-epigallocatechin gallate (EGCG).[32,33] Although numerous in vivo studies have demonstrated that polyphenols from green tea are absorbed in humans,[29,34−36] the percentage of polyphenols absorbed in plasma is low (0.07% of EGCG intake is absorbed).[29] Additionally, only one-third of chlorogenic acid from foods is absorbed in the human small intestine, reflecting the poor intestinal bioavailability of chlorogenic acid isomers such as CQAs and DCQAs.[17]

The aim of this study was to monitor the stability of major polyphenols from green tea, coffee, and gomchui tea using an in vitro digestion model, which simulates preabsorptive digestion events. Furthermore, we simultaneously investigated and compared the inhibitory effect of three beverages on free fatty acid formation during simulated digestion. The inhibitory activity of each major compound in the three beverages on pancreatic lipase was also evaluated to determine the role of tea and coffee polyphenols in lipid hydrolysis.

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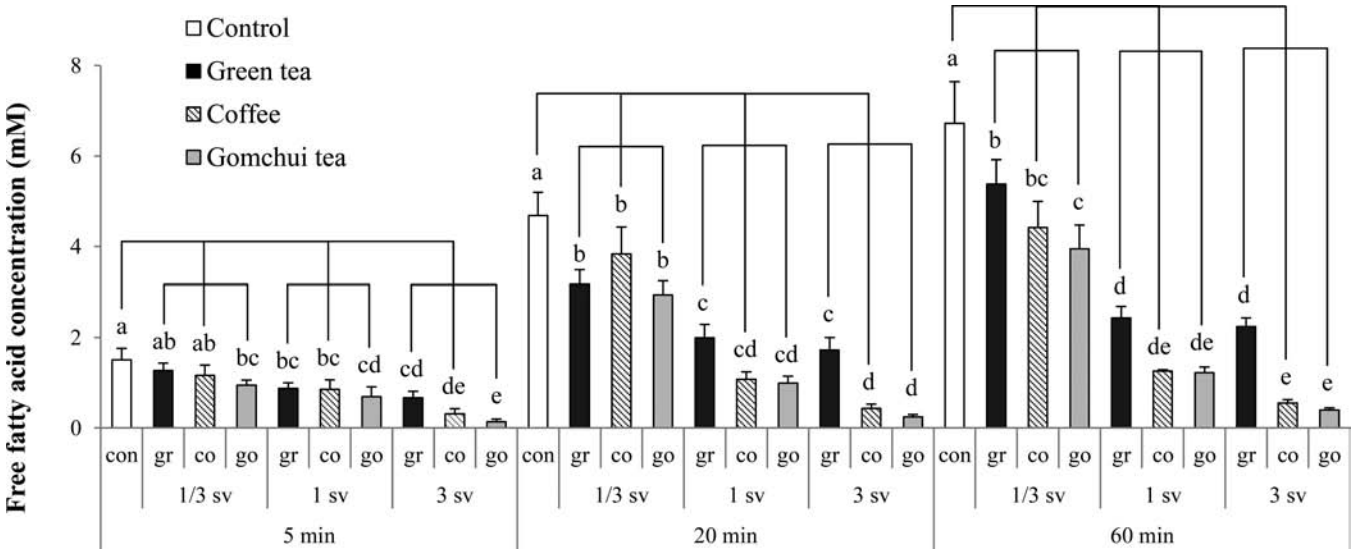

Figure 1. Inhibitory effect of green tea, coffee, and gomchui tea on lipolysis during simulated digestion. The reaction was stopped at the times indicated, and free fatty acid content was measured. Data represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences ($p < 0.05$) between samples at a given time point (5, 20, and 60 min). Abbreviations: con, control; gr, green tea; co, coffee; go, gomchui tea; sv, serving.

MATERIALS AND METHODS

Reagents. Pepsin, pancreatin, bile extract, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), EGCG, caffeine, 5-*O*-caffeoylquinic acid (5-CQA), 4-*O*-caffeoylquinic acid (4-CQA), and 3-*O*-caffeoylquinic acid (3-CQA) were acquired from Sigma-Aldrich (St. Louis, MO, USA). 3,4-Di-*O*-caffeoylquinic acid (3,4-DCQA), 3,5-di-*O*-caffeoylquinic acid (3,5-DCQA), and 4,5-di-*O*-caffeoylquinic acid (4,5-DCQA) were isolated from *L. fischeri*.[19] All chlorogenic acids presented in this study were named after the recommended IUPAC numbering system (axial hydroxyls on carbons 1 and 3 and equatorial hydroxyls on carbons 4 and 5 in the quinic acid moiety).[37] The purities of all isolated compounds from *L. fischeri* were >90%, according to high-performance liquid chromatography (HPLC) analysis. HPLC grade acetonitrile and water were obtained from Fisher Scientific (Springfield, NJ, USA). Triolein, trifluoroacetic acid (TFA), porcine pancreatic lipase (type II), and 4-methylumbelliferyl oleate (4-MUO) were supplied by Sigma-Aldrich. All other chemicals were purchased from Sigma-Aldrich unless otherwise indicated.

Sample Preparation. Commercially available green tea (Amore Pacific Corp., Seoul, Korea), instant coffee (Taster's Choice, Nescafé; Nestlé, Vevey, Switzerland), and gomchui tea (Gangwon-chinhwan-kyung Agricultural Cooperative, Gangneung, Korea) were purchased from local markets. One gram each of the green and gomchui teas was steeped in 100 mL of boiled water (90−95 °C) for 5 min for a one-cup serving. Similarly, coffee was prepared by adding 100 mL of boiled water to 1 g of powdered instant coffee according to the manufacturer's instructions. For the dose-dependency study, the amount of each raw material was varied (0.33 g for one-third serving and 3 g for three servings). Finally, samples were filtered and lyophilized using a freeze-dryer. All samples were prepared in triplicate.

In Vitro Digestion. The in vitro digestion procedure was performed according to the method of Garrett et al.[38] with minor modifications. The lyophilized samples, as described above, were dissolved with equal amounts (10 mL) of saline solution containing NaCl, KCl, and $CaCl_2$ (120, 5, and 6 mM, respectively). We eliminated the initial oral digestive phase, as the samples were beverages and thus swallowed immediately without further digestion. To mimic the gastric phase of human digestion, the pH was acidified to 2.0 with 1 N HCl, and 1 mL of porcine pepsin solution (0.04 g/mL HCl) was added, creating a final volume of 20 mL. The samples were overlaid with nitrogen gas and incubated at 37 °C for 1 h in a shaking water bath at 95 rpm. The intestinal phase involved increasing the pH to 5.3 with 0.9 M sodium bicarbonate followed by adding 200 μL of glycodeoxycholate (0.04 g in 1 mL of saline), taurodeoxycholate (0.025 g in 1 mL of saline), and taurocholate (0.04 g in 1 mL of saline) bile salts, 100 μL of pancreatic lipase (40 mg in 1 mL of 0.1 M sodium bicarbonate), and 100 μL of pancreatin (0.04 g in 500 μL of saline). The pH of each sample was increased to 7.4 with 1 N NaOH and overlaid with nitrogen. Samples were incubated for 2 h at 37 °C in a shaking water bath to complete the intestinal phase of the in vitro digestion process. After completing the small intestinal phase, samples were centrifuged at 10000*g* for 1 h at 4 °C. Aliquots of raw material and small intestinal digestate (5 mL) were collected, acidified with 2% aqueous acetic acid (1:1), and stored frozen at −80 °C under a blanket of nitrogen until HPLC analysis. To measure free fatty acid content, 60 mg of triolein was initially added to the beverages and then collected at 5, 20, and 60 min after the addition of pancreatic lipase.

Evaluation of Polyphenols and Free Fatty Acids. Aliquots of raw material and aqueous digestate were thawed and centrifuged at 14000*g* for 10 min. Supernatants of both the raw material and aqueous digestate were collected and filtered through a 0.45 μm PTFE filter prior to analysis. The analysis was conducted on a HPLC system (Varian, Walnut Creek, CA, USA) equipped with a Prostar 230 ternary gradient pump (Varian), a Prostar 430 AutoSampler (Varian), and a Prostar 335 photodiode array detector (Varian). A Prevail C_{18} column (250 × 4.6 mm, 5 μm particle size; Alltech Associates, Deerfield, IL, USA) preceded by a guard column packed with the same stationary phase was housed in a column heater thermostated at 40 °C. The mobile phase was composed of 100% acetonitrile (A) and 0.1% TFA aqueous solution (B) under the following conditions: isocratic at 15% A for 3 min followed by a linear gradient from 15 to 40% A in 25 min, a linear increase to 90% A in 5 min, and isocratic at 90% A for 2 min. The column was equilibrated to the starting conditions for 15 min before the injection of each sample. Major phenolic compounds were detected and tentatively identified using authentic standards by comparing the retention time and inline photodiode array data between 215 and 600 nm.[19,32,39] Calibration plots for quantification were constructed from 215 nm for caffeine and catechins (EC, EGC, and EGCG), and from 330 nm for chlorogenic acids (CQAs and DCQAs). Peaks through the fourth most intense peak were identified for each tea or coffee sample and assessed for the following experiments. Their concentrations were expressed as milligrams per 100 g of tea sample. The level of free fatty acids in digestate was determined with a free fatty acid quantification kit (Abcam, Cambridge, MA, USA) according to the manufacturer's instructions.

Measurement of Pancreatic Lipase Activity. Lipase activity was measured using 4-MUO as the substrate with a slight modification from a previous paper.[10] Lipase (100 mg) was dissolved in 10 mL of water to prepare the lipase working solution. Then, insoluble matter was discarded by centrifugation, and the supernatant was collected in a fresh tube. The protein concentration of the collected supernatant was 1 mg/mL according to the Bradford assay. Finally, the protein solution was diluted 1:100 using the reaction buffer solution [20 mM Tris-Cl (pH 8.0), 150 mM NaCl, and 1.3 mM $CaCl_2$]. In a 96-well microtiter plate, 50 μL of lipase working solution was mixed with 1 μL of appropriate concentration of purified or purchased polyphenolic compounds dissolved in dimethyl sulfoxide (DMSO). Orlistat, a lipase inhibitor,[40] was used as a positive control. The final concentrations of DCQAs and EGCG were 0.01−100 μM in 10-fold intervals. The final concentrations were 500, 100, 20, 4, 0.8, and 0.16 μM for the CQAs, EGC, EC, and caffeine. The final Orlistat concentrations were 0.1 nM−10 μM (10-fold intervals). The enzyme reaction was started by adding 50 μL of 0.1 mM 4-MUO prepared in 1% DMSO in distilled water. Then, the plate was immediately inserted into a microplate reader (Bio-Tek Instruments, Winooski, VT, USA), and the amount of released 4-MUO was monitored at an excitation wavelength of 360 nm and an emission wavelength of 460 nm for 6 min at 2 min intervals. The IC_{50} of the test sample was obtained from the least-squares regression line of the plots of the logarithm of the sample concentration (log) versus pancreatic lipase activity (%). A minimum of three independent experiments were performed, and the IC_{50} values were expressed as the mean ± standard deviation.

Data Analysis. Polyphenol content (mg/100 g tea sample) was determined for both the raw material and digestate of each formulation factor. The raw material provided a reference for the starting concentration of available phenolic compounds before the in vitro digestion procedure from which to compare the final phenolic compound content in the digestate. A minimum of three individual digestions was performed for statistical analysis. All data are expressed as the mean ± standard error of the mean (SEM). Significant differences were evaluated by analysis of variance (Student−Newman−Keuls test) using GraphPad Prism 4 software (San Diego, CA, USA).

RESULTS AND DISCUSSION

Inhibitory Effect of Green Tea, Coffee, and Gomchui Tea on Lipolysis during in Vitro Digestion. During the simulated digestion experiment, triolein was added as a substrate for lipase at the lipase addition phase, and the amount of released free fatty acids was measured. The lipolysis inhibitory effect of each beverage was measured in a dose- and time-dependent manner and compared to each other (Figure 1). First, as digestion time elapsed (5, 20, and 60 min), the free fatty acid concentrations continued to increase, indicating that triolein was readily digested throughout the lipase addition phase of simulated digestion. Second, the overall amount of free fatty acid decreased, whereas the serving amount increased (one-third to three servings). Thus, the inhibitory effect of lipolysis was exhibited by the beverages.

Finally, by comparing the concentration of free fatty acids released by the three beverages, the highest amount of free fatty acid release was observed with green tea. In other words, coffee and gomchui tea were better lipolysis inhibitors than green tea. In particular, the difference was clear at 20 and 60 min. At those time points, green tea was the only sample categorized into a different group according to the Student−Newman−Keuls test (Figure 1). At 20 min, green tea belonged to group c, whereas coffee and gomchui tea were in the same group, cd or d; at 60 min, green tea was in group d, whereas coffee and gomchui tea were in group de or e (Figure 1). Additionally, although coffee and gomchui tea were occasionally categorized in the same group, gomchui tea tended to exhibit a somewhat stronger inhibitory effect on lipolysis than coffee (particularly at the 5 min time point and one-third serving at 60 min). Thus, gomchui tea had the strongest lipolysis inhibitory effect among the three beverages.

Compositional Change in the Major Compounds during in Vitro Digestion. The effect of simulated digestion on the change in composition of the major polyphenolic compounds in green tea, coffee, and gomchui tea was determined by HPLC. Typical chromatograms before (gray line) and after (black line) the simulated digestion for each sample are shown in Figure 2. Additionally, the content of each major compound was quantified using the chromatograms with their respective standard compounds (Table 1). The identified peaks were as follows. In green tea (Figure 2A), the most abundant compound was EGC (**2**), followed by ECGC (**6**), caffeine (**7**), and EC (**5**). In coffee (Figure 2B), the most abundant was caffeine (**7**), followed by 5-CQA (**4**), 4-CQA (**3**), and 3-CQA (**1**). In gomchui tea (Figure 2C), 3,4-DCQA (**8**) was the most intense, followed by 5-CQA (**4**), 3,5-DCQA (**9**), and 4,5-DCQA (**10**). 4-CQA (**3**) and 3-CQA (**1**) were also found in gomchui tea. Among the four major compounds detected in green tea (Figure 2A), two catechins, EGC and EGCG from green tea, were degraded and not detectable after the simulated digestion (Table 1), which agreed with a previous paper.[32] In particular, the two major catechins, EGCG and EGC, were not detectable after the simulated digestion. EC, another catechin in green tea, was also markedly degraded after simulated digestion, and only 41.1% of the compound remained in the digestate. However, a non-catechin derivative, caffeine, was relatively more stable than the catechins, and 87.0% were preserved. This stability of caffeine was also observed in coffee. Furthermore, unlike catechin derivatives, other major polyphenols such as 5-CQA, 4-CQA, and 3-CQA were stable in coffee and preserved after simulated digestion (residual percentage, 87.9−92.0%). Isomers of DCQA in gomchui tea were also stable during the simulated digestion (residual percentage, 53.7−73.9%).

Moreover, despite the fact that 5-CQA was found in both coffee and gomchui tea, the stabilities were somewhat different from each other (90.4% of 5-CQA was reserved in coffee, whereas 78.1% was reserved in gomchui tea). Amaki et al. reported that the oxidative product of CQA, CQA quinone, interacts with catechin, thereby reverting CQA quinone to its original form, CQA.[41] Thus, CQA was protected from being oxidized by polyphenol oxidase. However, this study considered only catechins, which are not found in coffee.[42] Thus, factors that might have made 5-CQA in coffee more stable than in gomchui tea are yet to be determined.

From the assessment of the major compounds in the three beverages during simulated digestion, most compounds, other than catechin derivatives in green tea, were relatively stable during simulated digestion. Thus, lipase inhibitory activities of the individual major compounds from each beverage were further analyzed to evaluate their contributions.

Inhibitory Effect of Major Compounds from the Three Beverages on Lipase Activity and Their Contributions. The IC_{50} of each compound is listed in Table 2. The results showed that EGCG from green tea was the strongest inhibitor among those tested (IC_{50} = 1.8 ± 0.57 μM), followed by the gomchui tea polyphenols 4,5-DCQA, 3,4-DCQA, and 3,5-DCQA (IC_{50} of 12.7 ± 4.5, 33.2 ± 4.2, and 40.4 ± 2.3 μM, respectively). The major polyphenols from coffee, 5-CQA, 4-

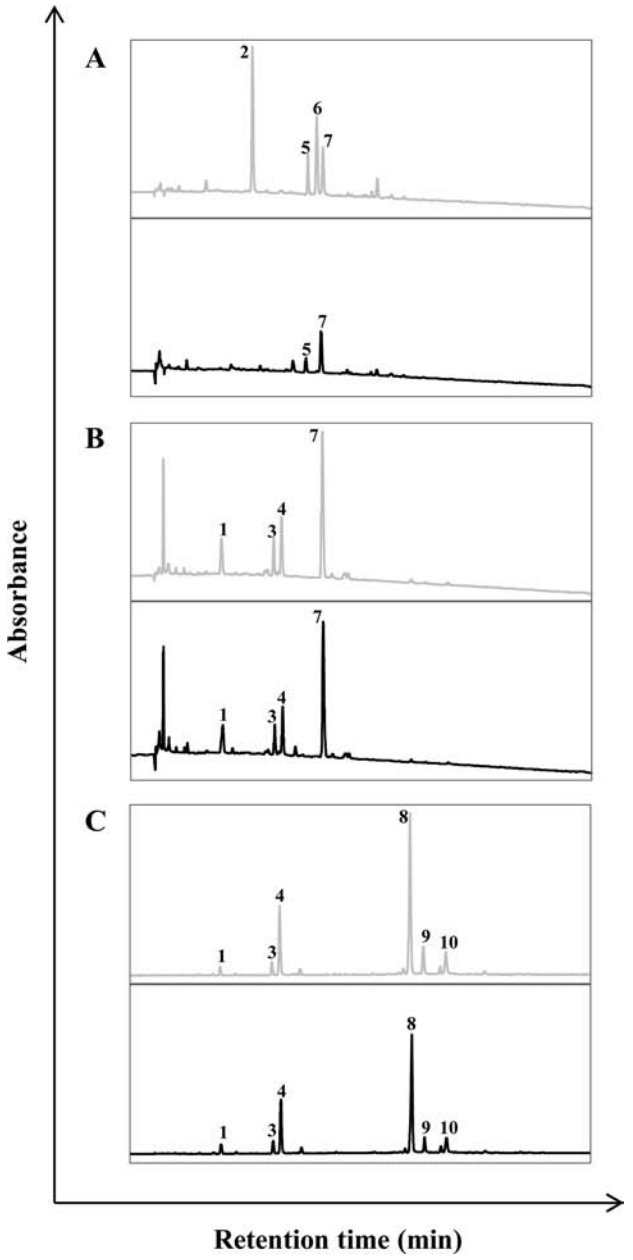

Figure 2. High-performance liquid chromatography (HPLC) profiles of catechin and caffeine; representative chromatograms from green tea (A) and coffee (B) at 215 nm and of chlorogenic acids in gomchui tea (C) at 330 nm. Major compounds in different tea samples before (gray line) and after (black line) simulated digestion were analyzed by comparing retention times and mass data with pure standards. The *y*-axis scales in each panel are identical. **1**, 3-CQA; **2**, EGC; **3**, 4-CQA; **4**, 5-CQA; **5**, EC; **6**, EGCG; **7**, caffeine; **8**, 3,4-DCQA; **9**, 3,5-DCQA; **10**, 4,5-DCQA.

CQA, and 3-CQA, were relatively weak inhibitors compared to the above tea compounds (IC_{50} of 286.5 ± 52.3, 304.8 ± 50.7, and 253.3 ± 31.2 μM, respectively). The EGC and EC from green tea, and caffeine from green tea and coffee, showed no lipase inhibitory effect even at the highest concentration tested.

Our results suggest that CQAs were weaker lipase inhibitors compared to DCQAs (Table 2). This result was consistent with a study by Murase et al.[16] However, in coffee, CQAs, the weak lipase inhibitors, were the major polyphenol. Thus, it was contradictory that the amount of free fatty acid released during simulated digestion of coffee was somewhat similar to that of gomchui tea (Figure 1). In the study by Murase et al.,[17] the percentage of DCQA isomers in coffee was 4.1−7.0%. However, in our study, even though we confirmed the existence of DCQA in coffee (data not shown), the composition of DCQA was not as high as shown in the study of Murase et al.[17] The differences may have arisen from the form of coffee; our specimen was an instant product. Furthermore, they also reported that polyphenols other than CQAs, such as feruloyl quinic acid isomers or ferulic acid, exhibit a lipase inhibitory effect. Taken together, the results suggest that the lipase inhibition activity of both CQA and DCQA drove the lowering effect of free fatty acid release during the simulated digestion in the instant coffee we tested.

Recalling that EGCG was the most potent lipase inhibitor among other compounds, we might expect that EGCG-containing green tea was the strongest lipolysis inhibitor followed by gomchui tea and coffee (green tea > gomchui tea ≥ coffee). However, on the basis of our results (Figure 1), the ranking of the lipolysis inhibitory effect of the three beverages during simulated digestion was gomchui tea ≥ coffee > green tea. This reversal might be explained by the fact that EGCG was degraded during simulated digestion.

During in vitro digestive conditions, EGCG is degraded and concurrently dimerized into theasinensin A or theasinensin D.[32,33] If one considers theasinensins A and D as dimerization products rather than degradation products, their lipase inhibitory effects were even higher than that of EGCG itself (respectively, about 2.4 and 3.5 times higher, according to IC_{50} values reported by Nakai et al.[10]). However, the total amount of theasinensins in the digestate is insufficient to exhibit a lipolysis inhibitory effect.[32] Therefore, because green tea was found to be the least effective lipolysis inhibitor in our study, EGCG might have undergone extensive cleavage or hydrolysis into a non-lipase-inhibiting derivative. No derivatives other than polymerization products such as theasinensins have been reported under in vitro digestive conditions. In contrast, Bonfili et al. identified an EGCG metabolite (molecular mass, 326 Da) in 50 mM Tris-Cl buffer, pH 8.0, conditions, or in cell culture medium.[43] Additionally, Chen et al. identified EGCG metabolites in an in vitro incubation with various mammalian hepatocytes.[44] However, both of these conditions are still not digestive tract conditions. It may be necessary to use other analytic equipment, such as liquid chromatography with electrospray ionization mass spectrometry,[36] gas chromatography with flame ionization detector,[45] gas chromatography with time-of-flight mass spectrometry,[45] or nuclear magnetic resonance to assess the cleaved or hydrolyzed EGCG product.[46] A functional assay such as an online ABTS assay method used in antioxidant screening[19,47] could be utilized for detecting unknown EGCG metabolites.

Until now, EGCG was thought to be a functional compound with anticancer[23] and antiobesity effects.[24] However, studies,[32] including ours, suggest that EGCG is almost totally degraded or cleaved during simulated digestion, thus resulting in loss of its beneficial effect. Compared to EGCG, DCQAs had a slightly lower lipase inhibitory effect. However, DCQA was more stable than EGCG during simulated digestion. Consequently, gomchui tea exerted a better lipolysis inhibition effect.

This study was conducted to investigate the lipolysis inhibition activity of three beverages during simulated digestion. Additionally, the contributions of major polyphenols on the inhibition of lipolysis were analyzed by determining both

Table 1. Major Compounds in Green Tea, Coffee, and Gomchui Tea before and after Simulated Digestion[a]

		concn (mg/100 g tea sample)		
	major compd	before digestion	after digestion	residual percentage
green tea	EGC	449.89 ± 16.28	ND[b]	
	EGCG	164.93 ± 9.49	ND	
	caffeine	154.55 ± 7.17	134.47 ± 2.50	87.0
	EC	91.73 ± 5.57	37.71 ± 5.18	41.1
coffee	caffeine	436.82 ± 32.35	410.44 ± 8.22	94.0
	5-CQA	256.59 ± 31.98	231.90 ± 3.36	90.4
	4-CQA	146.53 ± 14.48	128.78 ± 2.50	87.9
	3-CQA	135.74 ± 12.80	124.93 ± 2.25	92.0
gomchui tea	3,4-DCQA	448.38 ± 28.52	322.36 ± 18.91	71.9
	5-CQA	145.71 ± 7.51	113.86 ± 3.80	78.1
	3,5-DCQA	68.82 ± 4.83	36.97 ± 4.29	53.7
	4,5-DCQA	66.33 ± 4.27	49.03 ± 2.57	73.9

[a]All catechin, caffeine, CQA, and DCQA contents in each tea sample were calculated by high-performance liquid chromatography−diode array detection (HPLC-DAD) analysis. Data are the mean ± SEM of three independent experiments. [b]ND, not detected.

Table 2. Inhibitory Effect of Major Compounds from Green Tea, Coffee, and Gomchui Tea on Lipase Activity

compd	IC_{50}[a] (μM)
Orlistat[b]	1.2 ± 0.13 nM
EGC	>500
EGCG	1.8 ± 0.57
caffeine	>500
EC	>500
5-CQA	286.5 ± 52.3
4-CQA	304.8 ± 50.7
3-CQA	253.3 ± 31.2
3,5-DCQA	40.4 ± 2.3
3,4-DCQA	33.2 ± 4.2
4,5-DCQA	12.7 ± 4.5

[a]Half-maximal inhibitory concentration. Data represent the mean ± standard deviation of three independent experiments. [b]Positive control.

the inhibitory activity of lipase and stability during digestion. As a result, although EGCG itself was a potent lipase inhibitor, it was degraded during simulated digestion, leaving green tea as the weakest lipolysis inhibitor among the others. Moreover, gomchui tea was the strongest lipolysis inhibitor due to the contribution of DCQA. In conclusion, our results suggest that the lipolysis inhibitory effect of beverages is due to the lipase inhibitory activity and stability during digestion of their respective polyphenols.

AUTHOR INFORMATION

Corresponding Author

*Phone: +82-33-650-3652. Fax: +82-33-650-3679. E-mail: cheolpan@gmail.com..

Author Contributions

[‖]These authors contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

3-CQA, 3-*O*-caffeoylquinic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; 3,4-DCQA, 3,4-di-*O*-caffeoylquinic acid; 3,5-DCQA, 3,5-di-*O*-caffeoylquinic acid; 4,5-DCQA, 4,5-di-*O*-caffeoylquinic acid; EC, (−)-epicatechin; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; 4-MUO, 4-methylumbelliferyl oleate.

REFERENCES

(1) McCarthy, M. I. Genomics, type 2 diabetes, and obesity. *N. Engl. J. Med.* **2010**, *363*, 2339−2350.

(2) Highlander, P.; Shaw, G. P. Current pharmacotherapeutic concepts for the treatment of cardiovascular disease in diabetics. *Ther. Adv. Cardiovasc. Dis.* **2010**, *4*, 43−54.

(3) Tilg, H.; Moschen, A. R. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat. Rev. Immunol.* **2006**, *6*, 772−783.

(4) Wellen, K. E.; Hotamisligil, G. S. Inflammation, stress, and diabetes. *J. Clin. Invest.* **2005**, *115*, 1111−1119.

(5) Lowe, M. E. Pancreatic triglyceride lipase and colipase: insights into dietary fat digestion. *Gastroenterology* **1994**, *107*, 1524−1536.

(6) McClendon, K. S.; Riche, D. M.; Uwaifo, G. I. Orlistat: current status in clinical therapeutics. *Expert Opin. Drug Saf.* **2009**, *8*, 727−744.

(7) de la Garza, A.; Milagro, F.; Boque, N.; Campión, J.; Martínez, J. Natural inhibitors of pancreatic lipase as new players in obesity treatment. *Planta Med.* **2011**, *77*, 773−785.

(8) Yun, J. W. Possible anti-obesity therapeutics from nature − a review. *Phytochemistry* **2010**, *71*, 1625−1641.

(9) McDougall, G. J.; Kulkarni, N. N.; Stewart, D. Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors* **2008**, *34*, 73−80.

(10) Nakai, M.; Fukui, Y.; Asami, S.; Toyoda-Ono, Y.; Iwashita, T.; Shibata, H.; Mitsunaga, T.; Hashimoto, F.; Kiso, Y. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *J. Agric. Food Chem.* **2005**, *53*, 4593−4598.

(11) Yoshikawa, M.; Shimoda, H.; Nishida, N.; Takada, M.; Matsuda, H. *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. *J. Nutr.* **2002**, *132*, 1819−1824.

(12) Guo, Y.; Wu, G.; Su, X.; Yang, H.; Zhang, J. Antiobesity action of a daidzein derivative on male obese mice induced by a high-fat diet. *Nutr. Res. (N.Y.)* **2009**, *29*, 656−663.
(13) Karu, N.; Reifen, R.; Kerem, Z. Weight gain reduction in mice fed *Panax ginseng* saponin, a pancreatic lipase inhibitor. *J. Agric. Food Chem.* **2007**, *55*, 2824−2828.
(14) Lee, Y.-S.; Cha, B.-Y.; Yamaguchi, K.; Choi, S.-S.; Yonezawa, T.; Teruya, T.; Nagai, K.; Woo, J.-T. Effects of Korean white ginseng extracts on obesity in high-fat diet-induced obese mice. *Cytotechnology* **2010**, *62*, 367−376.
(15) Han, L. K.; Kimura, Y.; Kawashima, M.; Takaku, T.; Taniyama, T.; Hayashi, T.; Zheng, Y. N.; Okuda, H. Anti-obesity effects in rodents of dietary tea saponin, a lipase inhibitor. *Int. J. Obes. Relat. Metab. Disord.* **2001**, *25*, 1459−1464.
(16) Murase, T.; Yokoi, Y.; Misawa, K.; Ominami, H.; Suzuki, Y.; Shibuya, Y.; Hase, T. Coffee polyphenols modulate whole-body substrate oxidation and suppress postprandial hyperglycaemia, hyperinsulinaemia and hyperlipidaemia. *Br. J. Nutr.* **2012**, *107*, 1757−1765.
(17) Olthof, M. R.; Hollman, P. C.; Katan, M. B. Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* **2001**, *131*, 66−71.
(18) Richelle, M.; Tavazzi, I.; Offord, E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *J. Agric. Food Chem.* **2001**, *49*, 3438−3442.
(19) Shang, Y. F.; Kim, S. M.; Song, D.-G.; Pan, C.-H.; Lee, W. J.; Um, B.-H. Isolation and identification of antioxidant compounds from *Ligularia fischeri*. *J. Food Sci.* **2010**, *75*, C530−C535.
(20) Hashimoto, F.; Kashiwada, Y.; Nonaka, G.-I.; Nishioka, I.; Nohara, T.; Cosentino, L. M.; Lee, K.-H. Evaluation of tea polyphenols as anti-HIV agents. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 695−700.
(21) Pecorari, M.; Villaño, D.; Francesca Testa, M.; Schmid, M.; Serafini, M. Biomarkers of antioxidant status following ingestion of green teas at different polyphenol concentrations and antioxidant capacity in human volunteers. *Mol. Nutr. Food Res.* **2010**, *54*, S278−S283.
(22) Krul, C.; Luiten-Schuite, A.; Tenfelde, A.; van Ommen, B.; Verhagen, H.; Havenaar, R. Antimutagenic activity of green tea and black tea extracts studied in a dynamic in vitro gastrointestinal model. *Mutat. Res.* **2001**, *474*, 71−85.
(23) Han, C. Screening of anticarcinogenic ingredients in tea polyphenols. *Cancer Lett.* **1997**, *114*, 153−158.
(24) Uchiyama, S.; Taniguchi, Y.; Saka, A.; Yoshida, A.; Yajima, H. Prevention of diet-induced obesity by dietary black tea polyphenols extract in vitro and in vivo. *Nutrition (N. Y., NY, U. S.)* **2011**, *27*, 287−292.
(25) Almoosawi, S.; McDougall, G. J.; Fyfe, L.; Al-Dujaili, E. A. S. Investigating the inhibitory activity of green coffee and cacao bean extracts on pancreatic lipase. *Nutr. Bull.* **2010**, *35*, 207−212.
(26) Gondoin, A.; Grussu, D.; Stewart, D.; McDougall, G. J. White and green tea polyphenols inhibit pancreatic lipase in vitro. *Food Res. Int.* **2010**, *43*, 1537−1544.
(27) Sugiyama, H.; Akazome, Y.; Shoji, T.; Yamaguchi, A.; Yasue, M.; Kanda, T.; Ohtake, Y. Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. *J. Agric. Food Chem.* **2007**, *55*, 4604−4609.
(28) Grove, K. A.; Sae-tan, S.; Kennett, M. J.; Lambert, J. D. (−)-Epigallocatechin-3-gallate inhibits pancreatic lipase and reduces body weight gain in high fat-fed obese mice. *Obesity* **2011**, DOI: 10.1038/oby.2011.139.
(29) Henning, S. M.; Niu, Y.; Lee, N. H.; Thames, G. D.; Minutti, R. R.; Wang, H.; Go, V. L. W.; Heber, D. Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. *Am. J. Clin. Nutr.* **2004**, *80*, 1558−1564.
(30) Parada, J.; Aguilera, J. M. Food microstructure affects the bioavailability of several nutrients. *J. Food Sci.* **2007**, *72*, R21−R32.
(31) Green, R. J.; Murphy, A. S.; Schulz, B.; Watkins, B. A.; Ferruzzi, M. G. Common tea formulations modulate in vitro digestive recovery of green tea catechins. *Mol. Nutr. Food Res.* **2007**, *51*, 1152−1162.
(32) Neilson, A. P.; Hopf, A. S.; Cooper, B. R.; Pereira, M. A.; Bomser, J. A.; Ferruzzi, M. G. Catechin degradation with concurrent formation of homo- and heterocatechin dimers during in vitro digestion. *J. Agric. Food Chem.* **2007**, *55*, 8941−8949.
(33) Yoshino, K.; Suzuki, M.; Sasaki, K.; Miyase, T.; Sano, M. Formation of antioxidants from (−)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma. *J. Nutr. Biochem.* **1999**, *10*, 223−229.
(34) Lee, M.-J.; Maliakal, P.; Chen, L.; Meng, X.; Bondoc, F. Y.; Prabhu, S.; Lambert, G.; Mohr, S.; Yang, C. S. Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11*, 1025−1032.
(35) Kotani, A.; Miyashita, N.; Kusu, F. Determination of catechins in human plasma after commercial canned green tea ingestion by high-performance liquid chromatography with electrochemical detection using a microbore column. *J. Chromatogr., B* **2003**, *788*, 269−275.
(36) Renouf, M.; Redeuil, K.; Longet, K.; Marmet, C.; Dionisi, F.; Kussmann, M.; Williamson, G.; Nagy, K. Plasma pharmacokinetics of catechin metabolite 4′-*O*-Me-EGC in healthy humans. *Eur. J. Nutr.* **2011**, *50*, 575−580.
(37) IUPAC Commission on the Nomenclature of Organic Chemistry (CNOC) and IUPAC-IUB Commission on Biochemical Nomenclature (CBN). Nomenclature of cyclitols. Recommendations, 1973. *Biochem. J.* **1976**, *153*, 23−31.
(38) Garrett, D. A.; Failla, M. L.; Sarama, R. J. Development of an in vitro digestion method to assess carotenoid bioavailability from meals. *J. Agric. Food Chem.* **1999**, *47*, 4301−4309.
(39) Mullen, W.; Nemzer, B.; Ou, B.; Stalmach, A.; Hunter, J.; Clifford, M. N.; Combet, E. The antioxidant and chlorogenic acid profiles of whole coffee fruits are influenced by the extraction procedures. *J. Agric. Food Chem.* **2011**, *59*, 3754−3762.
(40) Hadváry, P.; Lengsfeld, H.; Wolfer, H. Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipstatin. *Biochem. J.* **1988**, *256*, 357−361.
(41) Amaki, K.; Saito, E.; Taniguchi, K.; Joshita, K.; Murata, M. Role of chlorogenic acid quinone and interaction of chlorogenic acid quinone and catechins in the enzymatic browning of apple. *Biosci., Biotechnol., Biochem.* **2011**, *75*, 829−832.
(42) Arts, I. C.; van De Putte, B.; Hollman, P. C. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J. Agric. Food Chem.* **2000**, *48*, 1752−1757.
(43) Bonfili, L.; Cuccioloni, M.; Mozzicafreddo, M.; Cecarini, V.; Angeletti, M.; Eleuteri, A. M. Identification of an EGCG oxidation derivative with proteasome modulatory activity. *Biochimie* **2011**, *93*, 931−940.
(44) Chen, W. W.; Qin, G.-Y.; Zhang, T.; Feng, W.-Y. In vitro drug metabolism of green tea catechins in human, monkey, dog, rat and mouse hepatocytes. *Drug Metab. Lett.* **2012**, DOI: 10.2174/1872212225945643128.
(45) Jumtee, K.; Bamba, T.; Fukusaki, E. Fast GC-FID based metabolic fingerprinting of Japanese green tea leaf for its quality ranking prediction. *J. Sep. Sci.* **2009**, *32*, 2296−2304.
(46) Lee, J.-E.; Lee, B.-J.; Hwang, J.-A.; Ko, K.-S.; Chung, J.-O.; Kim, E.-H.; Lee, S.-J.; Hong, Y.-S. Metabolic dependence of green tea on plucking positions revisited: a metabolomic study. *J. Agric. Food Chem.* **2011**, *59*, 10579−10585.
(47) Kim, C. Y.; Lee, H. J.; Lee, E. H.; Jung, S. H.; Lee, D.-U.; Kang, S. W.; Hong, S. A.; Um, B.-H. Rapid identification of radical scavenging compounds in blueberry extract by HPLC coupled to an on-line ABTS based assay and HPLC-ESI/MS. *Food Sci. Biotechnol.* **2008**, *17*, 846−849.