Effect of black tea on the fasting concentrations of chylomicrons and chylomicron remnants in humans

Cheryl A. Dane-Stewart, John C.L. Mamo, Jonathan M. Hodgson, Ian B. Puddey and Sebely Pal

Abstract

Objective: This study aimed to investigate effects of chronic black tea consumption on concentrations of chylomicrons and chylomicron remnants using plasma concentrations of apolipoprotein B-48 as a marker.

Subjects and study design: Twenty-two healthy men and women were recruited into a randomised, controlled crossover study of four weeks, consuming five cups per day black tea or hot water and then switched over for four weeks.

Main outcome measures: Plasma apolipoprotein B-48, lathosterol, and serum lipid measurements were taken from fasting blood samples at the end of each four-week period.

Statistical analysis: Paired t-tests were used to compare concentrations of apolipoprotein B-48, lathosterol and serum lipids after tea consumption with those after hot water control.

Results: Comparing ingestion of black tea to hot water, we found no changes in apolipoprotein B-48, lathosterol or serum lipids collectively.

Conclusions: We conclude there was no effect of five cups of black tea per day for four weeks on the plasma concentrations of chylomicrons and their remnants. (Nutr Diet 2002;59:240–3)

Key words: chylomicron remnants, cholesterol, apoB, catechins, atherosclerosis

Introduction

The initial stage of atherosclerosis is the formation of a fatty streak in arterial vessels, which involves the deposition of lipid, in particular cholesterol, in the subendothelial space of arterial intima (1). Cholesterol is transported in plasma mainly by low-density lipoproteins (LDL) and chylomicron remnants. Elevated LDL concentration has been established as a major risk factor for the development of cardiovascular disease (2). More recently, it has been suggested that chylomicron remnants also play an important role in the progression of atherosclerosis (3). Extensive studies in our laboratory have demonstrated that green tea polyphenolic compounds can penetrate arterial tissue and deposit lipid in arterial intima (4,5). One of the main strategies in preventing cardiovascular disease has been to lower the plasma concentration of LDL, but this suggestion has been extended to plasma chylomicron remnant concentrations (6). In this study, we examined the effects of black tea, a putative hypolipidaemic agent, on the concentrations of chylomicrons and their remnants in plasma.

Several epidemiological studies have suggested tea consumption has a protective effect against the risk of heart disease mortality and myocardial ischaemic events (7–9). Tea has been shown to have hypolipidaemic properties in animal studies (10–12), and cross-sectional studies, mainly in Japanese populations drinking green tea, have associated a higher tea intake with lower plasma total cholesterol concentration (13,14). However, intervention studies in humans have generally shown no effect of black tea (15–19) on plasma cholesterol concentrations.

The major group of polyphenolic compounds present in green and black tea, collectively called catechins, have been suggested to mediate any beneficial effects of tea consumption on plasma cholesterol concentrations (9). In particular, (-)-epigallocatechin gallate, the most abundant catechin in green tea, has been found to be most effective in lowering plasma cholesterol levels in animals (20). However, the mechanisms by which catechins act to lower lipid levels are presently unclear. Increasing the clearance of atherosclerotic lipoproteins from plasma may be one of the mechanisms by which catechins have a favourable effect on lipoprotein metabolism. Cell culture studies conducted in our laboratory have demonstrated that green tea catechins upregulate the expression of the LDL-receptor—the primary receptor responsible for the uptake and removal of LDL and chylomicron remnants from circulation (21,22).

The effect of black tea on the concentrations of chylomicron remnants has not been reported. Given that hepatic clearance of these lipoproteins via the LDL-receptor may be improved with tea consumption (21,22), we hypothesise that tea consumption will lower the levels of pro-atherogenic chylomicron remnants in plasma. The aim of the study therefore, was to investigate for the first time the effect of chronic black tea ingestion on the fasting concentrations of chylomicrons and chylomicron remnants in healthy human subjects. To measure chylomicron remnants, we have employed a sensitive Western Blot and Enhanced Chemiluminescence Assay, to quan-
tify apolipoprotein B-48 (apoB-48), a protein found exclusively on chylomicron remnants and their precursor (23).

Methods

Subjects

Twenty-two subjects (16 men and six women) were recruited from the general population via media advertisements. Excluded from the study were subjects who were current smokers or ex-smokers who had stopped for less than six months; whose alcohol intake was greater than 40 g alcohol daily (i.e. four standard drinks per day); pre-menopausal women and postmenopausal women on hormone replacement therapy; those with a body mass index (BMI) > 35 kg/m², subjects with heart, liver, renal or gastrointestinal diseases or diabetes; and those taking medication (24). The study was approved by the Royal Perth Hospital Ethics Committee and all subjects gave informed written consent.

Study design

Subjects commenced a randomised-controlled crossover trial consisting of an initial four-week baseline period, followed by two four-week intervention periods. During the four-week baseline period, subjects drank five cups (250 ml per cup) of hot water daily. They were then randomised to either five cups of black tea or hot water (control) for the following four weeks after which they were switched to the other beverage for the next four weeks. Fasting blood samples were taken at the end of each four-week period. Effects of black tea were compared to hot water. For the duration of the study, subjects were instructed not to consume caffeine-containing beverages including tea, coffee, chocolate drinks, cola and herbal teas. Apart from these restrictions, subjects did not alter their daily food intake, alcohol consumption or physical activity level during the study.

Tea preparation

The Tea Trade Health Research Association (Toronto) provided a ‘world blend’ leaf tea (blended black tea). Each subject was given an amount of leaf tea and a set of instructions on how it was to be prepared. This entailed placing 2 g tea leaves into a spring-handled infuser and then into 250 ml boiled water for one minute while stirring constantly. The tea was consumed without any other additives (e.g. milk or sugar). Subjects in the control arm of the study were instructed to consume the water while hot.

Measurement of apolipoprotein B-48

Plasma was separated from whole blood by centrifugation at 1500 x g for 10 minutes. ApoB-48 was quantitated from plasma using a Western Blotting/Enhanced Chemiluminescence procedure described previously (23). ApoB-48 bands were identified and compared to a purified apoB-48 protein standard of known mass. Interassay coefficient of variance (CV) was < 5%.

Plasma lathosterol, serum lipids and lipoproteins and 4-O-methylgallic acid

Plasma lathosterol was used as a marker of whole-body cholesterol synthesis (25). This was measured using gas chromatography mass spectrometry as described previously (26). Total serum cholesterol and triglyceride concentrations were determined by enzymatic colorimetric methods (Boehringer Mannheim Pty Ltd, Mannheim, Germany). Fasting HDL-cholesterol was estimated after precipitation of apoB-containing lipoproteins (Boehringer Mannheim Pty Ltd, Mannheim, Germany) and LDL-cholesterol derived from the Freidwald formula. Total apoB-100 was measured by an immunonephelometric assay (Behring Diagnostics, Kingsgrove, NSW). The 24-hour urinary excretion of 4-O-methylgallic acid (4OMGA), a marker of black tea intake, was measured in urine samples collected at the end of each four-week period using a method described previously (27). Interassay CVs for the above assays were all < 3.0%.

Statistics

Statistical analyses were performed with SPSS statistical software (SPSS Inc, Chicago, SPSS for Windows, version 8.0 1998). Data were expressed as mean (SEM) and triglyceride and lathosterol data log-transformed to normalise skewed distributions. Data were compared using a paired samples t-test with a level of significance of P < 0.05.

Results

The mean age was 59 ± 2 years with a range of 43 to 75 years. Baseline measurements of BMI (27 ± 0.6 kg/m²) and systolic and diastolic blood pressures (125 ± 3 and 73 ± 2 mmHg respectively) remained unchanged throughout the study. Excretion of 4OMGA was significantly higher during regular ingestion of black tea in comparison to water (1246 ± 118 µg/day vs 95 ± 95 respectively; P < 0.001). All subjects showed an increase in 4OMGA during the black tea period, consistent with ingestion of the tea and exposure to polyphenols derived from tea.

Table 1 shows the fasting serum lipid and lipoprotein, plasma lathosterol and apoB-48 concentrations following four weeks of black tea and water ingestion. There were no significant changes in serum total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol or apolipoprotein B-100 concentrations with black tea ingestion. There were also no changes in either plasma lathosterol, lathosterol:cholesterol or apoB-48 associated with four weeks’ black tea ingestion as compared with the four weeks with hot water.

Discussion

We found no effect of chronic black tea ingestion on the plasma concentrations of chylomicrons and chylomicron remnants as indicated by apoB-48 levels in healthy human subjects. There was also no change in the plasma lathosterol, serum apoB-100 or lipids with chronic black tea ingestion.

Previous investigations into the hypolipidaemic properties of black tea have focused on fasting lipid concentrations and have found mixed results. Some ani-
Table 1. Fasting serum lipids and lipoproteins, plasma lathosterol and apolipoprotein B-48 following four weeks of five cups per day of black tea and hot water ingestion

<table>
<thead>
<tr>
<th></th>
<th>Black tea</th>
<th>Water</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.74 ± 0.13</td>
<td>5.78 ± 0.12</td>
<td>0.658</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.07 ± 0.07</td>
<td>1.07 ± 0.07</td>
<td>0.870</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.94 ± 0.10</td>
<td>3.98 ± 0.10</td>
<td>0.623</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.30 ± 0.05</td>
<td>1.31 ± 0.06</td>
<td>0.788</td>
</tr>
<tr>
<td>Apolipoprotein B-100 (g/L)</td>
<td>1.05 ± 0.02</td>
<td>1.06 ± 0.02</td>
<td>0.413</td>
</tr>
<tr>
<td>Lathosterol (µM)</td>
<td>6.04 ± 0.62</td>
<td>5.78 ± 0.50</td>
<td>0.545</td>
</tr>
<tr>
<td>Lathosterol/ cholesterol (µmol/mmol)</td>
<td>1.07 ± 0.11</td>
<td>1.01 ± 0.09</td>
<td>0.417</td>
</tr>
<tr>
<td>Apolipoprotein B-48 (mg/L)</td>
<td>7.90 ± 0.63</td>
<td>7.86 ± 0.55</td>
<td>0.887</td>
</tr>
</tbody>
</table>

Our in vitro studies investigating the mechanisms involved in the hypolipidemic properties of tea have found catechin supplementation from green tea can lead to LDL-receptor upregulation in human liver HepG2 cells (21,22). LDL-receptor upregulation was suggested to be mediated by increased conversion of the sterol-regulated element binding protein (SREBP-1) in the presence of green tea (22). As pro-atherogenic particles such as LDL and chylomicron remnants require removal from circulation primarily by the LDL-receptor (34), we hypothesised that possible upregulation of the LDL-receptor with tea consumption may lead to increased removal of these particles from circulation. However, we found no change in the plasma concentration of chylomicron and chylomicron remnants with ingestion of 1.25 L black tea daily. A possible explanation for the lack of change in lipid concentrations in our study may be related to catechin concentrations. The in vitro studies used green tea, which contains a higher percentage of catechins than black tea (35). In particular, (-)-epigallocatechin gallate (EGCG) is thought to be one of the most active catechins in lowering plasma lipids (20). Green tea contains about four- to five-fold the EGCG concentration of black tea (35). Thus, it may be possible that the level of catechin supplementation from 1.25 L per day of black tea was not sufficient to elicit significant upregulation of LDL-receptors, with no consequent effect on circulating chylomicron remnants or LDL-cholesterol concentrations. An increase in cholesterol removal from circulation as a result of increased LDL-receptor activity is often reflected in a decrease in plasma lathosterol levels—a marker of whole-body cholesterol synthesis (25). We found no change in lathosterol further supporting our conclusion of no change in the removal of cholesterol-rich particles such as chylomicron remnants or LDL via the LDL-receptor with black tea ingestion.

In conclusion, we have found that chronic black tea ingestion (five cups daily) in healthy men and women has no effect on chylomicron and chylomicron remnants. We also found no change in serum lipids, lipoproteins or plasma lathosterol concentrations. Future investigations should focus on the effects of higher concentrations of catechin supplementation and/or for longer time periods in dyslipidaemic subjects to further resolve the question as to whether tea can reduce the fasting concentrations of chylomicrons and their remnants or improve the lipid profile in humans.

Acknowledgments

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