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## Research article

Effects of *Panax ginseng* extracts prepared at different steaming times on thermogenesis in ratsHyung Taek Cho<sup>☆</sup>, Jun Ho Kim<sup>☆</sup>, Jin Hyup Lee, Young Jun Kim<sup>\*</sup>

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## ABSTRACT

**Background:** *Panax ginseng* (PG) has a long history of use in Asian medicine because of its multiple pharmacological activities. It has been considered that PG in a type of white ginseng may induce undesirable thermogenic effects, but not in a type of red ginseng. However, there is a lack of evidence about the correlation between ginsenoside and thermogenesis.

**Methods:** We investigated the effects of PG with different ginsenoside compositions on body temperature, blood pressure, and thermogenesis-related factors in rats.

**Results:** With increasing steaming time (0 h, 3 h, 6 h, and 9 h), the production of protopanaxadiol ginsenosides increased, whereas protopanaxatriol ginsenosides decreased in white ginseng. In both short- and long-term studies, administration of four ginseng extracts prepared at different steaming times did not induce significant changes in body temperature (skin, tail, and rectum) and blood pressure of rats compared to saline control. In addition, there were no significant differences in the molecular markers related to thermogenesis ( $p > 0.05$ ), mRNA expressions of peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  and uncoupling protein 1 in brown adipose tissue, as well as the serum levels of interleukin-6, inducible nitric oxide synthase, and nitrite among the treatment groups.

**Conclusion:** These observations indicate that the potential undesirable effects of PG on body temperature could not be explained by the difference in ginsenoside composition.

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## 1. Introduction

*Panax ginseng* (PG) has long been widely used as a folk and conventional medicine for the prevention and/or treatment of many diseases. Ginsenosides, known as ginseng saponins, are the fundamental compounds responsible for the multiple physiological and pharmacological activities of ginseng [1]. With the increasing consumption of ginseng as a pharmacological agent, its influence on body temperature, particularly a “hot feeling,” has been considered a potential side effect, although there is a lack of scientific evidence. Recent animal studies have shown that PG has no effect on body temperature under normal conditions [2,3], whereas intraperitoneal injection of ginsenoside Rb1 increased thermogenesis in rats exposed to cold conditions, indicating that Rb1 improved cold tolerance [4].

Based on the presence of a hydroxyl moiety at C-6, most ginsenosides are categorized into two groups: protopanaxadiols (PPD) and protopanaxatriols (PPT) [5]. It has been shown that PPT ginsenosides, but not PPD ginsenosides, promote endothelium-dependent vasodilation by enhancing the release of nitric oxide in rats [6]. Because the activation of vasodilation induces convective heat transfer from the body core to the skin surface [7], this observation indicates that PPT ginsenosides could be responsible for the thermogenic effect of ginseng. In addition, it has been anecdotally considered that white ginseng (WG), which has a low PPD/PPT ratio, increases body temperature, whereas red ginseng, which has a high PPD/PPT ratio, does not. Thus, in this study, we investigated the short-term and long-term effects of ginseng with different PPD/PPT ratios on thermoregulation in rats. Because the steaming process causes extensive conversion of ginsenosides in

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PG [8], we prepared ginseng samples with various PPD/PPT ratios by altering the steaming time using a WG. Changes in body temperature (skin, tail, and rectum), blood pressure, and thermogenesis-related factors were observed in rats administered the ethanol extracts of ginseng.

## 2. Materials and methods

### 2.1. Sample preparations

Five-year-old fresh PG cultivated in Hong-Chun, Korea, was purchased from a local ginseng market. Ginseng was washed and dried at 60°C for 48 h using a force airflow oven (INBD-150e; Hansung Co., Jeonbuk, Korea) to obtain WG, which is produced by drying of fresh ginseng. WG samples were steamed for 3 h, 6 h, or 9 h at 95 ± 5°C and dried at 40°C for 24 h. All ginseng samples were divided into three batches, ground into powder, and extracted with 10 volumes of 70% ethanol at 80 ± 5°C for 4 h in reflux. The extracts were filtered, concentrated under reduced pressure, and then freeze-dried. Prepared samples were stored at 4°C until use.

### 2.2. Ginsenoside analysis

A high-performance liquid chromatography (HPLC) system (UltiMate 3000; Dionex Co., Sunnyvale, CA, USA) with an EC-C18 column (100 mm × 2.1 mm, Poroshell 120; Agilent, Santa Clara, CA, USA) was used for ginsenoside analysis. Gradient elution was conducted using 10% acetonitrile (Solvent A) and 90% acetonitrile (Solvent B) in distilled water at A/B ratios of 90:10, 79:21, 78:22, 77:23, 76:24, 63:37, 55:45, 54:46, 52:48, 0:100, and 90:10, with run times of 0 min, 22 min, 23 min, 40 min, 45 min, 53 min, 61 min, 66 min, 75 min, 80 min, 95 min, and 105 min, respectively. The flow rate was maintained at 1.3 mL/min and the detection wavelength was 203 nm.

### 2.3. Animal study

Male 6-wk-old Sprague–Dawley rats (Samtako Biokorea, Seoul, Korea) were housed at constant temperature (24 ± 2°C) and humidity (60 ± 10%) with a 12-h light–dark cycle. After an adaptation period of 1 wk, rats were divided into five treatment groups as follows: (1) control (normal saline), (2) WG, (3) 3 h-steamed ginseng (SG-3), (4) 6 h-steamed ginseng (SG-6), and (5) 9 h-steamed ginseng (SG-9). All compounds at 200 mg/kg were administered by gavage using an esophageal cannula once daily for 4 wk. Because total ginsenoside contents differed among ginseng samples, in the next short-term test, WG or SG-9 at 10 mg ginsenosides/kg was administered orally with a saline control for 5 d. All rats received a normal chow diet and water *ad libitum* during the experimental period. At the end of the study, rats were euthanized by 5% urethane, and the blood was collected by cardiac puncture. The serum was separated by centrifugation at 1,200g for 30 min at 4°C, and the brown adipose tissue (BAT) was collected and kept at –80°C until use. All animal work complied with the guidelines of the Institutional Animal Care and Use Committee at the Korea University (Seoul, Korea).

### 2.4. Measurement of body temperature

Body temperature was measured in three parts of the rats, including the rectum, skin, and tail. The rectal temperature was recorded using an SDT25 thermometer probe (Summit Co., Incheon, Korea) inserted 1 cm into the rectum. Skin and tail temperatures were captured by thermal imaging using a fluke Ti45 infrared thermal imaging camera (Fluke Co., Everett, WA, USA). For

long-term testing, body temperature was measured at 10-min intervals for 2 h after sample administration each week. For short-term testing, body temperature was measured at 1-h intervals for 24 h after sample administration on Day 3 and Day 5.

### 2.5. Measurement of blood pressure

A noninvasive computerized tail-cuff system (CODA-HT8; Kent Co., Torrington, CT, USA) was used to record blood pressure in rats. Rats were immobilized in an acrylic restrainer (HLD-RM-T; Kent Co.) and placed on a warming platform maintained 37°C. Occlusion and the volume–pressure recording-cuff (OCC-M, VPR-M; Kent Co.) were threaded through the tail. Systolic and diastolic pressures were monitored at 10-min intervals for 30 min after sample administration each week.

### 2.6. Biochemical parameters

Serum levels of interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS) were measured using colorimetric assay kits for IL-6 (R&D Systems, Minneapolis, MN, USA) and iNOS (Neo Bio Lab, Woburn, MA, USA) according to the manufacturer instructions. The serum level of nitrite was measured using a colorimetric assay kit (Promega, Madison, WI, USA) involving the Griess reaction.

### 2.7. Real-time polymerase chain reaction analysis

BAT was homogenized in 1 mL TRIzol reagent, and then total RNA was isolated according to the TRIzol protocol. Total RNA was reverse-transcribed into cDNA using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster, CA, USA) as described in the manufacturer's protocol. cDNA was used as a template for the relative quantitation of selected target genes using predesigned TaqMan gene expression assay kits (Applied Biosystems). Each 20- $\mu$ L reaction contained 100 ng cDNA, 2 $\times$  TaqMan Gene Expression Mastermix, forward and reverse primers, and a TaqMan probe. All reactions were carried out in triplicate using an ABI 7500 system (Applied Biosystems) under the following conditions: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The results were normalized to GAPDH as an internal standard, and the relative quantities of each gene were presented in terms of  $2^{-\Delta\Delta Ct}$ , calculated using the  $\Delta Ct$  and  $\Delta\Delta Ct$  values.

### 2.8. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) using SAS software for Windows release 9.2 (SAS Institute Inc., Cary, NC, USA) on the W32\_VSHOME platform. One-way ANOVA with repeated measures was performed to assess mean differences between groups for body temperature and blood pressure over time. The least squares means option using a Tukey–Kramer adjustment was used for the multiple comparisons among the treatment groups. Data are presented as the mean ± standard deviation. A *p* value < 0.05 was considered statistically significant.

## 3. Results

### 3.1. PPD/PPT ratio of ginsenosides in WG increased with steaming time

HPLC analyses revealed alterations in the ginsenoside profiles of WG samples treated with different steaming times (Table 1). The chemical structures of PPD and PPT ginsenosides are shown in

**Table 1**  
Ginsenoside contents of ginseng extracts (mg/g extract)

	WG	SG-3	SG-6	SG-9
PPD ginsenoside				
Rb1	8.02 ± 0.03 <sup>a</sup>	6.20 ± 0.01 <sup>b</sup>	3.35 ± 0.02 <sup>c</sup>	1.47 ± 0.02 <sup>d</sup>
Rb2	2.21 ± 0.01 <sup>a</sup>	1.72 ± 0.01 <sup>b</sup>	1.25 ± 0.01 <sup>c</sup>	0.59 ± 0.01 <sup>d</sup>
Rc	2.62 ± 0.01 <sup>a</sup>	2.15 ± 0.03 <sup>b</sup>	1.37 ± 0.02 <sup>c</sup>	0.64 ± 0.01 <sup>d</sup>
Rd	0.73 ± 0.01 <sup>a</sup>	—	0.46 ± 0.01 <sup>b</sup>	0.37 ± 0.02 <sup>c</sup>
Rg3	—	0.25 ± 0.01 <sup>c</sup>	0.94 ± 0.01 <sup>b</sup>	1.87 ± 0.02 <sup>a</sup>
Rg5	—	2.23 ± 0.01 <sup>c</sup>	11.18 ± 0.09 <sup>b</sup>	22.21 ± 0.22 <sup>a</sup>
Rk1	—	2.04 ± 0.01 <sup>c</sup>	9.38 ± 0.01 <sup>b</sup>	18.76 ± 0.04 <sup>a</sup>
PPT ginsenoside				
Re	3.74 ± 0.02 <sup>a</sup>	3.17 ± 0.02 <sup>b</sup>	1.48 ± 0.01 <sup>c</sup>	0.43 ± 0.01 <sup>d</sup>
Rf	0.91 ± 0.03 <sup>a</sup>	0.76 ± 0.02 <sup>b</sup>	0.74 ± 0.01 <sup>b</sup>	0.73 ± 0.01 <sup>b</sup>
Rg1	3.95 ± 0.01 <sup>a</sup>	2.38 ± 0.01 <sup>b</sup>	0.85 ± 0.01 <sup>c</sup>	0.13 ± 0.01 <sup>d</sup>
Rg2	0.28 ± 0.01 <sup>c</sup>	0.40 ± 0.01 <sup>b</sup>	0.40 ± 0.01 <sup>b</sup>	0.49 ± 0.01 <sup>a</sup>
Rh1	0.02 ± 0.01 <sup>d</sup>	0.07 ± 0.02 <sup>c</sup>	0.37 ± 0.02 <sup>b</sup>	0.51 ± 0.01 <sup>a</sup>
PPD total	13.57 ± 0.03 <sup>d</sup>	14.59 ± 0.01 <sup>c</sup>	27.93 ± 0.14 <sup>b</sup>	45.92 ± 0.24 <sup>a</sup>
PPT total	8.90 ± 0.02 <sup>a</sup>	6.77 ± 0.02 <sup>b</sup>	3.84 ± 0.03 <sup>c</sup>	2.29 ± 0.01 <sup>d</sup>
Total ginsenosides	22.47 ± 0.05 <sup>c</sup>	21.36 ± 0.02 <sup>d</sup>	31.77 ± 0.11 <sup>b</sup>	48.21 ± 0.25 <sup>a</sup>
PPD/PPT ratio	1.52 ± 0.01 <sup>d</sup>	2.15 ± 0.01 <sup>c</sup>	7.27 ± 0.10 <sup>b</sup>	20.06 ± 0.01 <sup>a</sup>

Values represent means ± standard error ( $n = 3$ ).

Means with different superscripts (<sup>a-d</sup>) within the same row are significantly different ( $p < 0.05$ ).

PPD, protopanaxadiols; PPT, protopanaxatriols; SG-3, 3 h-steamed ginseng; SG-6, 6 h-steamed ginseng; SG-9, 9 h-steamed ginseng; WG, white ginseng.

**Fig. S1.** PPD ginsenosides (Rb1, Rb2, Rc, and Rd) in WG decreased with increasing steaming time, whereas three newly produced ginsenosides (Rg3, Rg5, and Rk1) increased gradually during steaming, ultimately increasing the total amount of PPD ginsenosides with steaming. The major PPT ginsenosides (Re, Rf, and Rg1) in WG decreased with increasing steaming time, resulting in a

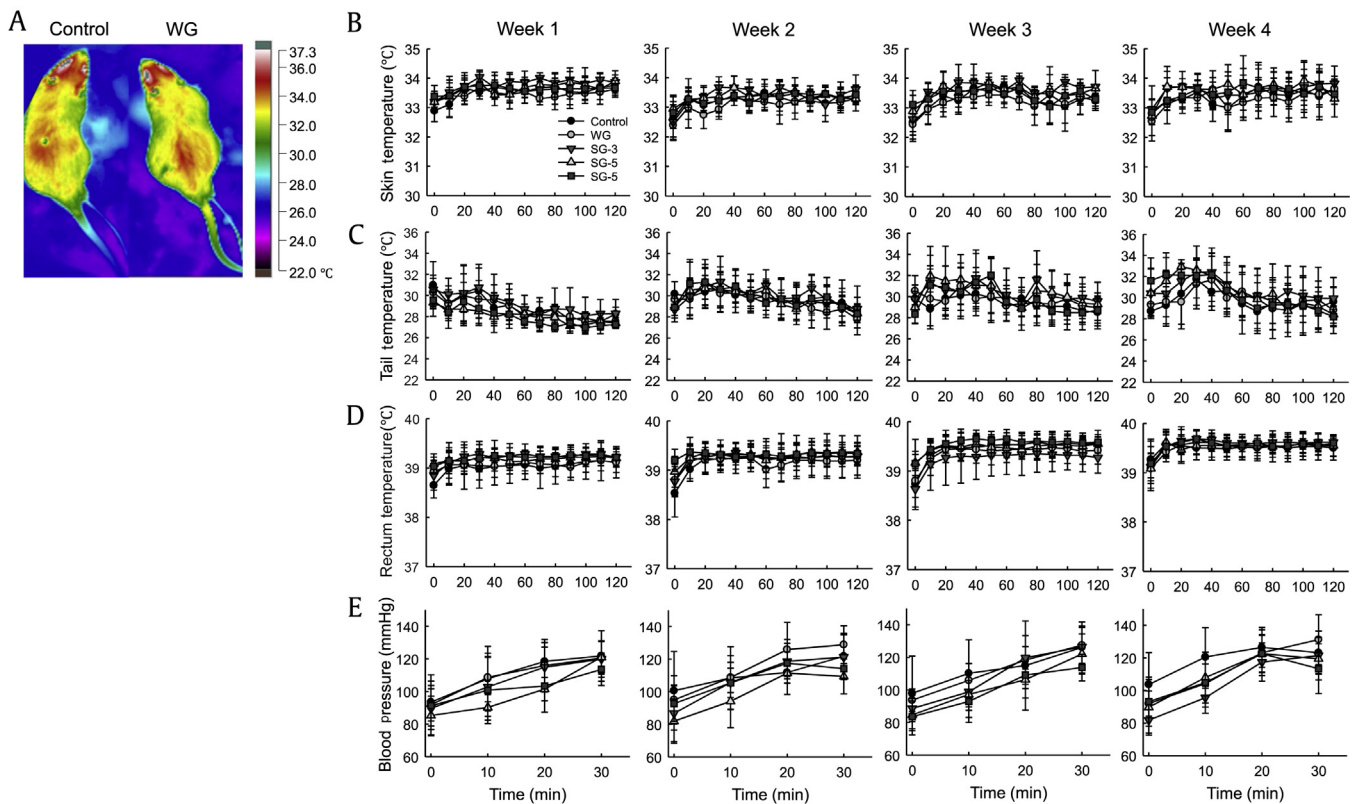
steady decrease of total PPT ginsenosides. This change in the ginsenoside profile dramatically increased the PPD/PPT ratio in WG with increasing steaming time for up to 9 h.

### 3.2. Ginseng with different PPD/PPT ratios of ginsenosides does not affect body temperature and blood pressure in rats

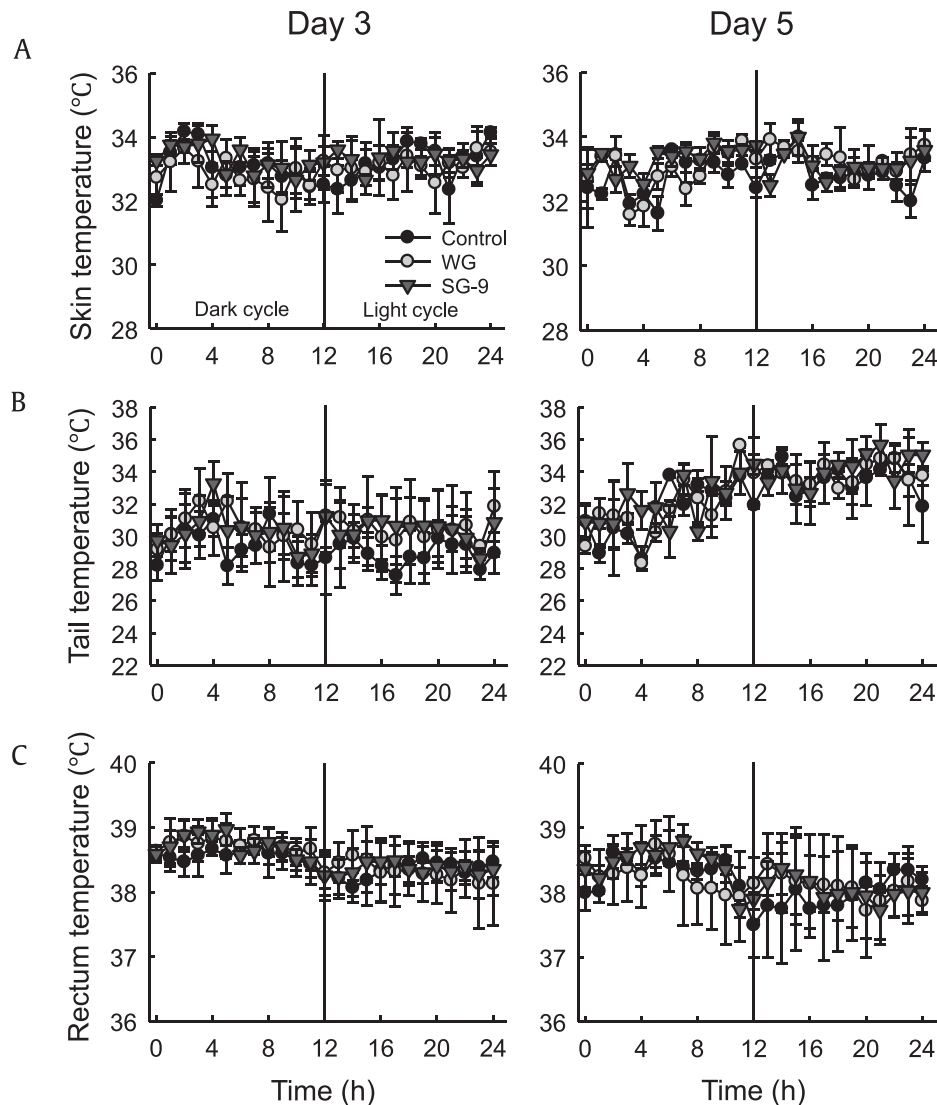
After the administration of ginseng extracts, body temperature (skin, tail, and rectum) was recorded for 2 h in the long-term study (Figs. 1A–E) and 24 h in the short-term study (Figs. 2A–2C). Blood pressure was recorded for 30 min in the long-term study (Fig. 1E). In both long- and short-term studies, no significant differences were observed in both body temperature and blood pressure in all regions examined among the treatment groups. The linear increase in blood pressure during the testing time in all groups may be associated with the use of a restrainer in this study [9]. These results indicate that PG with different ginsenoside profiles does not influence body temperature and blood pressure in rats.

### 3.3. Ginseng with different PPD/PPT ratios of ginsenosides does not alter thermogenesis-related factors in rats

To monitor compound responses involved in thermoregulation, we next measured the serum levels or mRNA expressions of key mediators of vasodilation and thermogenesis. In both long- and short-term feeding tests, no significant differences were observed in the serum levels of IL-6, iNOS, and nitrite (Fig. 3A) and gene expressions of peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) and uncoupling protein 1 (UCP1) in BAT (Fig. 3B) among the treatment groups. Combined with the data for



**Fig. 1.** Body temperature and blood pressure following ginseng administrations in long-term trial. All compounds were orally administered once daily for 4 wk with normal saline control. Body temperature and blood pressure were measured at 10-min intervals for 2 h or 30 min after sample administration each week. (A) Representative images of skin temperature. (B) Skin temperature. (C) Tail temperature. (D) Rectum temperature. (E) Blood pressure. Values represent means ± standard deviation ( $n = 8$ ). SG-3, 3 h-steamed ginseng; SG-6, 6 h-steamed ginseng; SG-9, 9 h-steamed ginseng; WG, white ginseng.



**Fig. 2.** Body temperature following ginseng administrations in short-term trial. All compounds were orally administered once daily for 1 wk with normal saline control. Body temperature was measured at 1-h intervals for 24 h after sample administration at Day 3 and Day 5. (A) Skin temperature. (B) Tail temperature. (C) Rectum temperature. Values represent means  $\pm$  standard deviation ( $n = 8$ ). SG-9, 9 h-steamed ginseng; WG, white ginseng.

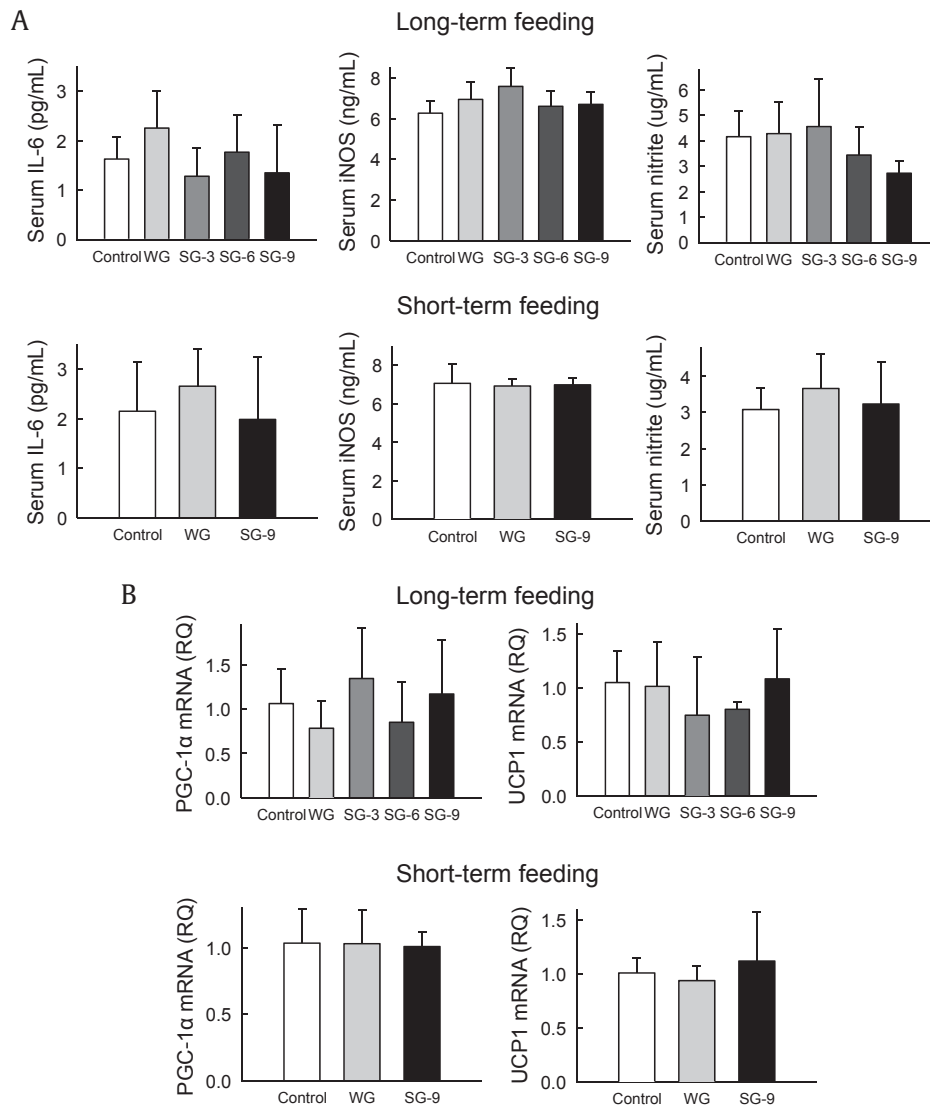
body temperature and blood pressure, these findings indicate no association between PG administration and thermogenesis.

#### 4. Discussion

This study demonstrates that short- and long-term administration of PG with different PPD/PPT ratios of ginsenosides does not alter body temperature, blood pressure, and thermogenesis-related factors in rats. Although recent evidence indicates that PG has no effect on body temperature in rodents [2,3], our study is the first report to investigate the effects of different ginsenoside profiles on body temperature.

Because the steaming process improves the biological activities of ginseng by altering the ginsenoside profile [10], previous studies have attempted to identify the optimal steaming condition for ginseng [10–12]. Our results showed that steaming for up to 9 h at 95°C gradually increased the PPD/PPT ratio of ginsenosides as well as total ginsenoside content. Given the specific structure–activity relationships of PPD and PPT ginsenosides [13], these changes in the ginsenoside profile and content in WG may improve its health-promoting activities, as shown for red ginseng.

It has been anecdotally considered that PG, particularly in a type of WG, may induce undesirable increases in body temperature. However, in this study, we did not observe any significant differences in body temperature of rats following ginseng administration. We examined the possibility of a thermogenic effect in terms of vasodilation and BAT function. Nitric oxide initiates and maintains vasodilation through a cascade of biological events that are partly regulated by iNOS and proinflammatory cytokines such as IL-1 and IL-6 [14,15]. Heat transfer from the body core to the skin surface is achieved through the vasodilation of skin blood vessels [16]. Additionally, in BAT, UCP1 uncouples mitochondrial oxidative phosphorylation from adenosine triphosphate production and dissipates chemical energy as heat, profoundly increasing energy expenditure [17]. PGC-1 $\alpha$  regulates BAT-mediated thermogenesis by directly inducing UCP1 expression [17]. Importantly, the serum or gene expressions of these regulators involved in vasodilation and BAT-mediated thermogenesis were not significantly altered in this study. Thus, the current results indicate that the undesirable effect of PG on body temperature could not be explained by differences in ginsenoside composition. However, we used an animal model, which does not reflect the different types of physical constitution in



**Fig. 3.** Thermogenesis-related factors in serum and brown adipose tissue. (A) Serum levels of IL-6, iNOS and nitrite in long- and short-term feeding studies. (B) BAT mRNA expressions of PGC-1 $\alpha$  and UCP1 in long- and short-term feeding studies. Values represent means  $\pm$  standard deviation ( $n = 8$ ). BAT, brown adipose tissue; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; PGC-1 $\alpha$ , peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$ ; SG-3, 3 h-steamed ginseng; SG-6, 6 h-steamed ginseng; SG-9, 9 h-steamed ginseng; UCP1, uncoupling protein 1; WG, white ginseng.

humans, and thus our results are rather limited. In traditional Asian medicine, known as “Sasang constitutional medicine,” it has been considered that the pyrogenic effect of ginseng may differ depending on four different types of physical constitution: Taeyang, Soyang, Taeum, and Soeum [18,19]. Thus, further clinical studies are necessary to investigate the pyrogenic effect of PG with different types of physical constitution and resulting physiological conditions.

#### Conflicts of interest

All contributing authors declare no conflicts of interest.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jgr.2016.07.001>.

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