Low dose of green tea catechins improves endothelial function and vascular smooth muscle cell reactivity in obese women

Daniel Alexandre Bottino, Débora Cherfan Goulart Nogueira, Ana Cláudia Lourenço, Vanessa Silveira Fortes, Andrea A. Berretta, Eliete Bouskela

BACKGROUND: The high prevalence of obesity in the world is associated with several health problems, with endothelial dysfunction figuring as a frequent feature. We investigated whether low dose consumption of green tea extract (catechins, < 200 mg/day) could modify endothelial function, lipid profile, fasting glucose and insulin, post load plasma glucose, inflammatory/oxidative stress biomarkers, and blood pressure in obese women.

METHODS: Sixteen obese women with body mass index (BMI) between 30 and 40 Kg/m^2, mean age 38 [33-40] years, consumed 600 ml green tea (3 x 200 ml) per day, containing 153.3 mg of catechins and 72.5 mg of caffeine, during three months. Endothelial function was evaluated through venous occlusion plethysmography by increment of peak forearm blood flow (FBF), after 5 min ischemia, during the reactive hyperemia response/baseline FBF. Endothelium-independent vasodilation was analyzed through peak FBF after 0.4 mg sublingual nitroglycerin/baseline FBF.

RESULTS: After 3 months, this consumption of green tea reduced BMI from 34.02 to 33.13, and diastolic blood pressure by 4 mmHg. The reactive hyperemia response/baseline FBF improved by 27%, and the endothelium-independent vasodilation by 12%. The blood biochemical profile, where all parameters were within the normal range, remained unaltered.

CONCLUSIONS: A low dose of green tea ameliorated the endothelial dysfunction present in obesity, indicating that its consumption should be encouraged in these patients, because endothelial dysfunction is an early marker of atherosclerosis.

KEYWORDS: Obesity; green tea; microcirculation; venous occlusion plethysmography; endothelial function.


Received for publication on July 10 2014; First review completed on July 22 2014; Accepted for publication on August 26 2014

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INTRODUCTION

Tea (Camellia sinensis), the most consumed beverage in the world after water, has been used by ancient cultures for its medicinal properties. Green tea has been widely consumed in Asia for many centuries and has now become one of the most consumed beverages in the world (20-22% of total consumed tea). It contains proteins, amino acids, carbohydrates, lipids, caffeine and is rich in polyphenols known as catechins, namely epicatechin (EC), epicatechin 3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin 3-gallate (EGCG), being the most abundant. Positive effects of green tea consumption on the cardiovascular system have been demonstrated in the literature. A study with 40,000 middle-aged Japanese showed that the use of more than 2 cups of green tea per day reduced cardiovascular risk around 27% compared to those who drank less than a half-cup per day. The catechins present in green tea exert anti-oxidant and biological effects, such as anti-inflammatory responses, reduction of platelet aggregation and body weight as well as improvement of vascular reactivity.

Obesity is today a major worldwide concern, and data from the World Health Organization show that its incidence has nearly doubled since 1980. In Brazil, temporal variation in the prevalence of overweight and obesity was investigated in 27 cities between 2006 and 2009: overweight increased from 43.0% to 46.6% and obesity from 11.4% to 13.8%.

Obesity is potentially a severe disease because it is accompanied by insulin resistance, glucose intolerance, dyslipidemia and other metabolic disorders associated to increased risk of cardiovascular disease or Type 2 Diabetes Mellitus. It has been associated with endothelial dysfunction, an early marker of atherosclerosis. It should be emphasized that any intervention that reverses or delays endothelial dysfunction may potentially prevent or delay the development of atherosclerosis. Therefore, the main objectives of this study were to determine if a low dose of green tea (less than 200 mg/day) would be enough to improve endothelial dysfunction. This was investigated at the brachial artery in obese women using venous occlusion plethysmography, and by studying changes in the lipid profile, inflammatory/oxidative stress biomarkers, blood pressure, weight, and waist and hip circumferences.
**MATERIALS AND METHODS**

Subjects and data collection

The study selected 50 out of a pool of 100 obese women (30 kg/m² < body mass index < 40 kg/m²) between 30 and 50 years old, in a consecutive order from Santa Casa de Misericórdia Hospital in Rio de Janeiro, Brazil, taking into account inclusion and exclusion criteria (Figure 1). All patients signed a written informed consent form approved by the Hospital Ethics Committee. The work was carried out according to the Helsinki Declaration.

Sample size calculation by free G Power software (Kiel University, Germany) was based on forearm blood flow during the reactive hyperemia response and after sublingual nitroglycerine. Previous studies indicate that a group with at least 15 patients was required. Exclusion criteria in our study were tabagism, Diabetes Mellitus, cardiovascular co-morbidities such as high blood pressure, coronary artery disease, heart failure, or other diseases that could compromise the microcirculation. Obesity, which can compromise the microcirculation, was obviously not an exclusion criterion.

Study design

In the present investigation, green tea extract with less than 200 mg/day of catechins, considered as low dose, was used. Patients drank 600 ml of green tea (Apis Flora Company, São Paulo, Brazil) per day [3 × cup of tea, 200 ml each: 1 tablespoon of powder (10.0 g) diluted in water, at room temperature] during 3 months. For compliance control, the product was delivered every 15 days, a questionnaire was filled every time, and the patient brought the empty can.

Cathechin Analyses in green tea

Catechin (C), Epicatechin (EC), Epigalocatechin 3-galate (EGCG) and Caffeine (CAF) in the powder extract were analyzed by High Performance Liquid Chromatography (HPLC) using as references epicatechin (L. 097K2568, 90% purity) and caffeine (L. 067K70S, ≥ 98.9% purity) from Sigma Aldrich (St. Louis, Missouri, USA), catechin hydrate (455,888L, ≥ 96.0% purity) from Fluka (Lausanne, Switzerland) and epigallocatechin-3-galate (L. 515-008, 92.9% purity) of ChromaDex (Boulder, Colorado, USA). HPLC analyzes were performed a on Shimadzu LC-20AT chromatograph (Kyoto, Japan) with a diode array detector SPC-M20A Shimadzu (Kyoto, Japan), operating at 275nm wavelength, and Shimadzu Shim-Pack CLC-ODS (M) (Kyoto, Japan) (4.6mm × 250mm, 5µm particle diameter, and pore size 100Å). Figure 2 shows the chromatographic profile of green tea extract components.

Each cup of tea contained of 5.0mg catechin, 38.0mg EGCG, 8.0mg epicatechin and 24.0mg caffeine as shown in Table 1.

Study protocol

Patients had three visits, both before and immediately after 3 months drinking of green tea:

1. **Visit 1 - Physical exam** - included weight, body mass index (BMI), Waist Circumference (WC), Hip Circumference (HC), Waist to hip ratio (WHR), blood pressure, and homeostatic model assessment. Values for insulin resistance (HOMA-IR, fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5) where values > 2.71 were considered as reference for the Brazilian population.11

2. **Visit 2 - Blood sample collection** after a 12 hours fast, the following parameters were determined: glucose, insulin, lipid profile (low density lipoprotein calculated by Friedwald’s equation),12 oral glucose tolerance test, soluble vascular cell adhesion molecule (sVCAM-1), soluble intercellular adhesion molecule (sICAM-1), soluble endothelium selectin (sE-Selectin), interleukin 6 (IL-6), Tumor Necrosis Factor α (TNF-α), hSC-reactive protein, and oxidized Low Density Lipoprotein (Ox-LDL).

3. **Visit 3 - Vasoreactivity (endothelial function)** Venous occlusion plethysmography.

Vasoreactivity (endothelial function) - Venous occlusion plethysmography

Subjects remained in a temperature controlled room (20-22°C) at supine position after a 6 hour fast. Forearm blood flow (FFB), in ml/min/100 ml tissue, was measured using a venous occlusion plethysmograph (Hokanson, EC6, DE, Bellevue, WA, USA) as described previously. Briefly, a mercury-in-silastic strain gauge was placed on the upper third of the forearm at its maximum circumference in the non-dominant arm, and kept at the heart level to detect dilatation. When venous drainage from the arms is briefly interrupted (cuff pressure of 50 mmHg), arterial inflow remains constant and blood can flow through the veins, and forearm blood flow increases due to endothelium-dependent vasodilatation. This increase in forearm blood flow is known as reactive hyperemia. The increase in blood flow is measured using the plethysmograph and is used to assess endothelial function.

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Figure 1 - Line plots for Weight (Box A) and Body Mass Index (Box B) considering all individual values. One can note that almost all patients presented decrease of weight/BMI after 3 months of green tea treatment. *p < 0.05.
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**Figure 2** - (A) Chromatographic profile of (1) catechin, (2) 3-epigallocatechin gallate, (3) caffeine and (4) epicatechin with their UV spectra and (B) green tea Extract (Apis Flora Ind. Com, Lot: 1730111), by HPLC.

**Table 1 - Daily Dosage of catechins from Green Tea extract.**

<table>
<thead>
<tr>
<th>Catechin</th>
<th>Green tea extract (1 tablespoon - 10.0 g)</th>
<th>Green tea extract (3 tablespoons - 30.0 g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>5.0 mg</td>
<td>15.0 mg</td>
</tr>
<tr>
<td>EGCG</td>
<td>38.0 mg</td>
<td>114.0 mg</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>8.0 mg</td>
<td>24.0 mg</td>
</tr>
<tr>
<td>Caffeine</td>
<td>24.0 mg</td>
<td>72.0 mg</td>
</tr>
</tbody>
</table>

EGCG - epigallocatechin gallate.

Statistical Analysis

Results are presented as mean ± SD or as median [first-third quartiles] when non-normal distribution occurred. Data were analyzed with Statistica 8.5 software (Statsoft, Tulsa, Oklahoma, USA) using paired Wilcoxon test for dependent samples in comparisons of variables before and after the 3-month period. Significance was set as p < 0.05.

**RESULTS**

During the 12 weeks, patients consumed 600 ml/day of green tea corresponding to 11.0mg of EGCG (total amount of catechins was 153.0 mg/day) and 72.0mg of caffeine, as shown in Table 1. Sixteen of fifty patients, (38 [33.5-40.0] years old), completed the study. The main reason given for the withdrawal was failure to lose weight.

Figure 1 shows Weight (Box A) and BMI (Box B) line plots for all individual values. There was a decrease of weight from 86.35 [83.00-94.25] to 86.00 [81.50-92.00] kg after 3 months of treatment with Green Tea (p = 0.03). BMI decreased from 34.02 [32.05-35.62] to 33.13 [32.28-35.05] (p = 0.03). Waist and Hip circumferences were also reduced (1cm each) accompanied by decrease of WHR (before: 0.89 [0.84-0.93], after: 0.88[0.83-0.93], p = 0.004). The cardiovascular

enter the forearm but cannot escape. This results in a linear increase in forearm volume over time which is proportional to arterial blood inflow. In order to avoid arterial-venous shunting in the hand, wrist cuff occlusion was performed, inflated 1 min before FBF and set 40 mmHg above systolic blood pressure.

The measuring sequence was basal flow 1, reactive hyperemia response after 5 min brachial artery occlusion, basal flow 2 (measured after 20 min) and flow 5 min after 0.4mg sublingual nitroglycerine (Nitrolingual BurnsAdler Pharmaceuticals Inc, Charlotte, NC, USA). For statistical analysis, %Hyper (blood flow during the reactive hyperemia response divided by basal flow 1) and %Nitro (blood flow after nitroglycerine divided by basal flow 2) were calculated.
system showed reduction of diastolic blood pressure from 75 [73-82] mmHg to 69 [67-72] mmHg (p = 0.002) as shown in Table 2. No differences were detected at baseline and after 3 months of green tea for biochemical parameters, fasting glucose and insulin, post-load plasma glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides. It is important to note that all of them were in the normal range as shown in Table 3. Although patients in this study were obese, they did not have insulin resistance as also shown in Table 3.

Analysis of pro-inflammatory biomarkers (sVCAM, sICAM-1, sE-Slectin, IL-6, TNF-α and hsC-reactive protein) and oxidative stress markers (oxidized-LDL) did not reveal any significant changes with green tea as shown in Table 4. On the other hand, two important changes in endothelial function could be seen: (i) brachial forearm blood flow increased during the reactive hyperemia response from 4.5 [4.54-5.01] to 5.83 [4.46-6.56], p < 0.01 (27% increase); and (ii) blood flow increased during the reactive hyperemia response from 1.26 [1.13-4.57 [3.54-5.01] to 5.83 [4.46-6.56], p < 0.01 (27% increase), as shown in Figure 3.

**DISCUSSION**

Obesity is a serious condition associated with increased cardiovascular morbidity and mortality. In this study, we have investigated obese women, without a diagnosis of metabolic syndrome, aged around 38 years, with few changes of anthropometric data after green tea consumption: BMI, weight and WHR slightly decreased over the three months of the study. Catechins and caffeine act synergistically stimulating the sympathetic nervous system resulting in increased energy expenditure and lipolysis which is hypothesized to explain the slimming effect of green tea.14,15 Probably our poor results were due to the low dose of catechins (153.0 mg/day) and caffeine (7.2 mg) used. Cardoso et al. used 40 mg/day caffeine and non-specified doses of catechins in green tea consumed by obese patients during 8 weeks, and resulting in 5.7 kg of weight loss.16 On the other hand, Jurgens et al conducted a meta-analysis of clinical trials with green tea during 2-3 months and found a small weight loss of -0.04 kg outside Japan, and ranging from -0.2 kg to -3.5 kg in Japan. They also concluded that weight loss was not maintained over time.17 Hsu et al did not show any statistical differences between green tea and placebo for BMI or body weight in obese patients.18 Green tea reduced diastolic pressure from 75 mmHg to 69 mmHg in our study. However the cardiovascular benefits of reducing normal blood pressure are yet to be determined.

Biochemical variables were within the normal range in our study and patients presented no insulin resistance (HOMA-IR < 2.71). Therefore, our patients presented low cardiovascular risk. Green tea failed to reduce these variables. One may speculate that catechins could have better effects under pathological situations. Indeed, green tea, given in capsules of 250 mg/day, was successfully used to reduce increased LDL-cholesterol and cholesterol levels after 8 weeks of treatment.19 Other studies have demonstrated positive effects of green tea on oxidative stress (Li et al., 2010)6 and in reducing glycaemia in type 2 diabetes.20

Pro-inflammatory and anti-oxidative biomarkers failed to decrease, in our group, after 3 months of green tea. Similar results were found by Basu et al in obese subjects with metabolic syndrome after 8 weeks of consumption of a green tea beverage (440 mg/day EGCG) or of an extract (two capsules with total 460 mg/day EGCG) showing no effect of catechins in decreasing inflammatory biomarkers.5

Probably higher (still to be determined) doses of catechins should be administered to decrease the inflammatory profile in obese patients. For example, Bogdanski et al. studied obese patients with hypertension, who were given 208 mg EGCG during 3 months: a decrease of TNF-α and C reactive protein was observed.21 Yang et al demonstrated an association between increasing habitual tea consumption (mainly green and oolong teas) and a gradual decrease of the risk of developing hypertension (<120 ml/day, risk decrease: 46%; >600 ml/day, risk decrease: 65%) in a community-based study in Taiwan.22

**Table 2 - Clinical data.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 16)</th>
<th>After 3 months treatment (n = 16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference (cm)</td>
<td>99 [93-107]</td>
<td>98 [91-105]</td>
<td>0.003**</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>115 [110-119]</td>
<td>114 [110-117]</td>
<td>0.004**</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.89 [0.84-0.93]</td>
<td>0.88 [0.83-0.93]</td>
<td>0.004**</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>117 [110-130]</td>
<td>115 [110-122]</td>
<td>0.163</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75 [73-82]</td>
<td>69 [67-72]</td>
<td>0.002**</td>
</tr>
</tbody>
</table>

BP = blood pressure. *p < 0.05, **p < 0.01.

**Table 3 - Biochemical data from obese patients.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 16)</th>
<th>After 3 months treatment (n = 16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>88 [83-94]</td>
<td>90 [82-96]</td>
<td>0.98</td>
</tr>
<tr>
<td>Fasting insulin, µU/ml</td>
<td>9 [7-13]</td>
<td>11 [8-12]</td>
<td>0.38</td>
</tr>
<tr>
<td>Postload Plasma Glucose, mg/dl</td>
<td>119 [105-137]</td>
<td>119 [106-128]</td>
<td>0.82</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.02 [1.52-2.85]</td>
<td>2.24 [1.54-2.73]</td>
<td>0.61</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>190 [164-200]</td>
<td>182 [154-211]</td>
<td>0.72</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>53 [45-62]</td>
<td>50 [46-61]</td>
<td>0.75</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>117 [91-132]</td>
<td>118 [92-135]</td>
<td>0.84</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>79 [61-93]</td>
<td>82 [62-114]</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Table 4 - Inflammatory and oxidative stress biomarkers.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 16)</th>
<th>After 3 months treatment (n = 16)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVCAM (ng/ml)</td>
<td>24.63 [21.26-28.64]</td>
<td>26.03 [23.66-28.01]</td>
<td>0.86</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>218.69 [192.14-263.18]</td>
<td>238.71 [188.78-261.18]</td>
<td>0.87</td>
</tr>
<tr>
<td>SE-SELECTIN (ng/ml)</td>
<td>3.24 [1.80-4.10]</td>
<td>3.21 [1.49-4.10]</td>
<td>0.92</td>
</tr>
<tr>
<td>Oxidized - LDL (U/l)</td>
<td>46 [40-54]</td>
<td>53 [44-55]</td>
<td>0.18</td>
</tr>
<tr>
<td>IL-6 HS (pg/ml)</td>
<td>2.10 [1.33-2.95]</td>
<td>1.98 [1.45-2.73]</td>
<td>0.78</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>3.55 [1.73-10.13]</td>
<td>3.47 [2.04-9.95]</td>
<td>0.71</td>
</tr>
<tr>
<td>hS C-reactive protein, mg/dl</td>
<td>0.42 [0.16-0.79]</td>
<td>0.46 [0.18-0.70]</td>
<td>0.81</td>
</tr>
</tbody>
</table>

sVCAM = soluble Vascular Cell Adhesion Molecule; sICAM = soluble Intercellular Adhesion Molecule; sE-SELECTIN = soluble endothelial selectin; IL-1 beta = Interleukin-1 beta; IL-6 HS = Interleukin-6 High Sensitivity; TNF-alpha = Tumor Necrosis Factor -alpha.

Obesity presents impaired endothelium-dependent vasodilatation, considered an independent risk factor for cardiovascular disease. Pasimeni et al have investigated endothelial dysfunction in uncomplicated obesity and found blunted endothelium-dependent vasodilatation in obese patients by forearm venous occlusion plethysmography compared to lean controls. In our study, green tea promoted a remarkable increase of forearm blood flow during the reactive hyperemia response. In this process, many metabolites are released such as nitric oxide (NO), adenosine, endothelium-derived hyperpolarizing factor (EDHF) and prostaglandins, and the participation of the endothelium via NO is inferred in the process. Green tea improved the endothelium-independent vasodilatation as well (from 1.26[1.13-1.38] to 1.41[1.15-1.50], p = 0.002). Endothelial dysfunction, expressed by reduced nitric oxide (NO) availability, is the first precursor of atherosclerosis, which produces cardiovascular diseases which are responsible for high mortality worldwide. When green tea improves vasoreactivity through nitric oxide, it protects the cardiovascular system from possible future environment aggressions. As our obese patients had no insulin resistance and we improved vasoreactivity with green tea, we may consider them in the healthy obesity group. Indeed, up to 30% of obese people do not display typical metabolic obesity-associated complications. Thus, we may say that catechins positively influence endothelial cells and vascular wall, and, consequently, promote health.

STUDY LIMITATION

Our study has limitations that should be pointed out: excessive number of drop-outs; VOP through the reactive hyperemia response investigates not only the endothelial function (30% of recorded vasoreactivity), but also prostaglandins, adenosine and endothelium-dependent hyperpolarizing factor, and the lack of a placebo group. However, the vasodilatation of the brachial artery was evident with high statistical significance (p = 0.001) discarding the possibility of a placebo effect.

In conclusion, this study has shown that green tea with 153.33 mg/day of catechins and 72.45 mg/day of caffeine improved endothelial dysfunction and vascular wall relaxation in obese women, but did not interfere with lipid metabolism, inflammatory or oxidative stress biomarkers.

AUTHOR’S CONTRIBUTION

Bottino DA: Data (collection and analysis), Research design, wrote the manuscript.
Nogueira DCG: Data (collection and analysis), Research Design.
Lourenço AC: Chemical extraction of green tea, chemical analysis of final product.
Fortes VS: Chemical analysis of extracts.
Berretta AA: Design of biochemical project, final approval of manuscript.
Bouskela E: Data (collection and analysis), Research design, wrote the manuscript.
Conflict of Interest: None.

RESUMO

OBJETIVO: A alta prevalência de obesidade no mundo traz vários problemas de saúde com a disfunção endotelial como um problema frequente. Investigamos se o consumo de baixa dose de extrato de chá verde (catequinas < 200 mg/dia) pode modificar a função endotelial, o perfil lipídico, a glicemia e a insulina de jejum, a glicose plasmática pós carga, todos biomarcadores inflamatórios de estresse oxidativo e a pressão arterial em mulheres obesas.
MÉTODOS: Dezesszeis mulheres obesas com índice de massa corporal (IMC) entre 30 e 40 kg/m², com idade média de 38 [33-40] anos, consumiram 600 ml (3 x 200 ml) chá verde por dia, contendo 153,3 mg de catequinas e 72,5 mg de cafeína, durante três meses. A função endotelial foi avaliada através da pleitismografia de oclusão venosa por aumento de pico de fluxo sanguíneo no antebraço, após 5 min de isquemia, durante a resposta hiperemia reativa comparada com a condição basal do fluxo sanguíneo de antebraço. A vasodilação endotélio-independente foi analisada através de pico de fluxo sanguíneo de antebraço após 0,4 mg de nitroglicerina a sublingual versus fluxo basal.

RESULTADOS: Após três meses, o consumo de chá verde reduziu o índice de massa corpórea 34,02 de 33,13 e pressão arterial diastólica em 4 mmHg; a resposta de hiperemia reativa versus fluxo basal de antebraço melhorou em 27%; a vasodilação endotélio-independente melhorou em 12%; o perfil bioquímico do sangue, onde todos os parâmetros estiveram sempre dentro da faixa de normalidade, permaneceu inalterado.

CONCLUSÕES: Uma dose baixa de chá verde melhorou o quadro de disfunção endotelial presente na obesidade, reconhecida como um marcador precoce da aterosclerose, indicando que o seu consumo deve ser incentivado nesses pacientes.

■ REFERENCES