Cholesterol-Lowering Effect of a Theaflavin-Enriched Green Tea Extract

A Randomized Controlled Trial

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Background: Tea consumption has been associated with decreased cardiovascular risk, but potential mechanisms of benefit are ill-defined. While epidemiologic studies suggest that drinking multiple cups of tea per day lowers low-density lipoprotein cholesterol (LDL-C), previous trials of tea drinking and administration of green tea extract have failed to show any impact on lipids and lipoproteins in humans. Our objective was to study the impact of a theaflavin-enriched green tea extract on the lipids and lipoproteins of subjects with mild to moderate hypercholesterolemia.

Methods: Double-blind, randomized, placebo-controlled, parallel-group trial set in outpatient clinics in 6 urban hospitals in China. A total of 240 men and women 18 years or older on a low-fat diet with mild to moderate hypercholesterolemia were randomly assigned to receive a daily capsule containing theaflavin-enriched green tea extract (375 mg) or placebo for 12 weeks. Main outcome measures were mean percentage changes in total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels compared with baseline.

Results: After 12 weeks, the mean ± SEM changes from baseline in total cholesterol, LDL-C, HDL-C, and triglyceride levels were −11.3% ± 0.9% (P = .01), −16.4% ± 1.1% (P = .01), 2.3% ± 2.1% (P = .27), and 2.6% ± 3.5% (P = .47), respectively, in the tea extract group. The mean levels of total cholesterol, LDL-C, HDL-C, and triglycerides did not change significantly in the placebo group. No significant adverse events were observed.

Conclusion: The theaflavin-enriched green tea extract we studied is an effective adjunct to a low-saturated-fat diet to reduce LDL-C in hypercholesterolemic adults and is well tolerated.

Arch Intern Med. 2003;163:1448-1453
include theaflavins, and the extract also failed to show any significant impact on lipids and lipoproteins.25

Because of the hypolipidemic effect of black tea poly-
phenols in animal studies and the lack of effect of a thea-
flavin-free extract in humans, we hypothesized that a tea
extract containing both black tea theaflavins and green
tea catechins might have a favorable impact on the lipid
profile of patients with high cholesterol. To test this
hypothesis, we performed a randomized, placebo-
controlled parallel study of a green tea extract enriched
with theaflavins in capsule form on the lipids and lipo-
proteins of individuals with mild to moderate hypercho-
lesterolemia consuming a low-fat diet. The main outcome
measures were the percentage changes in total
cholesterol, LDL-cholesterol (LDL-C), high-density li-
poprotein cholesterol (HDL-C), and triglyceride levels
compared with baseline.

**METHODS**

**PATIENTS**

Patients 18 years and older with mild to moderate hypercho-
lesterolemia (LDL-C, 130–190 mg/dL [3.4–4.9 mmol/L]) on a
low-fat diet were recruited from outpatient clinics in 6 urban
hospitals in China. Patients were excluded if their baseline tri-
glyceride level was 350 mg/dL or greater (≥4.0 mmol/L); if they
had uncontrolled hypertension (≥160/95 mm Hg), active pul-
monary, hematologic, hepatic, gastrointestinal or renal dis-
ease, premalignant or malignant disease, diabetes, thyroid dys-
function, a history of coronary heart disease or other atherosclerotic
disease, or any pathological values among routine clinical chemistry or hematological parameters; or if they
consumed greater than 32% of daily energy from fat or had a
body mass index of 35 or higher (calculated as weight in kilo-
grams divided by the square of height in meters). Subjects were also excluded if they were taking any lipid-lowering medica-
tions or drugs that might interfere with lipid metabolism or tak-
ing cardiac or other vasoactive medications including antihy-
pertensive drugs, thyroid hormones, oral contraceptives, cyclic
hormone replacement therapy, dietary supplements (eg, fish
oils, niacin at doses >400 mg/d, or dietary fiber supple-
ments), or antioxidants, and they were prohibited from taking
these medications during the course of the study. Patients were
required to maintain a stable diet and level of physical activity
during the 12-week intervention. Subjects were required to sign
an informed consent to participate in the trial. The protocol
was approved by the ethics committees in the 6 participating
hospitals in accordance with the guidelines of the Declaration
of Helsinki.

**STUDY DESIGN AND INTERVENTION**

The study was a double-blind, randomized, placebo-
controlled, parallel-group trial. Subjects were recruited from
April 15, 2001, to July 19, 2001. Two weeks after signing an
informed consent, those who met entry criteria were random-
ized to receive either a capsule containing a theaflavin-
enriched green tea extract (Nashat Biotech, LLC, Nashville,
Tenn) or placebo. Each active study capsule contained 75 mg of
theaflavins, 150 mg of green tea catechins, and 150 mg of other
tea polyphenols. The extract was manufactured accord-
ing to good manufacturing practice guidelines,30,31 with audits
performed by a senior manager of a major pharmaceutical com-
pany. The extract was produced from raw Camellia sinensis leaves through a controlled fermentation process in which catechins
are dimerized to form theaflavins. The placebo capsules were
made from inert ingredients and were identical to the active
capsules in weight, appearance, and odor.

Randomization was stratified by hospital. After random-
ization, subjects received either the tea extract capsule or pla-
cebo in a double-blind fashion and were instructed to take 1
capsule each morning for 12 weeks. After the randomization
visit, clinic visits were scheduled in the morning of weeks 4, 8,
and 12 after a 12-hour fast. Body weight was measured at each
visit. Subjects were asked to record study capsules taken, con-
comitant medications, and any adverse events in subject dia-
aries. Study capsules were dispensed at baseline, week 4, and
week 8. The period of intervention was from June 7, 2001, to
October 18, 2001.

**Food Records**

Subjects were asked to maintain their habitual, traditional Chi-
nese diet, including their customary intake of tea. Nutrient in-
take was assessed using 3-day food records. A registered dieti-
tian instructed subjects how to complete these records, and they
were collected prior to baseline and after 8 weeks of treat-
ment.

**Lipid and Lipoprotein Analysis**

Lipid and lipoprotein concentrations were measured at week
−2, week 0, week 4, and week 12 after a 12-hour fast. Lipids
and lipoproteins were analyzed enzymatically using standard
laboratory procedures. Low-density lipoprotein cholesterol con-
centrations were calculated according to the method of Friede-
wald et al.32 Prior to the trial, 40 reference samples were allo-
cated to each participating hospital to perform quality validation.
These samples represented 4 concentrations of LDL-C: low,
medium low, medium high, and high. Measurement of the samples
demonstrated tightly clustered test results, confirming high in-
trahospital and interhospital reliability of testing and standard-
dization of testing among the 6 hospitals. The intrahospital co-
efficients of variation for measurement of total cholesterol and
LDL-C were 1.9% or less and 4.1% or less, respectively. The
interhospital coefficients of variation for measurement of total
cholesterol and LDL-C were 2.0% and 1.0%, respectively. The
study, to achieve uniform quality control among the 6 hospitals, 2 different reference standards for each hospi-
tal were tested daily.

**Safety Monitoring**

Serum chemistry tests were performed at baseline and at weeks
4 and 12 to monitor for safety. These tests included assess-
ments of glucose, creatinine, uric acid, aspartate aminotrans-
ferase, total bilirubin, albumin, globulin, sodium, chloride, se-
rum urea nitrogen, calcium, lactate dehydrogenase, alkaline
phosphatase, total protein, albumin-globulin ratio, potassium,
gamma-glutamyltransferase, and phosphorus. A complete blood count with an automated differential was per-
formed at baseline and week 12.

**STATISTICAL ANALYSIS**

Changes in lipoprotein concentrations at weeks 4 and 12 were
analyzed in comparison with concentrations at week 0. Statis-
tical analysis was performed using the SAS 6.12 (SAS Institute
Inc, Cary, NC). Approximately 100 subjects per treatment group
were required to achieve 80% power to detect a treatment dif-
fERENCE of at least 0.15. This calculation is based on the as-
sumption that the true treatment difference is at least 0.20 and
the SD is 0.11.
Figure 1. Flowchart of trial participants.

Statistical analysis of the efficacy of the intervention was performed on all randomized subjects who had a baseline evaluation, at least 1 on-therapy evaluation, and no deviation from the study protocol. The efficacy analysis was based on the percentage change from baseline for total cholesterol, LDL-C, HDL-C, and triglyceride levels. Between-group comparisons were performed using analysis of covariance. Baseline characteristics of the 2 groups were compared by use of the χ² test for categorical variables and 2-tailed unpaired t test.

Evaluation of treatment safety, such as adverse events and abnormal findings from clinical laboratory tests, was performed using χ² or the Fisher exact test. Analysis of covariance was used to assess laboratory safety monitoring test parameters, using treatment as the main factor and baseline value as the covariate. All statistical tests on safety data were performed using 2-sided tests.

RESULTS

The present study comprised 240 randomized subjects, and 220 subjects completed the trial (Figure 1). The baseline characteristics are presented in Table 1. All subjects were Asian. Of subjects in the treatment and placebo groups, 95% and 88%, respectively, finished the study. Compliance with the study capsule was 99.6% in the treatment group and 99.9% in the placebo group. No subject in either group had a capsule intake of less than 80%.

FOOD RECORDS AND BODY MASS INDEX

Evaluation of the 3-day food diaries showed that both groups consumed a low-fat diet at baseline and at 8 weeks (Table 2). There were statistically significant increases in percentage of calories from protein, decreases in percentage of calories from total and polyunsaturated fat, and increases in cholesterol intake from baseline to week 8. In addition, there was an increase in total energy consumption in both groups, and this reached statistical significance in the placebo group. No statistical difference was observed for nutrient intake between the intervention and placebo groups at baseline or at 8 weeks for any of the dietary variables. Approximately half of the subjects in both groups consumed tea on a regular basis, with most subjects drinking 1 to 4 cups per day. At 8 weeks, subjects reported no changes in their tea-drinking habits compared with baseline. There were no statistically significant changes in body mass index in either group during the study (data not shown).

LIPID AND LIPOPROTEIN RESULTS

There were no significant differences in baseline lipid and lipoprotein values between groups. Mean percentage changes in lipid parameters from baseline to week 12 are presented in Figure 2. In the treatment group, after 4 weeks of intervention, total cholesterol decreased by 6.7%±0.8% and LDL-C decreased by 9.6%±1.1%; after 12 weeks, total cholesterol decreased by 11.3%±0.9% (P<.01) and LDL-C decreased by 16.4%±1.1% (P<.01). There were no significant changes in total cholesterol or LDL-C in the placebo group during the study. After 12 weeks, HDL-C rose 2.3%±2.1% (P=.27) and triglycerides rose 2.6%±3.5% (P=.47) in the tea extract group. In the placebo group, HDL-C fell 0.7%±1.6% (P=.67) and triglycerides rose 5.6%±3.8% (P=.14). The mean total cholesterol to HDL-C ratio fell from 4.61±1.03 to 4.05±0.91 (P<.001) from baseline to 12 weeks in the tea extract group, but did not change significantly in the placebo group (from 4.55±1.22 to 4.57±1.18; P=.85).

We attempted to identify any differential LDL-C-lowering effect of the tea extract according to habitual tea consumption or baseline LDL-C concentration, but found none. Among subjects randomized to the tea extract, LDL-C fell 16.5%±1.6% in those who did not drink tea compared with a 17.3%±1.8% reduction in subjects who drank 1 to 4 cups per day (P=.73 for comparison with nondrinkers) and a 13.9%±3.2% reduction in those who drank 5 to 8 cups.
10 cups per day (P = .39 for comparison with nondrinkers). Subjects in the active treatment group with baseline LDL-C in the upper half of the study sample (160-190 mg/dL [4.14-4.91 mmol/L]) experienced a mean 17.6% reduction in LDL-C compared with a mean 15.3% reduction in subjects in the active treatment group with baseline LDL-C in the lower half (130-159 mg/dL [3.36-4.13 mmol/L]), a difference that was not statistically significant.

**SAFETY MONITORING**

There were no significant differences in adverse events or routine chemistry and hematologic laboratory tests between the treatment and placebo groups, and no serious adverse events occurred. There were no statistically significant differences between baseline and week 12 laboratory results in either group.

**COMMENT**

We evaluated the lipid-lowering efficacy of a theaflavin-enriched green tea extract in subjects with mild to moderate hypercholesterolemia already consuming a low-fat diet, using a double-blind, randomized, placebo-controlled parallel-group design. The extract decreased serum total cholesterol and LDL-C by 11.3% and 16.4%, respectively. To our knowledge, this is the first human placebo-controlled trial to demonstrate an LDL-C-lowering effect from tea. Our results support the findings of several observational studies that indicate an association between tea drinking and a more favorable lipid profile and are consistent with animal experiments demonstrating the hypolipidemic effect of tea.

In observational studies as well as clinical trials with statin drugs, each 1% reduction in LDL-C results in approximately a 1.0% to 1.5% reduction in the relative risk of major cardiovascular events. By extrapolation, if the long-term use of the tea extract produced a persistent 16% reduction of LDL-C, this could reduce the risk of major cardiovascular events by 16% to 24%.

We did not examine the mechanism by which the theaflavin-enriched green tea extract lowered LDL-C in our study, but the biological plausibility of our finding has been established by the work of others, predominantly in animal models of hypercholesterolemia. Potential mechanisms include reduced micellar solubility and intestinal absorption of cholesterol, increased fecal excretion of fat and cholesterol, and hepatic cholesterol concentration, and up-regulation of the LDL receptor in liver cells.

The extract tested in this study contained 75 mg of theaflavins, 150 mg of green tea catechins, and 150 mg of other tea polyphenols in each daily capsule. Previous human tea-drinking trials tested daily exposure to theaflavins ranging in doses from 0 mg to 35 mg and to catechins in doses ranging from 50 mg to 850 mg. None of these studies found any significant impact on lipid and lipoprotein concentrations. We are aware of one published study in humans that tested...
the impact of a green tea extract in capsule form on lipids and lipoproteins. Princen et al.\textsuperscript{47} tested a capsule that contained 150 mg of green tea polyphenol and no theaflavins. Subjects ingested 24 capsules per day or 3.6 g of green tea polyphenol daily (equivalent to 18 cups of green tea per day and 2.5 g of catechins), and no effect on lipids was observed. While it is possible that the addition of theaflavins to catechins was responsible for the reduction in cholesterol seen in our study, it is not possible to conclude what role theaflavins, catechins, other tea polyphenols, or even the method of extract preparation may have had in the reduction of LDL-C. Additional research is necessary to determine the mechanism of action of this extract.

Several observational studies of human populations indicate that tea consumption is inversely associated with cardiovascular disease.\textsuperscript{2-7} In support of this observation, not only does tea lower cholesterol in animal models, but it also reduces atherosclerosis. Rabbits fed an atherogenic diet plus tea have less aortic atherosclerosis than rabbits fed the same diet without tea.\textsuperscript{16} Interestingly, green tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice fed an atherogenic diet without affecting plasma lipid levels.\textsuperscript{48} In addition to beneficial effects of tea on lipid levels and lipoprotein levels, other proposed mechanisms for the apparent cardioprotective effect of tea include antioxidant effects,\textsuperscript{1,2,3,41,42} improved endothelial function,\textsuperscript{43} an estrogenic effect,\textsuperscript{44} and anticoagulant effects.\textsuperscript{45} Our study did not investigate any of these potential lipid-independent mechanisms associated with tea.

The subjects in our study were all urban Chinese who consumed approximately 27% of total calories from fat, 6% of calories from saturated fat, and 400 mg of cholesterol daily. By comparison, the average US resident consumes 33% of total calories from fat, 11% of total calories as saturated fat, and 256 mg of cholesterol per day.\textsuperscript{46,47} National dietary surveys from the 1990s indicate the average urban Chinese consumed approximately 33% of calories from fat\textsuperscript{48} and 425 mg of cholesterol per day.\textsuperscript{49} The subjects in our study ate less dietary fat than the typical urban Chinese, perhaps because they had already been counseled to reduce their fat intake because of hypercholesterolemia. We do not know what effect the extract might have on the LDL-C level of other ethnic groups eating a diet with a different amount of fat or cholesterol.

The Third Report of the National Cholesterol Education Program Adult Treatment Panel reaffirms that in the primary prevention of coronary heart disease, diet therapy is the initial recommended intervention for lowering LDL-C.\textsuperscript{46} Prior to advancing to drug therapy, the guidelines recommend nonpharmacologic therapeutic options—specifically viscous fiber and plant stanols and sterols—to enhance LDL-C lowering if the LDL-C goal has not been achieved with dietary therapy.\textsuperscript{46} There is a need to identify additional nonpharmacologic therapeutic options for cholesterol lowering that have sufficient safety, efficacy, and product standardization data. There is also a need to find products that are more practical for the consumer than viscous fiber and plant stanols and sterols to permit widespread adoption. The present study represents the first step in establishing the practicality, safety, and LDL-C-lowering efficacy of a tea product. More research is needed to determine long-term safety, the effective dosing range, the optimal timing of administration, the effect of dietary fat content, drug interactions, impact when coadministered with lipid-altering medications (especially statins), and generalizability to other ethnic and patient groups.

In summary, we found that a theaflavin-enriched green tea extract administered once a day is an effective adjunct to a low-saturated-fat diet to reduce LDL-C in hypercholesterolemic adults and is well tolerated. Based on these results, we recommend larger and longer-term randomized controlled trials to confirm our findings in a more diverse population and to study the effect of this extract on other risk factors for atherosclerosis.

Accepted for publication October 31, 2002.
This study was sponsored by Nashai Biotech, LLC, Nashville, Tenn. Nashai Biotech LLC received a grant from Wyeth Consumer Healthcare Inc, Madison, NJ. Shanghai Kehao Biotechnology Co, Ltd, Shanghai received a grant from Nashai Biotech, LLC.

Robert V. Acuff, PhD, Center for Nutrition Research, East Tennessee State University contributed advice and consultation on nutritional science and informed consent. Stephanie Wang, BA, provided valuable assistance in organizing the study teams, coordinating organizational meetings with the 6 hospitals, and in managing the protocol timelines.

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