Assessment of human brown adipose tissue density during daily ingestion of thermogenic capsinoids using near-infrared time-resolved spectroscopy

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1 Introduction

Brown adipose tissue (BAT) is known to play a critical role in cold-induced nonshivering thermogenesis (CIT) to maintain body temperature. In adult humans, metabolically active BAT is potentially identified in the supraclavicular region by using 18F-fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG-PET/CT). Histological examination confirmed FDG deposits to be BAT. Recent studies using FDG-PET/CT have revealed that BAT is involved in adaptive energy expenditure, thereby contributing to the regulation of body fat. Moreover, BAT is also suggested to participate in glucose homeostasis and to improve blood lipid profiles in humans. Thus, BAT is expected to be a therapeutic target for obesity and related metabolic disorders in humans.

BAT activity and/or mass can be quantified by FDG-PET/CT after acute cold exposure, which is widely used as a standard method in humans. However, the FDG-PET/CT method has serious limitations, such as its inaccessibility, enormous cost, and patient exposure to ionizing radiation and uncomfortable cold. Particularly, inevitable radiation exposure makes it difficult to evaluate BAT repeatedly in the same subjects/patients in a longitudinal study. Recently, magnetic resonance imaging (MRI) and enhanced contrast ultrasonography were reported as less-invasive methods for the evaluation of BAT, but these are also limited by inaccessibility, enormous cost, and uncomfortable cold exposure.

Recently, we demonstrated in healthy humans that total hemoglobin concentration (total-Hb), evaluated by near-infrared time-resolved spectroscopy (NIRTRS) under thermoneutral conditions (i.e., without cold exposure), is positively correlated with FDG-PET/CT indices only in the supraclavicular region; which potentially contains BAT deposits. Considering abundant vascularity of BAT compared with other tissues, our results
suggest that [total-Hb] estimated by NIRTRS is an index of BAT density. Although the NIRTRS method does not precisely specify an area of BAT location but instead provides an ~4-cm² tissue focus, it is noninvasive, simple, inexpensive, and free of radiation exposure for evaluating tissue oxygenation in humans. Collectively, the NIRTRS method is expected to be suitable for evaluating BAT density in humans, particularly in longitudinal studies. To test this, in the present study, using the NIRTRS method we examined the changes in BAT induced by daily ingestion of thermogenic capsaicin-like compounds, capsinoids, which are known to activate and recruit BAT. 

2 Methods

Healthy volunteer subjects were recruited and given capsinoids every day for 6 to 8 weeks. Before and after the treatment, their BAT activity/density was assessed by FDG-PET/CT (Experiment 1) or NIRTRS (Experiment 2). The study design and protocols were approved by the Institutional Review Board of Ritsumeikan University and Tenshi College, in accordance with the ethical principles contained in the Declaration of Helsinki. Written informed consent was obtained from all participants. These studies were conducted from December 2014 to March 2015, the winter season in Japan.

2.1 Subjects

In Experiment 1, three healthy males (24- to 30-years old) were recruited by direct contact. In an independent Experiment 2, 10 healthy male and 10 healthy female college students were recruited by advertising on posters or by direct contact (Table 1). The participants were randomly allocated to the capsinoids or placebo group by a third party who did not participate in this study.

### 2.2 Capsinoids

Capsinoids were extracted from CH-19 Sweet (*Capsicum annuum* L.); consisted of capsiate, dihydrocapsiante, and nordihydrocapsiante in a 7:2:1 ratio; and were provided by Ajinomoto Co., Inc. (Tokyo, Japan). Each capsule contained 0 or 1.5 mg of capsinoids and 199 mg of a mixture of rapeseed oil and medium-chain triglycerides. Participants were instructed to take three capsules in each morning and evening of each day for 6 weeks (Experiment 1) or 8 weeks (Experiment 2).

### 2.3 Study Design

In Experiment 1, three male subjects were given six capsules containing 1.5 mg of capsinoids each day for 6 weeks. Before and after the treatment, their BAT activity was assessed by FDG-PET/CT.

In Experiment 2, 10 male and 10 female subjects were given either capsinoid (9 mg/day) or placebo capsules each day for 8 weeks in a double-blind design. Before and after the 8-week treatment, their anthropometric and circulatory parameters were measured. In addition, BAT density was measured every

### Table 1 Anthropometric parameters and blood pressure before and after the 8-week treatment, and after 8 weeks of follow-up period.

<table>
<thead>
<tr>
<th></th>
<th>Capsinoids (n = 10)</th>
<th>Placebo (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before the treatment</td>
<td>After the 8-week treatment</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.7 ± 1.2</td>
<td>—</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>60.1 ± 8.0</td>
<td>60.1 ± 8.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4 ± 1.8</td>
<td>21.4 ± 1.8</td>
</tr>
<tr>
<td>Body fat content (%)</td>
<td>21.3 ± 7.6</td>
<td>21.1 ± 7.6</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>12.2 ± 4.7</td>
<td>12.1 ± 4.7</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>45.3 ± 7.8</td>
<td>45.3 ± 7.7</td>
</tr>
<tr>
<td>Bone mass (kg)</td>
<td>2.7 ± 0.4</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>VFA (cm²)</td>
<td>40.5 ± 9.9</td>
<td>37.1 ± 10.7</td>
</tr>
<tr>
<td>SFA (cm²)</td>
<td>112.5 ± 34.6</td>
<td>115.4 ± 34.5</td>
</tr>
<tr>
<td>Supraclavicular subcutaneous fat thickness (cm)</td>
<td>0.22 ± 0.02</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>Deltoid muscle subcutaneous fat thickness (cm)</td>
<td>0.42 ± 0.06</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>118 ± 10</td>
<td>117 ± 12</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>68 ± 9</td>
<td>61 ± 8</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>67 ± 12</td>
<td>63 ± 7</td>
</tr>
</tbody>
</table>

BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; SBP, systolic blood pressure; DBP, diastolic blood pressure.
2 weeks by NIRTRS (Fig. 1). These parameters were measured again 8 weeks after stopping the capsinoid intake (follow-up period). The subjects were instructed to maintain their usual dietary intake and physical activity during the experimental period, and to record a dietary diary during the 16-week period.

2.4 Outcomes

The primary endpoint was the change in BAT density evaluated by [total-Hb] using NIRTRS after 8 weeks of capsinoid-treatment, and the secondary one was that after cessation of the treatment. An additional endpoint was the capsinoid-induced increase in BAT activity assessed by FDG-PET/CT.

2.5 18F-fluorodeoxyglucose Positron Emission Tomography Combined with Computed Tomography

FDG-PET/CT was performed as described previously. Briefly, after overnight fasting for ~12 h, the subjects were exposed to cold by being kept in an air-conditioned room at 19°C with standardized light clothing (a patient’s gown), and intermittently placed their feet on an ice block wrapped in cloth for ~4 min every 5 min to avoid cooling-associated pain. After 1 h, under these cold conditions, each was given an intravenous injection of 18F-FDG (1.66 to 5.18 megaBecquerel (10⁶ Bq) (MBq)/kg body weight) and kept under the same cold conditions. One hour after the 18F-FDG injection, FDG-PET/CT scans were performed by using a PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Japan). BAT activity in the supraclavicular fat deposits was quantified by calculating the maximal standardized uptake value of FDG (SUVmax), defined as the radioactivity per milliliter within the region of interest divided by the injected dose in MBq/g of body weight.

2.6 Near-Infrared Time-Resolved Spectroscopy

The [total-Hb] was measured using NIRTRS (TRS-20; Hamamatsu Photonics K.K., Hamamatsu, Japan) for 5 min at 27°C by placing the probes on the skin of the supraclavicular region potentially containing BAT deposits; and, as a reference, also in the deltoid muscle region, which is separated from the BAT deposits in the right side. The distance between the emitter and detector was set at 30 mm. The tissue was illuminated with a 200-μm core diameter optical fiber using pedometers (Omron Health Counter HJ-710IT; Omron Healthcare, Kyoto, Japan) after resting for 10 min.

2.7 Anthropometric and Circulatory Parameter Measurements

The body mass, fat mass, percent body fat, lean body mass, and bone mass were evaluated by a dual-energy x-ray absorptiometry scan (DXA, Lunar Prodigy; GE Healthcare, Buckinghamshire, UK). The visceral fat area (VFA) and subcutaneous fat area (SFA) at the abdominal level of L4–L5 were estimated using a 1.5-T MRI (Signa HDxt; GE Healthcare, Buckinghamshire, UK). During DXA measurements, subjects maintained a supine position. Then a series of transaxial MRI scans of abdominal sections were acquired (field of view = 420 × 420 mm, slice thickness = 10 mm, echo time = 7.3 ms, repetition time = one respiration). The images were exported and analyzed by the same investigator using an image analysis software program (SliceOmatic 4.3; Tomovision Inc., Magog, Canada). Subcutaneous fat thickness was measured by B-mode ultrasonography (SSD-3500SV; Hitachi Aloka Medical Co., Ltd, Tokyo, Japan) at the supracleavicular region potentially containing BAT and the deltoid muscle region, which is separated from BAT deposits. During ultrasonographic measurements, subjects maintained the same posture as during the NIRTRS measurement. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured using an automated sphygmomanometer (HBP-9020; Omron Corp., Kyoto, Japan) after resting for 10 min.

2.8 Dietary Diary and Records of Intakes

Dietary habits during the preceding month were assessed using a validated, brief, self-administered diet history questionnaire that contained questions about the consumption frequency of 56 foods and beverages and nine dishes that are commonly consumed in the general Japanese population. Daily intakes of energy, protein, fat, and carbohydrate were calculated before and after the 8-week treatment period, and after the 8-week follow-up period. Daily steps and activity energy expenditure were estimated using pedometers (Omron Health Counter HJ-710IT; Omron Healthcare, Kyoto, Japan), and the mean for 7 days was evaluated before and after the 8-week treatment period, and after the 8-week follow-up period.
2.9 Statistical Analyses

Data are expressed as mean ± standard deviation. In Experiment 1, to compare the SUVmax before and after the 6-week period, Wilcoxon signed-rank testing was conducted after results from the Shapiro–Wilks test proved significant. In Experiment 2, a two-way analysis of variance with repeated measures was used to test the interaction (group × time) and the main effects (group and time). If there was a significant interaction or main effect, the time or group differences of the variables were analyzed using the paired or unpaired t-test, respectively. Values were considered to be statistically significant if P was < 0.05. All statistical analyses were performed using SPSS version 19 (Chicago, Illinois).

3 Results

3.1 Experiment 1

Three male subjects were given 9 mg of capsinoids every day for 6 weeks, and their BAT activity was assessed by FDG-PET/CT. Figure 2 shows a typical FDG-PET/CT image in the supraclavicular region before and after the 6-week treatment. The calculated SUVmax in both sides was increased by 48.8% (2.2 ± 0.3 versus 3.3 ± 1.3, P < 0.05) by the treatment, being consistent with our previous results13 that the daily ingestion of capsinoids recruits BAT.

3.2 Experiment 2

Twenty subjects were randomly divided into two groups, and given either 9 mg of capsinoids or placebo for 8 weeks. Before and after the 8-week period, there were no significant changes, either in the anthropometric or circulatory parameters (Table 1) or for the physical activity levels (steps and physical activity, energy expenditure or dietary intake (energy, fat, protein, and carbohydrate intake) (data not shown). No apparent harmful incidents were observed in any individuals in the present study.

Figure 3 shows the [total-Hb] assessed by NIRTRS. There was a significant main effect of group on [total-Hb] in the supraclavicular region close to BAT deposits [Fig. 3(a)], but not in the deltoid muscle region separated from BAT deposits [Fig. 3(b)]. In the supraclavicular region, [total-Hb] increased by 46.4% after the 8-week capsinoid treatment (70.4 ± 14.8 versus 102.2 ± 27.2 μM; P < 0.01), despite large interindividual variations [Fig. 3(c)], while it did not change after the placebo treatment (71.3 ± 18.1 versus 81.9 ± 22.0 μM; P = 0.13) [Fig. 3(d)]. In contrast, the individual data of the deltoid muscle region separated from BAT deposits were stable in both the capsinoid [Fig. 3(e)] and placebo groups [Fig. 3(f)]. In the supraclavicular region, the change in [total-Hb] during the 8-week period was significantly greater in the capsinoid group than in the placebo group [Fig. 3(g)]. After stopping the capsinoid treatment, [total-Hb] in the supraclavicular region tended to decrease by 12.5%. The change in [total-Hb] during the 8-week follow-up period was insignificantly larger (P = 0.07) in the capsinoids group than in the placebo group [Fig. 3(h)].

4 Discussion

In this study, first, we confirmed, by FDG-PET/CT, increased BAT activity after daily ingestion of capsinoids by healthy humans. Then, we found that the capsinoid-induced increase in [total-Hb], a potential parameter for evaluating BAT vascularity, could be continuously monitored by NIRTRS.

Capsinoids, such as capsaicin, are capsaicin-like compounds found in a nonpungent type of red pepper called “CH-19 Sweet.”13,19,21 Capsinoids are known to have similar physiological effects to capsaicin. Animal studies have shown that capsinoids activate transient potential vanilloid 1 receptors in the gut,22,23,24 which in turn increase BAT thermogenesis and body fat mobilization via the sympathetic nervous system.22,23,24 Similar thermogenic effects of capsinoids were also found in humans: that is, single oral ingestion of capsinoids increases whole-body energy expenditure in subjects with active BAT, but not in those without it.25 Moreover, it was also reported that daily ingestion of capsinoids for 6 weeks resulted in an increased CIT.13 These results suggest that capsinoids not only activate but also recruit BAT in humans. Consistent with these previous findings, in Experiment 1 of the present study, FDG-PET/CT revealed a significant increase in BAT SUVmax in the supraclavicular region after the 6-week capsinoid treatment.

We reported previously a significant relationship between BAT density as evaluated by [total-Hb] in NIRTRS and BAT activity as evaluated by SUVmax in FDG-PET/CT.16 Thus, it was rational to expect that the capsinoid-induced change in BAT would be detected by NIRTRS. In fact, in Experiment 2 of the present study, we found that [total-Hb] in the supraclavicular region close to BAT deposits increased significantly after the 8-week capsinoid treatment, while it did not change after the placebo treatment. In contrast, no notable change was found in [total-Hb] in the deltoid muscle region separated from BAT deposits. Although the period of capsinoid treatment was different in the two experiments (6 and 8 weeks), the increases in [total-Hb] and SUVmax were almost similar (48.8% and 46.4%, respectively), supporting again our previous idea that [total-Hb] is an index of BAT density. We also found that [total-Hb] tended to decrease during the 8-week follow-up period after the capsinoid treatment. As there was no notable
change in the lifestyle such as food intake or physical activity of the participants during the 16-week test period, the change in [total-Hb] would be attributable to capsinoid ingestion. Taken together, the change in [total-Hb] evaluated by NIRTRS reflects those in BAT density induced by daily ingestion of capsinoids. It is thus evident that NIRTRS is a useful method for evaluating BAT density in humans, particularly in longitudinal intervention studies.

There are two distinct types of brown adipocyte: the classical brown adipocyte derived from the Myf-5 cell, and the beige adipocyte transformed from the white adipocyte in response to sympathetic stimulation.26,27 Based on the gene expression pattern, BAT in the supraclavicular region in adult humans was suggested to be mainly composed of beige adipocytes.27 It is to be noted, however, that neither NIRTRS nor FDG-PET/CT can distinguish these two types of adipocyte, and that BAT detected by these methods may contain both types of adipocytes.

In the present study, body composition did not change in the capsinoid group, although VFA tended to decrease. This conflicts with previous studies showing a significant reduction in VFA after prolonged ingestion of capsinoids in humans.10,28 This may be due to the difference in the adiposity of participants between the studies: i.e., the participants in the previous studies were obese, while ours were lean. Metabolically, it might be easier to induce a reduction in excess body fat in over-fat participants than it is to induce a reduction in body fat in healthy, lean persons possessing body fat levels within the physiologically healthy range. We reported previously that subcutaneous fat thickness affects NIR signal sensitivity.18 In our present studies, subcutaneous fat thickness in the supraclavicular region did not change during the testing period, supporting the observation that changes in [total-Hb] reflect those in BAT density more than those in subcutaneous fat.

5 Conclusion

The present study demonstrated a parallel change in BAT density, evaluated as [total-Hb] by NIRTRS or BAT activity evaluated as SUV$_{\text{max}}$ by FDG-PET/CT, after daily ingestion of thermogenic capsinoids in healthy humans, suggesting that NIRTRS is suitable for assessment of human BAT, particularly in longitudinal intervention studies where FDG-PET/CT is difficult to use. Because, in this study, the NIRTRS parameters were obtained from participants who did not undergo FDG-PET/CT, simultaneous assessment by the two methods would be helpful to further confirm our conclusion.
Acknowledgments

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References


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Takafumi Hamaoka, MD and PhD, has conducted research on muscle oxidative metabolism using near-infrared and phosphorus-magnetic resonance spectroscopies with Prof. Britton Chance. He has received a research awards, such as a Young Investigators Award, 1st Congress of European College of Sports Science in 1996. His research expertise is exercise medicine, control of muscle oxidative metabolism, and evaluation of human adipose tissue.