Prolonged Treatment with Grains of Paradise (*Aframomum melegueta*) Extract Recruits Adaptive Thermogenesis and Reduces Body Fat in Humans with Low Brown Fat Activity

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Summary

Increasing adaptive thermogenesis through the activation of brown adipose tissue (BAT) is a promising practical strategy for preventing obesity and related disorders. Ingestion of a single dose of 40 mg of an extract of Grains of Paradise (GP), a ginger family species, reportedly triggers BAT thermogenesis in individuals with high but not in those with low BAT activity. We hypothesized that prolonged treatment with GP might revive BAT in individuals who have lost active BAT. In the present study, we recruited 9 healthy young male volunteers with reduced BAT that was assessed by fluorodeoxyglucose positron emission tomography and computed tomography (FDG-PET/CT) following 2-h cold exposure at 19°C. The subjects ingested GP extract (40 mg/d) or placebo every day for 5 wk. Before and after the treatment with either GP or placebo, their body composition and BAT-dependent cold-induced thermogenesis (CIT)—a non-invasive index of BAT—were measured in a single-blinded, randomized, placebo-controlled cross-over design. Their whole-body resting energy expenditure at a thermoneutral condition remained unchanged following GP treatment. However, CIT after treatment was significantly higher in GP-treated individuals than in placebo-treated individuals. Body weight and fat-free mass did not change significantly following GP or placebo treatment. Notably, body fat percentage slightly but significantly decreased after GP treatment but not after placebo treatment. These results suggest that repeated ingestion of GP elevates adaptive thermogenesis through the re-activation of BAT, thereby reducing body fat in individuals with low BAT activity.

Key Words brown adipose tissue, energy expenditure, thermogenesis, obesity, Grains of Paradise

Obesity, characterized by an excess accumulation of body fat, constitutes a major risk factor for diabetes, liver steatosis, and associated diseases and mortality. The global pandemic of obesity may be due to difficulties and poor adherence to sustained lifestyle changes such as exercise (1), spurring a need for alternative approaches to increase energy expenditure (EE). To this end, attention has been paid to increasing adaptive thermogenesis, including cold-induced thermogenesis (CIT) and diet-induced thermogenesis (DIT) (2). One of the responsible organs for adaptive thermogenesis is brown adipose tissue (BAT) (3). BAT oxidizes glucose, fatty acids, and amino acids to generate heat in response to cold exposure or spontaneous overfeeding, thereby contributing to the control of body temperature and adiposity (3–6). A growing number of studies in humans have demonstrated apparent involvement of BAT in CIT and DIT (3). It is conceivable that increasing BAT-mediated adaptive thermogenesis would be a novel strategy to prevent obesity and related metabolic disorders. In fact, BAT recruitment through prolonged cold exposure increases CIT and DIT—concomitantly decreasing body fat mass and improving insulin sensitivity in healthy adults (7, 8). However, a considerable setback to long-term repeated cold exposure to obese/diabetic patients can potentially have unfavorable side effects on cardiovascular function (9). Thus, there is an urgent need to develop safe, practical regimens to activate BAT and adaptive thermogenesis.

The stimulatory effects of cold temperature on BAT via the sympathetic nervous system are initiated by peripheral activation of temperature-sensitive transient receptor potential (TRP) channels. We previously documented that thermogenic effects of cold exposure can be mimicked by chemical stimulation of TRP channels by food ingredients such as capsinoids which have ago-

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nistic activities at TRP channels (10, 11). Grains of Paradise (Aframomum melegueta [Rosco] K. Schum.) (GP) is another candidate that induces adaptive thermogenesis in BAT. GP is known as Guinea pepper or Alligator pepper and is rich in non-volatile pungent compounds such as 6-paradol, 6-gingerol, and 6-shogaol (12) which are capable of activating TRPV1 and TRPA1 (13–15). In fact, ingestion of a single dose of GP extract (40 mg) increases whole-body EE selectively in subjects with high BAT activity (12). Although no thermogenic effect of the single dose of GP was observed in subjects with low BAT activity (12, 16), we hypothesized that prolonged treatment with GP might revive BAT thermogenesis and decrease body fat even in individuals with low BAT activity, since prolonged exposure to cold has a similar effect (7). To test our hypothesis, in the present study, we selectively recruited healthy volunteers with low BAT activity assessed by fluorodeoxyglucose positron emission tomography and computed tomography before and after GP treatment in a single-blinded, randomized, placebo-controlled, cross-over design.

MATERIALS AND METHODS

Subjects. Japanese young male volunteers living in Sapporo for >3 y were recruited for this study to minimize possible effects of sex and intraindividual variation of age and geographical location. All subjects were carefully instructed regarding the study and gave their informed consent for their participation in this study. They underwent FDG-PET/CT after fasting for 12 h, the subjects were kept in an air-conditioned room at 19°C with light clothing for 2 h. FDG-PET/CT scans were performed using a PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Japan). BAT activity in the supraventricular region was quantified by calculating a standardized uptake value (SUV) of FDG, defined as the radioactivity per milliliter within the region of interest divided by the injected dose in megabecquerels per gram of body weight. According to the results, 9 subjects with low or undetectable activities of BAT, which was quantified in our previous study (7), were divided into two groups based on age and body composition. The first group ingested four GP capsules every day (GP 40 mg/d) for 5 wk, followed by four placebo capsules every day (0 mg/d) in the cross-over design, whereas the second group ingested the test capsules in the reverse order (Fig. 1B). Before and after the 5-wk period, EE and respiratory quotient (RQ) at 27°C and after 2-h cold exposure at 19°C were measured using a respiratory gas analyzer, and CIT was calculated as a predictive index of BAT activity, instead of repeated FDG-PET/CT, to avoid unnecessary radiation, fat oxidation, and ketosis.

FDG-PET/CT. BAT activity was quantified, as reported previously (6, 7). After fasting for 12 h, the subjects were kept in an air-conditioned room at 19°C with light clothing for 2 h. FDG-PET/CT scans were performed using a PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Japan). BAT activity in the supraventricular region was quantified by calculating a standardized uptake value (SUV) of FDG, defined as the radioactivity per milliliter within the region of interest divided by the injected dose in megabecquerels per gram of body weight. According to the results, 9 subjects with low or undetectable activities of BAT were assigned to a single-blinded, randomized, placebo-controlled, cross-over trial with a washout period of >2 wk to examine the effects of repeated ingestion of GP on BAT-dependent thermogenesis and body fat content.

Repeated ingestion of GP. GP extract was extracted from seeds of A. melegueta (Thiercelin Co., Paris, France) and encapsulated as described in our previous report (12, 16). Each capsule contained either no GP extract (placebo) or 10 mg GP extract including 6-gingerol (1.52 mg), 6-paradol (1.25 mg), 6-shogaol (0.17 mg), 6-gingeredione (0.40 mg), and 190 mg of a mixture of rapeseed oil (158 mg) and beeswax (32 mg) in a capsule.

Nine subjects with undetectable or low activities of BAT, which was quantified in our previous study (7), were divided into two groups based on age and body composition. The first group ingested four GP capsules every day (GP 40 mg/d) for 5 wk, followed by four placebo capsules every day (0 mg/d) in the cross-over design, whereas the second group ingested the test capsules in the reverse order (Fig. 1B). Before and after the 5-wk period, EE and respiratory quotient (RQ) at 27°C and after 2-h cold exposure at 19°C were measured using a respiratory gas analyzer, and CIT was calculated as a predictive index of BAT activity, instead of repeated FDG-PET/CT, to avoid unnecessary radiation.
exposure for the participants. None of the subjects ingested test capsules on the day of EE measurements to avoid acute thermogenic effects of GP. Body composition was also monitored before and after the experimental periods by employing the multifrequency bioelectric impedance method.

**Indirect calorimetry.** After fasting for 12 h, whole-body EE and RQ were estimated using a respiratory gas analyzer (O-Jiro, Alko System, Tokyo, Japan) at 27˚C and after 2-h cold exposure at 19˚C in light clothing in a sitting position. Fat oxidation was calculated from VO2 and VCO2, as described previously (17). CIT and cold-induced fat oxidation were calculated from the difference between the values before (27˚C) and after 2-h cold exposure (19˚C).

**Anthropometric and body fat measurement.** Body weight and body fat percentage were estimated by employing the multifrequency bioelectric impedance method (InBody 320 Body Composition Analyzer; Biospace, Seoul, Korea). The fat-free mass (FFM) was calculated as the difference between body weight and body fat mass.

**Data analyses.** Data are represented as means±SE and analyzed using statistical software (SPSS 18.0, IBM Japan, Tokyo). Comparisons between groups were analyzed by the paired t-test. Whole-body EE was adjusted for FFM using regression analysis, as described previously (17). A two-sided p-value <0.05 was considered statistically significant.

**RESULTS**

To investigate whether chronic GP treatment elevates the BAT-dependent thermogenic capacity in individuals who have lost active BAT, 9 young, healthy male volunteers with low or undetectable BAT activity were treated with either GP or placebo for 5 wk. Their BAT activities were assessed as FDG uptake into supraclavicular fat deposits, determined by FDG-PET/CT prior to the trial. Their FDG uptake was negligible or weak (Fig. 1A); the mean BAT activity (SUV 2.93±0.53) was significantly lower than individuals with high BAT activity (SUV 11.53±1.14, p<0.0001) in our previous study (6).

Body weight, BMI, and body fat percentage were within the normal range (Table 1).

To examine the effects of daily GP ingestion on EE and CIT, we measured whole-body EE at thermoneutral 27˚C and after 2-h of cold exposure at 19˚C before and after daily ingestion of either GP or placebo for 5 wk (Fig. 1B). Resting whole-body EE at 27˚C was significantly and positively correlated with FFM (r=0.78, p<0.001; Fig. 2A). Accordingly, whole-body EE at 27˚C and at 19˚C was adjusted for FFM to minimize possible effects of individual FFM fluctuations during the study on the results. FFM-adjusted EE demonstrated our reproducible EE measurement; the coefficient of variance (CV) of EE measurement at 27˚C, 4 times (before/after treatment with GP/placebo), was 0.056. Whole-body EE and FFM-adjusted EE at 27˚C were unchanged following GP or placebo treatment (Fig. 2B). The response of FFM-adjusted EE to cold exposure was the highest after GP treatment. In fact, CIT significantly increased after GP treatment (Fig. 2C, p<0.01). While CIT moderately increased following placebo treatment, it was significantly higher after GP treatment than after placebo treatment (p<0.05). Given that CIT is highly dependent on BAT activity and therefore is a predictive index of BAT (7), our results suggest that BAT thermogenic capacity increases in humans by repeated GP ingestion.

On the other hand, RQ and its response to cold exposure were unaffected by either GP or placebo treatment (Fig. 3A). Fat oxidation was also unchanged following

### Table 1. Subject profiles.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Mean±SE</th>
<th>Min–Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>22.4±0.6</td>
<td>20–25</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.1±1.6</td>
<td>166–184</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.6±3.7</td>
<td>49.8–89.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.0±0.9</td>
<td>17.9–26.4</td>
</tr>
<tr>
<td>Body fat percent (%)</td>
<td>14.3±1.5</td>
<td>9.5–23.5</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>9.6±1.6</td>
<td>4.7–21.0</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>55.1±2.4</td>
<td>45.1–68.4</td>
</tr>
<tr>
<td>BAT activity (SUV)</td>
<td>2.93±0.53</td>
<td>1.08–5.23</td>
</tr>
</tbody>
</table>

BMI, body mass index; BAT, brown adipose tissue; SUV, standardized uptake value.

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Fig. 2. Effects of repeated ingestion of GP on EE and CIT. (A) Relationship between FFM and whole-body EE at 27˚C. (B) FFM-adjusted EE at 27˚C and 19˚C before and after either GP or placebo treatment for 5 wk. n=9 per group. (B) BAT-dependent thermogenic capacity (CIT) before and after GP or placebo treatment. n=9 per group. Values are means±SE. *p<0.05, **p<0.01. FFM, fat-free mass.
GP or placebo treatment while fat oxidation at 19°C tended to be higher after GP treatment than after placebo treatment (Fig. 3B, p < 0.07). These results indicate no effect of GP on substrate preference in BAT thermogenesis.

We also monitored body weight, BMI and body fat percentage before and after either GP or placebo treatment to test the effects of daily GP ingestion on body fat. Body weight and BMI did not change significantly following treatment with either GP or placebo (Table 2, Fig. 4A). Body fat percentage decreased significantly following GP treatment (p < 0.05) and tended to be lower than after the placebo treatment (p = 0.057) (Table 2, Fig. 4B). In contrast, neither GP nor placebo affected FFM (Table 2, Fig. 4C).

**DISCUSSION**

Emerging evidence suggests that activating adaptive thermogenesis by BAT would be a promising strategy to prevent obesity and related metabolic diseases such as type 2 diabetes. The identification of novel tools to activate BAT thermogenesis is thus of great interest for developing practical anti-obesity regimens. Previously, we reported an apparent thermogenic response to a sin-
ingle ingestion of GP (40 mg) selectively in subjects with high BAT activity; however, the dose failed to increase EE in subjects with low BAT activity because of the decreased thermogenic capacity \((12)\). To investigate whether continuous treatment with GP is capable of reviving BAT thermogenesis in subjects with decreased BAT, in the present study, we selectively recruited healthy volunteers with low BAT activity and measured CIT as BAT-dependent thermogenic capacity before and after GP treatment in a single-blinded, randomized, placebo-controlled, cross-over design. Consistent with our previous investigation on the thermogenic effects of other food ingredients \((10, 11)\), we observed no impact of GP treatment on resting EE in thermoneutral condition at 27°C. Cold exposure \((19°C)\) significantly increased resting EE by \(\sim 10\%\), and the calculated CIT was greatly elevated up to \(\sim 20\%\) of resting EE following 5 wk of GP treatment. Although we observed a significant increase in CIT following placebo treatment, this could be attributed to the placebo effects or seasonal fluctuation of CIT during the 5-wk treatment period as outdoor temperature significantly decreased during the experimental period \((p = 0.015)\). However, outdoor temperature after the 5-wk treatment was indistinguishable between the treatments \((p = 0.70)\). The observed augmentation of CIT by the 5-wk GP treatment, compared to placebo treatment, clearly indicates apparent thermogenic effects of daily ingestion of GP. It should be noted that shivering of skeletal muscle is one of the dominant components of CIT during severe cold conditions, increasing the whole-body EE by 2-times \((18)\). In contrast, in mildly cold conditions, where CIT is \(\sim 10–20\%\) of resting EE, neither muscle shivering nor muscle metabolic activity is stimulated \((7, 19)\). It is thus highly likely that the contribution of skeletal muscle shivering to CIT was negligible, at least in our experimental condition, again supporting CIT as a predictive index of the BAT-dependent thermogenic capacity and thus BAT activity. Indeed, CIT reflects intra- and inter-individual fluctuations in BAT activity \((7, 17)\). Our results collectively suggest that long-term treatment with GP elevates thermogenesis and EE by recruiting BAT, even in individuals with decreased BAT. This is consistent with our previous reports that repeated ingestion of other thermogenic food ingredients such as capsinoids and catechins elicits CIT by increasing the thermogenic capacity and amount of BAT \((7, 11, 20)\).

An earlier study in rats showed that intragastric administration of GP extract enhanced the efferent discharges of sympathetic nerves to BAT and induced a significant increase in BAT temperature \((21)\). However, the substance responsible for BAT thermogenesis remains unclear because the GP extract contains various compounds with a vanilloid moiety such as 6-paradol, 6-gingerol and 6-shogaol. These compounds are capable of activating TRPV1 and TRPA1, which are involved in the thermogenic effects of capsinoids and catechins \((15)\). Notably, half-maximal effective concentration \((EC_{50})\) for activating TRPV1 are 0.2 mM and 0.7 mM for 6-shogaol and 6-paradol, respectively, showing a stronger channel activation potential than 6-gingerol \((EC_{50} = 3.3 \text{ mM})\) while the \(EC_{50}\) for activating TRPA1 is lower than that for TRPV1 \((13, 22)\). Given the content 6-shogaol content \((1.7\%)\) in the extract was much lower than 6-gingerol \((15.2\%)\) and 6-paradol \((12.5\%)\), 6-paradol seems to be responsible for the effects of GP on CIT. In fact, we used 40 mg GP extract that contained 5 mg 6-paradol, which sufficiently activates sympathetic nerves connecting to BAT to a similar extent as 30 mg of GP extract \((21)\). Thus, GP-induced BAT thermogenesis may be attributed to stimulation of TRP channels by 6-paradol followed by activation of sympathetic nerve activity in BAT.

It has been appreciated that the recruitment of human BAT through cold exposure or sympathomimetics results in a significant reduction of body fat content and/or an improvement of insulin sensitivity \((7, 23–25)\). Such beneficial effects can be mimicked by oral ingestion of food ingredients that have agonistic activities on temperature-sensitive TRP channels \((3, 15)\). As GP contains several TRP agonists, including 6-paradol, it is capable of decreasing visceral fat mass in healthy women \((16)\). Consistently, we observed a significant reduction in body fat percentage following GP treatment in healthy men, whereas no change was observed after placebo treatment. It should be noted that the body fat change following GP treatment is negatively correlated with the initial levels of visceral fat for the participants \((16)\). This implies that GP may have a stronger fat-reducing effect in individuals with more visceral fat. The present study subjects were non-obese males; we presume they exhibited a weaker fat-reduction response than what would be expected in obese subjects. Together, our results suggest that GP-enhanced BAT-dependent adaptive thermogenesis has the potential to reduce body fat content in humans.

In conclusion, our results suggest that repeated ingestion of GP extract incites CIT and reduces body fat by reviving BAT thermogenic capacity even in individuals with low BAT activity. Unlike cold exposure or pharmacological sympathomimetics, GP treatment would be applicable for sustained interventions to boost adaptive thermogenesis and suppress the consequences of obesity in humans.

**Authorship**

Research conception and design: TY, JS, MS; experiments: TY, MM, SA, TK; analysis and interpretation of the data: TY, HS, MS; writing of the manuscript: TY, MS.

**Disclosure of state of COI**

No conflicts of interest to be declared. Kao Corporation did not have any influence over the recruitment of subjects or on data collection, analysis, and interpretation in the study.

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