Kaempferia parviflora Extract Increases Whole-Body Energy Expenditure in Humans: Roles of Brown Adipose Tissue

Mami MATSUSHITA1, Takeshi YONESHIRO2, Sayuri AITA3, Tomoyasu KAMIYA4, Nobutaka KUSABA4, Kazuya YAMAGUCHI4, Kinya TAKAGAKI4, Toshimitsu KAMEYA3, Hiroki SUGIE3 and Masayuki SAITO1,*

1Department of Nutrition, School of Nursing and Nutrition, Tenshi College, Sapporo 065–0013, Japan
2Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, Japan
3Department of Food and Nutrition, Hakodate Junior College, Hakodate 042–0955, Japan
4Research and Development Division, Toyo Shingaku Co. Ltd., Tosu 841–0005, Japan
5LSI Sapporo Clinic, Kita13-Higashi1, Higashi-ku, Sapporo, Hokkaido 065–0013, Japan

Summary Kaempferia parviflora extract (KP) has been reported to have a preventive effect on obesity in mice, probably by increasing energy expenditure (EE). The aims of the current study were to examine the acute effects of KP ingestion on whole-body EE in humans and to analyze its relation to the activity of brown adipose tissue (BAT), a site of non-shivering thermogenesis. After an oral ingestion of an ethanol extract of KP, EE increased significantly, showing a maximal increase of $229 \pm 69$ kJ/d at 60 min, while it did not change after placebo ingestion. To evaluate BAT activity, the subjects underwent fluorodeoxyglucose-positron emission tomography, and divided into two groups with high- and low-BAT activities. A similar and greater response of EE to KP ingestion was observed in the high-BAT group ($351 \pm 50$ kJ/d at 60 min), but not in the low activity group. Placebo ingestion did not cause any significant EE change in either group. These results indicate that a single oral ingestion of the KP extract can potentially increase whole-body EE probably through the activation of BAT in healthy men, and may be useful as an anti-obesity regimen.

Key Words Kaempferia parviflora, brown adipose tissue, energy expenditure, human

The prevalence of obesity and associated metabolic diseases such as diabetes mellitus and dyslipidemia has been increasing over the past few decades. The cause of weight gain is explained by a chronic imbalance between whole-body energy expenditure (EE) and energy intake. During the last decade much attention has been paid on the role of brown adipose tissue (BAT) in the control of whole-body EE and body fatness (1, 2). BAT is known as the major site of sympathetically activated thermogenesis during cold exposure and probably after spontaneous overfeeding in small rodents. BAT thermogenesis is totally dependent on the uncoupling protein 1 (UCP1), which has the activity of uncoupling oxidative phosphorylation from ATP synthesis, thereby dissipating energy as heat. Recent radionuclide imaging studies using fluorodeoxyglucose (FDG)-positron emission tomography (PET) with computed tomography (CT) have revealed the existence of metabolically active BAT in adult human subjects (3–6). Human BAT is activated by acute cold exposure, being positively correlated to cold-induced thermogenesis (7–9). The activity and prevalence of cold-activated BAT are inversely related to body mass index (BMI), body fat and visceral fat (3–5, 10). Moreover, prolonged cold exposure recruits BAT in association with an increase in energy expenditure and decrease in body fat (9). It is thus likely that BAT contributes to the regulation of whole-body EE and body fatness in adult humans, as it does in small rodents.

A number of food ingredients have been proposed as tools for increasing EE and decreasing body fat. One of these is capsaicin, the pungent ingredient of hot pepper, which activates the adreno-sympathetic nervous system and BAT, increases EE, and fat oxidation and reduces body fat (11, 12). Recently, we reported that non-pungent capsaicin analogues (capsinoids) increase EE through the activation of BAT in human subjects (13, 14). Kaempferia parviflora (KP) is a plant of the Zingiberaceae family indigenous to Thailand and Laos, where it has been used as a folk medicine to improve blood flow and increase vitality. KP was demonstrated to have some beneficial effects such as stomach-protecting, anti-oxidant, and anti-inflammatory effects (15–17). Moreover, it has been reported that dietary supplementation of rhizome powder of KP suppressed body weight increase, body fat accumulation, and glucose intolerance in obese type II diabetic mice (18–20), suggesting anti-obesity effects of KP. As KP showed no notable effects on food intake, the observed anti-obesity effects may be attributable to increased EE. In line with this idea, Yoshino et al. demonstrated that KP ingestion increased urinary excretion of noradrenaline, UCP1 expression, and EE in...
mice (20). We also confirmed that intraduodenal administration of a KP extract increased sympathetic nerve activity in BAT of rats (unpublished observations).

Thus, it might be expected that KP, like capsaicin and capsinoids, activates BAT thermogenesis and increases EE in humans. To test this idea, in the present study, we examined the effects of KP ingestion on EE by indirect calorimetry in healthy human volunteers and analyzed its relation to BAT activity assessed by FDG-PET/CT.

MATERIALS AND METHODS

Subjects. Twenty healthy male volunteers aged 21–29 y were recruited and carefully instructed regarding the study, and gave their informed consent to participate in it. Each subject underwent a standardized health examination and FDG-PET/CT. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all the procedures were approved by the institutional review boards of Tenshi College. Written informed consent was obtained from all the subjects.

Test substances. KP extract was prepared in Toyoshinyaku Co., Ltd. (Tosu, Japan) as described previously (19). Briefly, dried rhizome purchased from Thailand and Laos was crushed into small particles, and soaked for extraction in 60% ethanol for a few hours. After the filtered ethanolic extract was concentrated, an equal amount (w/w) of dextrin (Matsutani Chemical Industry Co., Ltd., Itami, Japan) was added and dried with a spray drier. The chemical composition of the KP extract thus obtained was 5.2% protein, 5.1% lipid, 2.0% ash and 2.6% water. Carbohydrate content was not directly determined. HPLC analysis revealed that the KP extract contained 3,5,7,4′-tetramethoxyflavone (2.16%), 5,7-dimethoxyflavone (4.07%), and 3,5,7,3′,4′-pentamethoxyflavone (4.25%), but no detectable amounts of 6-gingerol or 6-shogaol, major pungent ingredients of ginger (Zingiberaceae). A measure of 100 mg KP extract was packed in a pullulan capsule (Capsugel Japan Inc., Sagamihara, Japan). The placebo capsule contained 100 mg dextrin but no KP extract.

FDG-PET/CT. FDG-PET/CT examination was performed as described previously (3). Briefly, after fasting overnight, the subjects entered an air-conditioned room at 19°C with light clothing (usually a T-shirt with underwear), and put their feet on an ice block wrapped in cloth intermittently (usually for 4 min every 5 min). After 1 h in these cold conditions, they were given an intravenous injection of ¹⁸F-FDG at doses of 1.66–5.18 MBq/kg body weight, and continued under the same cold conditions. One hour after the ¹⁸F-FDG injection, PET/CT scans were performed employing a PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Tochigi, Japan) in a room at 24°C.

PET and CT images were co-registered and analyzed using a VOX-BASE workstation (J-MAC Systems, Sapporo, Japan). Two experienced, blinded observers assessed the FDG uptake, particularly on both sides of the neck and paravertebral regions, by visually judging the presence of radioactivity greater than that of the background. BAT activity in the neck region was quantified by calculating the maximal standardized uptake value (SUVmax), defined as the radioactivity per milliliter within the region of interest divided by the injected dose in megabecquerels per gram of body weight. For dividing subjects into high- and low-BAT groups, the cutoff value of 2.0 was applied.

Anthropometric and body fat measurement. BMI was calculated as the weight in kilograms divided by the square of height in meters (kg/m²), and percentage body fat was estimated by the multi-frequency bioelectric impedance method (Full Body Sensor Body Composition Monitor and Scale HBF-361; Omron, Kyoto, Japan). The fat free mass was calculated as the difference between body weight and body fat mass.

Indirect calorimetry. Within 4 wk after the FDG-PET/CT examination, the responses of whole-body EE and skin temperature to oral ingestion of either KP or placebo were tested in a single-blind, randomized, crossover design with the high- and low-BAT groups. The two tests were conducted 1–3 wk apart. Whole-body EE was estimated by means of a respiratory gas analyzer connected to a ventilated hood (O-Jiro; Arco System, Chiba, Japan). In brief, after fasting for 6 to 12 h, the subjects relaxed on a bed while wearing light clothing in a room at 27°C, and oxygen consumption and carbon dioxide production were continuously recorded for 30 min. The stable value of the last 10-min period was used to calculate the resting energy expenditure and respiratory quotient. Then, the subjects ingested a KP or placebo capsule with 100 mL water in 1 min. After 15, 45, and 75 min, respiratory gas parameters were recorded for 20 min, and the energy expenditure and respiratory quotient during the last 10-min period were calculated.

Data analysis. Data are expressed as means±SE and analyzed by either t-test or ANOVA with post-hoc testing by Dunnett’s or Tukey’s tests using IBM SPSS Statistics 20.0 (IBM Japan, Tokyo, Japan). Values were considered to be statistically significant if p<0.05.
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RESULTS

Before the measurement of EE, subjects fasted overnight and underwent FDG-PET/CT examination after being in cold conditions at 19˚C for 2 h. Some subjects showed a clear and intense FDG uptake in adipose tissues in the supraclavicular and paravertebral regions, whereas other subjects showed no detectable FDG uptake into adipose tissues (Fig. 1). Based on these results, they were divided into two groups, high-BAT \((n=12)\) and low-BAT \((n=8)\) groups. No significant difference between the two groups was found in resting EE, or anthropometric parameters such as BMI, body fat content and fat-free mass (Table 1).

Within 4 wk after the FDG-PET/CT examination, subjects fasted overnight and underwent respiratory gas analysis. In resting conditions before ingestion of the KP extract, the mean EE calculated from oxygen consumption and carbon dioxide production was \(6,213\pm143\) kJ/d in all 20 subjects (Table 1). After oral ingestion of a placebo capsule, EE did not show any significant change (Fig. 2A). By contrast, ingestion of a capsule of the KP extract (100 mg) caused a significant increase at 30–90 min, showing a maximal rise of \(229\pm69\) kJ/d at 60 min, which was significantly higher than the value after placebo ingestion \((-1\pm56\) kJ/d).

The EE response was also analyzed separately in the high- and low-BAT groups. In the high-BAT group, EE increased markedly after KP ingestion, showing a maximal increase of \(351\pm50\) kJ/d at 60 min (Fig. 2B). Placebo ingestion did not cause any significant change. The difference between KP and placebo ingestions was highly significant at 30 and 60 min. In the low-BAT

### Table 1. Subjects profiles.

<table>
<thead>
<tr>
<th></th>
<th>All ((n=20))</th>
<th>High BAT ((n=12))</th>
<th>Low BAT ((n=8))</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y old)</strong></td>
<td>24.1±0.4 (21–29)</td>
<td>22.9±0.4 (21–25)</td>
<td>25.9±0.7 (21–29)</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>174.4±0.8 (163.0–183.5)</td>
<td>174.8±1.1 (168.0–183.5)</td>
<td>173.8±1.3 (163.0–182.0)</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>64.4±0.9 (56.1–77.5)</td>
<td>63.3±1.1 (56.1–74.6)</td>
<td>66.1±1.6 (59.0–77.5)</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>21.2±0.3 (19.4–25.9)</td>
<td>20.7±0.2 (19.4–23.3)</td>
<td>21.9±0.5 (19.5–25.9)</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Body fat content (%)</strong></td>
<td>15.6±0.6 (10.0–25.5)</td>
<td>14.5±0.6 (19.4–23.3)</td>
<td>17.2±1.2 (11.7–25.5)</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Fat-free mass (kg)</strong></td>
<td>54.2±0.6 (48.7–61.1)</td>
<td>54±0.8 (48.7–61.0)</td>
<td>54.5±0.9 (48.8–61.1)</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>REE before KP extract ingestion (kJ/d)</strong></td>
<td>6,213±143 (5,020–7,596)</td>
<td>6,076±184 (5,020–7,101)</td>
<td>6,418±223 (5,781–7,596)</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>REE before placebo ingestion (kJ/d)</strong></td>
<td>6,196±150 (5,028–7,398)</td>
<td>6,103±184 (5,028–7,168)</td>
<td>6,334±261 (5,473–7,398)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Values are means with SE and ranges in parentheses. Student’s t-test revealed no statistical difference (N.S.) between the high- and low-BAT groups, except age. BMI: body mass index, REE: resting energy expenditure.

![Fig. 2. Change in energy expenditure after oral ingestion of KP extract and placebo. Mean (±SE) ΔEE before (0 h) and after oral ingestion of KP (closed circles) and placebo (open circles) in all subjects (A; \(n=20\)), in the high-BAT group (B; \(n=12\)), and in the low-BAT group (C; \(n=8\)). a: \(p<0.05\) vs 0-time. b: \(p<0.05\) vs placebo. EE energy expenditure, ΔEE change in energy expenditure.](image)

![Fig. 3. The response of energy expenditure to oral ingestion of KP and placebo. The response of energy expenditure (EE) to KP (closed columns) and placebo (open columns) was calculated as the area-under-the-curve (AUC) between 0 and 90 min from the data in Fig. 2. Values are means±SE. *\(p<0.05\), **\(p<0.01\).](image)
In this study, we estimated the BAT activity of our subjects by FDG-PET/CT, and divided them into two groups, based on their BAT activity. The high-BAT group showed the mean SUVmax of 8.4±1.3, while the low-BAT group showed undetectably low activities of SUVmax less than 2. When compared to the low-BAT group, the high-BAT group showed comparable anthropometric parameters including BMI, body fatness and fat-free mass, as well as resting EE. Thus, it is rational to posit that the different EE responses to KP ingestion are largely due to the different BAT activities.

It is established both in small rodents and in humans that the thermic effect of capsaicin/capsinoids is largely attributable to the sympathetically mediated activation of BAT (11, 12). The primary action site of the BAT-dependent thermic effect of capsaicin/capsinoids seems to be transient receptor potential vanilloid 1 (TRPV1) in the gastrointestinal tract (14). The KP extract used in the present study contained large amounts of polymethoxyflavonoids, but no detectable amounts of vanilloid compounds, which are thought to be TRPV1 agonists. Capsinoids are known to have an agonistic activity on TRPA1 (22), another type of TRP, as well as TRPV1. Although there has been no report of the action of polymethoxyflavonoids on TRPA1, some flavonoid compounds including epigallocatechin are known to activate hormone-sensitive lipase in 3T3-L1 adipocytes. Moreover, 5,7-dimethoxyflavone, the major polymethoxyflavonoids in the KP extract, was reported to potentiate the inhibitory effect on phosphodiesterase, a cyclic AMP-degradating enzyme (23). As cAMP is a key intracellular signaling molecule for hormone-sensitive lipase activation in adipocytes and sympathetically activated BAT thermogenesis, it is possible that the BAT-mediated thermogenic effect of the KP extract occurs through the inhibition of phosphodiesterase in brown adipocytes. To confirm this idea, further studies are needed, particularly focusing on blood levels of candidate compounds in the KP extract.

In summary, our results indicate that a single ingestion of the KP extract may act directly on BAT after being absorbed from the gastrointestinal tract. In fact, Okabe et al. (24) demonstrated that some components of KP extract activate hormone-sensitive lipase in 3T3-L1 adipocytes. Moreover, 5,7-dimethoxyflavone, the major polymethoxyflavonoid in the KP extract, was reported to show a potent inhibitory effect on phosphodiesterase, a cAMP-degradating enzyme (25). As cAMP is a key intracellular signaling molecule for hormone-sensitive lipase activation in adipocytes and sympathetically activated BAT thermogenesis, it is possible that the BAT-mediated thermogenic effect of the KP extract occurs through the inhibition of phosphodiesterase in brown adipocytes. To confirm this idea, further studies are needed, particularly focusing on blood levels of candidate compounds in the KP extract.

Alternatively, it is also likely that some components of the KP extract may act directly on BAT after being absorbed from the gastrointestinal tract. In fact, Okabe et al. (24) demonstrated that some components of KP extract activate hormone-sensitive lipase in 3T3-L1 adipocytes. Moreover, 5,7-dimethoxyflavone, the major polymethoxyflavonoid in the KP extract, was reported to show a potent inhibitory effect on phosphodiesterase, a cAMP-degradating enzyme (25). As cAMP is a key intracellular signaling molecule for hormone-sensitive lipase activation in adipocytes and sympathetically activated BAT thermogenesis, it is possible that the BAT-mediated thermogenic effect of the KP extract occurs through the inhibition of phosphodiesterase in brown adipocytes. To confirm this idea, further studies are needed, particularly focusing on blood levels of candidate compounds in the KP extract.

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