Green tea catechin plus caffeine supplementation to a high-protein diet has no additional effect on body weight maintenance after weight loss1–3

Rick Hursel and Margriet S Westerterp-Plantenga

ABSTRACT
Background: Green tea (epigallocatechin gallate + caffeine) and protein each were shown to improve body weight maintenance after weight loss.

Objective: We investigated the effect of a green tea–caffeine mixture added to a high-protein (HP) diet on weight maintenance (WM) after body weight loss in moderately obese subjects.

Design: A randomized, placebo-controlled, double-blind parallel trial was conducted in 80 overweight and moderately obese subjects [age (mean ± SD): 44 ± 2 y; body mass index (BMI; in kg/m²): 29.6 ± 2.0] matched for sex, age, BMI, height, body mass, and with a habitually low caffeine intake. A very-low-energy diet intervention during 4 wk was followed by 3 mo of WM; during the WM period, the subjects received a green tea–caffeine mixture (270 mg epigallocatechin gallate + 150 mg caffeine/d) or placebo, both in addition to an adequate protein (AP) diet (50–60 g protein/d) or an HP diet (100–120 g protein/d).

Results: Subjects lost 7.0 ± 1.6 kg, or 8.2 ± 2.0%, body weight (P < 0.001). During the WM phase, WM, resting energy expenditure, and fat-free mass (FFM) increased relatively in both the HP groups and in the AP + green tea–caffeine mixture group (P < 0.05), whereas respiratory quotient and body fat mass decreased, all compared with the AP + placebo group. Satiety increased only in both HP groups (P < 0.05). The green tea–caffeine mixture was only effective with the AP diet.

Conclusion: The green tea–caffeine mixture, as well as the HP diet, improved WM independently through thermogenesis, fat oxidation, sparing FFM, and, for the HP diet, satiety; a possible synergistic effect failed to appear. Am J Clin Nutr 2009;89:822–30.

INTRODUCTION

The increasing incidence of obesity is a recognized medical problem in developed countries (1). Obesity is an important factor for a number of diseases, including coronary heart diseases, hypertension, type 2 diabetes, pulmonary dysfunction, osteoarthritis, and certain types of cancer (2–4). The main cause for the development of obesity is increased energy intake during sustained or decreased energy expenditure. Weight loss and loss of body fat can thus be achieved by reducing energy intake and at the same time sustaining energy expenditure. Treatment of obesity is beneficial in that weight loss reduces the risk of mortality and morbidity. Even modest weight loss, 5–10% of the initial body weight, already leads to beneficial health effects (5–7). Modest weight loss is a realistic goal for most subjects (5, 7). However, long-term maintenance of the body weight lost can be described as unsuccessful. Most studies on weight maintenance show an undesired weight regain (8–12), indicating that subjects did not change their eating and activity behaviors adequately (13). Interventions to improve long-term weight maintenance are needed to treat obesity effectively. A rapidly growing therapeutic area is the use of natural herbal supplements. One of these agents is a green tea–caffeine mixture [epigallocatechin gallate (EGCG) plus caffeine], whose claimed antiobesity properties have been ascribed to increased thermogenesis and fat oxidation (14–20). A green tea–caffeine mixture contains caffeine that may stimulate thermogenesis and fat oxidation through inhibition of phosphodiesterase, an enzyme that degrades intracellular cyclic AMP, and through antagonism of the negative modulatory effect of adenosine on increased noradrenaline release (14). Human studies have shown that caffeine stimulates thermogenesis and fat oxidation (15–17, 21, 22). In addition, green tea–caffeine mixtures contain large amounts of tea catechins that were shown to inhibit catechol-O-methyltransferase, an enzyme that degrades norepinephrine (18). Also in humans, a green tea–caffeine mixture was shown to stimulate thermogenesis and fat oxidation in the short term (19, 23, 24). Dulloo et al (19) showed that the effect of a green tea extract containing 90 mg EGCG + 50 mg caffeine was greater than that attributed to 50 mg caffeine alone. On the basis of these studies in humans, we hypothesized that a green tea–caffeine mixture would reduce body weight regain in humans after weight loss, possibly through a thermogenic effect (19, 23–25). Moreover, we have shown before that a relatively high-protein (HP) diet improves body weight maintenance after body weight loss (26, 27) (R Hursel and M Westerterp-Plantenga, unpublished observations, 2008). Thus, the question remains whether there may

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be a synergistic effect of a green tea–caffeine supplementation added to an HP diet. The following study addresses this research question, with the aim to investigate whether a green tea–caffeine mixture added to an HP diet may improve weight maintenance by preventing or limiting weight regain after weight loss of 5–10% in moderately obese subjects with a low habitual caffeine intake.

SUBJECTS AND METHODS

Subjects

The number of subjects needed was calculated according to the differences observed in fat mass (FM) in a previous study (28). With an observed difference of 2.2 kg and an SD of the population of 4.5 kg, it was calculated that after taking a 10% dropout into account ≥80 subjects were needed to achieve sufficient power (90%) to observe significant (P < 0.05) changes in body weight and FM as a result of treatment. Male and female overweight and moderately obese subjects (n = 100), aged between 18 and 60 y, with a body mass index (BMI; in kg/m²) between 25 and 35, were recruited from the local population in 2006. They underwent a medical screening. Selection was based on being in good health, being a nonsmoker, not using medication, ingesting ≤100 mg caffeine/d (from coffee, tea, chocolate, cola, or energy drinks; the main source of caffeine intake was coffee), not drinking green tea habitually, and being at most a moderate alcohol user. Ninety-two subjects were eligible for participation in the study. During the 4-wk weight-loss period, 12 subjects dropped out of the study, 8 because of moving, changing jobs, or going on vacation and 4 because of not being able to follow the very-low-energy diet (VLED). The remaining 80 subjects were further divided into 4 stratified groups according to, respectively, sex (11 women and 9 men per group), BMI (27.0 ± 2.6), age (44 ± 2 y), and weight loss (7.0 ± 1.6 kg). All subjects gave their written informed consent. The Medical Ethics Committee of the Academic Hospital in Maastricht approved of the study. Subsequently, subjects were randomly assigned to 4 groups: a green tea–caffeine mixture treatment + adequate protein (AP) group, a green tea–caffeine mixture treatment + relatively HP group, an AP + placebo group, and an HP + placebo group for the weight-maintenance phase (Figure 1).

During this weight-maintenance phase, the subjects received a green tea–caffeine mixture [45 mg EGCG + 25 mg caffeine + 380 mg placebo (vegetable oil)/capsule; 6 capsules/d; 2 capsules before each meal (corresponding to 2–3 cups of green tea/d and caffeine content alone is comparable to 1–2 cups of coffee/d)] or placebo (450 mg vegetable oil/capsule; 6 capsules/d; 2 capsules before each meal). The dosage of EGCG was the same as used by Dulloo et al (19); the dosage of caffeine was lower (29). The capsules were manufactured by Novartis CH, Nyon, Switzerland.

The capsules with a green tea–caffeine mixture or placebo were given in addition to an AP diet or an HP diet. With both diets, subjects received a prescription with examples of menus that represented a just-adequate energy intake, based on subjects' resting energy expenditure (REE) multiplied by their physical activity factor (for measurement description, see Baseline measurements) or, if the subjects’ physical activity factor was not measured, the group average of 1.6 was applied.

In the AP group, the prescribed energy intake consisted of 50–60 g protein (~10% of energy from protein); in the HP group, the prescribed energy intake consisted of 100–110 g of protein (~20% of energy from protein). The additional protein was given as 50 g calcium caseinate, a powder soluble in any fluid, provided by Novartis CH. The subjects were instructed by the dietitians how and when to prepare and consume the diets. Moreover, the subjects had to collect 24-h urine specimens before the weight-maintenance diet started and during the 6th and 12th week of the weight-maintenance phase.

Procedure

Baseline measurements

Anthropometry. Body weight was measured with a digital balance (model 707; Seca, Hamburg, Germany; weighing accuracy of 0.1 kg) with subjects in underwear, in a fasted state, and after emptying their bladder. Height was measured with the use of a wall-mounted stadiometer (model 220; Seca). BMI was calculated. The distribution of fat was investigated by measuring the waist circumference at the site of the smallest circumference between the rib cage and the ileac crest, with the subjects in standing position.

Body composition. Total body water (TBW) was measured with the use of the deuterium (2H2O) dilution technique (30, 31). In the evening, the subjects ingested a dose of deuterium-enriched water after collecting a background urine sample. After consumption of the deuterium-enriched water, no fluid or food was consumed. The following morning a urine sample from the

![Flow diagram of the progress through the phases of the study. VLED, very-low-energy diet; GT, green tea–caffeine mixture; PL, placebo.](https://academic.oup.com/ajcn/article-abstract/89/3/822/4596715)
second voiding was collected between 0800 and 1000. Deuterium concentration in the urine samples was measured with the use of an isotope ratio mass spectrometer (Micromass Optima, Manchester, United Kingdom). TBW was obtained by dividing the measured deuterium dilution space by 1.04 (30). Fat-free mass (FFM) was calculated by dividing the TBW by the hydration factor 0.73. By subtracting FFM from body weight, FM was obtained. FM expressed as a percentage of body weight gives the percentage of body fat.

**Attitude toward eating.** To determine whether attitude toward food intake changed during the experiment, the three-factor eating questionnaire was used (32). Factor 1 indicates cognitive dietary restraint, factor 2 indicates disinhibition of eating, and factor 3 indicates general hunger feelings. In addition, the Herman-Polivy questionnaire was used to determine the frequency of previous dieting (33). In addition to these questionnaires, subjects’ baseline caffeine intake was estimated during a food history questionnaire specific for caffeine-containing products.

**Postabsorptive appetite profile.** To determine the postabsorptive appetite profile, hunger and satiety were rated on anchored 100-mm visual analog scales (VASs) in the morning before breakfast. This time point was chosen because we found significant relations of body weight maintenance with changes in postabsorptive hunger or satiety ratings, ie, in the fasting state before breakfast, in previous studies (25, 26).

**Blood variables.** A fasted blood sample of 10 mL was taken and mixed using tubes containing EDTA to prevent clotting. Plasma was obtained by centrifugation, frozen in liquid nitrogen, and stored at −80°C until further analysis. Plasma glucose concentrations were measured with the use of the hexokinase method (Glucose HK 125 kit; ABX Diagnostics, Montpellier, France). The Wako NEFA C-kit (Wako Chemicals, Neuss, Germany) was used to determine free fatty acid (FFA) concentrations. Insulin concentrations were measured with the use of the radioimmunoassay kit (Insulin RIA-100; Kabi-Pharmacia, London, United Kingdom). The glycerol kinase method was used to measure glycerol concentrations (Boehringer Mannheim GmbH, Mannheim, Germany). Triacylglycerol were measured with the use of the GPO-Trinder kit (Sigma Diagnostics Inc, St Louis, MO). Concentrations of triacylglycerol were corrected for glycerol.

The β-hydroxybutyrate (BHB) dehydrogenase method (Sigma Diagnostics Inc) was used to determine BHB concentrations. Leptin concentrations were measured with the use of the human leptin radioimmunoassay kit (Linco Research Inc, St. Charles, MO).

**Adverse events.** Frequency and intensity of adverse events during treatment were recorded.

**REE and substrate oxidation.** REE and substrate oxidation were measured by means of an open-circuit-ventilated hood system. After 30 min of resting, to make sure that the subjects were rested, REE was measured in the morning with subjects in a fasted state while lying supine for 30 min. Gas analyses were performed by a paramagnetic oxygen analyzer (type 500A; Servomex, Crowborough Sussex, United Kingdom) and an infrared carbon dioxide analyzer (Servomex type 500A) (34). Calculation of REE was based on Weir’s formula (35). Respiratory quotient was calculated as carbon dioxide produced/oxygen consumed.

Alternatively, before or after each REE measurement, the function of the ventilated-hood system was checked with methanol burning during 20 min. The methanol burner has been set to burn 0–2 g/min, which is equivalent to the production of 150 mL CO₂/min and the consumption of 225 mL O₂/min. Because an error percentage depends on burn rate, the expression of the error limit in absolute mL/min is preferred; hence, the limit values of 7–5 mL CO₂ and 11 mL O₂/min, respectively (5% of 150 mL CO₂ and 5% of 225 mL O₂).

**Physical activity.** Physical activity was partly determined with the use of a CSA accelerometer (Computer Science and Applications Inc, Shalimar, FL) (36), partly with a triaxial accelerometer for movement registration (Tracmor, Philips, Eindhoven, Netherlands) during 1 wk. Half of the subjects from each group wore either the CSA (n = 40) or the Tracmor (n = 40) device. The Tracmor is a small device (7 × 2 × 0.8 cm; 30 g), which measures accelerations in the anteroposterior, mediolateral, and vertical directions of the trunk (37). Subjects were wearing the same type of accelerometer during waking hours in a belt at the back of the waist, during 3 different phases of the study.

**PAL (physical activity level)** was calculated with the following equations:

\[
\text{CSA: PAL} = \frac{0.000001379 \times (\text{counts/d} \times 5)}{1.113} (36)
\]

Tracmor: \[
\text{TEE} = -1.259 + (1.552 \times \text{REE}) + (0.076 \times \text{counts/min}) (37)
\]

\[
\text{PAL} = \frac{\text{TEE}}{\text{REE}}
\]

in which TEE (total energy expenditure) and REE are measured in MJ/d.

**Energy expenditure.** For subjects who had been wearing the CSA, TEE was calculated by multiplying REE by PAL. For those subjects who had been wearing the Tracmor, TEE was calculated as indicated above.

**Weight-loss period**

After determining the subject’s baseline measurements, a VLED (2.1 MJ/d) intervention was applied for 4 wk, to let the subjects lose weight. The diet (Modifast; Nutrition and Sante´, Breda, Netherlands) was supplied in 3 sachets daily, to be dissolved in water to obtain a milkshake, pudding, soup, or muesli. This diet provided 2.1 MJ/d and was a protein-enriched formula diet that provided 50 g carbohydrates, 52 g protein, 7 g fat, and a micronutrient content, which meets the Dutch recommended daily allowance. Vegetables and fruit were allowed in addition to the diet. The aim was a body weight loss of ≥4 kg over 4 wk. After this weight-loss period, the measurements described above were repeated.

**Weight-maintenance phase**

During the weight-maintenance phase, the subjects received a green tea-caffeine mixture (45 mg EGCG + 25 mg caffeine + 380 mg vegetable oil/capsule; 6 capsules/d) or placebo (450 mg vegetable oil/capsule; 6 capsules/d). This was given in addition to either the prescribed HP or AP diet.

The 4 groups were stratified for sex, BMI, age, and body weight loss. A double-blind administration of the supplementation (green tea-caffeine mixture or placebo) was performed.

**Compliance to protein intake**

Compliance to additional protein intake was checked by taking 24-h urine samples and analyzing them for nitrogen. The subjects...
had to collect 24-h urine specimens before the weight-maintenance diet started and during the 6th and 12th week of the weight-maintenance phase. Energy intake from protein was calculated from the 24-h nitrogen output according to the following formula of Isaksson (38):

\[
\text{Protein intake (in g)} = \left(\frac{\text{nitrogen output in 24-h urine samples in g/d}}{2g}\right) \times 6.25 \quad (3)
\]

Repeated measurements

Measurements as described under “Baseline measurements” were executed again 3 mo later. In addition, body weight was determined 1 and 2 mo after the start of the weight-maintenance phase.

Body weight maintenance was expressed as rate of regain over the first 3 mo after weight loss. The 3-mo period to measure weight maintenance was based on the stable rate of regain (0.67 kg/mo) that we showed before over the first 3 mo, and on the strong relation of rates of regain between 3 and 8 or 14 mo \((r = 0.9, P < 0.0001)\) (10–12).

Data analysis

The 4 groups were stratified according to the number of males and females, BMI, age, and weight loss, and the differences between these groups were checked with the use of a factorial analysis of variance (ANOVA). Stratification was successful when the \(P\) values of this comparison were high, indicating very small differences. \(P\) values for these differences were, respectively, >0.9 for sex distribution and BMI, >0.8 for age, and >0.5 for weight loss.

The effects of the 4 treatments were compared with the use of an ANOVA with time as the within-subject factor and group as the between-subject factor. Post hoc, a Scheffe’s \(F\) test was applied. The difference in nitrogen content of the urine between the AP and HP groups was determined with a factorial ANOVA, in which AP compared with HP was used as factor. The software used was STATVIEW 5.0 SE + GRAPHICS (Abacus Concepts, Berkeley, CA). Statistical significance was set at \(P < 0.05\).

RESULTS

General

No adverse events occurred. No different effects for men or women were observed; therefore, these data were pooled (Table 1).

Weight loss

During the VLED intervention, subjects lost a significant amount of their original body weight \((7.0 \pm 1.6 \text{ kg}, \text{ or } 8.2 \pm 2.0\%; \ P < 0.001)\). Furthermore, body composition changed during weight loss, as did waist circumference, attitude toward eating, Herman-Polivy restraint scores, hunger scores, and satiety scores. The PAL did not change during weight loss. Changes were not different between prospective treatment groups (Table 1).

The blood variables glucose, insulin, triacylglycerol, and leptin decreased during weight loss, whereas glycerol, FFAs, and BHB increased, without differences in changes between prospective treatment groups (Table 2).

Weight maintenance

The subjects were compliant to the treatments. The nitrogen content of the 24-h urine samples was significantly different between the HP and AP groups \((14.2 \pm 2.4 \text{ g compared with } 8.6 \pm 1.3 \text{ g}; \ P < 0.01)\). Calculated energy intake from protein showed that the AP group must have ingested \(\approx 10\%\) of energy as protein \((66.3 \pm 10 \text{ g})\), whereas the HP group had ingested \(\approx 20\%\) of energy as protein \((101.3 \pm 15 \text{ g})\). A possible inaccuracy in these calculations could have been caused by the estimation of the energy intake of the subjects, which was based on the Harris and Benedict equation (39), with a PAL of 1.6, and with that the difference of 1 kg weight loss or gain cumulative is 30 MJ. In addition, when returning capsules, only 3 \pm 2 capsules/mo were returned, without a difference between placebo and green tea + caffeine capsules.

During the weight-maintenance period, significant body weight regain appeared in the AP + placebo group, but not in the HP + placebo group, the green tea–caffeine mixture + AP group, or the HP + green tea–caffeine mixture group \((P < 0.01, 4\text{-factor treatment} \times \text{time ANOVA repeated measures, post hoc Scheffe’s }F\) test). Similarly, this was the case with BMI and waist circumference. FFM increased in all 4 groups with a comparable magnitude. FM increased only in the AP + placebo group \((P < 0.01)\); moreover, a treatment \times \text{time effect also appeared} \((P < 0.01, 4\text{-factor ANOVA repeated measures, post hoc Scheffe’s }F\) test). The increase in FFM and FM is the usual composition of the increase in body mass in the AP + placebo group. The increase in FFM and lack of increase, or even slight decrease, in FM are the FFM-sparing effects during weight maintenance in the HP groups and in the green tea–caffeine mixture + AP group.

Attitude toward eating did not change greatly during weight maintenance and did not differ between groups, except in the green tea–caffeine mixture + placebo group, in which factor 3, the general hunger score, had increased during weight maintenance. Similarly, the VAS ratings indicated a larger hunger and lower satiety in the green tea–caffeine mixture + AP group \((P < 0.01, 4\text{-factor ANOVA repeated measures, post hoc Scheffe’s }F\) test). Related to increases in FFM, REE increased in all groups \((P < 0.05)\) without differences between groups (Table 1).

Respiratory quotient increased in all groups \((P < 0.01)\), but more in the AP + placebo group \((P < 0.05, 4\text{-factor ANOVA repeated measures, post hoc Scheffe’s }F\) test).

PAL did not change, and TEE increased again in all groups \((P < 0.01)\) because REE had increased. A reversal effect of concentrations reversing in the direction of baseline concentrations was observed in glucose and to a lesser extent in insulin, BHB, glycerol, FFA, and triacylglycerol. For leptin, only the AP + placebo group reversed; in the other groups the concentrations remained low (Table 2).

DISCUSSION

No synergistic effect was seen from a green tea–caffeine mixture added to an HP diet on weight maintenance after weight loss. Interestingly, the green tea–caffeine mixture group showed...
Characteristics of subjects \( (n = 80) \) in the green tea + adequate protein (AP), green tea + high protein (HP), placebo + AP, and placebo + HP groups at baseline, after 4 wk of a very-low-energy diet, and after 13 wk of treatment

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline Green tea ( (n = 40) )</th>
<th>Baseline Placebo ( (n = 40) )</th>
<th>After 4-wk weight loss ( ^2 ) Green tea ( (n = 40) )</th>
<th>After 4-wk weight loss ( ^2 ) Placebo ( (n = 40) )</th>
<th>After 13-wk weight maintenance Green tea ( (n = 40) )</th>
<th>After 13-wk weight maintenance Placebo ( (n = 40) )</th>
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</thead>
<tbody>
<tr>
<td>BM (kg)</td>
<td>85.1 ± 7.6</td>
<td>85.0 ± 7.4</td>
<td>85.1 ± 7.8</td>
<td>85.0 ± 7.3</td>
<td>85.1 ± 7.4</td>
<td>85.0 ± 7.2</td>
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<tr>
<td>Height</td>
<td>1.70 ± 0.11</td>
<td>1.70 ± 0.10</td>
<td>1.70 ± 0.09</td>
<td>1.70 ± 0.10</td>
<td>1.70 ± 0.11</td>
<td>1.70 ± 0.10</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>29.6 ± 2.1</td>
<td>29.5 ± 2.0</td>
<td>29.6 ± 2.1</td>
<td>29.5 ± 1.9</td>
<td>29.6 ± 2.0</td>
<td>29.5 ± 2.1</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>94.0 ± 6.5</td>
<td>94.2 ± 6.4</td>
<td>94.1 ± 6.3</td>
<td>94.0 ± 6.0</td>
<td>94.0 ± 6.2</td>
<td>94.0 ± 6.1</td>
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<tr>
<td>FFM (kg)</td>
<td>53.4 ± 7.1</td>
<td>53.3 ± 7.3</td>
<td>53.4 ± 7.1</td>
<td>53.3 ± 7.0</td>
<td>53.4 ± 7.1</td>
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<tr>
<td>FM (kg)</td>
<td>31.7 ± 5.9</td>
<td>31.7 ± 5.0</td>
<td>31.7 ± 6.0</td>
<td>31.7 ± 4.6</td>
<td>31.7 ± 5.9</td>
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<tr>
<td>%BF</td>
<td>37.3 ± 4.2</td>
<td>37.3 ± 3.9</td>
<td>37.3 ± 4.7</td>
<td>37.2 ± 3.9</td>
<td>37.4 ± 3.8</td>
<td>37.3 ± 3.9</td>
</tr>
<tr>
<td>F1</td>
<td>6.5 ± 2.2</td>
<td>7.0 ± 2.7</td>
<td>7.0 ± 2.2</td>
<td>6.5 ± 3.1</td>
<td>6.5 ± 2.5</td>
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<tr>
<td>F2</td>
<td>4.7 ± 2.0</td>
<td>5.0 ± 2.0</td>
<td>5.1 ± 2.1</td>
<td>4.7 ± 2.2</td>
<td>4.7 ± 2.3</td>
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<tr>
<td>F3</td>
<td>3.9 ± 2.0</td>
<td>3.9 ± 2.1</td>
<td>3.9 ± 2.1</td>
<td>3.9 ± 2.2</td>
<td>3.9 ± 2.0</td>
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<tr>
<td>HP score</td>
<td>15.1 ± 2.5</td>
<td>15.3 ± 2.2</td>
<td>15.2 ± 2.6</td>
<td>15.2 ± 2.1</td>
<td>15.1 ± 2.5</td>
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<tr>
<td>Hunger score</td>
<td>36.1 ± 22.0</td>
<td>34.4 ± 25.2</td>
<td>33.6 ± 23.0</td>
<td>32.0 ± 24.2</td>
<td>31.0 ± 20.0</td>
<td>30.5 ± 18.2</td>
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<td>Satiety score</td>
<td>26.9 ± 21.2</td>
<td>25.3 ± 22.8</td>
<td>26.6 ± 21.0</td>
<td>26.3 ± 21.9</td>
<td>26.9 ± 21.2</td>
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</tr>
<tr>
<td>REE (MJ/d)</td>
<td>6.9 ± 0.9</td>
<td>7.0 ± 1.0</td>
<td>6.9 ± 0.9</td>
<td>7.0 ± 1.0</td>
<td>6.9 ± 0.9</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>RQ(^2)</td>
<td>0.84 ± 0.03</td>
<td>0.84 ± 0.04</td>
<td>0.84 ± 0.03</td>
<td>0.84 ± 0.03</td>
<td>0.84 ± 0.03</td>
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<tr>
<td>PAL</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
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</tr>
<tr>
<td>TEE (MJ/d)</td>
<td>11.1 ± 1.1</td>
<td>11.4 ± 1.2</td>
<td>11.1 ± 1.1</td>
<td>11.4 ± 1.5</td>
<td>11.1 ± 0.9</td>
<td>10.9 ± 0.8</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SDs. BM, body mass; FFM, fat-free mass; FM, fat mass; %BF, percentage of body fat; F1, factor 1 (cognitive restraint) of the three-factor eating questionnaire; F2, factor 2 (inhibition) of the three-factor eating questionnaire; F3, factor 3 (hunger) of the three-factor eating questionnaire; HP, Herman-Polivy restraint questionnaire; VAS, visual analog scale; REE, resting energy expenditure; RQ, respiratory quotient; PAL, physical activity level; TEE, total energy expenditure. All variables except PAL were measured in the fasting state before breakfast. ANOVA was used with time as the within-subject factor and group as the between-subject factor and with a post hoc Scheffe’s F test.

\(^2\) Variables, except height and PAL, changed significantly from baseline after 4 wk of a very-low-energy diet, \( P < 0.05 \) to \( < 0.0001 \); no differences were observed between groups.

\(^3\) Significantly different from the AP + placebo group after 13 wk of weight maintenance.

\(^4\) Significantly different from after weight loss, \( P < 0.05 \) to \(< 0.0001 \).

\(^5\) Significantly different from the AP + green tea group after 13 wk of weight maintenance.

\(^6\) Treatment × time effect: ANOVA with time as the within-subject factor and group as the between-subject factor, \( P < 0.05 \).
Blood variables of subjects (n = 80) in the green tea + adequate protein (AP), green tea + high-protein (HP), placebo + AP, and placebo + HP groups at baseline, after 4 wk of a very-low-energy diet, and after 13 wk of weight maintenance.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Green tea (n = 40)</th>
<th>Green tea (n = 40)</th>
<th>Green tea (n = 40)</th>
<th>Green tea (n = 40)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AP (n = 20)</td>
<td>HP (n = 20)</td>
<td>Placebo (n = 20)</td>
<td>Placebo (n = 20)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.5 ± 0.8</td>
<td>5.4 ± 0.5</td>
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<tr>
<td>Glycerol (mmol/L)</td>
<td>5.3 ± 0.5</td>
<td>5.3 ± 0.5</td>
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<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.5</td>
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<tr>
<td>FFA (mmol/L)</td>
<td>11.1 ± 3.6</td>
<td>11.0 ± 3.6</td>
<td>11.1 ± 3.6</td>
<td>11.1 ± 3.6</td>
<td>11.1 ± 3.6</td>
</tr>
<tr>
<td>BHB (mol/L)</td>
<td>8.6 ± 16.0</td>
<td>8.6 ± 16.0</td>
<td>8.6 ± 16.0</td>
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The only difference between the populations, because both consume mainly black tea, is that people in Great Britain add milk to their tea (48). Several studies examined the difference in antioxidative and antimutagenic capacity between tea with or without the addition of milk (49–57). In a recent study it was reported that the addition of milk to tea lowered the concentrations of catechins in vitro. They found a significant reduction in the endothelial function after tea with milk in comparison to tea alone. The added milk lowered the vascular protective effects of tea (53). In another study milk lowered the antioxidant

Some controversy still exists about whether the addition of milk to tea inhibits the beneficial effects of tea drinking. An epidemiologic study, Hertog et al (48) found in a Dutch population in which tea drinking was inversely associated with coronary heart diseases. The only difference between the populations, because both consume mainly black tea, is that people in Great Britain add milk to their tea (48). Several studies examined the difference in antioxidative and antimutagenic capacity between tea with or without the addition of milk (49–57). In a recent study it was reported that the addition of milk to tea lowered the concentrations of catechins in vitro. They found a significant reduction in the endothelial function after tea with milk in comparison to tea alone. The added milk lowered the vascular protective effects of tea (53). In another study milk lowered the antioxidant

Here, also compliance was present, as indicated by the nitrogen in urine. Although the postabsorptive VAS ratings have not been validated by, for instance, a relation with subsequent energy intake, the VAS ratings were supported by the factor 3 scores that were found in this study. It appears that the factor satiety is the main difference between an HP and green tea–caffeine mixture diet. As shown before, an HP diet is extremely satiating, because of either the amino acid kinetics, satiety hormone concentrations, or thermogenesis. A green tea–caffeine mixture not only fails to contribute to satiety but, on the contrary, seems to contribute to hunger feelings (41).

For the failure to show synergy between effects of a green tea–caffeine mixture and an HP diet, the following mechanisms may shed light on this issue. Already in 1963, Brown and Wright (42) reported that proteins formed complexes with the polyphenols in tea. Especially caseins, which are present in milk protein, tend to bind the polyphenols. In the absence of caseins, a- lactalbumin and -lactoglobulin can form complexes with the flavonoids (42). Several studies have confirmed the formation of such complexes (43–46). There are 3 different caseins; of these, -casein is usually preferred by different polyphenols because it is the casein richest in proline. Polyphenols and proteins can differ in preferences; for instance, EGCG prefers to bind to a proline-rich protein. The protein “wraps” itself around the catechins, a process named noncovalent cross-linking. This process might reduce the bioavailability and accessibility of the polyphenols (47).

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capacity of tea to a maximum of 28%. The authors also found that there was no difference between an addition of cow milk or soy milk, both lowered the antioxidant potential (50). Arts et al (52) found a 20% reduction of the total antioxidant capacity because of what they thought were the earlier-described interactions between polyphenols and caseins. The antioxidant capacity of a mixture between flavonoids and proteins was lower than the sum of the antioxidant potential of flavonoid and protein separately (52). This is supported by the results of the current study in which a possible synergistic effect of a green tea–caffeine mixture and an HP diet failed to occur as well. Krul et al (51) studied the influence of a food matrix on the anti-mutagenic capacity of tea in an in vitro gastrointestinal model that contained tea mixed with milk. Whole milk, semiskimmed milk, and skimmed milk were the 3 types of milk they used. All 3 reduced the antimutagenic capacity of black and green tea (51). Whole milk contained the largest amount of fat, and it reduced the beneficial effects of tea by 22%; skimmed milk contained no fat and abolished the antimutagenic capacity with a reduction of 78%. Semiskimmed milk reduced the beneficial effect by 58%. These results indicate that the fat content of milk might not be of importance; instead, they mention that the interactions between flavonoids and proteins impede the gastric hydrolysis and thereby reduced the absorption of the polyphenols (51). The first study that examined the decrease of antioxidant capacity after addition of milk was published by Serafini et al (49). They found that total antioxidant capacity was not lowered because of the addition of milk to tea, but the polyphenols were less available for absorption because the polyphenol-protein complexes were resistant to gastric hydrolysis. They also suggested that the absorption might be reduced because the pH of the stomach changed through the milk. The polyphenols have weak acid compounds that are easily absorbed in their nonionized form. If the pH in the stomach rises as a result of the addition of milk, this can increase the ionization, which impedes the passage of the polyphenols through the gastric mucosa (49). In addition, studies from Leenen et al (55) and Hollman et al (56) did not find a difference in the antioxidant capacity of tea after the addition of milk compared with tea alone; their results indicated a normal absorption. Reddy et al (57) did not find a lowered antioxidant potential but found that it was delayed during the interference with absorption by milk. van het Hof et al (54) did not find an effect of milk on catechin concentrations in vivo because they were comparable to the catechin concentrations in the blood after tea alone. In contrast to Krul et al (51), they believed that the fat content might play a role in a possible inhibition of antioxidant capacity and not the proteins. Catechins have a complex ring structure and are fat soluble, which causes the formation of complexes between fat and polyphenols (54).

Many explanations for the antioxidant-reducing effects have been given by the previous studies, but the most widely given explanation is the reduction in absorption after the formation of a protein-polyphenol complex that is resistant to gastric hydrolysis. The formation of such complexes takes place in the upper part of the digestive tract according to Serafini et al (49). If the complexes were resistant to gastric hydrolysis from the beginning of the gastrointestinal system and therefore cannot be absorbed, how is it then possible that the HP + green tea–caffeine mixture group has nearly the same effect as the HP + placebo group and the AP + green tea–caffeine mixture group from which the proteins and polyphenols are absorbed? Presumably, there is a surplus of proteins such as α-lactalbumin and β-lactoglobulin that only bind to flavonoids during the absence of caseins (42). Those proteins may still be absorbed when the rest has formed complexes with polyphenols, and they are known for their ability to reduce energy intake by a hunger-suppressive effect (58, 59), to increase diet-induced thermogenesis (R Hursel and M Westerterp-Plantenga, unpublished observations, 2008), and to preserve lean body mass at the expense of FM (60). The results of the blood variables show that they accompany the weight changes but are also independent from them. In accordance with Kovacs et al (25) and Diepvens et al (61), no effect of the green tea–caffeine mixture was seen on the blood variables, because all groups, except for leptin in the AP + placebo group, showed comparable results. Leptin is positively correlated with FM, which contributes to the explanation of the findings in this study (62). Fat oxidation increased during weight loss, because FFA and BHB increased and triacylglycerol decreased. During weight maintenance, a reversal effect was seen in these variables, which indicated that fat oxidation had decreased again. This means that there was no effect of green tea and caffeine on fat oxidation additional to the effect of weight loss.

In conclusion, no synergistic effect of green tea and caffeine supplementation was seen on an HP diet on body weight maintenance after weight loss. Weight maintenance after the HP + green tea–caffeine mixture group was comparable with the results of the HP + placebo group and the AP + green tea–caffeine mixture group. Formation of complexes presumably caused a reduction in absorption; therefore, a synergistic effect failed to occur. Beneficial effects of weight loss on the blood variables were reversed during the weight-maintenance period; except for leptin, only the AP + placebo group reversed and the others remained low.

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The authors’ responsibilities were as follows—RH and MSW-P: designed the study, analyzed the data, and wrote the manuscript; RH: contributed to the interpretation of the data; and MSW-P: supervised the execution of the study and reviewed the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES


