Regular ingestion of black tea improves brachial artery vasodilator function

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ABSTRACT

A higher intake of black tea has been associated with lower cardiovascular disease risk. The antioxidant effects of tea polyphenols may enhance endothelial function and thereby reduce the risk of coronary events. The objective of the present study was to determine whether regular ingestion of black tea can improve brachial artery vasodilator function. The effects of regular ingestion of 5 cups per day of black tea for 4 weeks were compared with control conditions (hot water ingestion) in 21 subjects with mild elevations in serum cholesterol or triacylglycerol (triglyceride) concentrations in a parallel designed study. Endothelial function of the brachial artery was assessed ultrasonographically by measurement of post-ischaemic (endothelium-dependent) dilatation of the brachial artery. Endothelium-independent dilatation of the brachial artery was measured following administration of 400 \( \mu \)g of sublingual glyceryl trinitrate. Regular ingestion of black tea resulted in a significant and consistent increase in endothelium-dependent dilatation (2.3\% \( P = 0.008 \)) and in a significant increase in endothelium-independent dilatation (4.2\% \( P = 0.03 \)), compared with ingestion of hot water. These differences remained after adjustment for age, sex and body mass index. These results suggest that one mechanism by which black tea may reduce cardiovascular risk is via improved vasodilator function of conduit arteries.

INTRODUCTION

A higher intake of black tea [1–4] and of polyphenols derived from black tea [3,5–7] has been associated with a lower risk of cardiovascular disease (CVD). The mechanisms responsible for the benefits of tea with regard to cardiovascular risk are, however, unclear.

Protective effects of drinking black tea against CVD may involve improvements in vascular function. Endothelial damage appears to be important in the development and progression of atherosclerosis and thrombogenesis. In addition, impaired vascular function is associated with CVD, and is believed to be an early indicator of vascular disease [8–13]. Dietary factors that improve vascular function may also reduce the risk of CVD.

Improvements in vascular function may be mediated by antioxidant polyphenols that are found in black tea. Decreased production of reactive oxygen species and increased antioxidant defences may give rise to reduced breakdown and/or enhanced synthesis and release of endothelial-derived nitric oxide, resulting in improved...
vascular function [14]. Black tea is a rich source of polyphenols, and can be a major contributor to total polyphenol intake in the human diet [15]. Many of the polyphenols present in black tea possess potent antioxidant activity [16,17], and vasodilatory effects of polyphenols found in tea have been demonstrated in vivo [18–21]. In addition, a recent study has shown that ingestion of black tea has acute and longer-term effects in improving vascular function [22].

The objective of the present study was to determine whether regular ingestion of black tea could improve vascular function in subjects with mild elevations in serum cholesterol or triacylglycerol (triglyceride) concentrations. Vascular function was measured ultrasonographically as flow-mediated and glyceryl trinitrate (GTN)-mediated dilatation of the brachial artery. Vasodilator responses in this artery provide a surrogate measure for the coronary circulation, and may predict coronary events [9].

METHODS

Subjects

A total of 21 healthy subjects (16 men and five postmenopausal women) were recruited from the general population. Subjects were regular tea drinkers (mean intake 3.8 ± 0.5 cups/day); had total cholesterol ≥ 5.0 mmol/l and/or triacylglycerols ≥ 1.8 mmol/l at screening; were non-smokers; had a usual alcohol intake < 40 g per day; had no history of chronic disease; were not taking medication; and women were not taking hormone replacement therapy. The project was approved by the Royal Perth Hospital Ethics Committee, and all subjects gave written informed consent.

Study design

A randomized controlled parallel designed study with a 4-week intervention period proceeded by a 4-week baseline period was performed. During baseline all subjects were instructed to drink 5 cups per day (250 ml each) of hot water. Subjects were then randomly allocated to drink 5 cups per day of black tea or to continue with 5 cups per day of hot water for 4 weeks. All staff performing measurements were blinded to the drink being consumed. However, because the subjects were unavoidably aware of the drink allocation, this does introduce the potential for expectation bias.

During the study, subjects were instructed to cease intake of caffeine-containing beverages (except those assigned), and not to make any changes to their usual food intake, alcohol consumption and physical activity. A dietitian monitored these behaviours throughout the intervention. Height was measured at baseline, and body weight was measured at the end of each period.

Preparation of tea

A ‘World blend’ leaf tea (a blended black tea) was obtained from the Tea Trade Health Research Association (Toronto, Canada). The method of tea preparation was standardized as far as possible. Tea leaves (2 g) were placed into a spring-handled infuser, then infused into approx. 250 ml of boiled water for 1 min with constant movement. Tea was consumed without additives, including milk and sugar. A consistent weight of tea was achieved by providing subjects with a container that, when filled, contained 2 g of tea leaves. The control drink was the same volume of boiled water consumed hot. Subjects were instructed to use a standard tea cup, which holds approx. 250 ml of water, to prepare the tea and hot water. Subjects did not drink tea on the morning before each blood sample in order to avoid the possibility of acute effects.

Brachial artery vasodilator function

Brachial artery vasodilator function was assessed non-invasively by measurement of brachial artery dilatation using ultrasonography. Subjects were each assessed at the same time of day and under the same conditions of fasting. A trained technician dedicated to this research protocol performed all measurements and analyses, blinded to the drink being consumed.

The method used has been described in detail elsewhere [23]. Duplicate measurements of endothelial function of the left brachial artery were obtained 1 week apart at the end of the baseline period and at the end of the intervention. The duplicate assessments at baseline and at the end of intervention were averaged to obtain a single value for each for baseline and post-intervention. This results in reduced variability and improves power to detect change. Endothelium-dependent flow-mediated dilatation (FMD) of the brachial artery was measured following an ischaemic stimulus. Pulse-wave Doppler flow velocities were used to derive resting flow rate and flow rate after reactive hyperaemia, calculated as the percentage increase in blood flow. Endothelium-independent dilatation of the brachial artery was measured following administration of 400 μg of sublingual GTN spray.

Analysis of FMD and of GTN-mediated dilatation was carried out using a semi-automated edge-detection software system [23]. Responses were calculated as the percentage change in brachial artery diameter from baseline. The analytical intra-observer coefficient of variation for the computerized technique in our hands is 6.7%, compared with 32.5% using more conventional visual estimation employing ultrasonographic callipers. The resolving power of the computerized technique tested on phantom arteries is 8.3 μm. Therefore the computerized image analysis system with edge-detection software used to analyse the data from the present study reduces observer error significantly compared with man-
Table 1  Effects of regular ingestion of black tea on body mass index, blood pressure, serum lipid concentrations, urinary F₂-isoprostane excretion and urinary 4-O-methylgallic acid excretion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hot water (n = 11) Mean ± S.E.M.</th>
<th>Black tea (n = 10) Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 ± 1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127 ± 5</td>
<td>123 ± 4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 ± 3</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.7 ± 0.2</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.25 ± 0.10</td>
<td>1.25 ± 0.09</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>F₂-isoprostane excretion (nmol/day)</td>
<td>3.4 ± 0.4</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>4-O-Methylgallic acid excretion (µg/day)</td>
<td>62 ± 16</td>
<td>87 ± 31*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.; *P < 0.001 for post-intervention differences between groups after adjustment for baseline values. LDL, low-density lipoprotein.

Blood pressure measurement

Blood pressure was measured using a Dinamap 1846SX/P oscillometric recorder (Critikon Inc., Tampa, FL, U.S.A.). Subjects rested in the supine position for 5 min. Blood pressure was then measured on the right arm on five occasions at 2-min intervals. The mean of the last two measurements was used in subsequent analyses. Blood pressure measurements were not disclosed to participants during the study.

Biochemical analyses

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol and triacylglycerol concentrations were analysed enzymically in fasting blood samples on a Cobas Mira analyser (Roche Diagnostics, Basel, Switzerland) with the use of reagents from Trace Scientific (Perth, Australia). Urinary creatinine concentrations were measured using a modified Jaffe reaction on a Hitachi 917 automated analyser (Boehringer Mannheim, Mannheim, Germany). Urinary F₂-isoprostane excretion was measured as a marker of in vivo oxidative stress. These compounds, which are formed by non-enzymic free-radical oxidation of arachidonic acid in membrane lipids, are thought to be one of the best available markers of in vivo lipid peroxidation [24]. F₂-isoprostanes were analysed in urine by GC-MS using negative chemical ionization. This method has been described in detail previously [25,26]. The intra-assay coefficient of variation was 8%. The 24-h urinary excretion of 4-O-methylgallic acid was measured as a marker of polyphenol intake from ingestion of black tea and as an indicator of compliance. The method used to measure 4-O-methylgallic acid has been described previously [27].

Statistics

Statistical analyses were performed using SPSS software (SPSS, Chicago, IL, U.S.A.). Results are presented as means ± S.E.M., and P < 0.05 was chosen as the level of significance. Pearson’s correlation coefficient (r) was used to determine the degree and direction of an association between two variables. The independent-samples t-test was used to compare baseline values between groups. The paired t-test was used to analyse within-group changes in dilatation. General linear models were used to examine differences in post intervention values after adjustment for baseline values. Logarithmic transformations were performed on variables that were not normally distributed.

RESULTS

There were 11 subjects in the hot water group (nine men and two women) and 10 subjects in the black tea group (seven men and three women). The mean age was not different between the groups (57.5 ± 2.8 and 60.9 ± 1.7 years respectively). At baseline, there were no significant differences between groups for any of the variables measured (Tables 1 and 2).

At baseline, endothelium-dependent FMD and endothelium-independent dilatation in response to GTN
Table 2  Baseline and post-intervention assessment of brachial artery function for subjects in the hot water and black tea groups
Values are means ± S.E.M.; *P = 0.008, **P = 0.03 for post-intervention differences between groups after adjustment for baseline values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hot water (n = 11)</th>
<th>Post-intervention</th>
<th>Black tea (n = 10)</th>
<th>Baseline</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting arterial diameter (mm)</td>
<td>3.97 ± 0.11</td>
<td>3.86 ± 0.13</td>
<td>3.76 ± 0.26</td>
<td>3.63 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Resting blood flow (ml/min)</td>
<td>139 ± 16</td>
<td>139 ± 22</td>
<td>140 ± 23</td>
<td>112 ± 19</td>
<td></td>
</tr>
<tr>
<td>Reactive hyperaemia (%)</td>
<td>616 ± 73</td>
<td>605 ± 92</td>
<td>481 ± 71</td>
<td>518 ± 43</td>
<td></td>
</tr>
<tr>
<td>FMD (%)</td>
<td>6.1 ± 0.9</td>
<td>5.7 ± 0.7*</td>
<td>5.1 ± 1.0</td>
<td>7.2 ± 1.2*</td>
<td></td>
</tr>
<tr>
<td>GTN-mediated dilatation (%)†</td>
<td>17.4 ± 1.1</td>
<td>16.7 ± 0.9**</td>
<td>20.3 ± 1.7</td>
<td>22.1 ± 2.0**</td>
<td></td>
</tr>
</tbody>
</table>

† n = 9 for black tea group.

Figure 1  Individual changes in endothelium-dependent FMD of the brachial artery (A) and endothelium-independent dilatation of the brachial artery in response to sublingual administration of 400 μg of GTN spray (B) from baseline to post-intervention for the hot water and black tea groups.

were significantly correlated (r = 0.54, P = 0.01). Age was significantly negatively associated with FMD (r = -0.43, P = 0.05), but not with the response to GTN (r = -0.31). The 24-h urinary excretion of 4-O-methylgallic acid was significantly positively associated with the response to GTN (r = 0.54, P = 0.01), but not with FMD (r = 0.27). Body mass index, blood pressure, serum total cholesterol and urinary F2-isoprostane excretion were not correlated with FMD or with the response to GTN.

Regular ingestion of black tea did not alter resting brachial artery diameter or reactive hyperaemia (Table 2). However, regular ingestion of black tea did result in significant increases in endothelium-dependent FMD (2.3%; P = 0.008) and in endothelium-independent GTN-mediated dilatation (4.2%; P = 0.03) of the brachial artery in comparison with regular ingestion of hot water (Table 2). These differences remained after adjustments for age, sex and body mass index.

The individual changes in endothelium-dependent and -independent dilatation of the brachial artery from baseline to post-intervention are presented in Figure 1. Increases in FMD were found for nine of the ten subjects drinking black tea. Increases in responses to GTN were found for four out of nine subjects drinking black tea (Figure 1). There was a large increase in FMD in one subject, but when this subject was excluded from the analysis the increase in FMD remained significant.

Regular ingestion of black tea also increased FMD from baseline (2.1%; P = 0.01) in absolute terms (Table 2), which represents a 41% improvement in FMD in terms of a percentage change. The increase in the response to GTN from baseline was not significant (P = 0.27). Both FMD and the response to GTN were not changed from baseline following regular ingestion of hot water.

The 24-h urinary 4-O-methylgallic acid concentrations increased during the regular ingestion of black tea in all 10 subjects. 4-O-Methylgallic acid excretion was increased with regular ingestion of black tea in comparison with hot water (P < 0.001). The 24-h urinary excretion of F2-isoprostanes was not altered by regular ingestion of black tea (Table 1).
DISCUSSION

We report that regular ingestion of black tea significantly improves brachial artery vasodilator function in people with mild elevations in serum cholesterol or triacylglycerol concentrations. Endothelium-dependent FMD of the brachial artery was significantly and consistently increased following regular ingestion of black tea.

Endothelial dysfunction is associated with the presence of CVD [11] and predicts cardiac events in prospective studies [8–10]. Furthermore, both endothelial cell and smooth muscle cell function can be impaired in patients with coronary heart disease [13]. Given the link between endothelial dysfunction and CVD, dietary factors that improve endothelial function may also lead to reduced risk of CVD.

The results of the present study are generally consistent with the suggestion that one mechanism by which black tea may reduce cardiovascular risk is via improved endothelial function. However, since endothelium-independent responses to GTN were also significantly improved, although less consistently, the effects of tea on brachial artery vasodilator function cannot be limited to an effect on endothelial function. Other possible benefits include an improvement in the bioavailability of endogenously released nitric oxide and/or an improvement in smooth muscle cell function.

We proposed that any improvements in vascular function might be mediated by antioxidant polyphenols found in black tea. Although we did not carry out a pharmacological study of the brachial artery response, it is recognized that the FMD response in peripheral conduit arteries is mediated principally by nitric oxide [28]. Reduced oxidative stress may result in enhanced synthesis and release, as well as reduced breakdown, of endothelial-derived nitric oxide [14]. An increase in the production and release of nitric oxide from the endothelium could result in an increase in FMD. Reduced breakdown of nitric oxide may result in an increase in the bioavailability of nitric oxide and increases in both FMD and the response to GTN.

We showed that 4-O-methylgallic acid, a marker of the absorption of black tea polyphenols [29], was significantly increased during the regular ingestion of black tea. This result is consistent with the ingestion of tea and the absorption of polyphenols from tea. Once absorbed, the polyphenols derived from tea circulate primarily as water-soluble compounds [30], but may be incorporated into cells, including endothelial and smooth muscle cells, and therefore have longer-term effects [31]. We found that F$_2$-isoprostane excretion was not altered by regular ingestion of black tea. Although this result is not consistent with the proposed mechanisms involved, F$_2$-isoprostanes are only one marker of oxidative stress, which may not have been sensitive or specific enough to detect any reduction in oxidative stress within the arterial wall.

Age was correlated with FMD at baseline in our study. The subjects involved in this study had a mean age of 59.8 ± 1.5 years, and had mild to moderate elevations in serum cholesterol or triacylglycerol concentrations. Age [32] and dyslipidaemia [33] are associated with endothelial dysfunction, and therefore the subjects involved in this study were likely to have had some degree of endothelial dysfunction. The mean baseline FMD in the present study was 5.6 ± 0.7%. In an age and sex-matched healthy normolipidaemic population we found a mean FMD of 6.4 ± 1.0% (results not shown). Endothelial dysfunction might be necessary in order for any reversible improvements to occur with appropriate interventions [34].

Studies that have examined the effects of water-soluble antioxidants on endothelial function in humans have generally shown improvements where some degree of endothelial dysfunction is likely to have been present at baseline [34–41]. Vitamin C given either as intravenous infusions [37–40] or as an oral supplement [34–36] has been found to reverse endothelial dysfunction. Improvements in ultrasound-assessed endothelial function have generally been endothelium-dependent [34–36]. Polyphenol-rich beverages have also been found to improve endothelial function in humans [41,42]. Agewall et al. [42] found that de-alcoholized red wine improved endothelial function acutely, but this study lacked the appropriate placebo control group. Stein et al. [41] demonstrated that regular ingestion of purple grape juice for 14 days improved both endothelium-dependent and -independent brachial artery dilatation, but again this was an uncontrolled observation. In addition, the results of experimental studies suggest that polyphenolic antioxidants can improve both endothelium-dependent and -independent vasorelaxation [18–21]. However, higher doses of polyphenols were needed to observe improvements in endothelium-independent relaxation than were required to improve endothelium-dependent relaxation [18,20]. Furthermore, results of a recent study have shown that ingestion of black tea has acute and longer-term effects in improving vascular function in patients with coronary artery disease [22].

In our study, the observed effects of black tea in significantly increasing endothelium-dependent FMD in comparison with water were quite consistent. The increase in FMD following ingestion of black tea relative to hot water represented an improvement in FMD of approx. 41%. Similarly, Duffy et al. [22] found that brachial artery FMD was improved by 65% following the acute ingestion of black tea, by 56% following regular ingestion of black tea for 4 weeks, and by 77% acutely when subjects were ingesting black tea chronically. In the present study, the effects of ingestion of black tea compared with hot water in increasing the response to
mechanism that may help to explain the association with oxides. These findings provide evidence for a novel enhancement in the availability and/or action of nitric oxide. These findings provide evidence for a novel mechanism that may help to explain the association between tea drinking and reduced risk of CVD.

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