Effects of α-Glucosylhesperidin on the Peripheral Body Temperature and Autonomic Nervous System

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Received October 7, 2009; Accepted January 12, 2010; Online Publication, April 7, 2010
[doi:10.1271/bbb.90742]

We studied the effects of α-glucosylhesperidin (G-Hsp) on the peripheral body temperature and autonomic nervous system in humans. We first conducted a survey of 97 female university students about excessive sensitivity to the cold; 74% of them replied that they were susceptible or somewhat susceptible to the cold. We subsequently conducted a three-step experiment. In the first experiment, G-Hsp (500 mg) was proven to prevent a decrease in the peripheral body temperature under an ambient temperature of 24 °C. In the second experiment, a warm beverage containing G-Hsp promoted blood circulation and kept the finger temperature higher for a longer time. We finally used a heart-rate variability analysis to study whether G-Hsp changed the autonomic nervous activity. The high-frequency (HF) component tended to be higher, while the ratio of the low-frequency (LF)/HF components tended to be lower after the G-Hsp administration. These results suggest that the mechanism for temperature control by G-Hsp might involve an effect on the autonomic nervous system.

Key words: α-glucosylhesperidin; cold sensitivity; peripheral body temperature; autonomic nervous system; heart-rate variability

Homeothermic animals use autonomic and behavioral effector responses to regulate their body temperature (known as thermoregulation) in both the heat and the cold.1–3) Autonomic thermoregulation and thermal comfort in response to cold stimuli differ even among healthy individuals, depending on such factors as age, gender, and body composition.4–7) Many Japanese women are troubled by a feeling of coldness; in fact, it has been reported that half of all adult women suffer from this problem.8) Many tend to feel cold, especially at the beginning of winter, but the problem seems to disappear with the arrival of spring. However, some feel unusually cold even in the summer months. Since most public transport facilities and offices are now air-conditioned, there are more situations involving exposure to cool air; as a result, women who are sensitive to cold temperatures suffer from these feelings more frequently throughout the year than they did in the past.9) It is recognized that this problem is not restricted to middle-aged and older women, but also affects younger women with cold sensitivity.8,9)

Hesperidin, one of the most prevalent flavanones, is found in the peel of such fruits as the Satsuma mandarin orange (Citrus unshu Marc.) and Valencia orange (Citrus sinensis Valencia) in the form of glycosides.10) The dried peel of Citrus unshu Marc., known as Chinpi, has been used as a traditional medicine in China. Hesperidin, so-called vitamin P, is known to exert many biological functions, including antioxidative, anti-inflammatory, antiviral, and anticarcinogenic activities.11) However, since hesperidin is not adequately soluble in an aqueous solution and is not absorbed well through the gastrointestinal tract, it has rarely been used clinically.

4'-α-glucopyranosylhesperidin (G-Hsp, Fig. 1) was synthesized from hesperidin by transglucosylation using cyclodextrin glucanotransferase (1,4-α-D-glucan 4-α-D-(1,4-α-D-glucano)-transferase (cyclizing), EC.2.4.1.19). G-Hsp is approximately 300 times more soluble than hesperidin in the aqueous phase12) and is absorbed more rapidly and efficiently than hesperidin.13) G-Hsp was therefore expected to rapidly exhibit strong biological effects.

We have studied the effects of G-Hsp on body temperature. A previous study showed that G-Hsp promoted the recovery of peripheral body temperature after a cooling stress by dilating blood vessels and increasing blood circulation.14) On the basis of our previous study, we aimed to elucidate whether G-Hsp could effectively prevent a fall in peripheral surface temperature during cold exposure and, if so, what its mechanism was. It has been reported that hesperidin acted as a direct vasodilator in endothelial cells. Chiou15) has shown that hesperidin inhibited strain-induced endothelin-1 secretion and enhanced nitric oxide (NO) production in human umbilical vein endothelial cells. Yamamoto16) has shown that hesperetin, a putative metabolite of G-Hsp, enhanced the endothelium-dependent relaxation induced by acetylcholine in isolated aortas of spontaneously hypertensive rats. Hesperidin is absorbed and appears in the serum in hesperetin.

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Abbreviations: G-Hsp, α-glucosylhesperidin; ANS, autonomic nervous system; HR, heart rate; LF, low-frequency component; HF, high-frequency component
glucuronidated and hesperetin sulfated forms. The hesperetin concentration in serum after hydrolyzing the glucuronide and sulfate conjugates is sufficient for favorable biological effects in cell and animal studies.\textsuperscript{13}

We found in our previous study\textsuperscript{7} that an oral administration of G-Hsp increased the interscapular brown adipose tissue-sympathetic nerve activity (BAT-SNA), but decreased the cutaneous sympathetic nerve activity (CASNA) in urethane-anesthetized rats. Predictably, we observed that the subcutaneous body temperature (BT) at the caudal end of the back after an oral administration of G-Hsp was significantly higher than subcutaneous BT after an oral administration of water in conscious rats. We aimed in this study to ascertain the effects of an oral administration of G-Hsp on the autonomic nervous system in humans. We measured the autonomic nerve activity to evaluate the indirect vasodilation effect of G-Hsp. Heart rate (HR) variability has been used to evaluate the autonomic nervous system, providing a quantitative, non-invasive measurement of the short-term cardiovascular system function.\textsuperscript{18,19} We measured HR variability to investigate the influence of G-Hsp on the autonomic nervous system.

We first conducted a survey on 97 female university students to assess excessive sensitivity to cold in the summer and winter. We subsequently conducted a three-step experiment as follows: we first investigated whether the ingestion of G-Hsp could raise the human peripheral body temperature in an air-conditioned room set at 24 ± 0.5 °C. We next studied whether the administration of a warm beverage (60 °C) containing G-Hsp could help maintain a high human peripheral body temperature. We recognized empirically that the body temperature would rise when a warm beverage was consumed. We expected that adding G-Hsp would increase this effect. We finally studied by a wavelet analysis of the HR variability whether there had been any change in the autonomic nervous system after the G-Hsp administration.

### Materials and Methods

**Questionnaire about coldness (survey about feeling cold).** We surveyed 97 female university students (18–25 years of age) to assess whether they had any unusual feeling of coldness, the frequency of this, and in which body parts felt cold in the summer and winter. We analyzed the results to determine whether they were suffering from hie-sho (cold sensitivity) by using Terasawa’s diagnostic criteria (Table 1).\textsuperscript{20} The subjects were diagnosed as suffering from hie-sho (cold sensitivity) if they answered “yes” to more than two important questions, to more than one important question and two reference questions, or to four or five reference questions.

![Fig. 1. Structure of 4'-α-Glucopyranosylhesperidin.](image)

<table>
<thead>
<tr>
<th>Table 1. Terasawa’s Cold Sensitivity (hie-sho) Diagnostic Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Sensitive to a reduction in environmental temperature than others?</td>
</tr>
<tr>
<td>(2) Feel cold and pain in waist, foot, hand or some parts of the body?</td>
</tr>
<tr>
<td>(3) Need an electric blanket or pocket warmer in winter?</td>
</tr>
<tr>
<td>(4) Feel colder in a cold environment than others do?</td>
</tr>
<tr>
<td>(5) Wear thicker socks in summer due to coldness?</td>
</tr>
<tr>
<td>(6) Feel cold in an air-conditioned room in summer where most people feel comfortable?</td>
</tr>
<tr>
<td>(7) Need thicker clothes than others do?</td>
</tr>
<tr>
<td>(8) Feel the temperature of the hand and the feet are lower than other parts of the body?</td>
</tr>
</tbody>
</table>

**Experiment 1. Effect of G-Hsp on the peripheral body temperature in an air-conditioned room.**

**Subjects.** We surveyed 20 healthy Japanese female volunteers (18–22 years of age) with an average body weight of 52.6 ± 5.0 kg (mean ± SD) and height of 159.2 ± 5.6 cm (mean ± SD) in 2005. We additionally surveyed 26 healthy Japanese female volunteers (18–22 years of age) with an average body weight of 51.7 ± 4.5 kg (mean ± SD) and height of 157.7 ± 5.0 cm (mean ± SD) in 2006. Informed consent was obtained from all the subjects according to the guidelines established by the declaration of Helsinki. This study was approved by the ethics committee of the University of Shiga Prefecture.

**Test samples and experimental conditions.** Test samples and experimental conditions are summarized in Table 2. The subjects received two hard capsules (Capsugel Japan, Kanagawa, Japan), each of which contained 500 mg of G-Hsp (Ezaki Glico Co., Osaka, Japan). A placebo hard capsule containing 500 mg of powdered sugar was used for comparison. The subjects each ingested a capsule with 50 ml of 37 °C water. Each subject took one of the two capsules on the first day and the other capsule on the second day. The treatment order was randomized.

**Measurements.** The body surface temperatures of the forehead, neck, wrist, annular finger of the left hand, left ankle (in 2005 and 2006), and middle toe of the left foot (only in 2006) were measured in an AM-7002 data collector (Adachi Keiki Co., Tokyo, Japan), and the tympanic temperature was measured every 3 min with an S-10 DC3V ear thermometer (Morishita Jintan Co., Tokyo, Japan).

**Experimental environment.** Measurements were taken in a quiet constant-temperature room (24 ± 0.5 °C) with a humidity of about 50%.

**Testing protocol.** We conducted a double-blind, placebo-controlled cross-over study. The testing protocol is shown in Fig. 2. The day and the time the experiments were conducted were specified. Each subject participated in this experiment within 2 weeks after the menstrual period to avoid fluctuation of the basal body temperature. Each measurement was taken between 15:00 and 17:00 to avoid the influence of the diurnal change in body temperature. The subjects abstained from food, drink, and exercise for 3 h before the experiment. Each subject wore the same clothing for every measurement to eliminate the influence of clothing on the body surface temperature. The subjects were instructed to first sit and rest quietly in an anteroom for more than 30 min (26 ± 0.5 °C). Within 10 min after a subject entered the measurement room (24 ± 0.5 °C), thermometer probes and blood flow sensors were placed on her body; the capsule was then taken within 1 min. Measurements were taken for 1 h after administration while the subject remained in a seated position. Each subject...
Table 2. Experimental Conditions

<table>
<thead>
<tr>
<th>Room temperature and room humidity</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anteroom (26 ± 0.5°C, 50 ± 5%)</td>
<td>Measurement room (22 ± 0.5°C, 50 ± 5%)</td>
<td>Measurement room (22 ± 0.5°C, 50 ± 5%)</td>
<td></td>
</tr>
<tr>
<td>Measurement room (24 ± 0.5°C, 50 ± 5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount of diet consumed</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg of G-Hsp, or 500 mg of powder sugar in hard capsule with 50 ml (37°C) of water</td>
<td>Warm beverage (60°C) containing 500 mg of G-Hsp (200 ml), or warm beverage (60°C) without G-Hsp (200 ml)</td>
<td>Beverage (37°C) containing 500 mg of G-Hsp (100 ml), or beverage (37°C) without G-Hsp (100 ml)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables measured</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body surface temperature and blood flow rate in wrist</td>
<td>Body surface temperature and blood flow rate in top of the finger</td>
<td>Heart rate</td>
<td></td>
</tr>
</tbody>
</table>

1st experiment

<table>
<thead>
<tr>
<th>Anteroom (26 ± 0.5°C)</th>
<th>Measurement room (22 ± 0.5°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature and blood flow sensors put</td>
<td>Relevant diet consumed</td>
</tr>
</tbody>
</table>

2nd and 3rd experiments

<table>
<thead>
<tr>
<th>Measurement room (22 ± 0.5°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature and blood flow sensors put</td>
</tr>
</tbody>
</table>

Fig. 2. Testing Protocol.

answered a questionnaire about her current physical condition during the test to identify any extraneous influences and to improve data reliability. This experiment was conducted from late June to late July in the years 2005 and 2006.

Experiment 2. Effects of a warm beverage containing G-Hsp on the peripheral body temperature.

Subjects. We surveyed 12 healthy Japanese female volunteers (18–22 years of age) with an average body weight of 52.2 ± 4.3 kg and height of 158.8 ± 4.6 cm. The other conditions were the same as in the previous experiment.

Test samples and experimental conditions. The test samples and experimental conditions are summarized in Table 2. Each subject drank 200 ml of a beverage containing 500 mg of G-Hsp at 60°C within 1.5 min. Flavoring had been added to the beverage to mask its smell and taste. A placebo beverage not containing G-Hsp was used for comparison. Each subject drank one of two beverages on the first day and the other beverage on the second day. The treatment order was randomized.

Measurements. The body surface temperatures at the neck, wrist, annular finger of the left hand, and middle toe of the left foot were measured as detailed in Experiment 1. The blood flow in the middle finger of the left hand was measured with an ALF21/21D laser blood flow meter (Advance Co., Tokyo, Japan).

Testing protocol. The testing protocol is shown in Fig. 2. The measurement room was kept at 22 ± 0.5°C with a humidity of about 50%, the other conditions being the same as those described in Experiment 1. The experiment was conducted in January 2008.

Experiment 3. Effect of G-Hsp on the autonomic nervous system.

Subjects. We surveyed 11 healthy Japanese female volunteers (18–22 years of age) with an average body weight of 49.5 ± 5.7 kg and height of 159.3 ± 4.7 cm. The other conditions were the same as the previous experiment 2.

Test samples and experimental conditions. The test samples and experimental conditions are summarized in Table 2. The test sample was 100 ml of a beverage containing 500 mg of G-Hsp at 37°C. The other conditions were the same as those in Experiment 2.

Measurements. We measured the HR response with a cardiac electromgram (Sanef Medical Co., Kumamoto, Japan). Using the Flucllet Jr instrument (Dainippon Sumitomo Pharma Co., Osaka, Japan), we made a wavelet analysis of the HR variability with a recently developed new algorithm that functions with accurate quantum resonance spectrometer complex recognition, accurate automatic rejection of outliers, baseline adjustment in periodograms, and digital filtering of each amplitude. The advantage of the method employed in this study is that it had a high time resolution of 1 s. The HF component was determined as the mean of every successive 1-s HF value. The LF and HF components are defined as the resolved components whose peak was within the respective range of 0.04–0.15 Hz and 0.15–2.00 Hz. The LF/HF ratio was also calculated. LF gives an estimate of the sympathetic activity with some influence from vagus nerve simulation, HF solely reflects the vagus nerve activity, and the LF/HF ratio provides a reasonable estimate of the cardiac sympathetic activity.

Testing protocol. The testing protocol is shown in Fig. 2. The time schedule was the same as that in Experiment 2, and the experiment was also conducted in December 2007.

Statistical analysis. Each result is expressed as the mean ± SEM. The time versus treatment effect was evaluated by repeated ANOVA. Comparisons between the G-Hsp and placebo means at certain time
Results

1. Paired points over 1 h after administration were evaluated by a two-tailed paired t-test. Statistical values were calculated with the StatView software package (Macintosh Version J5.0, Abacus Concepts, Berkeley, CA, USA). A p-value of <0.05 was considered significant.

2. The results of the time-course experiment (i.e., change in the surface temperatures at the forehead, neck, wrist, finger, left ankle, toe and tympanic membrane after administering G-Hsp or the placebo) are shown in Fig. 3. Differences between the respective basal values before administering G-Hsp or the placebo were not statistically significant. For example, the finger temperatures before administering G-Hsp or the placebo were 33.3 ± 0.3 °C and 31.2 ± 0.5 °C, respectively (p = 0.29 by the paired t-test), and the toe temperatures before administering G-Hsp or the placebo were 31.6 ± 0.6 °C and 31.2 ± 0.5 °C, respectively (p = 0.98 by the paired t-test). The surface temperature of the hand and foot tended to fall after taking the test samples, regardless of being G-Hsp or the placebo, in the air-conditioned room set at 24 °C. Compared with the placebo, G-Hsp maintained the peripheral surface temperature of the finger (time × treatment effect, p = 0.011 by repeated measures ANOVA, 45–60 min, p < 0.01–0.05 by the paired t-test), and of the toe statistically higher (time × treatment effect, p = 0.027 by repeated measures ANOVA, 45–60 min, p < 0.05 by the paired t-test).

In addition, G-Hsp maintained the tympanic temperature higher compared with the placebo (9–15, 39–42, and 51–54 min p < 0.01–0.05 by the paired t-test).

Table 3. Study of the Awareness of an Unusual Feeling of Coldness in the Summer and Winter

<table>
<thead>
<tr>
<th>Questionnaire about coldness</th>
<th>In summer</th>
<th>In winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1. Do you think you have unusual feelings of coldness?</td>
<td>42</td>
<td>74</td>
</tr>
<tr>
<td>1-2. Do you worry about unusual feelings of coldness?</td>
<td>17</td>
<td>31</td>
</tr>
<tr>
<td>2. Result of Terasawa’s diagnostic criteria test</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>3-1. Do you suffer from coldness in an air-conditioned room in the summer or winter?</td>
<td>19</td>
<td>74</td>
</tr>
<tr>
<td>3-2. Do you feel coldness in your whole body or in some body parts?</td>
<td>41</td>
<td>67</td>
</tr>
<tr>
<td>3-3. In which parts do you feel coldness?</td>
<td>40</td>
<td>39</td>
</tr>
</tbody>
</table>

Sensitivity to cold

Seventy-four percent (42% “almost always” and 32% “sometimes”) of the subjects replied that they were susceptible or somewhat susceptible to the cold; 76% (17% “seriously” and 59% “a little”) were worried about it.

Diagnosis of hie-sho (cold sensitivity)

Sixty-nine percent of the subjects were diagnosed with hie-sho (cold sensitivity) on the basis of Terasawa’s diagnostic criteria test. This figure was similar to the level of subject awareness.

Frequency and body parts of feeling coldness

Seventy-nine percent of the subjects replied that they felt cold either almost always (74%) or sometimes (32%) in the summer. Ninety-seven percent of the subjects replied that they felt cold either almost always (74%) or sometimes (23%) in the winter. More subjects replied that they felt cold in specific parts of the body rather than their whole body. The results of this study show that 70% of the subjects felt cold in the tips of their fingers and toes.

Experiment 1. Effect of G-Hsp on the peripheral body temperature in an air-conditioned room

Surface temperature

The results of the time-course experiment (i.e., change in the surface temperatures at the forehead, neck, wrist, finger, left ankle, toe and tympanic membrane after administering G-Hsp or the placebo) are shown in Fig. 3. Differences between the respective basal values before administering G-Hsp or the placebo were not statistically significant. For example, the finger temperatures before administering G-Hsp or the placebo were 33.3 ± 0.3 °C and 31.2 ± 0.5 °C, respectively (p = 0.29 by the paired t-test), and the toe temperatures before administering G-Hsp or the placebo were 31.6 ± 0.6 °C and 31.2 ± 0.5 °C, respectively (p = 0.98 by the paired t-test). The surface temperature of the hand and foot tended to fall after taking the test samples, regardless of being G-Hsp or the placebo, in the air-conditioned room set at 24 °C. Compared with the placebo, G-Hsp maintained the peripheral surface temperature of the finger (time × treatment effect, p = 0.011 by repeated measures ANOVA, 45–60 min, p < 0.01–0.05 by the paired t-test), and of the toe statistically higher (time × treatment effect, p = 0.027 by repeated measures ANOVA, 45–60 min, p < 0.05 by the paired t-test).

In addition, G-Hsp maintained the tympanic temperature higher compared with the placebo (9–15, 39–42, and 51–54 min p < 0.01–0.05 by the paired t-test).
Evaluation of the subjects’ perception of the effectiveness of G-Hsp

While 54% of the subjects replied that they felt warmer after the administration of G-Hsp, 27% of the subjects replied that they felt warmer after administering the placebo. Nineteen percent of the subjects replied that they felt the same degree of warmth after administering either G-Hsp or the placebo.

Experiment 2. Effects on the peripheral body temperature of a warm beverage containing G-Hsp

Surface temperature and blood flow

The results from the time-course experiment conducted on the finger after administering G-Hsp or the placebo are shown in Fig. 4A. Differences in temperature between the respective basal values before administering G-Hsp (28.2 ± 1.1 °C) or the placebo (28.1 ± 1.3 °C) were not statistically significant (p = 0.40, paired t-test). The surface temperature of the finger rose by 3–5 °C and that of the wrist rose by about 1 °C immediately after administering the warm beverage, and then gradually decreased. The surface temperature rise after administering the beverage containing G-Hsp was greater and lasted longer than that after administering the placebo. The G-Hsp beverage tended to keep the temperature of the finger higher for 18–33 and 42–45 min (p < 0.05 by the paired t-test). Changes in temperature at the other surface parts like the

Fig. 3. Time-Course Changes in Temperature.

The temperatures of the forehead (A), neck (B), wrist (C), finger (D), left ankle (E), toe (F), and tympanic membrane (G) after administering 500 mg of G-Hsp (●) or a placebo (○) with water (37 °C, 50 ml) are shown. Each value is expressed as the difference from the mean temperature before administration, mean ± SEM, n = 46 (A, B, C, D, E, and G), and 26 (F). *p < 0.05 (ANOVA), †p < 0.01 (paired t-test), ‡p < 0.05 (paired t-test).
are shown. Each value is expressed as the latter half of the experiment (HF, which only reflects the vagus nerve activity, was statistically higher in the latter half of the experiment (time \times treatment effect, p = 0.0014 by ANOVA) and 32, 37, and 38 min (p < 0.05 by the paired t-test) after administering the G-Hsp beverage.

**Discussion**

It has so far been reported that hesperidin had a direct vasodilating effect by enhancing the NO production in endothelial cells.\textsuperscript{15,16} The present study is the first to demonstrate an indirect vasodilating effect of G-Hsp by influencing the autonomic nervous system. G-Hsp increased the vagal activity and decreased the cardiac sympathetic activity. In addition, the results of this study clearly show that G-Hsp could prevent a decrease in the peripheral surface temperature during exposure to cold.

We conducted a survey of 97 female university students to collect basic data regarding excessive sensitivity to cold during the summer and winter. Like the results of the previous study,\textsuperscript{8} more than half of the subjects replied they were susceptible or somewhat susceptible to the cold and that they felt cold both in the summer and winter (Table 3). About 70% of subjects were diagnosed as having *hie-sho* (cold sensitivity) based on Terasawa’s diagnostic criteria test.\textsuperscript{20} More subjects replied that they felt cold in specific parts of their body rather than over their entire body, and 70% of the subjects felt cold in the tips of their fingers and toes as reported in a previous study.\textsuperscript{9} Although a feeling of coldness is rarely addressed in medical treatment because it is not a life-threatening problem, it is nevertheless a serious problem for those who suffer from it, as the quality of life would be likely to be improved if this problem were relieved.

Traditional Chinese medicine considers some foods to have a physiological effect of warming or cooling the human body,\textsuperscript{22} although this classification is based on the perception and experience of ancestors and lacks scientific justification. We have already shown by measuring body surface temperature that Japanese persimmon (kaki, *Diospyros kaki*) had the effect of lowering the peripheral human body temperature and that ginger had the effect of raising this temperature by increasing the blood flow.\textsuperscript{24}

We studied the effect of G-Hsp on the body temperature. The results of a previous experiment show that G-Hsp promoted recovery of the peripheral body temperature after cooling stress by dilating the blood vessels and increasing the blood circulation.\textsuperscript{14} In the first experiment of the present study, G-Hsp was proven to prevent a decrease in the peripheral body temperature compared with a placebo under an ambient temperature of 24°C (an air-conditioned room) (Fig. 3). Although we did not measure the temperature at the tip of the toe in 2005, we later recognized that G-Hsp was effective in the peripheral body parts and added it in 2006 as a measurement to elucidate the effectiveness of G-Hsp. In addition, G-Hsp kept the tympanic temperature statistically higher than the placebo could. It is possible that the oral administration of G-Hsp increased heat production.
In the second experiment of the present study, the administration of a warm beverage containing G-Hsp was proven to promote blood circulation and keep the body surface temperature at the finger higher for a longer time than the placebo could (Fig. 4A). Experience shows that our body temperature rises when we drink a warm beverage. This experiment revealed that adding G-Hsp to a warm beverage enhanced the effect of increased blood flow for a longer time than the placebo could.

As shown in Table 3, many women in Japan feel intolerable coldness in their fingertips and/or toes in both a warmed room in the winter and in an air-conditioned room in the summer. It is highly likely that ingesting G-Hsp would have the effect of alleviating their complaint of excessive coldness.

Thermogenesis is controlled by parallel networks in the central nervous system that respond to feed-forward afferent signals from cutaneous to core body thermosensitive neurons and to feedback signals from brain thermosensitive neurons that activate the appropriate sympathetic and somatic efferents. 1–3) We measured the HR variability by a wavelet analysis to investigate the influence of G-Hsp on the autonomic nervous system after drinking G-Hsp.

We found in our previous study 17) that an oral administration of G-Hsp increased the interscapular brown adipose tissue-sympathetic nerve activity (BAT-SNA), but decreased the cutaneous sympathetic nerve activity (CASNA) in urethane-anesthetized rats. We assessed in the present study the effects of an oral administration of G-Hsp on the autonomic nervous system in humans. A power spectrum analysis of HR variability is a useful non-invasive technique for investigating neural mechanisms. 18,19) The power spectrum analysis is based on the fact that data are present with a stationary organization and a combination of sinusoidal functions. Unlike the power spectrum analysis, the wavelet is usually used for analyzing non-stationary signals and provides a reliable assessment of the dynamics of the analyzed signal. As a recently developed method to analyze HR viability, it offers two interesting features that complement a power spectrum analysis: first, it allows a temporarily localized sliding analysis of the signal, thus giving access at any time to the status of HR variability; second, the shape of the wavelet analyzing equation differs from the fixed sinusoidal shape of the power spectrum analysis and can be designed to better fit the shape of the mother spectrum signal, thus allowing a better quantitative measurement. 25)

The HR response after consuming the G-Hsp beverage was slightly lower than that after consuming the placebo. HF, which only reflects the vagal activity, was higher after administering G-Hsp than after administering the placebo (Fig. 5A), while the LF/HF ratio, which provides a reasonable estimate of the cardiac sympathetic activity, was lower after administering the G-Hsp beverage than after administering the placebo (Fig. 5B). It has been shown that sympathetic nerves mediate vasoconstriction and depress peripheral temperature. 1,26) This suppressed sympathetic nerve activity is one of the mechanisms that regulates blood flow and prevents a decrease in the peripheral body surface temperature during exposure to cold. The same result has been

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**Fig. 5.** Time-Course Change in HR Variability.

A, HF; B, LF/HF. The HR variability for 20 min before administering the beverage and for 60 min after administering a beverage (37°C, 100 ml) containing 500 mg of G-Hsp (●) or a beverage (37°C, 100 ml) without G-Hsp (○) is shown. Each value is expressed as the difference from that before administration, mean ± SEM, n = 11. *p < 0.05 for the latter half of the experiment (ANOVA). **p < 0.01 for the latter half of the experiment (ANOVA). *p < 0.05 (paired t-test).
shown when the cutaneous blood flow was significantly increased by CO\textsubscript{2}-water immersion in comparison to fresh water: HF was significantly higher but LF/HF was significantly lower.\textsuperscript{27} HR variability can be used not only as an index of cardiac autonomic balance, but also as an index of stress and emotional state.\textsuperscript{28,29} Numerous studies have indicated that a stressor induced a reduction in HF and an augmentation of LF/HF.\textsuperscript{30,31} Hassan \textit{et al.}\textsuperscript{32,33} have shown that mental stress significantly induced peripheral arterial vasoconstriction with subsequent increases in HR and blood pressure. On the other hand, during relaxation by yoga training, sedative music, or lavender fragrance stimulus, HF increased and LF/HF was concomitantly reduced.\textsuperscript{34–36} Yamamoto \textit{et al.}\textsuperscript{37} have reported that relaxation was induced by an increase in peripheral temperature. The present study has demonstrated that G-Hsp promoted the blood circulation and increased the peripheral temperature. Relaxation might be induced after taking G-Hsp as a result.

As shown in Fig. 6, our results provide a purported scenario for the bioactivity of G-Hsp: (i) decreased CASNA, increased peripheral blood flow, and elevated peripheral temperature; (ii) enhanced NO production in endothelial cells, increased peripheral blood flow, and elevated peripheral temperature; (iii) induction of relaxation by the thermal effect (affirmation by the change of HR variability); (iv) vasodilation by reduction of the cardiac sympathetic activity; and (v) enhancement of NO production led by acetylcholine released by parasympathetic neurons. The influence of acetylcholine on vasodilation is very much doubted, because acetylcholine released from parasympathetic cholinergic synapses is hydrolyzed by acetylcholinesterase into choline and acetyl coenzyme A.\textsuperscript{38,39} However, when the acetylcholine concentration is high, acetylcholinesterase is less effective in governing such thermoregulation as the sweat rate.\textsuperscript{41} The indirect vasodilating effect might be related to the direct one, and both effects are considered to be cyclical processes under homeostatic conditions. The possibility exists that other thermogenic mechanisms such as behavioral regulation, shivering thermogenesis, and non-shivering thermogenesis regulate the body surface temperature.

Hesperidin has been recognized as good for human health and is used as a traditional Chinese medicine. Previous studies have demonstrated its biological effectiveness, although only some parts of its mechanism of action have been elucidated. This present study has uncovered a safe and available mechanism by observing the change in HR variability after administering G-Hsp. We therefore expect that the use of G-Hsp would be safe and would help to promote health.

The results of the present study demonstrate that G-Hsp was effective for promoting recovery from cold stress as well as for preventing a decrease in the peripheral surface temperature during exposure to cold. It is further suggested that one mechanism for the temperature control by G-Hsp might be an effect on the autonomic nervous system. On the basis of this result, it would be useful to further investigate whether G-Hsp could reduce the associated mental stress.

\textbf{Acknowledgments}

The authors thank Mr. Shinya Nagata of Dainippon Sumitomo Pharma Co., Ltd. for the wavelet analysis, and all the volunteers who made this study possible.

\textbf{References}

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