Applied nutritional investigation

Association between soy and green tea (*Camellia sinensis*) diminishes hypercholesterolemia and increases total plasma antioxidant potential in dyslipidemic subjects

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Abstract

Objective: To evaluate the hypolipemic and antioxidant effects of soy and green tea alone and/or in association in dyslipidemic subjects.

Methods: One hundred dyslipidemic individuals were allocated into four groups. The soy group ingested 50 g of soy (*kinako*) daily, and the green tea group ingested 3 g of green tea in 500 mL of water per day. A third group ingested 50 g of soy and 3 g of green tea daily, and the control group had a hypocholesterolemic diet. Evaluations were performed at baseline and after 45 and 90 d. Plasma levels of total cholesterol, high-density lipoprotein, and triacylglycerols were evaluated by automated methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. LDL was isolated by ultracentrifugation. Total plasma antioxidant capacity and plasma levels of total lipid hydroperoxides and those linked to LDL were evaluated by chemiluminescence. The results were expressed as median values and their 25th to 75th percentiles, with a 5% level of significance.

Results: No significant difference occurred in LDL, high-density lipoprotein cholesterol, and triacylglycerol levels across groups. However, a statistically significant difference in total cholesterol occurred within the soy/green tea group 45 and 90 d after intervention. No statistically significant difference occurred in plasma levels of lipid hydroperoxides or those linked to LDL in any of the groups studied. All the groups that used soy and/or green tea presented increased total plasma antioxidant potential. All the groups that used soy and/or green tea presented increased total plasma antioxidant potential.

Conclusion: Soy and green tea, alone or in combination, increased the total antioxidant potential of hypercholesterolemic patients, whereas only the combination decreased total cholesterol levels. © 2008 Elsevier Inc. All rights reserved.

Keywords: Soy; Isoflavones; Green tea; Catechins; Antioxidants; Dyslipidemic human subjects

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Introduction

In the past 10 y, numerous researchers and the food industry have demonstrated growing interest in polyphenols. The main reason is the recognition of their antioxidant properties, their great abundance in nature, and their probable role in the prevention of several pathologies associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative disorders. The beneficial effects of these substances depend on the quantity consumed and their bioavailability [1].

Several studies have considered that the hypocholesterolemic effect of vegetable proteins, in particular soy, is due to the high fecal excretion of steroids as a consequence of intestinal absorption reduction. A large fecal excretion of steroids could lead to increased biliary acid production from plasma cholesterol, thus reducing its own levels [2]. Moreover, soy protein amino acids and isoflavones appear to exert synergic action on improving lipid metabolism with the activation of receptors for low-density lipoprotein (LDL) [3].

From the chemical viewpoint, polyphenol compounds (such as isoflavones or flavanoids found in soy, tea, or wines) are the most promising natural antioxidants with regard to the inhibition of atherogenic modifications of LDL [4]. Even though the action mechanism of these polyphenols is not fully understood, the hypolipemic and protective actions against LDL oxidation are attributed to genistein and daidzein, in the case of soy, and to catechins, in the case of tea [5,6]. Considering the lack of consensus regarding these actions in the use of soy and green tea and, to our knowledge, the lack of studies that have made use of this association in humans to evaluate the lipid profile and analyze the total plasma antioxidant potential, the present study is justified.

Materials and methods

Casuistic

One hundred four hypercholesterolemic patients, 40 male and 64 female, were selected at a cardiology clinic in Londrina, Paraná, Brazil. Four patients, one male and three female, left during the development of the protocol for personal reasons. Therefore, 100 individuals, 39 male and 61 female, with a mean age of 52.2 ± 12.2 y, participated in the study. The patients were sedentary and oriented to make no changes in their habitual lifestyle. This was monitored by means of a questionnaire that was filled out every time the patients returned to the clinic for blood collection. Furthermore, compliance was measured by this questionnaire and counting green tea and soy packages when patients returned for their clinical and nutritional evaluations. Body weight was measured to the nearest 0.1 kg by using an electronic scale, with individuals wearing minimal clothing; height was measured to the nearest 0.1 cm by using a stadiometer and was used to calculate body mass index (BMI; kilograms per square meter). The baseline BMIs (mean ± SD) for each group (green tea, soy, soy/green tea, and control) were 26.2 ± 3.3, 25.4 ± 3.2, 26.1 ± 3.3, and 27.1 ± 3.3 kg/m², respectively. Subjects in each comparison consumed similar dietary fat.

The inclusion criteria were patients >18 y old, with total cholesterol plasma levels ≥220 mg/dL. Individuals who presented with diabetes mellitus, hypothyroidism, or other causes of secondary dyslipidemia were excluded from the study. Women receiving hormone replacement therapy and patients who were using hypolipemic medication, isoflavones, soy, and/or green tea for ≥40 d before entry into the protocol were also excluded. No modification occurred in the treatment of patients receiving hypertensive medication.

All participants were informed regarding the procedures they would undergo and signed a term of free informed consent. This study and the term were approved by the ethics in human research committee of the Londrina University Hospital.

Study design: randomized controlled trial

Patients were randomized to one of four groups (green tea, soy, soy/green tea, and control) using sealed opaque envelopes indicating treatment allocation. Randomization envelopes were prepared at study inception and a random number sequence was obtained from a table of randomized numbers. After orientation by a nutritionist, participants used the products they were offered for 90 d. The soy group (n = 25) ingested 50 g/d of kinako (toasted ground soy bean, with 31.2 mg of isoflavones) throughout their daily meals: breakfast, lunch, and dinner. The green tea group (n = 25) ingested 500 mL of tea (dry leaves 3 g/vol, prepared by brewing for 10 min in 500 mL of water containing 145 mg of epigallocatechin-3-gallate [EGCG]) throughout the day. The soy/green tea group (n = 25) ingested the same as the soy and green tea groups. The control group (n = 25) received a standard hypocholesterolemic diet low in saturated fat and cholesterol contents and high in polyunsaturated fatty acids (PUFAs) [7]. From knowledge of the habitual intake of each individual, the control diet consisted of decreased red meat intake (beef and pork, a daily habit in Brazil), eggs, bacon, and replacing milk and butter with skimmed milk and margarine.

All groups were evaluated on three occasions by a blind assessor: at baseline and after 45 and 90 d. The soy was provided by the Brazilian Farming Research Company (EMBRAPA-SOJA, Londrina, Paraná, Brazil) and processed by the Kinasoy Company of Londrina. The green tea brand used was Yamamotoyama (Midori Indústria de Chá Ltda., São Paulo, Brazil) and donated by the manufacturer.
The batches and procedures of the products used by these groups were the same.

**Analytical procedures**

**Extraction and separation of isoflavones for high-performance liquid chromatography**

Separation and quantification of the isoflavones was performed using a Waters liquid chromatograph (model 2690, Watters & Associates, Milford, MA, USA) with an automatic sample injector [8]. The patients ingested the following concentrations of aglycone isoflavones in milligrams-percent (m/m) per 100 g of *kinako*: daidzein 23.2, glycitein 31.2, and genistein 8.0.

**Determination of catechin EGCG by high-performance liquid chromatography in green tea samples**

Separation and quantification of EGCG was performed using a Waters liquid chromatograph (model 2690) with an automatic sample injector [9]. The concentration of EGCG in 100 g of green tea leaves used by the patients was 4.84 g (m/m). The amount of EGCG present in the green tea ingested by the patients was 145 mg/500 mL.

**Blood collection**

Approximately 20 mL of blood was collected in vacuum tubes containing ethylene-diaminetetra-acetic acid or without a coagulant. Immediately after collection, the samples were chilled on ice. To obtain the plasma, the tubes were placed in a Fanem refrigerated centrifuge (Model 280 R, Guarulhos, São Paulo, Brazil) at 4°C and rotated at 3000 × g.

**LDL separation**

To obtain the LDL level, the following gradient was prepared: 0.5 mL of saline (density [d] = 1.182 g/mL), 3.0 mL of plasma (d = 1.090 g/mL), 1.0 mL of saline (d = 1.056 g/mL), 1.0 mL of saline (d = 1.045 g/mL), 1.5 mL of saline (d = 1.034 g/mL), 1.5 mL of saline (d = 1.024 g/mL), and 1.0 mL of saline (d = 1.019 g/mL). The pH of all these saline solutions was adjusted to 7.4 [10]. This gradient was centrifuged in a Sorvall ultracentrifuge (model Pro-80, Sorvall, Newtown, CT, USA), at 284 000 × g for 24 h at 4°C.

From the top of the tube, aliquots were removed in the following order: 1.0 mL, the aliquot considered to be very LDL; 6.0 mL, the aliquot considered to be LDL; and 3.5 mL, the aliquot considered to be infranatant.

Next, the LDL aliquot was dialyzed in 0.9% NaCl solution for 12 h to remove the excess KBr.

**Plasma parameters**

Biochemical quantifications were measured in a biochemical autoanalyzer (Dade AR, Newark, NJ, USA) using Dade Behring kits. Total cholesterol was quantified by the cholesterol oxidase technique. Whenever possible, LDL cholesterol was calculated by the Friedewald equation [11]. High-density lipoprotein (HDL) cholesterol was quantified by the selective precipitation method using a buffered phosphotungstate reagent. Triacylglycerols were analyzed by means of the bichromatic enzymatic technique, using lipase and glycerol dehydrogenase.

**Total plasma antioxidant capacity**

Total plasma antioxidant capacity (TRAP) was evaluated by chemiluminescence [12]. This methodology detects hydrophilic antioxidants in the plasma. The 2,2 azobis produces peroxyl radicals rapidly, through an interaction centered on carbon and molecular oxygen, causing the oxidation of lipids and proteins in biomolecules [13]. These free radicals react with luminol, which acts as a signal amplifier, producing chemiluminescence. This reaction is inhibited by superoxide dismutase, catalase, and vitamin E analogs. The results were expressed as micromol Trolox. This experiment was conducted in a Beckman β counter (model LS 6000, Beckman, Fullerton, CA, USA) in non-coincident counting mode for 30 min and with a response range of 300–620 nm.

**Lipid hydroperoxides**

Evaluation of the formation of lipid hydroperoxides was also realized by chemiluminescence [14]. The reaction medium was placed in 20-mL plastic flasks for scintillation. The medium consisted of 1750 µL of 30 mM phosphate buffer, pH 7.4, and 20 mM (v/v) KCl added to 250 µL of plasma or LDL and 20 µL of tert-butylhydroperoxide at a final concentration of 3 mM in 2.0 mL of reaction medium.

Chemiluminescence was measured in a Beckman β counter (model LS 6000) in non-coincident counting mode for 30 min and with a response range of 300–620 nm. The scintillation flasks were maintained in darkness and the reaction was realized in a dark environment to avoid phosphorescence of the flasks. This experiment was conducted at a temperature of 30°C and the results were expressed in counts per minute.

**Statistical analysis**

Initially, a descriptive analysis of the data was performed. Because the variables did not confirm to the assumptions of normality (lipid and antioxidant profiles), the data were presented as median and 25th to 75th quartiles. The percentage of change of outcome measurements was performed between baseline and final assessment (90 d). To compare the variables and percentage of change among and within groups, at three moments in time, analysis of variance for repeated measures was used. Mauchly’s sphericity test was applied, and when it was violated, technical corrections were performed using the Greenhouse-Geisser test. When the F test was significant, complemental analysis was performed using Bonferroni’s test for multiple comparisons. A statistical significance of 5% was adopted (P ≤
Results

Mean ages were 51.3 ± 13.4 y in the green tea group (9 men and 16 women), 52.7 ± 10.8 y in the soy group (11 men and 14 women), 57.0 ± 11.2 y in the soy/green tea group (8 men and 17 women), and 48.0 ± 12.4 y in the control group (11 men and 14 women).

The TRAP presented statistically significant differences within all groups and between the green tea and control groups. After the 90-d intervention, the tea group presented a significant difference compared with the control group and with percentage of change (17% of improvement in the green tea group versus 30.9% of worsening in the control group, P < 0.0001). After 45 and 90 d of treatment with green tea, there was a significant difference (P = 0.007) from baseline values. In relation to the soy group, significance was found when comparing baseline and 45-d interventions (P < 0.001) with 19% of change. In the soy/green tea group, significant differences were found between the baseline evaluation and the 45-d intervention (P = 0.006) and between the baseline evaluation and the 90-d intervention (P = 0.007) with 14% of change. With the exception of the control group, at the end of intervention, all groups presented an increase in antioxidant defenses. Statistically significant increases in TRAP occurred for the soy, green tea, and soy/green tea groups and a significant decrease in the control group (Table 1).

Regarding the pro-oxidant profile, no statistically significant differences occurred in plasma lipid hydroperoxide levels (23%, 45%, −14%, and 26% in each group, respectively) or in isolated LDL hydroperoxide levels (15%, −3%, −11.7%, and −16%) at any evaluation moment for all groups studied.

No statistically significant differences occurred between BMI and abdominal circumference when comparing the groups studied (data not shown).

Regarding the lipid profile, no statistically significant differences were observed in the concentrations of triacylglycerols (−12%, 13%, 36%, and 36%), HDL cholesterol (1.1%, 5%, 5%, and 0.6%) and LDL cholesterol (−6%, 6%, 3%, and −1.6%) among groups. However, total cholesterol presented a statistically significant reduction within the soy/green tea group (P = 0.002) between the baseline evaluation and after the 45-d intervention and 90 d (P = 0.028) with 6% of change (Table 2). The percentages of change in the other groups were −2%, 6.9%, and 3.7%.

Discussion

Diets based on polyphenols, substances renowned for their antioxidant power, have been associated with a decrease in cardiovascular disorders [15]. Among soy-based products, kinako (toasted ground soy beans) possess a large concentration of isoflavones [16]. The consumption of approximately 50 mg of isoflavones per day is recommended for ideal hypocholesterolemic action [17]. In this study, the soy and soy/green tea groups ingested 31.2 mg of isoflavones per day. In the tea ingested by the patients, the daily concentration of EGCG was 145 mg/500 mL (3 g of leaves in 500 mL of water). In the groups under study (soy, green tea, soy/green tea), statistically significant increases in TRAP were observed. Similar results in relation to soy use were

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Green tea group</th>
<th>Soy group</th>
<th>Soy/green tea group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><strong>TRAP</strong></td>
<td>45 d</td>
<td>50 d</td>
<td>50 d</td>
<td>50 d</td>
</tr>
<tr>
<td><strong>LOOH</strong></td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><strong>Median (25–75)</strong></td>
<td>21 900 (16 050–26 280)</td>
<td>21 932 (17 125–23 650)</td>
<td>17 450 (13 837–24 685)</td>
<td>14 000 (9600–14 750)</td>
</tr>
<tr>
<td><strong>LDL-LOOH</strong></td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><strong>Median (25–75)</strong></td>
<td>9927 (8669–13 522)</td>
<td>11 371 (8357–17 549)</td>
<td>14 172 (8093–17 393)</td>
<td>4471 (2967–6984)</td>
</tr>
</tbody>
</table>

**TRAP:** total plasma antioxidant capacity

**LOOH:** low-density lipoprotein lipid hydroperoxides

**LDL-LOOH:** lipid hydroperoxides

* Statistically significant difference between green tea and control groups (P < 0.0001).

† Statistically significant difference within green tea group, baseline versus 45 d (P = 0.007) and baseline versus 90 d (P = 0.007).

§ Statistically significant difference within soy/green tea group, baseline versus 45 d (P = 0.006) and baseline versus 90 d (P = 0.007).

| Statistically significant difference within control group, baseline versus 45 d (P = 0.02) and baseline versus 90 d (P = 0.001).
found by other investigators in a study involving women who ingested 40 g of soy-based products with 110 mg of isoflavones for 4 wk [18].

In a study involving healthy volunteers who ingested 300 mL (5.0 g of leaves) and 450 mL (7.5 g of leaves) of green tea, a significant increase in TRAP was also obtained [19]. Similarly, other researchers have observed an expressive improvement in TRAP when offering five cups of green tea per day over 4 wk to their patients [20].

According to the investigators, the increase in plasma antioxidant defenses verified by TRAP was important for the prevention of oxidation compounds present in the blood [20].

In the control group, in which a reduction in the ingestion of cholesterol and saturated fatty acids and an increase in PUFAs was planned, a statistically significant reduction in antioxidant defenses occurred, also verified by TRAP. This may be due to increased ingestion of PUFAs because the individuals of this group maintained their BMI after 90 d, revealing that no alteration in caloric ingestion occurred during the study.

Diet rich in monounsaturated fatty acids generate particles of LDL that are less susceptible to oxidation when compared with the LDL isolated from individuals who ingest a PUFA-rich diet [21]. Some studies have indicated a large concentration of oxidation products, such as conjugated dienes or plasma hydroperoxides, after the consumption of diets involving a large quantity of PUFAs compared with diets rich in monounsaturated fatty acids. The formation of hydroperoxides in PUFA-rich diets is directly proportional to the quantity of double bonds contained in the fatty acids [22].

In a study involving young pregnant women, a high level of isoprostane (a product of lipid peroxidation) excretion was detected. These patients ingested diets rich in PUFAs, specifically linoleic acid. Because these acids can serve as substrate for lipid peroxidation, PUFA-rich diets could contribute to increased oxidative stress, which is an imbalance between the production of pro-oxidant and antioxidant substances in the organism [23]. Although all groups that ingested polyphenols presented increased TRAP, no reduction in the formation of lipid hydroperoxides was observed. In contrast, other investigators found significant reductions in lipid hydroperoxide levels in women who ingested soy protein for 4 wk [17].

The present study also presented no statistically significant reduction in hydroperoxide levels in relation to isolated LDL. Given the hydrophilic characteristics of isoflavones, they probably present certain difficulties when incorporating into LDL particles [24]. Thus, only a slight interiorization of isoflavones occurs in these lipoproteins. This fact could have contributed to the absence of lower hydroperoxide levels in the isolated LDL of the patients.

After studying the inhibition of lipid peroxidation in vivo by polyphenol-rich beverages, such as green and black teas and red and white wines, researchers observed that these increased plasma antioxidant potential by different degrees in healthy individuals. The investigators concluded that the association of diverse polyphenol classes could act synergistically, leading to a significant increase in antioxidant defenses in vivo [25].

In a recent meta-analysis of 17 studies, it was concluded that the risk of myocardial infarct diminished 11% with the ingestion of three cups of green tea per day [26]. The individuals who made up part of the present study presented moderate hypercholesterolemia, with the me-

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**Table 2**

Medians and their quartiles (25th–75th) of total plasma cholesterol, HDL, LDL, and TG in the groups studied at each stage

<table>
<thead>
<tr>
<th></th>
<th>Green tea group</th>
<th>Soy group</th>
<th>Soy/green tea group</th>
<th>Control group</th>
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<tbody>
<tr>
<td><strong>Total cholesterol</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>25 240 (228.5–274.5)</td>
<td>25 263 (247.5–292.5)</td>
<td>25 257 (244–290)</td>
<td>25 257 (244.5–276)</td>
</tr>
<tr>
<td>45 d</td>
<td>25 250 (228–269.5)</td>
<td>25 262 (240.5–309.5)</td>
<td>25 245 (224.5–261)*</td>
<td>25 250 (234–264)</td>
</tr>
<tr>
<td>90 d</td>
<td>25 264 (230–278.5)</td>
<td>25 259 (240–306.5)</td>
<td>25 246 (237.5–269)</td>
<td>25 255 (232–279.7)</td>
</tr>
</tbody>
</table>

| **HDL**              |                 |           |                     |               |
| Baseline             | 25 44 (35.5–54.5) | 25 44 (32.75–53.0) | 25 45.5 (37.75–53.50) | 25 44 (38–53.5) |
| 45 d                 | 25 43 (34–53.5) | 25 44.5 (33.50–59.25) | 25 44 (39.25–58.0) | 25 46 (38–54.5) |
| 90 d                 | 25 45 (34.5–56.5) | 25 45 (38.25–58.75) | 25 49.5 (44.5–56.0) | 25 45 (35.7–51.0) |

| **LDL**              |                 |           |                     |               |
| Baseline             | 25 163 (143.50–202) | 25 181 (160.5–215) | 25 187 (167–196) | 25 178 (148.5–196.5) |
| 45 d                 | 25 172 (156–181.75) | 25 177 (161.2–199.75) | 25 170.5 (156.75–192.25) | 25 173 (153.5–189.5) |
| 90 d                 | 25 180 (166–206) | 25 177.5 (154–205.75) | 25 183 (162.25–193.25) | 25 184.5 (146.5–203.7) |

| **TG**               |                 |           |                     |               |
| Baseline             | 25 132 (88–198) | 25 177 (122.5–242) | 25 170 (109.5–295.5) | 25 147 (122.5–226.5) |
| 45 d                 | 25 149 (100.5–198) | 25 176 (136–215) | 25 141 (92.5–247.5) | 25 159 (118–201) |
| 90 d                 | 25 144 (98–168.5) | 25 174 (114–242.5) | 25 128 (78–241.5) | 25 127 (92.75–203.25) |

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triacylglycerol
* Statistically significant difference within soy/green tea group, baseline versus 45 d (∗P = 0.002).
† Statistically significant difference within soy/green tea group, baseline versus 90 d (∗∗P = 0.028).
dian varying from 240 to 263 mg/dL. A significant reduction in total plasma cholesterol occurred only in the soy/green tea group after 45 and 90 d of intervention compared with the baseline value. However, when comparing among total cholesterol, LDL, HDL, and triacylglycerols in all groups, no statistically significant differences were observed.

Some researchers have stated that soy-based diets possess no significant hypocholesterolemic action in normocholesterolemic individuals. Moreover, they have reported that the hypocholesterolemic effect is directly proportional to cholesterol concentration before intervention [27–29]. In a study assessing three soy protein bars per day to normolipidemic individuals for 2 wk, no alterations in total cholesterol, LDL, HDL, and triacylglycerols were observed at the end of the protocol [30].

In this study, no significant difference occurred in the green tea group in relation to the lipid profile. In a large populational study, after adjustment by multivariable linear models, some investigators concluded that healthy Japanese workers showed a 2% reduction in plasma cholesterol after ingesting 10 cups of green tea per day. However, no alterations occurred in HDL cholesterol and triacylglycerol levels [6]. According to the investigators, despite the minimal decrease, a 1% reduction in total cholesterol means a 2–3% diminished risk of cardiovascular disease in Western countries and 5% for the Chinese. In contrast, other investigators have found no association between green tea consumption and improvement in lipid profile [6]. Corroborating these results, in 13 epidemiologic studies involving green tea ingestion to verify its hypocholesterolemic action, only four showed a significant reduction in lipids [31].

In this work, improvement in the lipid profile of patients was not observed in those who ingested soy or green tea alone but only in the soy/green tea group.

Considering the present data, it is possible that no significant improvement in plasma lipid levels was observed in the soy and green tea groups due to the moderate dyslipidemia of these patients. The association was shown to be more effective in this respect.

The results of this research demonstrate that soy flour and green tea, alone or in association, increased TRAP. When combined, these substances presented a synergy in relation to the hypocholesterolemic property.

In conclusion, the present study could not prove that the products used by the patients help avoid cardiovascular disorders; however, it contributed by providing a clearer understanding of how they might assist toward this end. If soy and green tea in isolation were shown to be important for increasing TRAP, the same was not verified in relation to the lipid profile, where the combined ingestion of soy and green tea appeared to provide more satisfactory results.

References


