J Nutr Sci Vitaminol, **60**, 22–27, 2014

Daily Ingestion of Grains of Paradise (*Aframomum melegueta*) Extract Increases Whole-Body Energy Expenditure and Decreases Visceral Fat in Humans

Jun SUGITA[1,2], Takeshi YONESHIRO[3], Yuuki SUGISHIMA[4], Takeshi IKEMOTO[2], Hideyo UCHIWA[2], Isao SUZUKI[4,*] and Masayuki SAITO[1,**]

[1] *Department of Nutrition, School of Nursing and Nutrition, Tenshi Collage, Kita-13, Higashi-3, Higashi-ku, Sapporo 065–0013, Japan*
[2] *Innovative Beauty Science Laboratory, Kanebo Cosmetics Inc., Odawara 250–0002, Japan*
[3] *Department of Anatomy, Hokkaido University Graduate School of Medicine, Sapporo 060–8638, Japan*
[4] *Environmental and Symbiotic Science, Prefectural University of Kumamoto, Kumamoto 862–8502, Japan*

(Received May 28, 2013)

Summary We reported previously that a single ingestion of an alcohol extract of grains of paradise (GP, *Aframomum melegueta*), a species of the ginger family, increases energy expenditure (EE) through the activation of brown adipose tissue, a site of sympathetically mediated metabolic theromogenesis. The present study aimed to examine a daily ingestion of GP extract on whole-body EE and body fat in humans. Whole-body EE and body fat content were measured before and after daily oral ingestion of GP extract (30 mg/d) for 4 wk in 19 non-obese female volunteers aged 20–22 y in a single-blind, randomized, placebo-controlled, crossover design. Four-week daily ingestion of GP and a placebo decreased and increased slightly the visceral fat area at the umbilicus level, respectively. The GP-induced change was significantly different from that induced by the placebo ($p<0.05$), and negatively correlated with the initial visceral fat area ($r=-0.64$, $p<0.01$). Neither GP nor placebo ingestion affected subcutaneous or total fat. The daily ingestion of GP, but not the placebo, increased whole-body EE ($p<0.05$). These results suggest that GP extract may be an effective and safe tool for reducing body fat, mainly by preventing visceral fat accumulation.
Key Words grains of paradise, 6-paradol, energy expenditure, visceral fat

The global increase in obesity and associated metabolic disorders underscores the need for effective treatments. In principle, obesity can be treated by reducing energy intake and/or increasing energy expenditure (EE). Some food ingredients have been proposed as tools for increasing EE and decreasing body fat. A prominent example is capsaicin, a pungent principle of hot pepper that activates the adreno-sympathetic nervous system and brown adipose tissue (BAT) thermogenesis, increases EE and fat oxidation, and reduces body fat (*1–5*). Our group recently (*6*) reported that a non-pungent capsaicin analog (capsinoids) increases EE through the activation of BAT in humans. Slight but significant fat-reducing effects of capsinoids are also reported in mildly obese human subjects (*7–9*). Significantly, the effects of capsaicin and capsinoids are much attenuated in mice lacking the transient receptor potential vanilloid 1 (TRPV1) (*10*), a capsaicin receptor. This suggests that the thermic and fat-reducing effects of capsaicin and capsinoids are elicited by activation of the pathway of TRPV1, the sympathetic nervous system, and BAT.

Grains of paradise (*Aframomumu melegueta* [Rosco] K. Schum.) (GP), also known as Guinea pepper or Alligator pepper, belong to the Zingiberaceae family native to west Africa. GP seeds are used as a spice for food and as an agent for wide-ranging ethnobotanical uses, for example, as a remedy for treating stomachache, diarrhea, and snakebite (*11*). GP seeds are very rich in non-volatile pungent compounds such as 6-paradol, 6-gingerol, 6-shogaol and related compounds (*12–14*). These compounds share an important structural feature with capsaicin, namely, a vanilloid moiety. This feature may equip them with the power to activate the pathway of TRPV1 (*15*, *16*), the sympathetic nervous system, and BAT, and thereby to increase EE. In fact, Iwami et al. found that the intragastric administration of an alcohol extract of GP and 6-paradol to rats enhanced the efferent discharges of sympathetic nerves to BAT and induced a significant rise in BAT temperature (*17*). In a previous study by our group, a single ingestion of GP extract increased EE through the activation of BAT in men (*18*). We can thus speculate that a repeated ingestion of GP extract will result in a sustained elevation of EE and a consequent reduction of body fat. In the present study we tested this hypothesis by examining the effects of a daily ingestion of GP extract on EE and body composition, particularly the subcutaneous and visceral fat content, in healthy human volunteers.

*Present address: Department of Human Life Science, Nagoya Keizai University, Aichi 484–8504, Japan
**To whom correspondence should be addressed.
E-mail: saito@tenshi.ac.jp

Table 1. Body compositions before and after 4 wk of daily ingestion of GP extract or placebo.

	GP (n=19)		Placebo (n=19)	
	0 wk	4 wk	0 wk	4 wk
Body weight (kg)	51.9±1.0	51.7±1.0	52.0±1.4	51.7±1.5
BMI (kg/m^2)	20.7±0.3	20.5±0.3	20.7±0.5	20.6±0.5
Body fat (%)	26.4±0.6	25.9±0.6	26.0±0.9	25.7±0.9
Visceral fat (cm^2)	41.2±2.7	38.3±2.1	38.7±2.2	43.4±3.7
Subcutaneous fat (cm^2)	164.3±12.6	160.6±12.9	155.7±13.0	152.8±12.9
Total fat (cm^2)	205.5±14.6	198.9±14.3	194.4±14.7	196.2±15.9

Mean values with their standard errors.

MATERIALS AND METHODS

Subjects. Nineteen healthy female volunteers aged 20–22 y (20.2±0.2) were recruited and carefully instructed on the procedures of the study. Every subject underwent a standardized health examination. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the institutional review boards of Prefectural University of Kumamoto. Written informed consent was obtained from every subject.

Test substances. GP extract was extracted from seeds of *Aframomum melegueta* and encapsulated as described in our previous report (*18*). HPLC analysis of the extract revealed peaks respectively identified as 6-gingerol (15.2%), 6-paradol (12.5%), 6-shogaol (1.7%), and 6-gingerdione (4.0%). Other components included in the GP extract were caryophyllene and α-humulene (5%), triglyceride (20%) and palmitic and oleic acid (3%). It also contained various phenolic glycosides of which the content was not quantitated. Each capsule contained 0 mg (placebo) or 10 mg of a GP extract and 190 mg of a mixture of rapeseed oil and beeswax. The GP and placebo soft gels also contained caramel (7 mg/capsule) to equalize the color. A preliminary safety assessment confirmed that daily oral ingestion of the test capsule (4 capsules after breakfast, 3 capsules after lunch, 3 capsules after dinner) for 4 wk caused no noticeable symptoms or adverse events.

Test protocol. Each subject was given either 0 or 30 mg GP extract daily for 4 wk with a wash-out period of 2 wk according to a randomized, single-blinded crossover design. Three capsules were given orally per day, 1 capsule 30 min before each of three regular meals. Anthropometric and body composition measurements, indirect calorimetry, and blood analyses were performed before and after the 4-wk period.

Anthropometric and body composition measurements. BMI was calculated as the body weight in kilograms divided by the square of height in meters (kg/m^2). The percentage body fat was estimated by the multifrequency bioelectric impedance method (InBody 230 Body Composition Analyzer, Biospace, Seoul, South Korea).

The body fat distribution was determined by a computed tomography (CT) scan according to the procedure described by Tokunaga et al. (*19*). The total cross-sectional area, subcutaneous fat area, and visceral fat area were measured at the level of the umbilicus. All CT scans were performed in the supine position with a HiSpeed NX/i CT scanner (General Electric Medical Systems, Milwaukee, WI). Digital Imaging and Communications in Medicine (DICOM) uncompressed images were exported to "Image J" software (National Institutes of Health, Rockville, MD) for further analyses. The intraperitoneal area with the same density as the subcutaneous fat layer was defined as the visceral fat area.

Indirect calorimetry. Whole-body EE was estimated with a respiratory gas analyzer connected to a tight-fitting breathing mask (Oxycon Delta ERICHJAEGER B.V., Bunnick, Netherlands). After fasting for 6–12 h, the subjects were asked to relax on a bed in light-clothing in a room at 22°C, and oxygen consumption and carbon dioxide production were continuously recorded for 30 min. The stable value of the final 10-min period was used to calculate the resting EE.

Blood analyses. Blood samples were taken in the clinic after overnight fasting for measurement of the following in peripheral blood: blood properties (leucocyte count, erythrocyte count, hemoglobin, platelet count), aspartate aminotransferase, alanine aminotransferase, γ-glutamyltranspeptidase, total protein, albumin, alkaline phosphatase, urea nitrogen, creatinine, blood glucose, hemoglobin A1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, TAG, and free fatty acid. The blood was sampled after a 10-min rest in a sitting position. All measurements were taken by the Japanese Red Cross Kumamoto Hospital according to appropriate methods.

Data analysis. Values were expressed as means with their standard errors. A paired t-test was used to compare each group with the baseline or placebo. Correlations between initial values and changes of the abdominal fat area were assessed using Pearson's correlation coefficient. Statistics were calculated using SPSS software, version 18 (IBM, Tokyo, Japan). A p value of <0.05 was considered statistically significant.

RESULTS

Nineteen healthy female subjects (20.2±0.2 y old) were recruited and given an oral dose of either GP

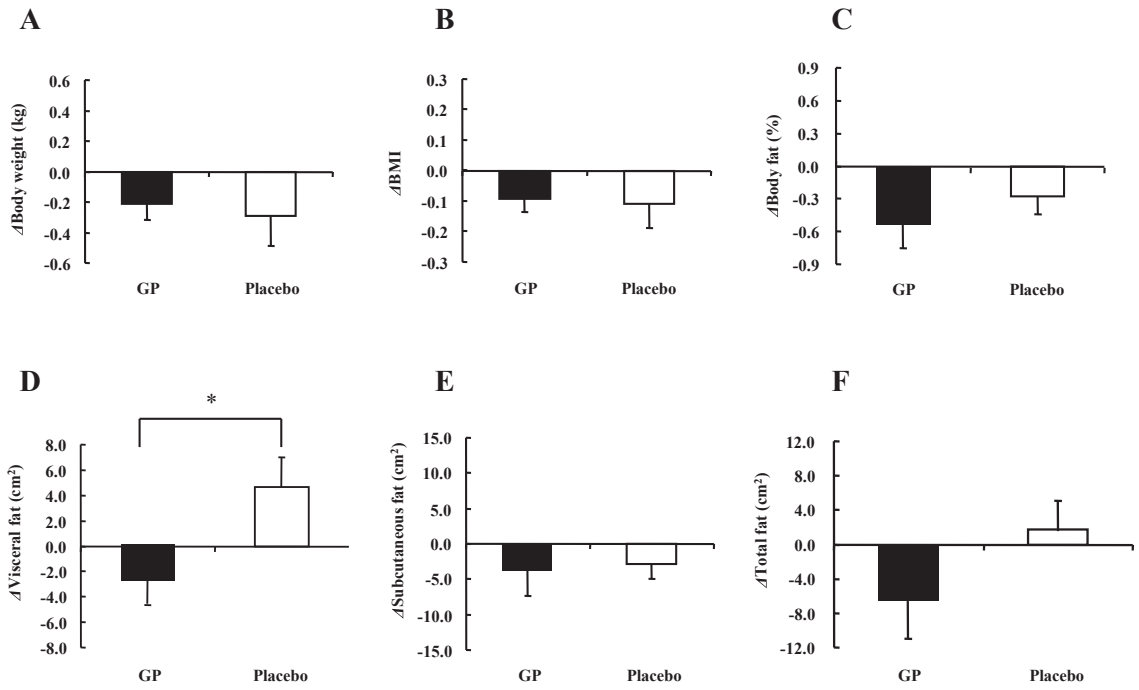

Fig. 1. Body composition changes after daily ingestion of GP extract or placebo. Body composition changes before and after oral ingestion of 30 mg GP extract. Changes in body weight (A), body fat mass (BMI) (B), body fat percentage (C), visceral fat area (D), subcutaneous fat area (E), and total fat area (F). $^{*}p<0.05$ (vs. placebo). Mean values with their standard errors represented by vertical bars.

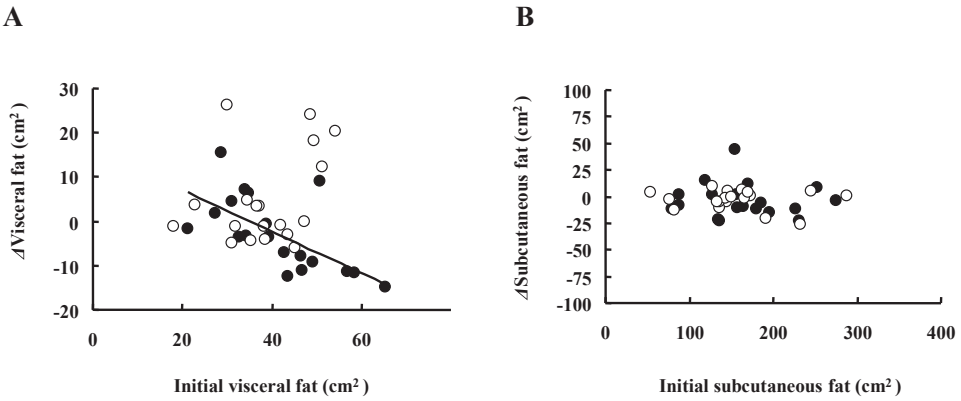

Fig. 2. Fat-reducing effect of GP in relation to the initial visceral fat and subcutaneous fat. A: Correlation between the induced change in visceral fat and the initial visceral fat before ingestion of GP extract (closed circles, $R=-0.64$, $p=0.003$) or placebo (open circles, $R=0.34$, $p=0.15$). B: Correlation between the induced change in subcutaneous fat and the initial subcutaneous fat before ingestion of GP extract (closed circles, $R=-0.007$, $p=0.77$) or placebo (open circles, $R=-0.12$, $p=0.62$).

extract or a placebo every day for 4 wk in a single-blinded, randomized, crossover study. The height, body weight, body fat content, and fat area at the level of the umbilicus were measured in every subject before and after the 4-wk period of GP or placebo ingestion. As summarized in Table 1, there was no significant change in body weight, BMI, body fat content, or fat areas after the 4-wk treatment period. The pre-versus-post-treatment differences in the GP group were compared with those in the placebo group after the 4-wk ingestion period. As shown in Fig. 1, the differences in body weight, BMI, body fat content, subcutaneous fat area, and total fat area were almost the same after GP and placebo ingestion. The visceral fat area decreased slightly in the GP group (-2.9 ± 1.9 cm^2) but rose in the placebo group (4.7 ± 2.4 cm^2). These changes in visceral fat differed significantly between the two groups ($p<0.05$).

The correlation between the fat area before GP ingestion and the fat area change induced by GP ingestion was examined to confirm the effects of GP on visceral fat. As shown in Fig. 2, the GP-induced change showed a significant negative correlation with the initial visceral fat ($r=-0.64$, $p=0.003$). In contrast, no correlation was found between the initial visceral fat and the placebo-induced change ($r=0.34$, $p=0.15$). The initial subcutaneous fat was uncorrelated with the change in

subcutaneous fat induced by GP ($r=-0.007$, $p=0.77$) or placebo ($r=-0.12$, $p=0.62$) ingestion.

Whole-body EE was also measured under a resting condition before and after the 4-wk period of GP or placebo ingestion (Fig. 3). The mean EE calculated from oxygen consumption and carbon dioxide production rose significantly from 1,402±24.7 kcal/d at baseline to 1,499±33.7 kcal/d after 4 wk of GP ingestion. It also rose in the placebo group, but only slightly (1,444±44.0 kcal/d). Table 2 shows the effects of GP ingestion on blood parameters. Glucose and γ-GTP decreased slightly and significantly after 4 wk of ingestion in both the GP and placebo groups, but all of the other parameters remained approximately unchanged, at their normal levels.

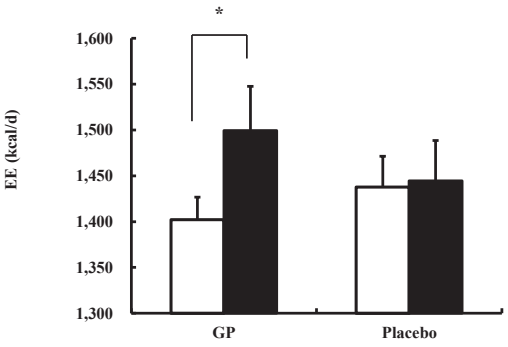

Fig. 3. Whole-body energy expenditure (EE) before and after 4 wk of daily ingestion of GP or placebo. Whole-body energy expenditure under a resting condition was measured before (open columns) and 4 wk after daily ingestion of 30 mg GP extract or placebo (closed columns). *$p<0.05$ (vs. before). Mean values with their standard errors represented by vertical bars.

DISCUSSION

The present study demonstrated that daily ingestion of GP resulted in a significant reduction of visceral fat in humans. Earlier studies have shown that the ingestion of hot pepper, its pungent principle (capsaicin), and capsinoids (non-pungent capsaicin analogs) activate TRPV1 (*1–6*, *9*), the adreno-sympathetic nervous system, and BAT thermogenesis, increase EE and fat oxidation, and reduce body fat, particularly visceral fat, in both humans and small rodents. Our results and earlier results on GP extract share a common finding with the earlier results on capsaicin and capsinoids, namely, that GP is rich in 6-paradol, 6-gingerol, 6-shogaol, and other pungent compounds that have the potential to activate TRPV1 (*15*, *16*). Intragastric administration of either GP extract or 6-paradol enhances the efferent discharges of sympathetic nerves to BAT and significantly increases BAT temperature in rats (*17*). Our group previously reported that a single ingestion of GP-extract increased EE through the activation of BAT in men (*18*). Here, in the present study, we have found that a daily ingestion of GP-extract brings about a slight but significant increase in whole-body EE that may contribute, at least in part, to the fat-reducing effects of GP extract.

As the test sample is an ethanol extract of GP seeds, the compounds responsible for the observed effect of

Table 2. Blood parameters before and after 4 wk of daily ingestion of GP extract or placebo.

	GP (n=19)		Placebo (n=19)	
	0 wk	4 wk	0 wk	4 wk
Glucose (mg/dL)	84.2±0.9	81.5±1.1*	85.7±4.2	82.8±0.9*
HbA1c (%)	4.92±0.04	4.91±0.04	4.91±0.04	4.95±0.04
TC (mg/dL)	169.8±6.0	171.4±6.5	176.3±7.7	172.2±7.1
TAG (mg/dL)	64.7±4.4	63.8±4.4	61.2±5.0	61.1±3.3
HDL-C (mg/dL)	67.2±2.4	66.5±2.8	68.0±3.1	66.6±2.8
LDL-C (mg/dL)	95.0±5.3	96.1±5.4	101.9±6.4	98.5±6.4
RBC ($10^4/\mu$L)	432.1±6.4	427.0±5.6	435.2±7.0	425.5±6.3
WBC ($10^4/\mu$L)	5,267±382	5,547±287	5,536±286	5,320±388
Hb (g/dL)	12.3±0.2	12.0±0.2*	12.3±0.3	12.0±0.3
Free fat acid (mg/dL)	360.4±35	424.4±37	359.6±42	431.5±41
Total protein (g/dL)	7.32±0.08	7.34±0.05	7.37±0.09	7.31±0.08
Albumin (g/dL)	4.5±0.06	4.5±0.06	4.5±0.04	4.5±0.06
ALP (U/L)	172.6±5.8	175.3±6.6	184.1±6.8	177.4±7.2
ALT (U/L)	13.1±1.0	11.7±0.7	11.7±0.8	11.5±0.8
AST (U/L)	17.4±0.8	16.8±0.8	16.4±0.8	16.4±0.8
γ-GTP (U/L)	13.8±1.5	12.2±1.4*	13.9±1.2	12.3±1.2*
Urea nitrogen (mg/dL)	11.3±0.4	12.7±0.6	12.0±0.5	11.8±0.6
Creatinine (mg/dL)	0.65±0.01	0.66±0.01	0.64±0.02	0.66±0.01

HbA1c: hemoglobin A1c, TC: total cholesterol, Hb: hemoglobin, ALP: alanine aminotransferase, ALT: alkaline phosphatase, AST: aspartate aminotransferase.

Mean values with their standard errors. *$p<0.05$ vs. 0 wk.

GP extract are not known at present. The GP extract contained various compounds with a vanilloid moiety such as 6-paradol, 6-gingerol and 6-shogaol (*15*, *16*). All these compounds are capable of activating TRPV1, which is involved in the thermic and anti-obesity effects of capsaicin and capsinoids. The thermic effect of capsaicin and capsinoids are known to be mediated through the activation of TRPV1 in the gastrointestinal tract. Therefore, the effects of GP extract may also be via gastrointestinal TRPV1, although it cannot be ruled out that some vanilloid compounds are absorbed from intestinal tract and directly activate BAT and some other energy-consuming processes. Further studies are needed to identify the compounds responsible for the thermic and fat-reducing effects of GP extract, and to clarify the action mechanism including their bioavailability.

Based on the acute stimulatory effect of GP extract on BAT thermogenesis (*18*), it might be rational to consider that the daily ingestion of GP extract results in a sustained increase in the thermogenic activity of BAT and thereby whole-body EE. In the present study, BAT activity could not be measured because of an ethical restriction: the activity of human BAT can be accessed by ^{18}F-fluorodeoxyglucose-positron emission tomography in combination with computed tomography (*20*), which involves inevitable radiation exposure, and thereby its use is strictly limited, particularly for normal young females.

The fat-reducing effects of GP extract were observed only in visceral fat, not in subcutaneous or total body fat. The effects seem similar to those of capsinoid ingestion, which significantly reduces visceral fat, but not total body fat (*7*, *8*). The selective effects of these agents may be attributable to the different metabolic properties of visceral and subcutaneous fats. Specifically, visceral fat is more sensitive to nutritional and hormonal challenges than subcutaneous fat. We note, with interest, that the reducing effect of GP extract is negatively correlated to the initial levels of visceral fat. This implies that GP extract may have a stronger fat-reducing effect in individuals with more visceral fat. The subjects in the present study were non-obese females, so we presume they exhibited a weaker fat-reduction response than what could be expected in obese subjects.

In conclusion, daily ingestion of GP extract increases whole-body EE and decreases visceral fat in young non-obese females. Although further studies on obese subjects are needed, the present results suggest that GP extract has the potential to become an effective and safe tool for reducing body fat, mainly by preventing visceral fat accumulation.

REFERENCES

1) Kawada T, Watanabe T, Takaishi T, Tanaka T, Iwai K. 1986. Capsaicin-induced β-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. *Proc Soc Exp Biol Med* **183**: 250–256.

2) Kawada T, Hagihara K, Iwai K. 1986. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J Nutr* **183**: 1272–1278.

3) Yoshioka M, Lim K, Kikuzato S, Kiyonaga A, Tanaka H, Shindo M, Suzuki M. 1995. Effects of red-pepper diet on the energy metabolism in men. *J Nutr Sci Vitaminol* **41**: 647–656.

4) Lejeune MP, Kovacs EM, Westerterp-Plantenga MS. 2003. Effects of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *Br J Nutr* **90**: 651–659.

5) Ludy MJ, Moore GE, Mattes RD. 2012. The effects of capsaicin and capsiate on energy balance: critical review and meta-analyses of studies in humans. *Chem Senses* **37**: 103–121.

6) Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M. 2012. Non-pungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. *Am J Clin Nutr* **95**: 845–850.

7) Kawabata F, Inoue N, Yazawa S, Kawada T, Inoue K, Fushiki T. 2008. Effects of CH-19 sweet, a non-pungent cultivar of red pepper, in decreasing the body weight and suppressing body fat accumulation by sympathetic nerve activation in humans. *Biosci Biotechnol Biochem* **70**: 2824–2835.

8) Snitker S, Fujishima Y, Shen H, Ott S, Pi-Sunyer X, Furuhata Y, Sato H, Takahashi M. 2009. Effects of novel capsinoid treatment on fatness and energy metabolism in humans: possible pharmacogenetic implications. *Am J Clin Nutr* **89**: 45–50.

9) Saito M, Yoneshiro T. 2013. Capsinoids and related food ingredients activating brown fat thermogenesis and reducing body fat in humans. *Curr Opin Lipidol* **24**: 71–77.

10) Kawabata F, Inoue N, Masamoto Y, Matsumura S, Kimura W, Kadowaki M, Higashi T, Tominaga M, Inoue K, Fushiki T. 2009. Non-pungent capsaicin analogs (capsinoids) increase metabolic rate and enhance thermogenesis via gastrointestinal TRPV1 in mice. *Biosci Biotechnol Biochem* **73**: 2690–2697.

11) Akendengué B, Louis AM. 1994. Medicinal plants used by the Masango people in Gabon. *J Ethnopharmacol* **41**: 193–200.

12) Connell DW. 1970. Natural pungent compounds. III. Paradols and associated compounds. *Aust J Chem* **23**: 369–376.

13) Connell DW, McLachlan R. 1972. Natural pungent compounds. IV. Examination of the gingerols, shogaols, paradols, and related compounds by thin-layer and gas chromatography. *J Chromatogr A* **61**: 29–35.

14) Tackie AN, Dwuma-Badu D, Ayim JSK, Dabra TT, Knapp JE, Slatkin DJ, Schiff PL Jr. 1975. Constituents of West African medicinal plants. VIII. Hydroxyphenylalkanones from *Amomum melegueta*. *Phytochemistry* **14**: 853–854.

15) Riera CE, Menozzi-Smarrito C, Affolter M, Michlig S, Munari C, Robert F, Vogel H, Simon SA, le Coutre J. 2009. Compounds from Sichuan and Melegueta peppers activate, covalently and non-covalently, TRPA1 and TRPV1 channels. *Br J Pharmacol* **157**: 1398–1409.

16) Morera E, De Petrocellis L, Morera L, Moriello AS, Nalli M, Di Marzo V, Ortar G. 2012. Synthesis and biological evaluation of [6]-gingerol analogues as transient receptor potential channel TRPV1 and TRPA1 modulators. *Bioorg Med Chem Lett* **22**: 1674–1677.

17) Iwami M, Mahmoud FA, Shiina T, Hirayama H, Shima T, Sugita J, Shimizu Y. 2011. Extract of grains of paradise and its active principle 6-paradol trigger thermogenesis of brown adipose tissue in rats. *Auton Neurosci* **161**:

63–67.

18) Sugita J, Yoneshiro T, Hatano T, Aita S, Ikemoto T, Uchiwa H, Iwanaga T, Kameya T, Kawai Y, Saito M. 2013. Grains of paradise (*Aframomum melegueta*) extract activates brown adipose tissue and increases whole-body energy expenditure in men. *Br J Nutr* **4**: 733–738.

19) Tokunaga K, Matsuzawa Y, Ishikawa K, Tarui S. 1983. A novel technique for the determination of body fat by computed tomography. *Int J Obes* **7**: 437–445.

20) Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M. 2009. High incidence of metabolically active brown adipose tissue in healthy adult humans. Effects of cold exposure and adiposity. *Diabetes* **58**: 1526–1531.