Effect of capsinoids on energy metabolism in human subjects

Jose E. Galgani, Donna H. Ryan and Eric Ravussin*
Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808, USA
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Capsinoids are non-pungent compounds with molecular structures similar to capsaicin, which has accepted thermogenic properties. To assess the acute effect of a plant-derived preparation of capsinoids on energy metabolism, we determined RMR and non-protein respiratory quotient (NPRQ) after ingestion of different doses of the capsinoids. Thirteen healthy subjects received four doses of the capsinoids (1, 3, 6 and 12 mg) and placebo using a crossover, randomised, double-blind trial. After a 10 h overnight fast as inpatients, RMR was measured by indirect calorimetry for 45 min before and 120 min after ingesting capsinoids or placebo. Blood pressure and axillary temperature were measured before (−55 and −5 min) and after (60 and 120 min) dosing. Before dosing, mean RMR was 6247 (SE 92) kJ/d and NPRQ was 0·86 (SE 0·01). At 120 min after dosing, metabolic rate and NPRQ remained similar across the four capsinoids and placebo doses. Capsinoids also had no influence on blood pressure or axillary temperature. Capsinoids provided in four doses did not affect metabolic rate and fuel partitioning in human subjects when measured 2 h after exposure. Longer exposure and higher capsinoids doses may be required to cause meaningful acute effects on energy metabolism.

Capsinoids: Thermogenesis: Respiratory quotient: Fat oxidation

Obesity is pandemic and continues to increase in developed, developing and even some societal strata in emerging economies. At present, energy restriction and increased physical activity are advocated in most weight control programmes; however, sustained changes in diet and physical activity are difficult to achieve.

Growing interest exists to find natural substances or extracts that modify energy balance. Capsaicinoids (i.e. capsaicin) are naturally present in chilli pepper. Capsaicin increases the activity of the sympathetic nervous system, in parallel with increased catecholamine secretion, energy expenditure and fat oxidation. However, given its strong pungency, not all people feel comfortable consuming it. Capsaicin-like compounds such as capsinoids (i.e. capsiate, dihydrocapsiate and nordihydrocapsiate) are much less pungent and have also been shown to increase the activity of the sympathetic nervous system, adiPose tissue uncoupling protein content, energy expenditure and fat oxidation in mice. In human subjects, Ohnuki et al. found increased body temperature and higher VO₂ after ingestion of a single dose of capsinoids-rich peppers (CH-19 sweet; Capsicum annuum L.). Additionally, an increased VO₂ (per kg of body weight) associated with a small reduction in body weight was observed in overweight individuals after ingesting 10 mg/d of encapsulated capsinoids for 4 weeks. Very recently, Snitker et al. have reported that overweight individuals ingesting 6 mg/d of encapsulated capsinoids for 12 weeks had increased fat oxidation and reduced abdominal fat compared with placebo-treated subjects. Unfortunately, in these studies, energy expenditure was not normalised for the metabolically active mass, making the comparison within and between groups difficult with changes in body weight and body composition. Therefore, we investigated the acute effect of encapsulated capsinoids on metabolic rate and substrate oxidation in a crossover study.

We conducted a double-blind, placebo-controlled, single centre, randomised, crossover clinical trial to test the impact of different doses of encapsulated, plant-derived capsinoids (1, 3, 6 and 12 mg) on RMR and fat oxidation measured by indirect calorimetry. The primary objective of this randomised trial was to test the hypothesis that consumption of capsinoids increases resting energy expenditure and/or fat oxidation in human subjects. The secondary objective was to evaluate the effect of capsinoids on blood pressure and adverse events.

Methods

Subjects

Thirteen young (28·4 (SE 1·4) years), non-smoking men were recruited by advertising in local newspaper, television and the Pennington Biomedical Research Center website. They were healthy as indicated by physical examination and routine medical laboratory tests and their body weight was stable over the past month. Participants had an average BMI of 27·1 (SE 4·7) kg/m² ranging from 22·3 to 34·8, body weight of 83·9 (SE 4·7) kg (range: 60·5–117·5) and body fat content of 22·4 (SE 1·6) % (range: 11·9–31·3). None of them exercised more than twice a week for the past 6 months and none participated in regular resistance exercise. Exclusion criteria

Abbreviation: NPRQ, non-protein respiratory quotient.
* Corresponding author: Dr Eric Ravussin, fax +1 225 763 2525, email eric.ravussin@pbrc.edu
also included attempt to diet to increase or decrease body weight, allergy to chilli pepper, consuming more than two cups of tea or coffee per day or more than three standard alcohol drinks per day, use of weight loss drugs, drugs affecting energy metabolism and drugs for depression. The protocol was approved by the Pennington Biomedical Research Center Institutional Review Board and all subjects provided written informed consent.

**Experimental design**

The present study was a double-blind, placebo-controlled, randomised, crossover clinical trial. After completing the screening process, participants presented at 18.00 hours at the Pennington inpatient unit for 5 d. At 18.30 hours, they received a standardised dinner and, at 20.00 hours, a snack. This provided 50% of the basal energy requirement, calculated according to published equations\(^{16}\). The macronutrient composition of the diet was 50% from carbohydrates, 30% from fat and 20% from protein. At 21.00 hours, subjects were interviewed for the occurrence of symptoms within the last 12 hours\(^{17}\). On the next day, in order to minimise physical activity, subjects remained in their bed for the entire duration of the RMR assessment. RMR was measured for 45 min (baseline) before ingestion of twelve gel capsules followed by another 120 min of RMR measurement. Doses (placebo or capsinoids at 1, 3, 6 or 12 mg) were randomly assigned using a randomised number sequence. Urine was collected before and after the 165 min RMR measurement for nitrogen excretion rate. Blood pressure and body temperature were measured at –50, –5, 60 and 120 min of capsules ingestion. At the end of the 165 min RMR measurement, occurrence of symptoms for the last 3 h was assessed according to a symptoms’ checklist\(^{17}\). Total body fat content was measured by dual energy X-ray absorptiometry scan on one of the mornings after RMR testing. Finally, 72 h after the last testing day, subjects were contacted by phone and interviewed for the presence of symptoms and adverse events. Physical activity and consumption of tea, coffee and alcohol were not permitted during the week of metabolic evaluation.

**Capsinoids capsules**

Capsules contained an extract from pepper fruit variety CH-19 sweet (C. annuum L.). Capsinoids oil was extracted as follows: dried fruit was treated with hexane, and fruit sediment was removed by filtration, followed by evaporation and distillation with medium-chain TAG and column chromatography to yield purified capsinoids. Capsinoids consisted of capsiate, dihydrocapsiate and norhydrocapsiate in a 70:23:7 ratio (HPLC). The purified capsinoids were dissolved in rapeseed oil and encapsulated in vegetarian softgel capsules made of modified maizestarch, vegetable glycerine and carageenan; each capsule contained 1 mg capsinoids and 199 mg of a mixture of rapeseed oil and medium-chain TAG. All capsules were manufactured in one batch. Stability tests determined that the product would be stable beyond the duration of the trial. The same amount of capsules (twelve) was given every testing day, but with different proportions of capsules containing placebo or capsinoids. For example, with the 6 mg capsinoids dose, six capsules contained placebo and six capsules contained capsinoids. Capsules were ingested with 150 ml of tap water in less than 2 min, and they are expected to be absorbed within 10 min.

**Indirect calorimetry and fuel oxidation**

RMR was measured in fasting conditions with subject in supine position, awake, in a quiet environment (soft music was permitted) and with a room temperature of 22°C. RMR was determined from gas exchange measurements using a Deltatrac II metabolic cart (Deltatrac II, Datex-Ohmeda, Helsinki, Finland). The analyser was calibrated before each study with standardised gases containing 5 % CO\(_2\) and 95 % O\(_2\). A transparent plastic hood connected to the device was placed over the head of the participant. \(V_O_2\) and \(V_CO_2\) were calculated from continuous measurements of CO\(_2\) and O\(_2\) concentrations in inspired and expired air diluted in a constant air flow of approximately 40 litres/min. \(V_O_2\), CO\(_2\) production and energy expenditure standardised for temperature, pressure and moisture were calculated at 1 min intervals. Energy substrate oxidation was calculated using Jequier et al.\(^{18}\) equations\(^{18}\) taking into account urinary nitrogen excretion. The RMR response to the drug was evaluated as the net incremental area under the curve\(^{19}\).

**Body composition**

Body fat mass and fat-free mass were measured on a Hologic Dual Energy X-ray Absorptiometer in the fan beam mode (QDR 4500; Hologic, Waltham, MA, USA).

**Blood pressure and body temperature**

Blood pressure was measured at –50, –5, 0, 60 and 120 min of capsule ingestion using a manometer (Baumanometer; W.A. Baum Co., Inc., Copiague, NY, USA). Axillary body temperature was measured using an electronic thermometer (Sure Temp 679, Welch Allyn, Skaneateles Falls, NY, USA).

**Symptoms and adverse events**

Assessment of the incidence of symptoms and adverse events, which might possibly be induced by capsinoids, was done using a validated symptom questionnaire\(^{17}\). This questionnaire assesses thirty-four different symptoms (e.g. energetic, tired, hungry, fresh, alert, sleepy, etc.) in four different intensities: not at all; slight amount; moderate amount; great amount. The questionnaire was applied the night before the metabolic testing, immediately after the 165 min metabolic testing and 72 h after the last metabolic testing.

**Statistical analysis**

Data are expressed as mean ± SE. All statistical analyses were done with SAS software version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). Data were analysed using a two-way repeated-measures ANOVA (SAS PROC MIXED) with dose and time as the fixed effects and subject as the random effect. The statistical significance for multiple comparisons was adjusted with respect to the Tukey–Kramer method.
to control for type I error rate. *P*<0.05 was considered statistically significant.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Pennington Biomedical Research Center Institutional Review Board (#27034). Written informed consent was obtained from all subjects.

## Results

All subjects completed the 5 d metabolic rate assessment. No significant changes in body weight were detected during this period (0.15 (SE 0.18) kg; range: ‒1.1 to 1.3; *P*=0.41). Capsinoids’ capsules were well tolerated and adverse events were similar across treatments (*P*=0.09). Likewise, systolic and diastolic blood pressures were unchanged over time and unaffected by capsinoids. A small but statistically significant increase in body temperature was observed when body temperature was compared at 50 and 120 min after ingestion of the capsules (35.9 (SE 0.1) to 36.0 (SE 0.0) °C; *P*=0.02), but was not affected by treatment (*P*=0.16).

### Energy metabolism

RMR before dosing had an intraindividual CV of 4.1% (SD = 259 kJ/d). After capsinoids ingestion, no differences in metabolic rate were noted as a function of time (*P*=1.00), capsinoids dose (*P*=0.91) or its interaction (*P*=1.00; Fig. 1(A)). The RMR incremental area under the curve (kJ min/d) was also similar between doses (*P*=0.48; Table 1). In addition, the change in metabolic rate in response to dosing did not show differences when compared with placebo (*P*>0.53; Table 1).

The intraindividual variability in non-protein respiratory quotient (NPRQ) before dosing was 3.1% (SD = 0.03). NPRQ was not affected by treatment (*P*=0.36) or treatment × time interaction (*P*=1.00). However, a significant time effect was noted (*P*=0.002; Fig. 1(B)) with a decrease in NPRQ 30 and 60 min after dosing. After that time, NPRQ values were similar to those observed during the baseline period (*P*=0.19; Fig. 1(B)).

Fat oxidation before capsules ingestion had an intraindividual CV of 45% (SD = 18 g/d). No significant effect of capsinoids dose was observed on fat oxidation (*P*=0.24). However, fat oxidation was increased 30 min (*P*=0.0002) and 60 min (*P*=0.007) after dosing, when compared with baseline fat oxidation (baseline, 30 and 60 min: 40 (SE 3), 62 (SE 4) and 57 (SE 3) g/d, respectively). Mirror image results were observed for carbohydrate oxidation, which was reduced 30 min after dosing, when compared with baseline values (118 (SE 9) v. 156 (SE 8) g/d, respectively; *P*=0.01).

We also evaluated the influence of the chronological order (days 1, 2, etc.) on energy metabolism, and no differences were detected among days on baseline RMR (days 1, 2, 3, 4 and 5 in kJ/d: 6166 (SE 166), 6247 (SE 211), 6190 (SE 222), 6260 (SE 199) and 6379 (SE 262), respectively; *P*=0.96) or NPRQ (days 1, 2, 3, 4 and 5: 0.85 (SE 0.01), 0.87 (SE 0.01), 0.86 (SE 0.01), 0.87 (SE 0.01) and 0.86 (SE 0.02), respectively; *P*=0.59). Similar results were found when the 2 h post-pill RMR and NPRQ were assessed.

## Discussion

Using a crossover design, we failed to demonstrate a significant effect of capsinoids on metabolic rate and fuel partitioning in human subjects. With our design, we did not have to normalise energy expenditure data for body composition, since each subject was his own control with probably no change in body composition over the 5 d of testing. In addition to these results, blood pressure and axillary temperature were not affected by capsinoids.

One potential target for obesity treatment and/or prevention is metabolic rate. It is proposed that even a small decrease in energy intake (<200 kJ/d) may be able to prevent weight gain<sup>[20]</sup>, although this concept has been vigorously challenged<sup>[21–23]</sup>. Similarly, one can make the case that a small but consistent increase in energy expenditure will help individuals to maintain body weight or even lose weight.

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![Fig. 1. RMR (A) and non-protein respiratory quotient (B) before and for 120 min after 12 mg capsinoids (C) or placebo (●) ingestion. Data for lower doses (1, 3 and 6 mg) of capsinoids are not shown. *P*<0.05 compared with baseline.](image-url)
The best option to increase energy expenditure is by increasing physical activity; however, the current obesogenic environment makes this option often difficult or impossible for most people\(^{(24)}\). Alternatively, ingestion of compounds with thermogenic properties in combination with energy restriction might eventually lead to higher body weight loss.

Capsinoids share a similar molecular structure with capsai-
cin. These compounds may therefore have a capsainc-like effect on metabolic rate in human subjects since animal studies support such a thermogenic effect\(^{(11,12)}\). In human sub-
jects, Inoue et al.\(^{(14)}\) assessed the effect of 3 and 10 mg/d of encapsulated capsinoids for 4 weeks on metabolic rate and respiratory quotient. No changes in these variables were observed. However, when only overweight individuals were included in the analysis, a larger increase in \(V_O_2\) with the highest capsinoids dose \(v\). placebo was detected. A similar but NS increase in metabolic rate and fat oxidation was observed \((P<0.10)\), but without changes in respiratory quotient. Unfor-
nately, the lack of normalization of metabolic rate for the metaboli-
cally active body mass did not allow firm conclusions. In a recent study in overweight men, encapsulated capsinoids (6 mg/d for 12 weeks) did not modify metabolic rate, although a higher but NS increase in fat oxidation was detected after the 12-week treatment period when compared with the placebo group. However, the authors failed to nor-
malise their data for the metabolically active tissue before and after intervention\(^{(15)}\).

Several factors may explain the present results, including the low doses and short exposure to capsinoids, a lack of power to find significant differences or simply the absence of thermogenic effect of capsinoids. The present study was designed with the power to detect a 5 % effect in RMR or approximately 300 kJ/d. After 2 h of metabolic rate measure-
ment, the differences in RMR after capsinoids ingestion compared with placebo were well below our detection limit and more importantly of no physiological importance on body weight. Therefore, we are confident that the lack of significant difference is not due to a type II error.

Another possibility is that the measurement of energy expenditure was not of sufficient duration (only 120 min after dosing) to detect an effect. Such an observation has been made with sibutramine, a medication that is approved for obesity and which has a small effect on energy expendi-
ture. Seagle et al.\(^{(25)}\) did not find an effect of sibutramine \((10 \text{ mg or } 30 \text{ mg/d})\) in obese women when they measured RMR for only 3 h. However, Hansen et al.\(^{(26)}\) measured energy expenditure for 5-5 h after dosing with 30 mg sibutra-
mine compared with placebo in fed and fasted men. There was a sibutramine-induced increase in energy expenditure of about 3–5 %. Data from mice also support the idea that longer exposure to high doses of capsinoids may be required to increase metabolic rate, since the thermogenic effect may be mediated by upregulation of uncoupling proteins\(^{(12)}\).

Alternatively, capsinoids might influence food intake by suppressing hunger and increasing satiety in human sub-
jects\(^{(27–29)}\). We did not specifically assess food intake in the present study, although the symptoms’ questionnaire given to the volunteers included a question about hunger feelings. We found no difference in hunger across treatments.

In conclusion, a combination of longer exposure time and higher capsinoids dose may be required to increase metabolic rate in a physiologically meaningful way. Therefore, there is still a need to investigate the potential long-term effects of capsinoids on metabolic rate in human subjects. In conclusion, the present study does not support the hypothesis that capsi-


dnoids can be helpful in increasing energy expenditure and fat oxidation in human subjects, at least over 2 h after exposure. Long-term use of capsinoids needs to be further tested to establish them as a potential weight control agent.

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### References

FAO expert consultation on diet, nutrition and the prevention of 
chronic diseases: process, product and policy implications. 

sympathetic efferent nerve and catecholamine secretion

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**Table 1. RMR area under the curve and change from baseline to post-capsinoids ingestion**

<table>
<thead>
<tr>
<th>Capsinoids effect ((P) value)</th>
<th>Placebo</th>
<th>1 mg</th>
<th>3 mg</th>
<th>6 mg</th>
<th>12 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>RMR before pills ingestion (kJ/d)</td>
<td>6238 ± 201</td>
<td>6305 ± 230</td>
<td>6180 ± 163</td>
<td>6247 ± 205</td>
<td>6276 ± 268</td>
</tr>
<tr>
<td>RMR IAUC (kJ/min/d)</td>
<td>11 163 ± 8004</td>
<td>11 485 ± 11 485</td>
<td>8435 ± 9335</td>
<td>4134 ± 8017</td>
<td>-9858 ± 13 636</td>
</tr>
<tr>
<td>(\Delta) RMR(_{\text{mean}}) (kJ/d)</td>
<td>109 ± 84</td>
<td>-79 ± 113</td>
<td>63 ± 92</td>
<td>-46 ± 79</td>
<td>-92 ± 134</td>
</tr>
<tr>
<td>(\Delta) RMR(_{\text{peak}}) (kJ/d)</td>
<td>322 ± 92</td>
<td>142 ± 117</td>
<td>293 ± 109</td>
<td>205 ± 92</td>
<td>155 ± 117</td>
</tr>
</tbody>
</table>

RMR IAUC, RMR incremental area under the curve for 120 min after capsinoids or placebo ingestion; \(\Delta\) RMR\(_{\text{mean}}\), mean RMR after capsinoids ingestion – baseline RMR; \(\Delta\) RMR\(_{\text{peak}}\), maximal RMR after capsinoids ingestion – baseline RMR.

* The mixed model with repeated measures was used with dose as the fixed effect and subject as the random effect.