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ORIGINAL CONTRIBUTION

Purified black tea theaflavins and theaflavins/catechin supplements did not affect serum lipids in healthy individuals with mildly to moderately elevated cholesterol concentrations

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Abstract
Background Ingestion of tea flavonoids found in both green and black tea is linked to cardiovascular health benefits such as lowering serum lipids. Evidence for a cholesterol-lowering benefit of green or black tea consumption from human intervention studies is, however, conflicting and active components responsible for the effect have not yet been clearly identified.
Aim of the study In a randomized, double-blind, placebo-controlled, parallel design study the effects of ingesting a purified black tea theaflavins (TFs) powder alone or in combination with catechin (TFs/catechins) on lowering serum total (TC) and LDL-cholesterol (LDL-c) were assessed.
Methods In total, 102 mildly to moderately hypercholesterolemic (TC and LDL-c: 5.70 ± 0.74 and 3.97 ± 0.61 mmol/L, respectively) subjects (67 men and 35 women) were randomly assigned to consume once daily one capsule of one of the 3 treatments: TFs (providing 77.5 mg), TFs/catechins (providing 75.0 mg TFs plus 150.0 mg catechins and 195.0 mg of other polyphenols), or placebo (cellulose).
Results Serum TC and LDL-c concentrations did not differ significantly among the 3 treatments as assessed at 4, 8, and 11 weeks using analysis of covariance ($p = 0.1187$ and $p = 0.1063$, respectively). Although changes over time from baseline to week 11 were significant for TC and LDL-c ($p = 0.0311$ and $p = 0.0269$, respectively), this decrease over time was seen in the TFs and placebo groups.
Conclusion In this human intervention study, no statistically significant LDL-c lowering effect was seen with either TFs alone or the TFs/catechins combination as compared to placebo. Based on these findings it cannot be concluded that tea flavonoids such as theaflavins and catechins are responsible for a putative cholesterol-lowering effect of black tea, at least not with the daily dose applied in the present study.

Keywords Black tea · Theaflavins · Catechin · Cholesterol-lowering · Healthy individual

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Introduction

Tea has been consumed as a beverage for more than 2000 years with the worldwide consumption of tea being second only to water. Drinking tea is associated with several health benefits, in particular related to a reduced risk of cardiovascular disease (CVD) [9]. Epidemiological studies provide evidence to support that tea consumption reduces the risk of CVD although evidence is not consistent [11, 20]. Furthermore, it is not well-understood whether green or black tea differs in this respect. The CVD benefits of tea are believed to be linked largely to the presence of polyphenols, primarily present in the form of flavonoids. Green and black tea both are rich in flavonoids comprising up to 30% of its dry content [23]. While the total flavonoid

content of green and black tea is comparable, green tea is particularly rich in catechins such as epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate. In contrast, black tea contains 1–2% theaflavins (TFs) including theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate and 6–12% of thearubigins, which are polymerization products of catechins.

One proposed mechanism by which tea flavonoids contribute to a reduced risk of CVD is related to a beneficial effect on lowering blood lipids. A number of animal studies demonstrated that tea consumption significantly lowered blood total cholesterol (TC) and reduced atherosclerotic lesion formation in various animal models [23]. The underlying mechanisms of action of the cholesterol-lowering effect of tea flavonoids are not fully elucidated, but there is some evidence that the first site of action could be inhibition of intestinal cholesterol absorption by interfering with the formation of mixed micelles [12, 13, 22, 25].

Despite the beneficial effects observed in animal studies, results from human intervention studies regarding a cholesterol-lowering effect of tea consumption or tea flavonoid intake are conflicting. In several human studies consumption of either green or black tea or the intake of tea extracts rich in flavonoids given as a supplement revealed no beneficial effects on blood lipid profiles [1, 7, 14, 18, 21, 24]. In contrast, a human study investigating moderate green tea consumption of two cups per day containing approximately 250 mg of total catechins reported a modest, but statistically significant, reduction in LDL-cholesterol (LDL-c) compared to baseline [5]. However, LDL-c concentrations were not different from control treatment. The blood lipid-lowering effect of black tea consumption was tested in a controlled dietary intervention study with mildly hypercholesterolemic adults [2]. Daily intake of five servings of tea for 3 weeks as part of a diet moderately low in fat resulted in a significant reduction in TC and LDL-c concentration as compared with control [2]. Furthermore, a beneficial cholesterol-lowering effect of a Chinese fermented black tea extract ingested in tablet form has recently been reported [6].

In another study, Maron et al. [17] investigated the properties of a TFs-enriched green tea extract in subjects with mild to moderate hypercholesterolemia. After 12 weeks, TC and LDL-c concentrations were significantly lowered by −11.3 and −16.4%, respectively, in the group receiving the tea extract as compared to the placebo group. This decrease in TC and LDL-c is significantly larger than effects found in any of the other studies reporting a beneficial effect of tea flavonoids. As the daily ingested tea extract tested was especially rich in TFs (75 mg) next to containing 150 mg of green tea catechins and 150 mg of other polyphenols, this could suggest that TFs and/or catechins are the active components in tea or at least enhance the cholesterol-lowering effect.

Therefore, it was hypothesized that ingestion of a TF-extract derived from black tea could result in lowering serum cholesterol concentrations. Thus, the aim of this randomized, placebo-controlled study was to investigate whether purified black tea TFs or a TFs/catechin mix consumed in capsule form for 11 weeks could lower serum TC and in particular LDL-c concentrations in subjects with mildly to moderately elevated cholesterol concentrations.

Methods

Study design

The study was approved by the Research Ethics Committee of Unilever Shanghai, China. All study participants signed an informed consent form.

The study had a randomized, double-blind, placebo-controlled, parallel arm design, and was conduced following the guidelines of Good Clinical Practice (ICH-GCP).

Study participants were randomly assigned to one of the three treatments: TFs, TFs/catechin, or placebo capsules. The study had a 9-day run-in period, in which all participants used the placebo capsules followed by an 11-week intervention period, during which one of the study capsules was consumed once daily directly after the dinner meal.

Study participants

The study was conducted at the Comprehensive Outpatient Department of Sinopec Shanghai Petrochemical Company Limited, Shanghai, China. In total, 235 subjects were pre-screened from a subject-database by six factory physicians during a face-to-face interview and 224 subjects joined the screening phase, and signed an informed consent form before any measurements were performed. Inclusion criteria were defined as age between 18 and 65 years, TC between 4.80 and 7.00 mmol/L, LDL-c between 2.50 and 4.90 mmol/L and fasting triglycerides (TG) < 4.50 mmol/L. Results of hematological tests and blood glucose as well as liver and kidney function tests had to be within the normal reference range as assessed by the research physician. Subjects smoking <5 cigarettes per day were included but they were encouraged to keep smoking habits similar during the study. Exclusion criteria were reported coronary heart disease, uncontrolled hypertension, diabetes and any medical treatment or conditions that may affect bioavailability of the test ingredients and/or affect study measurements as assessed by the research physician. Subjects had to refrain from any cholesterol-lowering supplements such as fish oil,

niacin supplements, and dietary fiber supplements 6 week before the start of the study and during the study. Further, subjects were excluded when they reported excessive tea drinking with >3 fresh brewed cups per day, body weight gain or loss >10% in the past 6 months, and alcohol consumption more than 21 units per week for women and more than 28 units per week for men or reported intense sporting activities (more than 10 h per week).

Of the 224 subjects screened, 119 were rejected mainly due to not meeting the inclusion criteria. Of the remaining 105 subjects, 3 subjects did not show up at the baseline visits due to unknown reasons. The 102 remaining participants that showed up at the baseline visit were randomized into the three study groups (Fig. 1). Randomization was stratified for gender and LDL-c (2.5–3.2, 3.2–4.0, and 4.0–4.9 mmol/L) with a total of six strata.

During the intervention period, one subject complained about whole body discomfort, and another subject about stomach discomfort and both withdrew after completing their baseline visits. A third person withdrew after the visit at 4th week due to personal reasons. Drop-outs during or after the first intervention day were not replaced.

All included subjects were apparently healthy and had normal to mildly elevated serum lipid concentrations (LDL-c: 2.5–4.9 mmol/L, TC: 4.8–7.0 mmol/L, and TG < 4.5 mmol/L).

Anthropometric measurements and general health and compliance

Height was measured at screening and body weights were measured at screening, baseline and 3 times during the intervention at 4, 8, and 11 weeks. Height and weight were recorded without shoes in light clothing. Study participants recorded illnesses, medicine use, and important deviations from their lifestyle, dietary, and activity pattern in a health and lifestyle questionnaire, which was filled in at baseline and during the intervention at 4, 8, and 11 weeks.

Adverse events (AEs) during the study period were recorded by physicians and classified according to ICD-10. In case of any (medical) questions prior to or during the study, volunteers could contact the physicians.

Compliance of the capsule ingestion was checked by means of a daily record form in which subjects were asked to daily report the time of capsule intake and the time of dinner. The form had to be brought to the study facility at each visit and was checked for completeness by the study staff. Further, study participants were asked to return any unused and spare capsules they had received.

During the total study period (run-in and intervention period), study participants were encouraged to minimize changes in the composition of their habitual diet and lifestyle such as activity patterns.

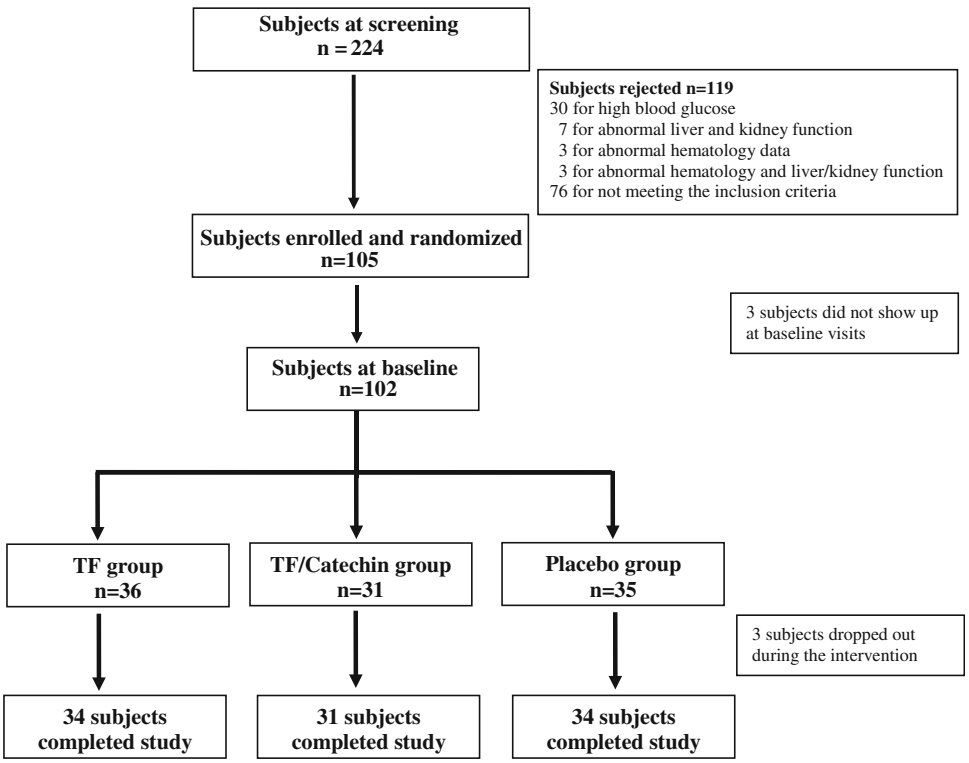

Fig. 1 Overview of study recruitment

Study products

The capsules used in this human study are not commercially available and were specifically made for this study. The three types of study capsules TFs, TFs/catechins, and placebo were prepared at GMP-certificated ISP Laboratory, Shanghai, China. Although the three capsules contained different amounts of materials and differed in weight, they looked identical and the capsules were fully filled. The TFs capsules contained a mixture of TFs-90 powder (90% pure) and cellulose. Each capsule weighed about 238.0 mg and contained 88.0 mg TFs-90 powder equivalent to 77.5 mg TFs. Based on individual component analysis TF capsules contained 10.7 mg theaflavin, 16.4 mg theaflavin-3-gallate, 21.5 mg theaflavin-3′-gallate, and 28.9 mg theaflavin-3,3′-digallate.

TFs-90 powder was produced at lab scale by Unilever China. TF-90 powder was made by column chromatography from TFs-60GF (60% pure), which was a commercially available TFs powder product from a local supplier (Hai Nan Group Force Company, Ltd, China).

The TFs/catechins capsules contained a mixture of Sunphenon 80SKNG (about 80% of total polyphenols, a product from Taiyo Green Power Co., Ltd, Wuxi, China), TFs-60GF and cellulose. Each capsule weighed about 320.0 mg and contained 75.0 mg TFs (15.1 mg theaflavin, 16.2 mg theaflavin-3-gallate, 19.3 mg theaflavin-3′-gallate, and 24.5 mg theaflavin-3,3′-digallate) and 149.4 mg catechins. The placebo capsules contained only cellulose and weighed about 320.0 mg.

The empty gelatin capsules were provided by Coni-Snap Capsules (Suzhou Capsugel Ltd, China).

Capsules were packed in white little plastic bottles and labeled with the instruction “One capsule daily directly after dinner”. For practical reasons, the test products were provided in batches, which were distributed at start of the run-in period, at baseline and 2 times during the intervention periods at 4 and 8 weeks. Subjects were asked to return unused samples.

Blood sample processing and analysis

To determine TC, LDL-c, HDL-cholesterol (HDL-c), and TG concentrations, two blood samples for serum lipid analyses were taken with 1 day in between at baseline (start of intervention) and at week 4, 8, and 11 of the intervention period. At the eight blood drawing occasions, 5 mL of blood was collected and blood samples were centrifuged for 10 min at 3000 rpm at 22 °C to separate serum from red blood cells. Serum was then stored at −70 °C until analyzed. At screening, 10 mL blood was taken to determine serum TC, LDL-c, HDL-c, and TG concentrations as well as blood glucose concentrations, and liver and kidney function makers in serum and hematological markers in blood.

Before blood sampling, subjects were fasted and had refrained from food for at least 10 h.

Serum lipids (TC, LDL-c, HDL-c, and TG) were measured by the clinical department of Zhong Shan Hospital, Shanghai, China. Serum lipids were analyzed in duplicate with standardized reagents using a Hitachi 7170 autoanalyzer (Hitachi Ltd, Tokyo, Japan). All samples from a subject were analyzed within the same batch in order to minimize batch differences in analysis. Serum lipids were determined by enzymatic assays using commercially available kits (CHOD-PAP for TC, GPO-PAP for TG, and a direct method for LDL-c and HDL-c, Roche Diagnostics, Basel, CH). All measurements were performed according to local standard procedures that were certified with the Chinese Cholesterol Reference Method set by the Ministry of Health.

The mean of the duplicate analysis was used for data evaluation. The autoanalyzer was calibrated before each run based on a standardization protocol.

Statistical analysis

The number of study participants needed was calculated taking into account a critical difference in LDL-c of 0.4 mmol/L between one of the two test groups and the control group at the end of intervention with alpha = 5% and a power = 90% (SD of 0.6). Under these constraints, 30 subjects per treatment arm were required. Assuming a 20% dropout rate, 36 subjects per arm or 108 subjects in total were required to start the study.

The statistical analysis plan was defined as follows: for the primary analysis, the population analyzed was the intention-to-treat (ITT) population, which consisted of the entire population enrolled, randomized and having taken at least one dose of the study treatments. Thereby, 102 subjects who were randomized and joined the baseline visit were included. Additionally, a secondary per-protocol (PP) analysis which only consisted of the subjects who complied with the protocol and whose blood samples were qualified was carried out. For the PP analysis, only the 99 subjects who completed the study were included and additionally blood lipid data from 12 samples at a particular day were excluded from the analysis. The decisions on non-compliance and exclusion were made before de-blinding of the study data. The statistical analysis was performed using SAS software (SAS Institute, Cary, NC, version 9.1).

In order to compare the difference in serum lipids between treatments, a mixed model analysis of variance (ANOVA) that included fixed terms for treatment (TF, TF/catechin, placebo) and time was performed. The model was adjusted for baseline concentrations while considering

treatment and baseline interactions. Other covariates than baseline lipids that could have an effect on the outcome parameters such as gender, age, body weight at baseline, change in body weight and smoking status were also included in the model. Only covariates that potentially affected the outcome ($p < 0.1$) were selected into the final model.

Differences between treatments were analyzed using the multiple comparisons test according to Dunnett. Data are presented as least-square means ± standard deviation (SD) unless otherwise stated. Values are considered statistical significant at $p \leq 0.05$.

Results

There were no differences in baseline characteristics of subjects in the three treatment groups (Table 1). Data from 102 subjects (67 males and 35 females) were statistically analyzed (ITT population). The mean age of study participants was 48.1 ± 6.1 years and their mean BMI was 23.2 ± 2.36 kg/m^2. Baseline serum TC and LDL-c concentrations were 5.70 ± 0.74 and 3.97 ± 0.61 mmol/L, respectively.

All subjects returned the daily record form in which they reported every day the time of intake, the time of dinner, and any deviations from the background diet. All participants consumed the test products as instructed and no deviations were reported.

The study treatments were well-tolerated and the overall health of the subjects during the study was good. In total, 14 AEs were reported during the whole study period, with 6 occurring in the TFs group, 2 in the TFs/catechins group, and 6 in the placebo group. Of the 14 AEs, 13 AEs were not related to the study, while one AE in the form of stomach discomfort was considered as possibly related to study treatment. Other AEs reported were common cold, throat pain, headache, whole body discomfort and one subject was taking traditional Chinese medicine for a gynecological-related reason. None of the subjects reported more than one AE.

There were no indications that subjects had changed their lifestyle habits during the duration of the study. There was also no significant difference between the three groups body weights ($p = 0.1405$), and no significant time effect ($p = 0.4277$) or treatment by time interaction ($p = 0.0856$) was observed. Only baseline body weight was a significant covariate.

Descriptive mean serum lipid concentrations at week 4, 8, and 11 of consumption of the placebo, TFs, and TFs/catechins treatments are shown in Table 2. Treatment effects as investigated using an ANOVA-based mixed model for effects of treatment, week, and treatment by week interaction are presented in Table 3. There were no statistically significant differences in TC, LDL-c, HDL-c, and TG concentrations between the three treatments. No gender-specific effects on plasma lipids were noted. In addition, the treatment by week interaction was not statistically

Table 1 Baseline characteristics of study participants

Parameter	Treatment groups			
	TFs ($n = 36$)	TFs/catechins ($n = 31$)	Placebo ($n = 35$)	All ($n = 102$)
Age, years	47.54 ± 6.70 (45.24, 49.84)	50.07 ± 3.58 (48.76, 51.38)	46.85 ± 7.07 (44.42, 49.28)	48.08 ± 6.16 (46.86, 49.29)
Gender				
Males (%)	23 (63.9)	20 (64.5)	24 (68.6)	67 (65.7)
Females (%)	13 (36.1)	11 (35.5)	11 (31.4)	35 (34.3)
Height, m	1.66 ± 0.05 (1.64, 1.68)	1.66 ± 0.07 (1.63, 1.68)	1.67 ± 0.07 (1.64, 1.69)	1.66 ± 0.06 (1.65, 1.67)
Body weight, kg	65.79 ± 8.24 (63.00, 68.58)	63.16 ± 8.84 (59.92, 66.40)	63.57 ± 8.52 (60.65, 66.50)	64.23 ± 8.52 (62.56, 65.90)
BMI, kg/m^2	23.82 ± 2.45 (22.99, 24.65)	22.96 ± 2.42 (22.07, 23.85)	22.79 ± 2.13 (22.06, 23.52)	23.20 ± 2.36 (22.74, 23.67)
Smoking status				
Yes	9 (25.0)	4 (12.9)	8 (22.9)	21 (20.6)
No	27 (75.0)	27 (87.1)	27 (77.1)	81 (79.4)
TC, mmol/L	5.71 ± 0.81 (5.43, 5.98)	5.78 ± 0.83 (5.47, 6.08)	5.63 ± 0.56 (5.44, 5.82)	5.70 ± 0.74 (5.56, 5.85)
LDL-c, mmol/L	4.02 ± 0.69 (3.78, 4.25)	3.90 ± 0.67 (3.65, 4.14)	3.99 ± 0.49 (3.82, 4.15)	3.97 ± 0.61 (3.85, 4.09)
HDL-c, mmol/L	1.33 ± 0.35 (1.21, 1.44)	1.30 ± 0.39 (1.16, 1.45)	1.36 ± 0.34 (1.25, 1.48)	1.33 ± 0.36 (1.26, 1.40)
TG, mmol/L	1.96 ± 1.02 (1.62, 2.31)	2.30 ± 1.03 (1.92, 2.68)	1.81 ± 0.84 (1.53, 2.10)	2.01 ± 0.98 (1.82, 2.20)

Values are expressed as means ± SD with 95% confidence intervals (CI) in brackets

One-way ANOVA or Chi-square test showed no significant differences in baseline parameters between the three treatment groups

TFs theaflavins, *TFs/catechins* theaflavins/catechins, *TC* total cholesterol, *LDL-c* LDL-cholesterol, *HDL-c* HDL-cholesterol, *TG* triglycerides

Table 2 Descriptive serum lipid concentrations in the TFs, TFs/catechins, and placebo groups at 4, 8, and 11 weeks of treatment (ITT population)

Serum lipids mmol/L	Time (weeks)	Treatment groups		
		TFs ($n = 36$)	TFs/catechins ($n = 31$)	Placebo ($n = 35$)
TC	4	5.61 ± 0.72 (5.36, 5.86)	5.92 ± 0.85 (5.60, 6.23)	5.64 ± 0.64 (5.42, 5.86)
	8	5.62 ± 0.70 (5.38, 5.86)	5.91 ± 0.87 (5.59, 6.23)	5.65 ± 0.55 (5.46, 5.84)
	11	5.55 ± 0.86 (5.24, 5.85)	5.74 ± 0.85 (5.43, 6.06)	5.59 ± 0.56 (5.40, 5.79)
LDL-c	4	3.93 ± 0.60 (3.72, 4.14)	3.97 ± 0.89 (3.64, 4.29)	4.01 ± 0.58 (3.81, 4.21)
	8	3.91 ± 0.57 (3.71, 4.11)	3.99 ± 0.78 (3.70, 4.27)	4.02 ± 0.49 (3.85, 4.19)
	11	3.72 ± 0.73 (3.47, 3.98)	3.92 ± 0.71 (3.66, 4.18)	3.92 ± 0.46 (3.76, 4.07)
HDL-c	4	1.39 ± 0.38 (1.25, 1.52)	1.36 ± 0.41 (1.21, 1.51)	1.41 ± 0.31 (1.30, 1.51)
	8	1.36 ± 0.35 (1.24, 1.48)	1.34 ± 0.41 (1.19, 1.49)	1.42 ± 0.39 (1.28, 1.55)
	11	1.36 ± 0.42 (1.21, 1.50)	1.34 ± 0.43 (1.18, 1.50)	1.38 ± 0.36 (1.25, 1.50)
TG	4	1.63 ± 0.73 (1.37, 1.88)	2.18 ± 1.54 (1.61, 2.74)	1.57 ± 0.77 (1.31, 1.83)
	8	1.75 ± 0.94 (1.42, 2.08)	2.10 ± 1.17 (1.68, 2.53)	1.63 ± 0.76 (1.37, 1.90)
	11	2.09 ± 1.55 (1.55, 2.64)	2.01 ± 1.07 (1.62, 2.40)	1.73 ± 0.85 (1.44, 2.03)

Values are expressed as means ± SD with 95% confidence intervals (CI) in brackets

TFs theaflavins, *TFs/catechins* theaflavins/catechins, *TC* total cholesterol, *LDL-c* LDL-cholesterol, *HDL-c* HDL-cholesterol, *TG* triglycerides, *ITT* intention-to-treat

Table 3 Serum lipid concentrations of study participants receiving TFs, TFs/catechins, or placebo treatment for a duration of 11 weeks

Serum lipids mmol/L	Treatment groups			*p* Values		
	TFs ($n = 36$)	TFs/catechins ($n = 31$)	Placebo ($n = 35$)	Treatment	Weeks	Treatment × week interaction
TC	5.61 (5.47, 5.75)	5.81 (5.67, 5.95)	5.65 (5.52, 5.79)	0.1187	0.0311	0.8223
LDL-c	3.83 (3.70, 3.96)	4.01 (3.87, 4.15)	3.96 (3.83, 4.09)	0.1750	0.0269	0.7686
HDL-c	1.37 (1.33, 1.41)	1.38 (1.34, 1.42)	1.37 (1.33, 1.41)	0.8822	0.2456	0.7557
TG	1.91 (1.72, 2.10)	1.83 (1.63, 2.03)	1.83 (1.64, 2.02)	0.7935	0.2063	0.0729

Data are expressed as least-square means and 95% confidence intervals in brackets following a mixed model analysis of variance (ANOVA) for effects of treatment, weeks, and treatment by weeks interaction with statistical adjustment for covariance with corresponding baseline lipid values plus additionally age (for TG) and change in body weight (for TC)

TF theaflavins, *TF/catechins* theaflavins/catechins, *TC* total cholesterol, *LDL-c* LDL-cholesterol, *HDL-c* HDL-cholesterol, *TG* triglycerides

significant for any of the serum lipids demonstrating that the effect did not depend on the time of comparison. However, changes over time were significant for TC and LDL-c ($p = 0.0311$ and $p = 0.0269$, respectively). In general, TC and LDL-c concentrations decreased in subjects consuming the TFs capsules from baseline to week 11, however, the same trend was also seen in the placebo group. The results on serum lipids from the per-protocol (PP) analysis were consistent with those from the ITT analysis (data not shown).

Discussion

The aim of the present human study was to investigate whether consumption of black tea TFs either alone or in combination with catechins administered as a capsule would affect serum LDL-c concentrations in healthy normo- to modestly hypercholesterolemic men and women. After 11 weeks, daily intake of either 77 mg of TFs alone or 75 mg TFs combined with 150 mg catechins did not significantly lower serum TC and LDL-c as compared to placebo. The TFs capsule contained a mix of the four typically found TFs. In the combination capsule, the ratio of TFs to catechins was in the range of what is typically found in freshly brewed black tea.

Showing no effect on serum cholesterol concentrations, the outcome of the present study is in line with other previous studies which failed to demonstrate a clear cholesterol-lowering effect of black or green tea consumption [1, 7, 14, 18, 21, 24]. There are, however, two controlled human intervention studies that reported a significant cholesterol-lowering effect in subjects with mild to moderate hypercholesterolemia of either a TFs-enriched green tea extract [17] or a lyophilized powdered black tea preparation [2]. Consumption of 5 servings of black tea daily as

studied by Davies et al. [2] providing a daily intake of 3.5 g of tea solids with about 30 mg TFs and 120 mg catechins lowered TC and LDL-c concentrations by −6.5 and −11.1%, respectively, after 3 weeks compared with a placebo drink with a similar caffeine content as the black tea. The present study was a background diet controlled intervention with participants consuming the majority of the study drinks under supervision. Whether such strict control of background diet and hence tea consumption may explain why such a significant reduction in TC and LDL-c was observed remains to be elucidated.

In another study, Maron et al. [17] tested a tea extract supplement consisting of 75 mg TFs, 150 mg catechins, and 150 mg of other tea flavonoids including thearubigins per capsule. Consumption of one capsule daily for 12 weeks lowered TC by −11.3% and LDL-c by −16.4% from baseline, while no significant changes were reported in the placebo group. Interestingly, the effect observed after 12 weeks of intervention was about 1.7 times the effect of 4 weeks treatment where TC was lowered by −6.7% and LDL-c by −9.6%. This observation suggests that the cholesterol-lowering effect apparently amplified after continued intake, which seems surprising. In general, a 3–4 week period is considered sufficient for reaching a new metabolic steady state in cholesterol metabolism following dietary interventions [16].

In contrast, our study found no significant changes in TC and LDL-c concentrations at 4, 8, or 11 weeks of treatment demonstrating that treatment duration had no impact on efficacy. Both studies used comparable amounts of tea flavonoids with 75 mg of TFs alone or a combination of 75 mg TFs with 150 mg catechins and also the study duration were similar in length with 11 vs. 12 weeks. One possible reason for the differing results could be the apparently different methods used for preparation of the tea extracts. As described by Maron et al. [17], their extract was produced from raw *Camellia sinensis* leaves through a controlled fermentation process in which catechins are dimerized to form TFs. The TFs-rich powder used in the present study was purified from a commercially available TFs powder under lab scale conditions. This could result in differences in the composition of the TFs preparation. A TF mix typically contains different TFs such as theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate, which may differ in their cholesterol-lowering action as was previously demonstrated in an in vitro micellar assay study [25]. Unfortunately, the composition of the TFs mix used in the study by Maron et al. [17] is not specified and therefore it remains hypothetical whether differences in the TFs composition may explain the conflicting study outcomes in view of a cholesterol-lowering effect. Also, whether the other polyphenols, i.e., the thearubigins are contributing to a cholesterol-lowering effect is not clear. Differences in subject characteristics seem unlikely to offer an explanation for the different outcome seen in these studies as the study of Maron et al. [17] and ours were both carried out with a Chinese study population ruling out any genetic differences.

A limitation of the present study is that no background diet and nutrient intake data are available. Nevertheless, subjects were counselled not to make dietary changes and it can be assumed that they kept their habitual diets with dietary fat intakes typically as high as 30% of energy [4]. Whether changes in background diet, particularly in fat and cholesterol intake, could explain the lack of a clear cholesterol-lowering effect of TFs in the present study cannot be answered. The fact that no changes in body weight were observed is supportive that habitual diet was not significantly changed. In the study by Maron et al. [17], study participants having mild to moderate hypercholesterolemia and were advised to lower their fat intake and consumed a diet low in total and saturated fat with about 23 and 6% of total energy, respectively. The reported fat and fatty acid intakes are far lower than reported data for the average Chinese population [8]. It cannot be completely ruled out that this may have had an impact on the observed cholesterol-lowering effect of the TF mix.

Although subjects consuming more than 3 cups of tea per day were excluded from our study, intake data on regular tea consumption in the study population are not available. It could be possible that regular tea intake was higher than anticipated and this could have diminished the likelihood to detect an effect of a supplemental intake of TFs and a TFs/catechins mix.

Based on typical amounts of catechins (up to 84 mg) in a cup of green tea and TFs (up to 12 mg) in a cup of black tea, a daily consumption of 3 cups of tea would result in an intake of about 250 mg catechins and 36 mg of TFs [26]. Considering that Chinese populations are mainly drinking green tea, an intake of TFs is negligible. With the capsules 75–77.5 mg TFs and 150 mg catechins were daily ingested, which clearly increases the consumption beyond the amounts being consumed with the typical background diet.

The underlying mechanism of action for the cholesterol-lowering effect of TFs is not fully understood, but one potential mechanism is thought to be through a partial inhibition of cholesterol absorption in the intestine. Tea flavonoids, especially tea catechins have been shown to reduce the absorption of lipids and cholesterol and consequently increasing fecal excretion of fat and cholesterol [10, 12, 13, 15, 22, 25]. Catechins, and especially gallate ester-containing catechins such as epigallocatechin gallate are effective in inhibiting micelle formation and hence lowering the solubility of cholesterol in mixed micelles [12, 22]. Since most of the TFs also contain gallate moieties, it seems plausible that they work in a similar way

affecting the incorporation of cholesterol into micelles. In fact, as was shown recently, TFs and in particular theaflavin-3-gallate affect intestinal cholesterol absorption via inhibiting the incorporation of cholesterol into mixed micelles [25]. Moreover, as the bioavailability of TFs is rather low as shown by human plasma concentrations after black tea consumption [19, 27], the gut is considered to be most likely the main site of action on TFs. In the present study, subjects were asked to take the TFs and TFs/catechins capsules directly after eating their dinner meal. Intake with a meal is required for an optimal cholesterol-lowering efficacy as was shown for plant sterols, which lower serum cholesterol by partly inhibiting intestinal cholesterol absorption via competing with cholesterol for micellar solubilization [3, 28]. Since TFs are thought to lower cholesterol by a similar mechanism of action as plant sterols taking the capsule close to a meal was considered important. One could, however, argue that taking TFs and the TFs/catechins mix with a meal could have affected their bioavailability. However, optimal bioavailability would only be an important factor if TFs and catechins would work via other mechanisms of action than affecting cholesterol absorption in the gut. Furthermore, it cannot be ruled out that the bioavailability of TF and catechins when ingested in capsule format once a day versus drinking tea at multiple daily occasions may be different and may so provide an explaining for the lack of a cholesterol-lowering effect observed in this study as compared to study with green or black tea consumption. Further studies directly comparing tea flavonoid consumption after drinking tea or taking supplements enriched with tea flavonoid extracts would be needed.

Conclusion

In this double-blind, placebo-controlled, parallel human intervention study daily intake of a TFs-enriched capsule delivering 77.5 mg TFs or a capsule delivering a combination of 75.0 mg TFs plus 150 mg catechins did not show a significant effect on lowering total and in particular LDL-c concentrations as compared to placebo. Therefore, based on the outcome of the present study it cannot be concluded that black tea flavonoids such as TFs are responsible for a putative cholesterol-lowering effect of tea. More research is needed to determine whether green or black tea consumption has an unequivocal cholesterol-lowering benefit and especially which components in tea may be responsible for this effect.

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Conflict of interest statement All authors are employed by Unilever Foods, The Netherlands or China.

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