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Effects of encapsulated green tea and Guarana extracts containing a mixture of epigallocatechin-3-gallate and caffeine on 24 h energy expenditure and fat oxidation in men

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It has been reported that green tea has a thermogenic effect, due to its caffeine content and probably also to the catechin, epigallocatechin-3-gallate (EGCG). The main aim of the present study was to compare the effect of a mixture of green tea and Guarana extracts containing a fixed dose of caffeine and variable doses of EGCG on 24 h energy expenditure and fat oxidation. Fourteen subjects took part to this randomized, placebo-controlled, double-blind, cross-over study. Each subject was tested five times in a metabolic chamber to measure 24 h energy expenditure, substrate oxidation and blood pressure. During each stay, the subjects ingested a capsule of placebo or capsules containing 200 mg caffeine and a variable dose of EGCG (90, 200, 300 or 400 mg) three times daily, 30 min before standardized meals. Twenty-four hour energy expenditure increased significantly by about 750 kJ with all EGCG–caffeine mixtures compared with placebo. No effect of the EGCG–caffeine mixture was observed for lipid oxidation. Systolic and diastolic blood pressure increased by about 7 and 5 mmHg, respectively, with the EGCG–caffeine mixtures compared with placebo. This increase was significant only for 24 h diastolic blood pressure. The main finding of the study was the increase in 24 h energy expenditure with the EGCG–caffeine mixtures. However, this increase was similar with all doses of EGCG in the mixtures.

Green tea: Body weight: Energy balance

Green tea is one of the most widely consumed beverages in the world and is currently perceived as a healthy drink. Green tea contains a large amount of catechins (30 to 42 % dry weight), a group of very active flavonoids (Yang & Landau, 2000; Dusfresne & Farnworth, 2001). The catechins, which are antioxidants, have been attributed beneficial health properties such as protection against CVD and certain types of cancer. Also, some attention has recently been given to the possible beneficial effects of green tea on the treatment of obesity.

The catechins epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG) are the major components of green tea leaves. EGCG is the most abundant catechin and has received the most attention (Yang & Landau, 2000). Caffeine represents approximately 3 to 5 % of the dry weight of green tea (Yang & Landau, 2000; Dusfresne & Farnworth, 2001). Caffeine consumption has been related to an increase in energy expenditure (Astrup *et al.* 1990; Dulloo *et al.* 1989), which explains why the thermogenic effect of green tea is generally attributed to its caffeine content. However, Dulloo *et al.* (2000) reported that, in rats, a green tea extract stimulates brown adipose tissue thermogenesis to a much greater extent than that which can be attributed to its caffeine content *per se*. In another study, ten healthy men were assigned to three treatments: green tea extract containing 50 mg caffeine and 90 mg EGCG, caffeine (50 mg) or placebo. A capsule of green tea extract, caffeine or placebo was taken with each meal. Ingestion of green tea extract increased 24 h energy expenditure by 4 % (328 kJ), reflecting its stimulatory effect on thermogenesis. The study also found a reduction in RQ during the green tea extract treatment, suggesting an increase in fat oxidation (Dulloo *et al.* 1999). On the other hand, the caffeine treatment did not produce any effect on these variables.

In addition, the thermogenesis and fat oxidation stimulation obtained in that study was not accompanied by an increase in heart rate that may be seen when patients are treated with sympathomimetic anti-obesity drugs. Since obese individuals are at greater risk of developing cardiac problems, the increase in heart rate and blood pressure frequently observed when treated with sympathomimetic agents is a matter of concern for health professionals. In this context, the green tea extract and caffeine mixture seems to have potential as an effective alternative to these anti-obesity drugs.

Since it was shown that a total daily dose of 270 mg (3×90 mg) EGCG combined with a total daily dose of 150 mg (3×50 mg) caffeine has the potential to produce an increase in energy expenditure, augmenting the amount of caffeine in the blend could possibly accentuate this increase. Moreover, augmenting the amount of EGCG in the green tea extract mixture may produce a greater increase in energy expenditure. If so, the most effective level of EGCG to use in combination with caffeine to produce a significant increase in energy expenditure and fat oxidation without producing negative cardio-stimulatory side-effects would deserve specific investigation.

Abbreviation: EGCG, epigallocatechin-3-gallate.

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Therefore, the first aim of the present study was to assess the impact of four mixtures of green tea and Guarana (a plant that contains caffeine) extracts containing a fixed 600 mg daily (3 × 200 mg) dose of caffeine and different amounts of EGCG (270 mg/d: 3 × 90 mg; 600 mg/d: 3 × 200 mg; 900 mg/d: 3 × 300 mg; 1200 mg/d: 3 × 400 mg) on 24 h energy expenditure, RQ and substrate oxidation in comparison with a placebo. The second aim of the study was to determine whether there is a dose-related effect of EGCG, and if so, which dose produces a greater increase in energy expenditure and fat oxidation without inducing significant cardio-stimulatory effects when combined with caffeine.

Materials and methods

Subjects

Healthy, non-smoking and sedentary men (*n* 14), from 20 to 50 years of age and with BMI between 20 and 27 kg/m^2, were selected to participate in the study (Table 1). Subjects on a particular diet (vegetarian), subjects consuming a diet rich in capsaicin (e.g. red pepper), subjects using anorectic or related compounds (sympathomimetic compounds), athletes or regularly active individuals (>30 min of intense physical activity three times weekly) and subjects with a caffeine intake >200 mg/d (about two small cups of coffee daily) were all excluded from the study. In addition, all fourteen participants had a stable weight (±3 kg) for at least 3 months before the protocol and no history of weight loss (≥4·5 kg). They gave their written consent to participate in this study, which received approval of the Laval University Medical Ethics Committee.

Study design and randomization

The present study has a randomized, placebo-controlled, double-blind, cross-over design. Subjects came to the laboratory for a first visit during which anthropometric and metabolic rate measurements were performed. Each subject then spent 24 h in a metabolic chamber on five separate occasions and was randomly assigned to receive one of the following five treatments, three times daily:

(1) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 90 mg EGCG (270 mg/d);
(2) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 200 mg EGCG (600 mg/d);
(3) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 300 mg EGCG (900 mg/d);
(4) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 400 mg EGCG (1200 mg/d);
(5) Placebo: inert filler of cellulose.

Table 1. Characteristics of the subjects

(Mean values with their standard deviation for fourteen subjects)

	Mean	SD
Age (years)	34·7	8·0
Weight (kg)	78·6	12·9
BMI (kg/m^2)	25·7	2·7
% Body fat	19·9	7·9
Fat mass (kg)	16·0	7·0
Fat-free mass (kg)	62·7	10·0

The capsules of the four pre-existing mixtures and placebo were developed and standardized by Iovate Health Sciences Research Inc. (previously known as Muscle Tech Research and Development; Mississauga, Ontario, Canada). The four mixtures contained a green tea extract in which EGCG represented 45 % dry weight. Therefore these mixtures also contained unknown amounts of other catechins and possibly caffeine. The mixtures were also composed of a Guarana extract that contained caffeine and other possibly unknown components. The amounts of green tea and Guarana extracts were adjusted to obtain the desired dose of EGCG (90, 200, 300 and 400 mg/mixture) and caffeine (fixed dose of 200 mg for each mixture).

Subjects received all of the treatments but in a different order depending on the randomization to which they were assigned. The first dose was given at 08.00 hours, followed by the standardized breakfast 30 min later. The second dose was at 12.00 hours, followed by a standardized lunch 30 min later. The third dose was at 18.00 hours, followed by a standardized dinner 30 min later. Before each standardized meal, immediately after and every hour until the next meal, levels of hunger and satiety were evaluated with visual analogue scales. At each metabolic chamber visit, the 24 h ambulatory blood pressure monitor was installed. The 24 h urine collection was also performed at these visits. It is to be noted that subjects did not change their diet or activity pattern during the study. The various sessions in the metabolic chamber were administered within an interval of 5–10 d of each other for each subject.

Measurements

Anthropometric measurements. Body weight was taken with a standard beam scale. Waist and hip circumferences were taken according to Harrison *et al.* (1988). Body density was determined by hydrodensitometry (Behnke & Wilmore, 1974). The closed-circuit He dilution method (Meneely & Kaltreider, 1949) was used to assess residual lung volume. The Siri formula (Siri, 1956) was then used to estimate the percentage of body fat from body density, while fat mass and fat-free mass were calculated from the derived percentage of body fat and total body weight.

Measurement of resting metabolic rate and substrate oxidation. Resting energy expenditure (RMR) was measured by indirect calorimetry after a 12 h overnight fast. After resting for 15 min, expired gas collection was performed through a mouthpiece for 15 min while the nose was clipped during the whole measurement. O_2 and CO_2 concentrations were determined by nondispersive IR analysis (Uras 10 E; Hartmann & Braun, Frankfurt, Germany) whereas pulmonary ventilation determination was assessed with an S430A measurement system (KL Engineering, Ventura, CA, USA). The Weir formula (Weir, 1949) was used to determine the energy equivalent of O_2 volume. The determination of substrate oxidation was assessed through the calculations previously described by Frayn (1983) while assuming that protein oxidation contributes 10 % of total energy expenditure measured under these conditions.

Measurement of 24 h energy expenditure and substrate oxidation. Twenty-four hour total energy expenditure was measured with a whole-body indirect calorimeter, which has

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been shown to provide highly reproducible data in our laboratory (White *et al.* 1996). Subjects entered the calorimeter at about 07.30 hours after an overnight fast (12 h). During this stay, subjects were maintained in energy balance by using the resting energy expenditure performed at the initial visit and by extrapolating this value over a 24 h period and then multiplying this value by an activity factor of 1·32 (White *et al.* 1997). The same energy intake was maintained for the five measurements of 24 h energy expenditure. Moreover, the nutrient composition of the diet (daily food quotient 0·85), the sedentary life-style pattern (watching television, computer, reading, etc.) and the meal pattern, as well as the period of sleep were also standardized in each session. It was not permitted to eat or drink any other foods than those provided and therefore no foods or beverages containing caffeine were allowed during the metabolic chamber stay. Before each metabolic chamber visit, subjects were asked to refrain from exercise and eliminate consumption of foods or beverages containing caffeine for 24 h prior to the measurements.

Twenty-four hour blood pressure and heart rate monitoring. To determine the 24 h means (overnight + daily) for systolic and diastolic blood pressure and heart rate, subjects were asked to wear an ambulatory blood pressure monitor in the metabolic chamber. The ambulatory blood pressure device (model #90 207; Space Labs Medical, Redmond, WA, USA), which was installed by the investigator, consists of a programmed console that is worn on the belt with an appropriate size cuff (depending on arm circumference) worn on the non-dominant arm and a cable connecting the console to the cuff. Data were recorded at frequent intervals throughout the day (every 30 min from 08.00 to 22.00 hours) and at night (every hour from 22.00 to 08.00 hours) and were then analysed with a computerized system (FT1000A).

Twenty-four hour urine collection (urinary nitrogen and catecholamine excretion). While in the metabolic chamber (five visits), patients were asked to collect urine for a 24 h period and a sample was taken for analysis to measure urinary N and catecholamine excretion. The extraction and separation of urinary catecholamines was done using C_{18} solid-phase extraction sorbent and HPLC (Talwar *et al.* 2002).

Levels of hunger and satiety. The levels of hunger and satiety were evaluated with visual analogue scales. Before the standardized breakfast, levels of hunger and satiety were evaluated and the measures were repeated immediately after and 60, 120 and 180 min after the breakfast. The same pattern was used for the standardized lunch and dinner.

Statistical analysis

JUMP Software 3.1.6.2 (SAS Institute Inc., Cary, NC, USA) was used for all analyses. ANOVA for repeated measures was performed to determine if there were differences between the effects of the five treatments on 24 h energy expenditure, sleeping metabolic rate, RQ, carbohydrate oxidation, lipid oxidation, heart rate, systolic and diastolic blood pressure (24 h, day and night), and noradrenaline, adrenaline and dopamine excretion. When ANOVA was significant ($P<0·05$), paired *t* tests were performed to compare each pair of treatments in order to detect the treatments between which there were the differences. As there were multiple comparisons, Bonferonni correction was applied and results of the paired *t* tests were considered statistically significant at $P<0·005$ (ten comparisons). The effects of the five treatments on visual analogue scale ratings were also determined by an ANOVA for repeated measures. Fasting ratings (before breakfast), area under the curve for the entire day (from after breakfast to 240 min after dinner) and mean rating for the three hours following each meal were compared. Results are presented as means with their standard deviation.

Results

Within all the variables measured, the ANOVA for repeated measures revealed that the EGCG–caffeine mixtures had an effect on 24 h energy expenditure, 24 h diastolic blood pressure and carbohydrate oxidation (Table 2). Paired *t* tests were then performed on these three variables to determine between which treatments there were differences. Table 2 also indicates that the EGCG–caffeine mixtures favoured an increase in sleeping metabolic rate and 24 h systolic blood pressure but the effect

Table 2. Effect of treatment on the variables measured in the metabolic chamber

(Mean values with their standard deviation for fourteen subjects)

	Placebo		270 mg EGCG/d (3 × 90 mg)		600 mg EGCG/d (3 × 200 mg)		900 mg EGCG/d (3 × 300 mg)		1200 mg EGCG/d (3 × 400 mg)	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
24 h energy expenditure (kJ/d)**	9421	1446	10 081	1585	10 134	1435	10 209	1705	10 249	1716
Sleeping metabolic rate (kJ)	7136	1181	7321	1094	7464	1156	7542	1195	7827	1330
24 h RQ	0·89	0·03	0·87	0·05	0·91	0·05	0·90	0·08	0·86	0·04
Carbohydrate oxidation (g/d)*	352·9	59·9	334·2	100·4	404·5	87·2	396·4	149·3	327·3	104·5
Lipid oxidation (g/d)	63·2	34·3	83·6	48·5	59·2	45·8	65·9	70·7	94·3	43·4
24 h heart rate (beats/min)	63	9	63	9	62	7	63	10	65	10
24 h systolic blood pressure (mmHg)	116	6	122	8	123	8	123	8	123	7
24 h diastolic blood pressure (mmHg)*	70	4	74	4	75	5	75	5	75	5
Noradrenaline excretion (nmol/d)	216	117	241	87	211	98	231	98	222	93
Adrenaline excretion (nmol/d)	<90	37	<104	29	<124	32	<110	36	<94	33
Dopamine excretion (nmol/d)	1950	871	1937	615	1856	586	1510	650	1674	583

EGCG, epigallocatechin-3-gallate.

Statistically significant effect of treatment (ANOVA for repeated measures for difference between groups): *$P<0·05$, **$P<0·001$.

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did not reach standard statistical significance. Thus, no *a posteriori* comparison was performed for these two variables.

The EGCG–caffeine mixtures increased 24 h energy expenditure by about 750 kJ (8 %) compared with placebo (Fig. 1). Increasing the dose of EGCG in the mixtures induced a mild increase in 24 h energy expenditure, but these differences were not significant, even between the lowest (270 mg/d: 3 × 90 mg) and the highest (1200 mg/d: 3 × 400 mg) doses. On the other hand, the paired *t* tests for carbohydrate oxidation did not reveal an *a posteriori* significant difference for any pair of treatments. Contrary to what was expected, the intake of EGCG–caffeine mixtures had no effect on RQ, lipid oxidation or catecholamine excretion.

As the different doses of EGCG induced the same increase in blood pressure for the day, the night and for the entire 24 h period, only the 24 h results are presented. Twenty-four hour systolic blood pressure was increased by about 7 mmHg, independently of the dose of EGCG (Fig. 2(a)). However, the effect of the EGCG–caffeine mixtures on 24 h systolic blood pressure did not reach significance. Twenty-four hour diastolic blood pressure was increased by about 5 mmHg by the EGCG–caffeine mixtures compared with placebo (Fig. 2(b)). The paired *t* test revealed a significant difference between placebo and the 270, 600 and 900 mg daily doses of EGCG, but there was no difference between placebo and the 1200 mg daily dose of EGCG or between the different doses.

Fasting visual analogue scale ratings did not differ between the five visits of the subjects in the metabolic chamber. The intake of EGCG–caffeine mixtures did not modify the response levels of hunger or satiety of the visual analogue scale, either for the entire day or for each meal analysed separately (results not shown).

Discussion

The objective of the present study was to investigate the impact of the mixture of green tea and Guarana extracts on energy metabolism with a design focused on a clinical outcome, but not intended to discriminate the independent effect of each extract. In fact, the main preoccupation in this study was to verify the possibility that increasing the EGCG content of a compound containing a fixed dose of caffeine (Guarana extracts) and EGCG (green tea extracts) enhances the impact of the compound on 24 h energy expenditure. As expected, the four different EGCG–caffeine mixtures tested in the study induced a significant increase in 24 h energy expenditure compared with the placebo. There were no statistically differences among the EGCG–caffeine mixtures with the different amounts of EGCG. Indeed, the difference between the highest (1200 mg/d: 3 × 400 mg) and lowest (270 mg/d: 3 × 90 mg) doses reached a difference of only about 168 kJ. In this regard, our results are clear and innovative in that they demonstrate that, beyond a certain threshold, the EGCG content of a compound only produces a small non-significant additional increase in 24 h energy expenditure. Therefore, from a clinical standpoint it does not appear relevant to increase the EGCG content of the reference mixture in order to produce a substantial increase in daily energy expenditure.

No effects of the EGCG–caffeine mixtures on RQ and macronutrient oxidation were observed. This is possibly related to the high variability observed in the results with the different doses of EGCG. This absence of effect of the EGCG–caffeine mixture on RQ is concordant with the results obtained by Kovacs *et al.* (2004) during weight maintenance with green tea (323 mg/d: about 108 mg EGCG three times daily; 104 mg/d: about 35 mg

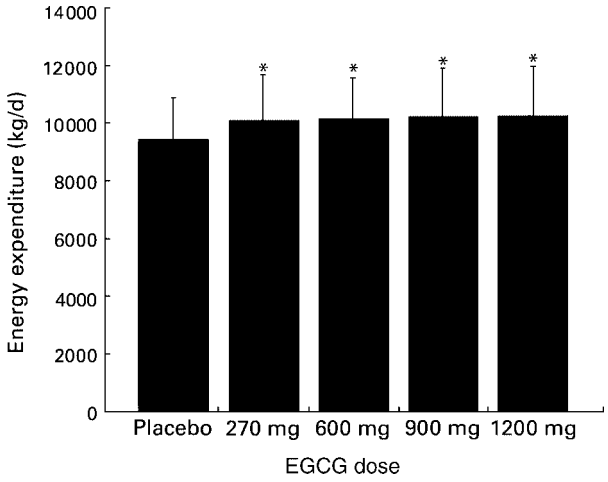

Fig. 1. Twenty-four hour energy expenditure with the placebo and the epigallocatechin-3-gallate (EGCG)–caffeine mixtures containing 200 mg caffeine (600 mg/d) and different daily doses of EGCG. Values are means with their standard deviation shown by vertical bars. Mean values were significantly different from placebo (paired *t* test with Bonferonni correction): **P*< 0·005.

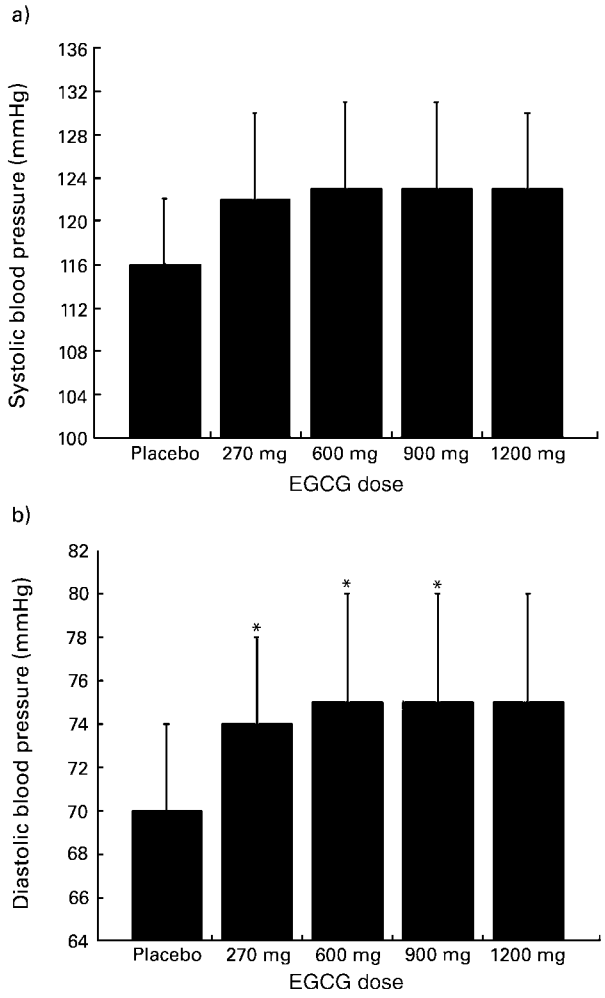

Fig. 2. Twenty-four hour systolic (a) and diastolic (b) blood pressure with the placebo and the epigallocatechin-3-gallate (EGCG)–caffeine mixtures containing 200 mg caffeine (600 mg/d) and different daily doses of EGCG. Values are means with their standard deviation shown by vertical bars. Mean values were significantly different from placebo (paired *t* test with Bonferonni correction): **P*< 0·005.

caffeine three times daily) after weight loss. However, when comparing the EGCG–caffeine mixture with the lowest dose of EGCG (90 mg) with the placebo, there appears to be a decrease in RQ and an increase in lipid oxidation. This is in accordance with results obtained with the same EGCG dose in the study by Dulloo *et al.* (1999). Therefore, it could be hypothesized that the 90 mg (270 mg/d) EGCG dose is the optimal concentration to produce an effect on macronutrient oxidation. It is to be noted that substantial fluctuations in RQ were observed in the present study, which is concordant with the fact that RQ is a less stable variable than energy expenditure and is characterized by a lower reproducibility than 24 h energy expenditure (White *et al.* 1996). In this regard, we cannot exclude the possibility that increased fluctuation in 24 h RQ might have prevented the demonstration of a significant EGCG effect.

The EGCG–caffeine mixtures did not produce significant increases in heart rate as was observed in the Dulloo *et al.* (1999) study. However, a non-significant increase in 24 h systolic blood pressure accompanied by a significant increase in 24 h diastolic blood pressure was observed. It is possible that the EGCG–caffeine treatment used by Dulloo *et al.* (1999) produced a slight increase in blood pressure even if there was no change in heart rate. However, blood pressure measurements were not tested and/or presented in that study. Since regular physical activity results in a decrease in resting heart rate and blood pressure (Seals & Hagberg, 1984; McArdle *et al.* 1996), we can suppose that adding regular exercise when taking the EGCG–caffeine mixture could be helpful to prevent the slight cardio-stimulatory effects produced by this mixture. This beneficial effect has been observed with the weight-loss medication Meridia™, which is known to produce increases in blood pressure and heart rate. Indeed, it was shown that combining physical activity with Meridia™ prevented the cardio-stimulatory effects that were observed when the drug was combined with diet alone (Bérubé-Parent *et al.* 2001).

The EGCG–caffeine mixture should be considered as a good complement in a weight-loss programme. Indeed, this EGCG–caffeine mixture appears to have potential benefits in the treatment of obesity and should be further tested in a clinical context where nutrition counselling and supervision are offered with regular physical activity participation. Such an approach would be expected to attenuate the decrease in energy expenditure related to body-weight loss while preventing cardio-stimulating effects.

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