European Journal of Clinical Nutrition (2005) 59, 733–741
 0954-3007/05 $30.00
www.nature.com/ejcn

ORIGINAL COMMUNICATION

Bioactive food stimulants of sympathetic activity: effect on 24-h energy expenditure and fat oxidation

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Objective: Bioactive food ingredients influence energy balance by exerting weak thermogenic effects. We studied whether the thermogenic effect of a combination of capsaicin, green tea extract (catechins and caffeine), tyrosine, and calcium was maintained after 7-day treatment and whether local effects in the gastric mucosa were involved in the efficacy.
Design: The present study was designed as a 3-way crossover, randomised, placebo-controlled, double-blinded intervention.
Setting: Department of Human Nutrition, RVAU, Denmark.
Subjects: A total of 19 overweight to obese men (BMI: $28.0 \pm 2.7\,kg/m^2$) were recruited by advertising locally.
Intervention: The subjects took the supplements for a period of 7 days. The supplements were administrated as a simple supplement with the bioactive ingredients, a similar enterocoated version, or placebo. In all, 24-h energy expenditure (EE), substrate oxidations, spontaneous physical activity (SPA), and heart rate were measured in respiration chambers on the seventh day of each test period.
Results: After adjustment for changes in body weight and SPA, 24-h EE was increased by 160 kJ/day (95% CI: 15–305) by the simple preparation as compared to placebo, whereas the enterocoated preparation had no such effect (53 kJ/day, −92 to 198); simple *vs* enterocoated versions ($P = 0.09$). The simple preparation produced a deficit in 24-h energy balance of 193 kJ/day (49–338, $P = 0.03$). Fat and carbohydrate oxidation were equally increased by the supplements.
Conclusion: A supplement containing bioactive food ingredients increased daily EE by ~200 kJ or 2%, without raising the heart rate or any observed adverse effects. The lack of effect of the enterocoated preparation suggests that a local action of capsaicin in the gastric mucosa is a prerequisite for exerting the thermogenic effect.
Sponsorship: Supported by Science, Toxicology & Technology, San Francisco, CA, USA.
European Journal of Clinical Nutrition (2005) **59,** 733–741. doi:10.1038/sj.ejcn.1602121
Published online 4 May 2005

Keywords: energy expenditure; capsaicin; caffeine; green tea; tyrosine; calcium

Introduction

In an attempt to understand the development of the current obesity, epidemic studies have estimated the magnitude of surplus in daily energy balance required for the apparent chronic weight gain at population level. Hill *et al* (2003) found that 90% of the population consumes an excess of 209 kJ/day or less. This implies that an intervention that reduces the positive energy balance by 209 kJ/day could offset weight gain in about 90% of the population.

It has previously been shown that the effects of ephedrine and caffeine on appetite and thermogenesis are rather weak when administered separately, but are potentiated in a synergistic fashion when given in combination (Dulloo, 2002). The principal mode of action is an enhancement of activation of the sympathetic nervous system (SNS), which causes suppression of hunger, enhanced satiety, and stimulation of energy expenditure (EE), covered in part by increased fat oxidation (Astrup *et al*, 1991). A 6-month clinical trial has confirmed that a combination of ephedrine/caffeine can produce greater weight loss in obese subjects than the agents given separately (Astrup *et al*, 1992). However, during the last

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Received 1 June 2004; revised 18 November 2004; accepted 21 December 2004; published online 4 May 2005

decade, the safety of ephedrine has been questioned (Haller & Benowitz, 2000; Greenway, 2001; Shekelle *et al*, 2003). We therefore found it pertinent to examine the possibility of achieving a synergistic effect of well-known harmless food ingredients to increase SNS activity.

The synthesis of norepinephrine (NE) in SNS may be enhanced by increasing the substrate supply of the NE precursor tyrosine. Diet supplementation with tyrosine will not increase SNS activity to any detectable degree, but there is experimental evidence to suggest that tyrosine may potentiate the effect of ephedrine and other SNS stimulants on energy balance (Hull & Maher, 1990).

Capsaicin is the major pungent principle in hot red pepper. It has been shown in human studies that the addition of capsaicin to meals increases SNS activity, EE, and, in some studies, fat oxidation (Yoshioka *et al*, 1998, 1999; Lejeune *et al*, 2003). However, decreased fat oxidation and increased respiratory quotient (RQ) have previously been observed when capsaicin is added to meals (Yoshioka *et al*, 1995; Lim *et al*, 1997). Capsaicin is thought to activate the sympathetic nerves via specific receptors by stimulating the release of NE into the synaptic cleft, where NE interacts with the adrenergic receptors. (Caterina *et al*, 2000; Vogel, 2000). In addition, capsaicin has been shown to possess appetite suppressant qualities (Yoshioka *et al*, 1998, 1999). The adrenergic receptor stimulation of NE produces increased thermogenesis, causes release of fatty acids for combustion, or in the case of a central action, mimics the natural effect of satiety on appetite control. However, the signal is usually short lived because NE is rapidly removed or degraded from the synaptic cleft by catechol *O*-methyltransferase (COMT) (Durand *et al*, 1977). Various compounds, such as polyphenols from tea, inhibit this enzyme (Borchardt & Huber, 1975; Rhodes, 1996; Dulloo *et al*, 2000). A green tea extract rich in the polyphenol catechin epigallocatechin gallate has been shown to increase SNS activity acutely, and to increase 24-h EE and fat oxidation in humans (Dulloo *et al*, 1999). Furthermore, a recent study has shown that catechins and caffeine interact synergistically in suppression of fat accumulation in mice (Zheng *et al*, 2004).

The intracellular signal producing increased lipolysis in adipose tissue, heat production in skeletal muscle, and putative satiety signals in the liver is dependent on the production of cyclic adenosine-mono-phosphate (cAMP). The increased cAMP response is short-lived, because cAMP is rapidly degraded by phosphodiesterase. The intracellular signal can be sustained for a longer time by the inhibition of phosphodiesterase by methylxanthines. Methylxanthines are a group of related agents including caffeine, paraxanthine and others, occurring naturally in numerous food products such as coffee and tea. Methylxanthines have weak effects on thermogenesis and appetite, but they are potent amplifiers of thermogenesis in humans when given in conjunction with agents stimulating SNS. Apart from their inhibition of phosphodiesterase, methylxanthines are also antagonists of the inhibitory effect of adenosine on NE release (Dulloo *et al*, 1994). Finally, there is some evidence to suggest that high calcium intake produces weight loss in humans and rodents (Zemel *et al*, 2000; Zemel, 2002; Papakonstantinou *et al*, 2003; Parrikh & Yanovski, 2003). The mode of action may be through a decrease in the intracellular Ca^{2+} levels in adipocytes, followed by a decrease in lipogenic gene expression and activity, thereby promoting lipolysis (Zemel *et al*, 2000; Zemel, 2002; Parrikh & Yanovski, 2003). Furthermore, some studies suggest that dietary calcium can bind fatty acid in the gut and thereby decrease fat absorption (Welberg *et al*, 1994; Jacobsen *et al*, 2005). However, there are some inconsistencies in the evidence of the weight-reducing effect of calcium, (Barr, 2003) and further studies are needed.

The purpose of the present study was to examine whether a combination of five bioactive food ingredients (capsaicin, catechins, caffeine, tyrosine, and calcium) taken as a daily supplement could increase 24-h EE after 7 days of intake, and also if local effects of capsaicin in the gastric mucosa were involved in efficacy or caused side effects.

Subjects and methods

Subjects

A total of 19 healthy but overweight to obese men (age: 40.8 ± 13.1 y, BMI: $28.0 \pm 2.7\,kg/m^2$) participated in the study. They were weight stable (± 3 kg in last 3 months), nonsmoking, nonathletic, and had no use of dietary supplements or frequent use of medication. The subjects followed a normal Danish habitual diet, with rare use of hot spices, and avoided extreme intake of dairy products or coffee/tea. Frequency of caffeine intake was not an exclusion or inclusion criterion. The subjects were recruited by advertisement in local newspaper. All subjects gave their written consent after having received verbal and written information about the study. The study was approved by The Municipal Ethical Committee of Copenhagen and Frederiksberg, and it was in accordance with the Helsinki II Declaration.

Experimental design

The present study was designed as a 3-way crossover, randomised, placebo-controlled, double-blinded study with each supplementation period of 7 days separated by a >6-day washout period. Both supplements (verum treatment) were administered as one tablet containing green tea extract (250 mg — whereof 62.5 mg catechins and 25 mg caffeine), tyrosine (203 mg), anhydrous caffeine (25.4 mg), and capsaicin (0.2 mg ~ 40.000 heat units). One supplement contained capsaicin in a simple release formulation, and the other supplement contained capsaicin in a controlled (enterocoated) release formulation. Both supplements were taken together with one tablet containing biolactacal calcium (655 mg) (Table 1). The two tablets were taken three times a day (t.i.d.). The placebo tablets contained microcrystalline cellulose and could not be distinguished from the verum

Table 1 Description of daily dose of ingredients in the bioactive study supplements

Ingredient	*Daily dosage*
Green tea extract	750 mg (whereof 188 mg catechins)
L-tyrosine	609 mg
Caffeine	151 mg (whereof 75 mg from green tea and 76 mg anhydrous caffeine)
Cayenne[a]	225 mg (whereof 0.6 mg capsaicin or 120.000 heat units)
Biolactacal calcium[b]	1965 mg (whereof 550 mg elementary calcium)

[a]Cayenne was the enterocoated ingredient in the supplement with enterocoated release formulation, and untreated in the simple release supplement. [b]Taken as a separate tablet together with the experimental tablets containing green tea, tyrosine, caffeine and capsaicin (simple *vs* enterocoated release) t.i.d.

supplements with respect to colour, taste, smell, or appearance. Both verum and placebo supplements were taken 30 min before breakfast, lunch, and dinner. The capsaicin-containing tablets were of similar dosage, but differed in release form. The capsaicin compound in the simple release formulation was released in the stomach, whereas the controlled release formulation of the capsaicin component was enterocoated in order to delay uptake until the small intestine. The subjects were not allowed to change their dietary and beverage habits (including intake of coffee and tea), use of spices, level of physical activity, smoking habits, and use of medication, throughout the study period.

Respiratory measurements

On the seventh and last day of each supplementation period EE and substrate oxidation rate were assessed by 24-h indirect whole-body calorimetry in a 14.7 m^3 respiratory chamber (previously described in detail (Astrup *et al*, 1990; Vasilaras *et al*, 2001)). The within-subjects coefficient of variation for repeated measurements of 24-h EE was 1.4%.

Body temperature was assessed by digital thermometer (Becton Dickinson, Franklin Lakes, NJ, USA) at 08:00 prior to the respiratory measurement, in order to detect possible infections. A urine sample, collected at the start of the respiratory measurement, was tested for proteinuria, haematuria, and glucoseuria. Urine was subsequently collected throughout the 24-h measurement and used to adjust the respiratory measurements for nitrogen excretion.

The protocol included standardised respiratory measurement of 24-h EE, which was assessed from the start of the respiratory measurement at 09:00 and continued for 24 h. The subjects had fasted for 10 h and abstained from physical activity and use of alcohol and medication for 24 h before each chamber measurement. The basal metabolic rate (EE_{BMR}) was measured in the last hour of the chamber stay, after 13 h of fasting. EE during sleep was measured from 01:00 to 06:00. Other parameters measured were 24-h oxidation of macronutrients (carbohydrate, fat, and protein), 24-h RQ, and 24-h energy balance (energy balance = energy intake − energy expenditure).

Heart rate was registered by a portable ECG devise (Dialogue 2000 type 2070-14 XTNJ, Dania Electronics, Rødovre, Denmark) attached to the subjects. Spontaneous physical activity (SPA) was assessed by microwave radar detectors (Sisor Mini-Radar, Static Input System SA, Lausanne, Switzerland). SPA indicates the percentage of time in which the subjects are active to a detectable degree.

The 24-h EE protocol included scheduled physical activity: two sessions of 15 min cycling on an ergometer bicycle (Monark 814E, Monark AB, Varberg, Sweden) (75 W), and two sessions of walking back and forth 25 times in the chamber. Only sedentary activities were otherwise allowed.

Body weight, blood pressure, and body composition were assessed after completion of the chamber stay. Body weight was measured to the nearest 0.05 kg on a decimal scale (Lindeltronic 8000, Copenhagen, Denmark) and height to the nearest 0.5 cm. Blood pressure was measured by an automatically inflating cuff (digital blood pressure meter model UA-743, A&D Company Ltd, Tokyo, Japan). Body composition, fat-free mass (FFM), and fat mass (FM) were estimated by bioelectrical impedance analysis using an Animeter (HTS-Engineering Inc, Odense, Denmark) and calculated as described previously (Lukaski *et al*, 1986).

Respiration chamber diets

The subjects were given three main meals and one snack during the 24 h. These were identical on each chamber stay. The individual diet was designed to match requirements in a controlled weight-maintenance diet, estimated after Klausen *et al* (1997). Energy from protein, carbohydrate, and fat were 17, 56, and 27%, respectively, calculated using The Danish Nutrient Database, Dankost 2000® (version 1.4C, National Food Agency of Denmark, Søborg, Denmark). The subjects were allowed to drink water and decaffeinated coffee/tea *ad libitum* during the chamber stay. Subjects recorded the amount and type of beverage consumed on each chamber stay, and this proved to be similar on all three occasions.

On completion of respiratory measurements, the subjects were given an *ad libitum* breakfast 2 h after intake of one-third of the daily supplement. The meal was composed of 325 g cheese sandwiches and 150 ml water. The subjects were instructed to eat at a constant pace and to stop eating when they felt satiated. *Ad libitum* energy intake (EI_{ad}) was assessed from the amount of the meal consumed.

Questionnaires

Visual analogue scales (VAS) were used to monitor each subject's appetite sensations before and after completion of the *ad libitum* meal. Composition of VAS has been described previously (Hill *et al*, 1984). The scales contained questions about subjective sensations of hunger, satiety, prospective consumption, fullness, thirst, well-being, and desire to eat

something sweet, salty, rich in fat, or meat/fish. The subjects were instructed to complete VAS immediately before the start of the meal and 10, 20, and 30 min after finishing the meal. To monitor the subjective opinion of organoleptic quality of the meal, VAS of appearance, smell, taste, aftertaste, and general palatability were completed immediately after the meal.

Subjects were also supplied with a booklet containing identical questions for each day to be completed consecutively during the supplementation periods. The questionnaires included questions about compliance to treatment, side effects, and general well-being.

Statistical analysis

All results are given in mean and 95% confidence interval (95% CI). The significant level is set at <0.05. Statistical analyses were performed using SAS 8.2 (SAS Institute, Cary, NC, USA). All data were, prior to the statistical analysis, tested for normality by the Shapiro–Wilk *W*-test, and variance homogeneity and data-transformed if necessary. Differences between supplements were tested by analysis of mixed linear models, with or without adjusting for various confounders. *Post hoc* comparisons were made, with Turkey–Kramer adjustment of significance levels for the pair-wise comparison, using unpaired *t*-test when the analysis indicated significant treatment effect.

Estimates of the difference between verum and placebo in 24-h EE were calculated by subtracting placebo from the active treatments ($EE_{treatment}-EE_{placebo}$) to investigate the influence of various confounders. Data were analysed by analysis of mixed linear models. The difference between verum and placebo was significant if zero was not included in the 95% CI. The relationship between 24-h EE and 24-h heart rate (both placebo-subtracted) was tested in a Pearson correlation test.

The ratings of VAS were calculated as an area under the curve (AUC). Difference between supplements was tested by the analysis of mixed linear models adjusted for baseline.

Difference between treatments in the prevalence of self-reported side effects was tested by the homogeneity test.

Results

EE and substrate oxidations

There were no significant differences within or between group in unadjusted body weight, FFM, FM, or SPA on the seventh day of treatment (Tables 2 and 3), or in unadjusted 24-h EE, BMR, EE_{sleep}, and energy balance (Table 3). There was no periodic effect on 24-h EE, or any interaction between supplements and the previous supplement (carry-

Table 2 Physical characteristics of the 19 subjects measured after the 24-h respiratory chamber stay

	Simple supplement	*Enterocoated supplement*	*Placebo*
Body weight (kg)	90.2 (86.2:94.3)	90.1 (86.2:94.3)	89.9 (86.2:94.3)
BMI (kg/m^2)	27.7 (26.6:28.8)	27.6 (26.6:28.8)	27.6 (26.5:28.7)
Fat-free mass (kg)	65.4 (63.3:68.0)	65.6 (63.3:68.0)	65.5 (63.3:68.0)
Fat mass (kg)	24.7 (22.4:27.5)	24.4 (21.9:26.9)	24.3 (21.9:26.9)

Mean (95% CI), $n=57$ observations. Data were analysed in mixed linear models.

Table 3 Energy expenditure (EE), energy balance, physical activity level (SPA), systolic and diastolic blood pressure (SBP and DBP, respectively), and heart rate measured for 24-h in a respiratory chamber

	Simple supplement	*Enterocoated supplement*	*Placebo*
24-h EE (MJ/day)	11.1 (10.7:11.5)	10.9 (10.5:11.5)	10.9 (10.5:11.5)
24-h EE_{adj} (MJ/day)[a]	11.1 (10.8:11.3)[b]	11.0 (10.7:11.2)	10.9 (10.7:11.2)
BMR-EE (kJ/h)	386 (363:407)	379 (354:398)	378 (358:398)
BMR-EE_{adj} (kJ/h)[a]	386 (374:397)	384 (373:395)	383 (372:395)
Sleep-EE (kJ/h)	342 (325:359)	337 (320:354)	342 (325:358)
Sleep-EE_{adj} (kJ/h)[a]	340 (331:348)	337 (329:346)	338 (330:347)
Energy intake (MJ/day)	10.2 (9.8:10.5)	10.2 (9.8:10.5)	10.2 (9.8:10.5)
24-h energy balance (kJ/day)	−959 (−1336:−582)	−790 (−1167:−413)	−758 (−1135:−381)
24-h energy balance$_{adj}$ (kJ/day)[a]	−947 (−1308:−587)[c]	−806 (−1167:−446)	−754 (−1114:−393)
SBP (mmHg)	125 (119:131)	119 (113:126)	121 (115:127)
DBP (mmHg)	75 (70:80)	72 (67:77)	73 (68:78)
Heart rate (bpm)	67 (63:71)	66 (62:69)	66 (63:69)
Heart rate$_{adj}$ (bpm)[a]	67 (64:71)	66 (62:70)	66 (63:70)
24-h respiratory quotient	0.84 (0.83:0.85)	0.84 (0.83:0.85)	0.84 (0.82:0.85)
24-h SPA (%)	8.4 (7.8:9.0)	8.4 (7.7:8.9)	8.4 (7.8:9.0)

Mean (95% CI), $n=57$ observations. Nonadjusted and adjusted variables analysed in mixed linear models. Pair-wise comparisons between supplements were adjusted with the Turkey–Kramer test.
[a]Adjusted for weight and SPA.
[b]Tendency towards significant difference between the simple formulation and placebo, $P=0.06$.
[c]Significant difference between the simple formulation and placebo, $P<0.05$.

Table 4 Placebo-subtracted 24-h energy expenditure before and after adjustment for various confounders

Covariate adjustment	*Significance of covariable(s)*	*Simple* vs *placebo*	*Enterocoated* vs *placebo*	*Difference between supplements*
No adjustment		181 (−19:380)	32 (−168:232)	$P=0.07$
BW	$P=0.001$	167 (−1:336)	45 (−123:214)	$P=0.08$
24-h SPA	$P=0.001$	168 (4:331)	45 (−118:208)	$P=0.1$
BW/24-h SPA	$P=0.03/0.04$	160 (15:305)	53 (−92:198)	$P=0.09$

BW: placebo-subtracted body weight (kg), 24-h SPA: placebo-subtracted 24-h spontaneous physical activity (%), EI: placebo-subtracted energy intake (kJ/day). Mean (95% CI), $n=38$ observations. Data were analysed in mixed linear models.

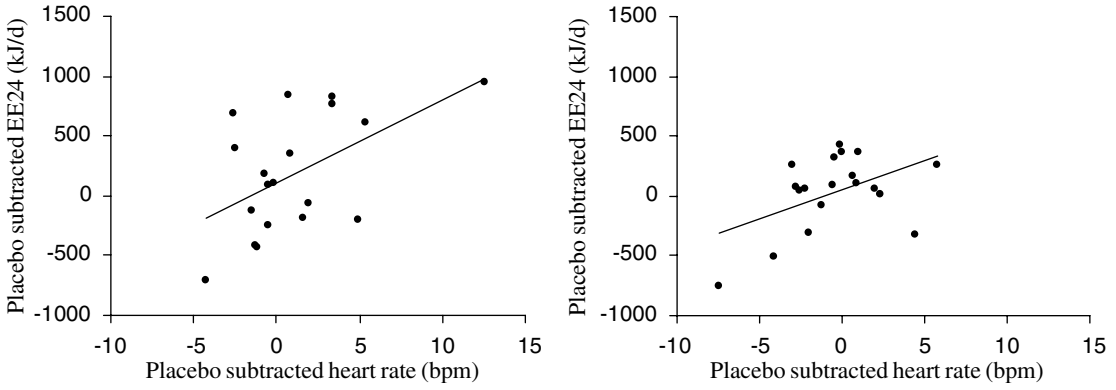

Figure 1 Relationship between placebo-subtracted estimates of 24-h EE and 24-h heart rate in 19 overweight men. Data were analysed by Peareson coorelation: left panel — simple supplement ($r=0.53$, $P=0.02$), Right panel — enterocoated supplement ($r=0.47$, $P=0.04$).

over effect). However, the small group differences in body weight and SPA influenced EE. We therefore adjusted 24-h EE for both covariates, that is, body weight and SPA (Table 4). After adjustment, 24-h EE was increased significantly by 160 kJ/day (95% CI: 15–305) by the simple preparation as compared to placebo, whereas the enterocoated preparation had no effect (53 kJ/day, −92 to 198) On average, energy balance was slightly negative during the chamber stays with all supplements. The simple formulation produced a significant deficit in 24-h energy balance, 193 kJ/day (49–338, $P=0.03$) compared to placebo. There was no indication of significant difference in unadjusted or adjusted BMR, EE_{sleep}, or 24-h respiratory quotient (Table 3).

There was observed positive correlation between the placebo-subtracted 24-h EE and the placebo-subtracted 24-h heart rate for both verum treatments (simple formulation, $r=0.53$, $P=0.02$; enterocoated formulation, $r=0.47$, $P=0.04$) (Figure 1).

There was no treatment effect on 24-h protein, carbohydrate, or fat oxidation, and this was not altered by adjustment for energy balance and body weight (Figure 2).

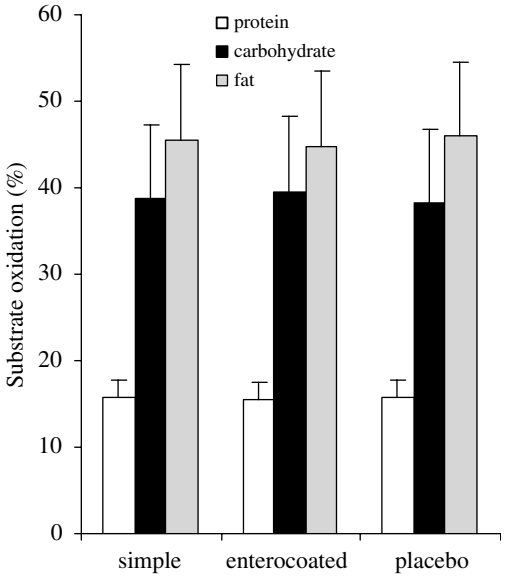

Figure 2 Substrate oxidation (%) of 19 overweight men measured during the 24-h chamber stay. Data are presented as mean ± s.d. and were analysed by mixed linear models adjusted for body weight and energy balance. No significant difference was found between supplements.

Spontaneous physical activity, heart rate and blood pressure

Total unadjusted 24-h SPA was similar with both treatments and with placebo. The same applied for heart rate, and diastolic and systolic blood pressure (Table 3).

Appetite sensations and *ad libitum* intake

Treatment period exerted a strong confounding effect ($P=0.0005$) on EI_{ad}. There were no significant effects of

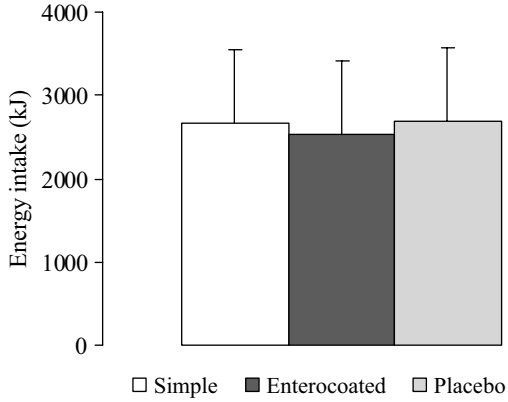

Figure 3 *Ad libitum* energy intake (kJ) of 19 overweight men. The *ad libitum* meal was served as breakfast after completion of the chamber stay, and 2 h after intake of the supplements. Data are presented as mean ± s.d. and were analysed by mixed linear models adjusted for period. No significant difference was found between supplements.

verum *vs* placebo before and after adjustment for treatment period. There was no significant difference between the enterocoated formulation and placebo. However, EI_{ad} decreased by 6% following the enterocoated formulation as compared to placebo (−180 kJ (−458:98), adjusted −154 kJ (−385:76)) (Figure 3). EI_{ad} was similar on the simple formulation and on placebo (−78 kJ (−356:201), adjusted 27 kJ (−282:228)). There was no significant effect of either meal sequence or time on energy intake.

VAS ratings of the appetite sensations showed no significant difference between supplements. Subjects rated the organoleptic quality of the meal as mediocre, and there was no significant difference between supplements.

Adverse effects

The frequency of self-reported side effects was similar with all supplements (P = NS). However, the placebo supplement gave rise to a higher frequency of headaches than did verum (Table 5). Borborygmia and flatulence were reported by 16 and 11% of the subjects on the simple and enterocoated versions, respectively.

Discussion

In rodents it has been shown that injection of capsaicin (Kawada *et al*, 1986; Watanabe *et al*, 1987) and treatment with capsaicin (10–100 mg/kg body weight/day) (Watanabe *et al*, 1988) can increase the activity of SNS by enhancing the release of NE. In previous human studies, capsaicin has been given as red pepper in single meals. The addition of about 30 mg capsaicin to meals increased SNS activity and EE (Lim *et al*, 1997; Yoshioka *et al*, 1998). We used a very low dose of capsaicin (0.2 mg before each meal), but combined it with other food ingredients thought to enhance the effect of capsaicin on sympathetic activation. The low dose was chosen because initial tolerability studies showed that capsaicin can be given in quite high amounts as red pepper in meals, but the same doses in capsules give rise to gastric discomfort and burning sensation in the anal mucosa when voiding. The estimated maximum tolerable dose given as capsules should not exceed more than 240–300.000 Scoville heat units per day when given to subjects with infrequent habitual intake of hot peppers (Belza *et al*, unpublished results).

The daily dose of caffeine was equivalent to 150 mg caffeine, which corresponds to a cup of Italian espresso coffee. Yoshioka *et al* (2001) have previously found that the acute administration of a combination of a daily dose of 27.8 g red pepper (*Saemaul Kongjang*) with an estimated capsaicin content of 3 mg/g red pepper and 800 mg caffeine increases 24-h EE by 3% compared to red pepper administrated alone. However, a comparison cannot be made with the present results, as an accurate dose in milligram capsaicin or in Scoville heat units was not reported. Despite the much lower dose of capsaicin, we were able to detect a significant effect of the simple version of the supplement on 24-h EE 160 kJ/day (95% CI: 15–305) compared to the placebo treatment. As we gave a combination of five different ingredients to act synergistically to enhance sympathetic activity, the present study could not evaluate the contribution of each of the ingredients separately. It would require a very large study to provide the statistical power to pick up very small effects on 24-h EE. However, the lack of effect of the enterocoated version suggests that a local action of capsaicin in the gastric mucosa is required to provide the essential stimulation of SNS, probably through afferent reflexes acting via vanilloid-1 receptors (Holtzer,

Table 5 Number of subjects reporting side effects during 7-day treatment with the simple version of the supplement, the enterocoated supplement, and the placebo

Side effects	*Simple supplement*	*Enterocoated supplement*	*Placebo*
Stomach pain	2	1	2
Watery faeces	2	1	4
Blood in faeces	1		
Increased defecation frequency	1	2	1
Constipation/inspissated faeces	2	1	1
Painful urination/defecation	2	1	1
Borborygmia/flatus	3	2	
Heartburn	1	2	1
Headache	1	2	5
Nausea/vertigo	1	1	1
Increased sweating		1	2
Decreased appetite		1	
Increased appetite	1		1
Increased thirst		3	1
Total[a]	17	18	20

[a]Homogeneity test was used when testing the difference between supplements in the prevalence of total self-reported side effects (P = NS).

2002; Ward *et al*, 2003). If there is a dose-response relationship, it is possible that 2–3 times higher doses of the simple version of the supplement may increase the observed 2% increase in 24-h EE to 4–5%. A study examining the effect of double the dose of the ingredients is planned.

It is interesting that many efforts to develop β_3-agonists with thermogenic properties have failed to increase 24-h EE in humans during repeated use. The highly selective and potent β_3-agonist at the cloned human β_3-receptor L-796568 was found to increase EE quite markedly in rodents and non-human primates, and acutely in humans (van Baak *et al*, 2002). By contrast, when it was administered chronically over 28 days in humans, no effect on 24-h EE could be detected (Larsen *et al*, 2002). In this context, we find it encouraging that a significant thermogenic effect of the test compounds in the present study was still present after 7 days of chronic treatment.

The 7-day supplementation by capsaicin, catechins, caffeine, tyrosine, and calcium did not change RQ, indicating that the relative mixture of macronutrients being oxidised was unchanged, or that the changes were too small to be detected. However, there are inconsistencies in the evidence of changes in fat utilisation with capsaicin supplementation (Yoshioka *et al*, 1995, 1998; Lim *et al*, 1997; Lejeune *et al*, 2003). Other compounds such as caffeine and green tea extract (catechins and caffeine) have also been shown to increase lipid oxidation by enhancing SNS activity (Bracco *et al*, 1995; Dulloo *et al*, 1999) but studies confirming these findings are lacking. Tappy *et al* (1995) have shown that increasing SNS activity stimulates plasma NE by 27%, and fat oxidation by 72% in the postprandial state. The failure to increase the proportion of EE covered by fat oxidation in the present study was probably due to the low dose. Unfortunately, no data on NE levels are available to support the present results. However, assessment of NE levels will be included in future studies.

An activation of the sympathetic nervous system is thought to suppress hunger and enhance satiety (Astrup *et al*, 1992; Raben *et al*, 1996). However, we found only an insignificant decrease of 6% in EI_{ad} with 7-day supplementation with the enterocoated formulation. Previous findings by Yoshioka *et al* (1999) suggest that a reduction in energy intake may be mediated by an increase in SNS when capsaicin is added to single meals. Furthermore, the same group has reported that capsaicin and caffeine additionally reduce EI_{ad} in a synergistic fashion (Yoshioka *et al*, 2001). One could speculate that the reduction in energy intake could partly be attributed to a reduced palatability of the diet when red pepper was added. However, two studies have found that the EI reducing effect of capsaicin may occur both in the mouth and in the gastrointestinal regions by stimulating sensory pathways activating SNS in a dose-dependent manner (Westerterp-Plantega *et al*, 2004; Yoshioka *et al*, 2004). Therefore, the trend towards a reduced energy intake seen in the present study may lead to weight loss with higher doses taken over a longer period.

The supplements with the five bioactive ingredients would be expected to possess a good safety profile, but the interactions between the ingredients could theoretically produce side effects not seen when the individual components are given separately. However, tolerability was good and we found no increase in blood pressure or heart rate. Although the study was not powered to assess frequency of adverse events, the number of subjects reporting adverse events was similar in all three groups (Table 5). More subjects tended to report headache during the placebo supplement period, which is a well-known caffeine withdrawal symptom and may be related to the lower caffeine intake in the placebo group as compared to the two verum supplements. On the other hand, more subjects tended to report borborygmia and flatulence during the verum supplement periods. However, previous studies have shown that the capsaicin binding receptor, vanilloid receptor-1 is associated with pain sensations (Caterina *et al*, 2000; Vogel *et al*, 2000; Holtzer, 2002). Therefore, it is possible that capsaicin supplementation could cause pain or burning sensations when released in the gastrointestinal region. However, these side effects were reported with similar frequency in verum and placebo treatments. Only the fresh blood in faeces of one subject in the simple supplement group was of concern, although the causal relation to the treatment could not be established. In our tolerability study of 15 subjects, no such events were reported (Belza *et al*, unpublished results), but tolerability and safety need to be addressed in a clinical trial.

In conclusion, a supplement composed of a mixture of low doses of capsaicin, green tea extract (including catechins and caffeine), tyrosine, and calcium taken three times a day for 7 days increased 24-h EE by 2% ($\sim$200 kJ/day) in overweight subjects. The current dose may be of value in the prevention of weight gain and weight regain, and a higher dose may have clinical relevance in the treatment of obesity.

Acknowledgements

We thank John Lind for his expert technical assistance. The study was supported by a grant from Science, Toxicology & Technology, San Francisco, California. The dietary supplements containing the ingredients examined in the present paper were manufactured by Alpine Health Products, Salt Lake City, Utah, and are not commercially available.

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