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Effects of Glucosyl Hesperidin on Skin Blood Flow and Temperature:

A Randomized, Double-blind, Placebo-controlled, Crossover Study

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● **Summary**

Objective: The objective of this study was to evaluate whether the intake of 100 mg of glucosyl hesperidin (GHES) promotes the recovery of blood flow and the temperature of the skin after cold water loading.
Method: GHES or placebo was administered in a double-blind crossover manner to 24 healthy adult men and women. Cold water (15℃) was then loaded onto the subject's hand for 5 minutes, and the blood flow and temperature of the skin were measured over time to evaluate the recovery status of each parameter.
Results: The intake of GHES significantly promoted the recovery of skin blood flow and temperature that were reduced (temporarily) by cold water loading compared with the placebo. The effects of GHES may be mediated by improving peripheral blood flow, which has been previously reported.
Conclusion: GHES at a dose of 100 mg facilitates the recovery of skin blood flow and temperature following cold stress, such as cold water loading.

Key word: Glucosyl hesperidin, Cold water loading, Blood flow, Skin temperature

INTRODUCTION

The feeling of cold is a consistent complaint of menopausal women, and it is often complicated with stiff shoulders, lower back pain, malaise, and others[1]. It is reported that the feeling of cold is caused by local circulatory insufficiency and decreased autonomic activity[2,3].

Recently, the prevalence of refrigerated air conditioning has increased the occurrence of the feeling of cold, even in the summer[4], and the number of people who complain of subjective symptoms of the feeling of cold is increasing yearly, regardless of gender and age[5]. Therefore, the feeling of cold is considered to be one of the causes contributing to the decreased quality of life in people throughout the year.

Hesperidin is a type of flavonoid that is often found in citrus peels. It has been known to inhibit increased vascular permeability[6] and to have various physiological functions, including blood flow-improving effects[7] and antihyperlipidaemic effects[8]. However, hesperidin cannot be used in beverages or other liquids because of its low water solubility. α-Glucosyl hesperidin (GHES), which is produced by adding glucose to hesperidin, has resolved this problem. It has been shown that hesperidin and GHES have similar pharmacokinetics in the body since GHES is readily hydrolyzed to hesperidin and glucose by α-glucosidase in the small intestine[9]. Also, GHES is highly soluble in water and has a better absorption efficiency[9], and therefore, GHES exerts its physiological actions more effectively than hesperidin.

We have previously demonstrated that GHES has physiological actions similar to hesperidin[10,11,12]. Specifically, we found that the long-term intake of GHES reduces the feeling of cold and improves blood flow[11,12].

In addition, the anti-coldness effect of a single dose of GHES has been reported, demonstrating that the single dose of GHES promotes the recovery of skin blood flow and temperature that were reduced in a cold-water loading test, in which a transient cold stress was given[13,14]. The dose of GHES that was required to be effective in these reports was 250 or 500 mg, but the minimum effective dose has not been investigated.

Therefore, this study investigated the effects of a single 100 mg dose of GHES, which is lower than the previously reported effective GHES doses, on skin blood flow and temperature after cold water loading.

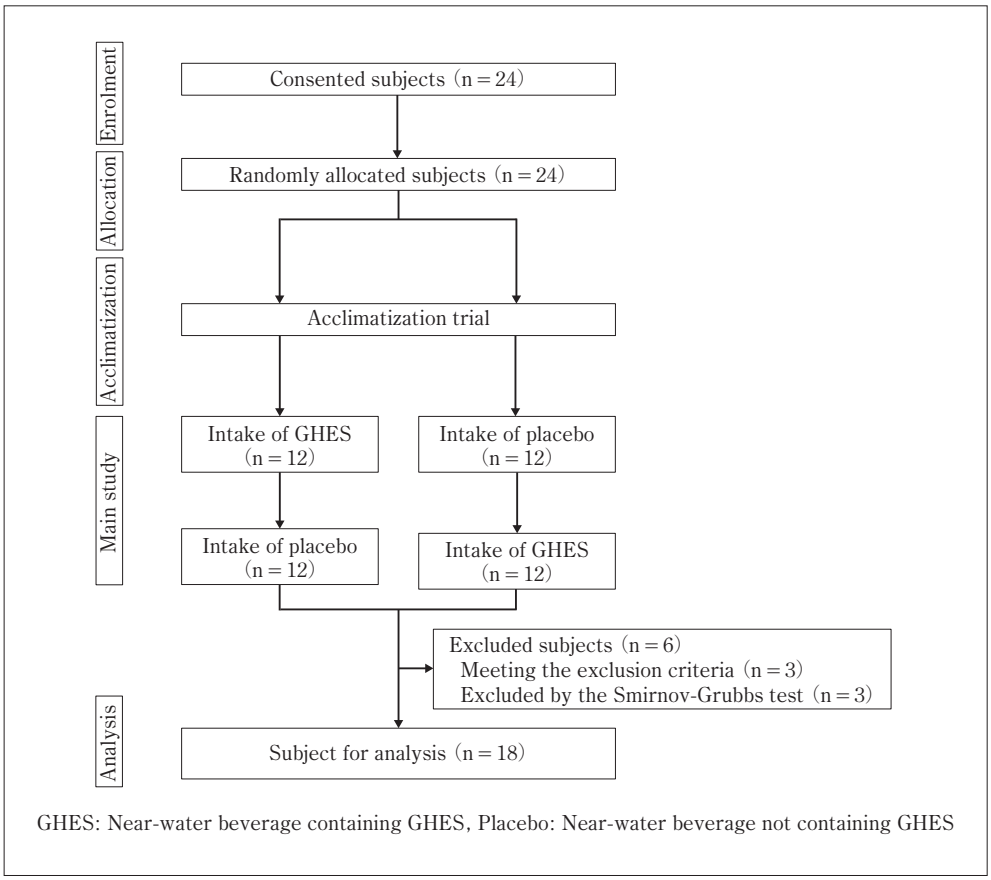

Fig. 1 Subject flow

I SUBJECTS AND METHODS

1. Subjects

Employees of Hayashibara Co., Ltd. who were 20 to less than 60 years old and did not meet the exclusion criteria described below were recruited. Among them, 24 healthy male (10) and female (14) subjects were considered eligible for the study by the investigator and were selected. Prior to the start of the study, the subjects were fully informed of the nature of the study and that participation in the study was on a voluntary basis. Their informed consent was obtained in writing.

Exclusion criteria: (1) Pregnant or possibly pregnant and lactating women, (2) Men/women with a smoking habit, (3) Men/women who have difficulty in following the instructions of the investigator, and (4) Men/women who are judged by the investigator to be inappropriate for the study.

This study was performed in accordance with the ethical principles based on the Declaration of Helsinki and the Ethical Guideline for Clinical Research (Ministry of Health, Labour and Welfare Announcement). This study was conducted after receiving approval by the ethics committee of Hayashibara Co., Ltd. (date of approval: February 9, 2018; approval No. 212) and being pre-registered in the University Hospital Medical Information Network Clinical Trials Registry System (UMIN-CTR).

2. Study method

(1) Test beverage

The test beverage (GHES beverage) was prepared by dissolving 100 mg of GHES (Hayashibara Co., Ltd., Okayama, Japan) in 100 mL of a commercially available orange-flavored near-water beverage (COCA-COLA (JAPAN) COMPANY, LIMITED, Tokyo, Japan). The near-water beverage without GHES was used as the placebo.

Subjects were provided with 2 types of test beverages in a manner that they were not distinguishable by appearance and taste.

(2) Study design

The study was conducted as a randomized, double-blind, placebo-controlled, crossover study from February to September 2018 (**Fig. 1**). Twenty-four subjects were randomized using random numbers and split into 2 groups, each containing 12 subjects with an equal number of males and females. In addition, the order of taking the test beverage was decided for each subject group (GHES beverage → placebo beverage or placebo beverage → GHES beverage). The beverages were allocated by a person responsible for allocation who was not directly

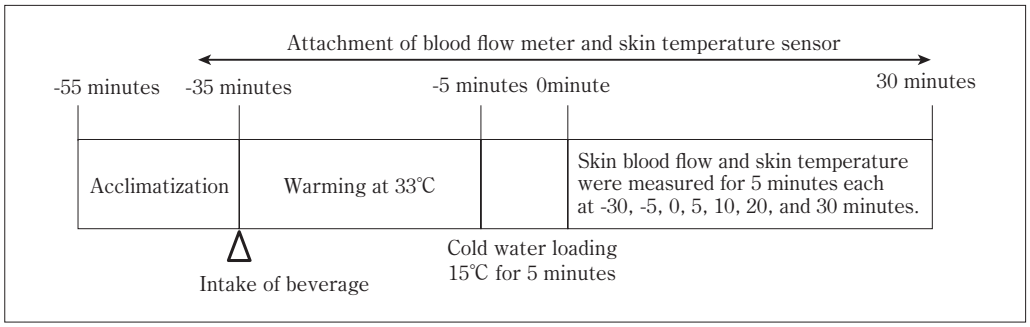

Fig. 2　Schedule of cold-water loading test

Table 1　Subject characteristics

Item	Overall	Men	Women
Number of subjects	18	8	10
Age（years）	41.0±9.7	37.0±10.3	48.4±7.7
Height（cm）	163.1±8.1	170.3±3.9	157.3±5.9
Weight（kg）	53.5±7.2	57.7±5.0	50.16±7.1
BMI（kg/m^2）	20.1±2.1	19.9±1.5	20.9±2.7

（mean ± standard deviation）

involved in the study, and the sealed allocation table was stored in a secure place until the end of the study.

After 2 acclimatization trials, 4 main studies（twice each for GHES and placebo beverages）were conducted. There was a washout period of at least 1 day between each study. On the day of the study, the same breakfast was served to control the condition of the subjects, and their clothing during measurements was also standardized.

（3）Endpoint

In this study, peripheral blood flow and skin temperature were measured as the primary endpoints with reference to the method of Takumi H. et al[15]. As part of the subject background survey, a questionnaire survey on the subjects' awareness of the feeling of cold（Terasawa's diagnostics criteria[16]）was also conducted. The study was performed in a constant temperature and humidity room set at 24 ± 1℃ and 50 ± 5% .

To stabilize the skin blood flow and temperature to be measured, subjects were instructed to comply with the following conditions:（1）no alcohol drinking on the day before the study,（2）no eating and drinking 1 hour before starting the study, and（3）no stand-up work 1 hour before starting the study.

In the study, the subjects were acclimated for 20 minutes after entering the constant temperature and humidity room, and then consumed 100 mL of the test beverage at room temperature. The subject's left hand was then immersed in a warm bath set at 33℃ for 30 minutes to warm their hands. Subsequently, the warmed hand was immersed in a water bath set at 15℃ for 5 minutes to temporarily decrease the peripheral blood flow and temperature（cold water loading）. The subjects were then instructed to gently place the hand that was subjected to cold water loading on a desk and wait for 30 minutes in a sitting posture. Skin blood flow and temperature were measured as described below during the study（**Fig. 2**）.

Skin blood flow; Measurements were performed with a research laser blood flow meter（RBF-101, PIONEER CORPORATION, Tokyo, Japan）. Finger-tip skin blood flow was measured during acclimatization and cold water loading at 0, 5, 10, 20, and 30 minutes, setting the time of withdrawal from cold water as t=0.

Skin temperature; A temperature logger（LT-8A, Gram Corporation, Saitama, Japan）was used to measure temperature. Finger-tip skin temperature was measured during acclimatization and cold water loading at 0, 5, 10, 20, and 30 minutes, setting the time of withdrawal from cold water as t=0.

3. Statistical analysis

Subject characteristics were presented as the mean ± standard deviation, and measured values were presented as the mean ± standard error. The mean values of each of the 2 measurements were used as individual data for analysis. Outliers were tested by the Smirnov-Grubbs test at a level of significance of 0.05, and the data for analysis were extracted. Then, the pattern of change over time in each test beverage group was compared using a two-way ANOVA. A paired t-test was used for the comparison of values at each measurement point. The

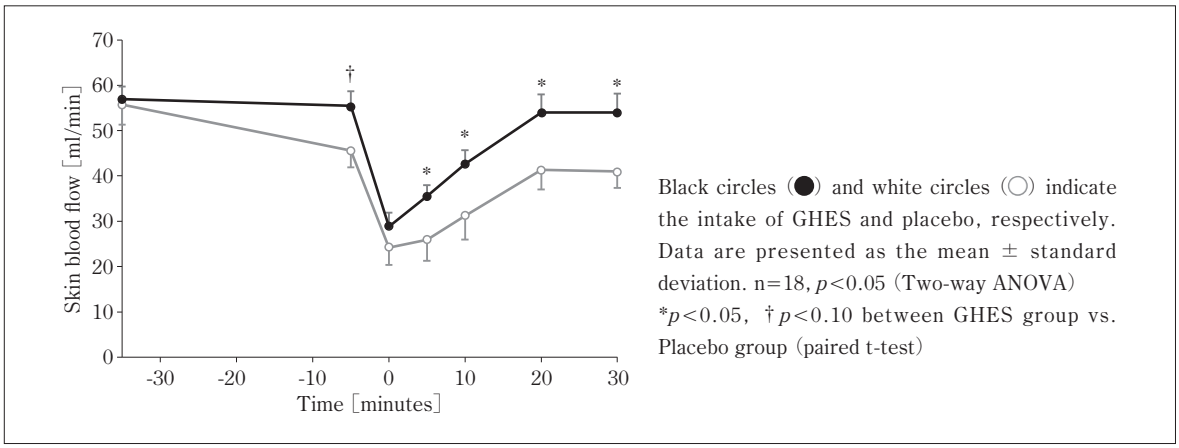

Fig. 3 Time-dependent changes in skin blood flow after cold water loading

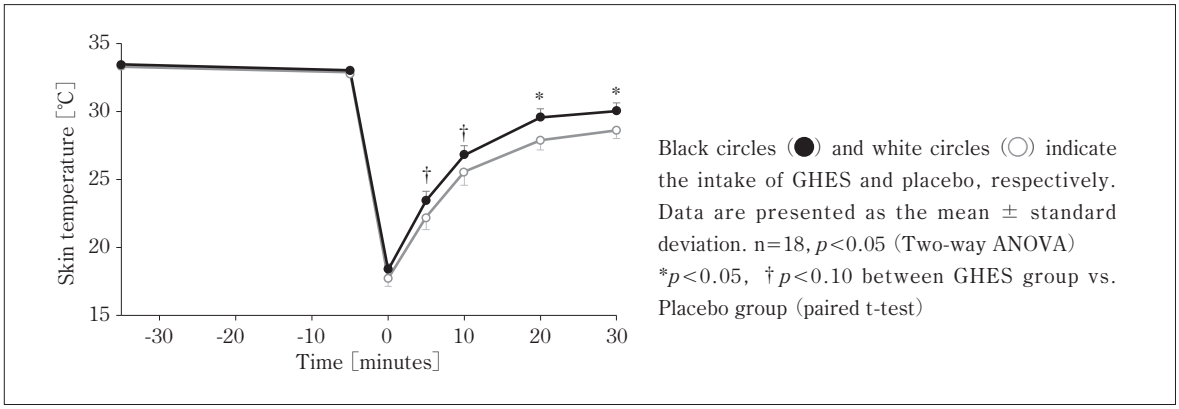

Fig. 4 Time-dependent changes in skin temperature after cold water loading

significance level was set at less than 0.05 in a two-sided test, and p-values less than 0.1 were considered to indicate a trend. SPSS (Ver. 25.0, IBM Japan) was used as the test software.

II RESULTS

1. Subjects

Twenty-four subjects were enrolled at the start of the study, and all of them completed the scheduled measurements. Of these, 3 subjects who met the exclusion criteria were excluded from the analysis. In addition, 3 subjects with low skin blood flow just before the cold-water loading, which were judged to be outliers by the Smirnov-Grubbs test, were excluded from the analysis.

Finally, the analysis was performed with the data obtained from 18 subjects (8 men and 10 women) (**Table 1**). The investigation of the presence or absence of the feeling of cold based on the results of the questionnaire survey administered before the start of the study showed that the number of subjects with and without the feeling of cold was 8 (6 men and 2 women) and 10 (2 men and 8 women), respectively.

No carry-over effect of the test beverages was observed in any of these tests. Therefore, it was judged that the crossover method worked appropriately in this study.

2. Skin blood flow

The measurements of skin blood flow are shown in **Fig. 3**. An interaction was observed between the placebo and GHES groups ($p<0.05$). The significance test at each measurement point showed that the finger-tip skin blood flow at 5, 10, 20, and 30 minutes after cold water loading was significantly higher in the GHES group than in the placebo group ($p<0.05$). Immediately before cold water loading (-5 minutes), the blood flow decreased in the placebo group, while it remained constant in the GHES group ($p<0.10$).

3. Skin temperature

The measurements of skin temperature are shown in **Fig. 4**. An interaction was observed between the placebo and GHES groups ($p<0.05$). The significance test at each measurement point showed that the finger-tip skin temperature was significantly higher ($p<0.05$) at 20 and 30 minutes and tended to be higher ($p<0.10$) at 0 and 5 minutes after cold water loading in the GHES group than in the placebo group.

4. Adverse events

No adverse events were observed throughout the study, including in subjects that were excluded from the analysis.

Ⅲ Discussion

This study examined if the ability of GHES to promote the recovery of skin blood flow and temperature after cold water loading, which has been confirmed at 250 or 500 mg, was observed at a lower dose of 100 mg. The study evaluated the effects of 100 mg of GHES on the recovery of skin blood flow and temperature that were transiently reduced by cold water loading in healthy adult men and women. We found that the reduced skin blood flow and temperature after cold water loading was markedly recovered in the GHES group compared with the placebo group. Previously, we demonstrated that a single dose of 50, 100, and 200 mg of GHES inhibited the decrease of skin blood flow in a dose-dependent manner in a slow-cooling study conducted at 24℃ where blood flow decreased in a resting state[17]. In addition, a dose-dependent effect on the feeling of cold has been reported for hesperetin（from 17 mg）, which is the aglycone of GHES[15]. This study, which was conducted based on these results, showed a similar effect at a dose of 100 mg, which is lower than the previously reported effective GHES doses that promoted the recovery of skin blood flow and temperature after cold water loading. In 3 subjects who were judged as outliers by the Smirnov-Grubbs test, the blood flow in the skin was much lower compared with other subjects before cold water loading and did not decrease further following cold water loading that was performed to temporarily decrease blood flow. For these subjects, the pre-set temperature of the warm bath or duration of bathing（33℃ for 30 minutes）performed before cold water loading to reduce variations in skin blood flow and temperature may have been insufficient.

Two potential mechanisms are proposed that may have contributed to the ability of GHES to improve blood flow in this study. One is through autonomic nerves, and the other is via nitric oxide（NO）production, which have been previously reported. For the former, it is generally known that increased sympathetic activity causes vasoconstriction and decreased blood flow, whereas increased parasympathetic activity causes vasorelaxation and increased blood flow. In the human clinical study conducted by Takumi H et al., the analysis of heart rate variability revealed that the intake of GHES inhibits sympathetic activity and enhances parasympathetic activity compared with the intake of a placebo[14]. On the other hand, Takumi H et al. investigated the effects of hesperetin on the production of NO, a vasodilator, using human umbilical vein endothelial cells and reported that increased NO production was observed following administration of hesperetin. They concluded that GHES promotes NO production in vascular endothelial cells and improves blood flow through capillary dilation[15]. Therefore, in this study, we suggest that changes in skin blood flow affected skin temperature, and it is likely that the intake of GHES promoted the increase in skin blood flow, which resulted in the recovery of skin temperature. To date, the effect of GHES has been reported only in groups of subjects who have the feeling of cold, but this study was conducted in subjects regardless of the presence or absence of the feeling of cold. These results confirm that GHES promotes the recovery of skin blood flow and temperature in all subjects. If the magnitude and characteristics of the effect of GHES depending on differences in gender, age, and the degree of the feeling of cold are clarified in the future, GHES can be used more effectively as an anti-coldness agent. We would like to further investigate these issues.

CONCLUSION

The results of this study showed that the intake of 100 mg of GHES significantly accelerates the recovery of skin blood flow and temperature that was transiently decreased by cold water loading compared with the placebo. Since similar effects were observed at 100 mg, which is lower than the previously reported effective doses of GHES（250 or 500 mg）, a wider range of food applications is expected in the future. Furthermore, since it was shown that GHES exerts its effect in people other than women who have the feeling of cold, it may relieve the discomfort associated with reduced body temperature and blood flow under cooled conditions and help improve the quality of life for more people.

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COI

Hayashibara Co., Ltd. performed all operations related to the conduct of the study.

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