Research Article Open Access

The beneficial effects of monoglucosyl hesperidin and monoglucosyl rutin on vascular flexibility: A randomized, placebo-controlled, double-blind, parallel-group study

Yushi Hashizume, Mahamadou Tandia*

Toyo Sugar Refining Co., Ltd. Yoto Bldg., 18-20, Nihombashi-Koamicho, Chuo-ku, Tokyo 103-0016, Japan.

***Corresponding Author:** Yushi Hashizume, Toyo Sugar Refining Co., Ltd. Yoto Bldg., 18-20, Nihombashi-Koamicho, Chuo-ku, Tokyo 103-0016, Japan.

Submission Date: March 11th, 2024; **Acceptance Date:** May 10th, 2024; **Publication Date:** May 23rd, 2024

Please cite this article as: Hashizume Y., Tandia M. The beneficial effects of monoglucosyl hesperidin and monoglucosyl rutin on vascular flexibility: A randomized, placebo-controlled, double-blind, parallel-group study. *Functional Foods in Health and Disease* 2024; 14(5): 346-365. DOI: https://doi.org/10.31989/ffhd.v14i5.1319

ABSTRACT

Background: There is mounting evidence that the intake of polyphenols can help prevent cardiovascular complications. Furthermore, multiple intakes of different polyphenol compounds are expected to have a synergistic effect. However, few studies have examined the preventive effect of simultaneous polyphenol intake on cardiovascular complications in healthy adults.

Objective: The present study aimed to evaluate the efficacy of daily intake of 70 mg of monoglucosyl hesperidin (MH) alone, or in combination with 140 mg of monoglucosyl rutin (MR), in improving vascular flexibility.

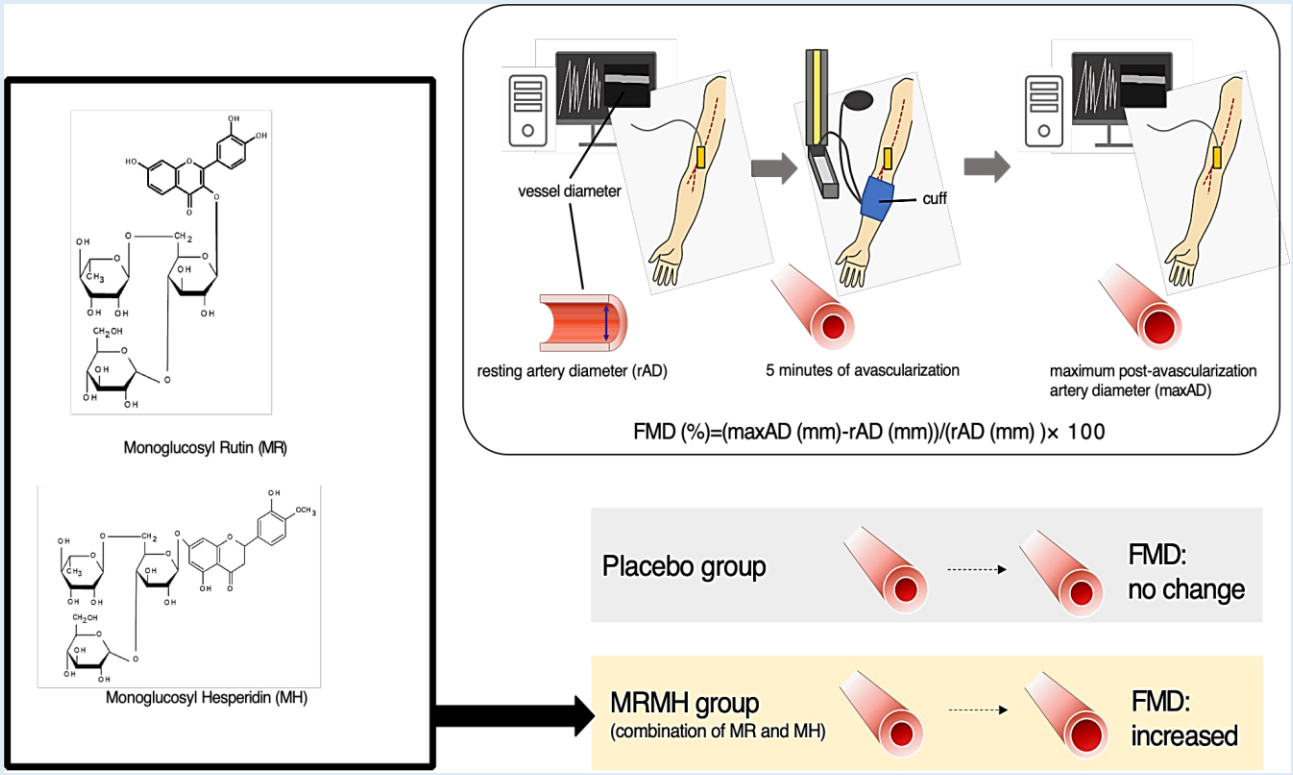
Methods: This 8-week study was conducted on 66 healthy male and female participants with a relatively high body mass index (BMI) and low vascular flexibility. The participants were randomly assigned to an MH group (MH alone), MHMR group (combination of MH and MR), or placebo group by a computer-generated list (each n = 22). Participants took two tablets per day of either MH, MHMR or placebo during the intervention. Outcomes included vascular function indices, capillary flow, and inflammatory markers. Assessment points were at 4 weeks (4w) and 8 weeks (8w) of the intervention.

Results: The primary outcome was flow-mediated dilation. In an analysis including participants whose left brachial-ankle pulse wave velocity (baPWV) was ≥1232.5 cm/s at baseline in the per-protocol set, the MHMR group showed significant improvement compared with the placebo group in flow-mediated dilation, maximum post-avascularization artery diameter, baPWV (each and average of both legs), and E-selectin at 8w.

Conclusion: These results indicated that MH, in combination with MR, may act on vascular endothelial cells to improve

vascular flexibility in healthy adults with a relatively low vascular flexibility. (UMIN000046054).

Keywords: Monoglucosyl hesperidin; Monoglucosyl rutin; flow-mediated dilation; E-selectin; pulse wave velocity, body mass index, vascular flexibility



INTRODUCTION

Rutin is a flavonol glycoside and a polyphenol found in plant-derived products, such as buckwheat [1], tea, apples [2], and asparagus [3]. Another polyphenol, hesperidin, is found primarily in citrus fruits [4]. Despite the antioxidant properties of rutin and hesperidin (sometimes referred collectively as vitamin P) and their potential in preventing lifestyle-related diseases, such as heart disease, arteriosclerosis, and hypertension [5,6], they have a low water solubility and largely limited usage due to their poor bioavailability. Following oral ingestion, a low amount of hesperidin and rutin is absorbed in the small and large intestine after being reduced to their aglycons by β-glucosidase and intestinal bacteria [7].

α-glycosyl-rutin (4G-α-D-glucopyranosyl rutin, monoglucosyl rutin [MR]) and α-glycosyl-hesperidin (4G-α-D-glucopyranosyl hesperidin, monoglucosyl hesperidin [MH]) are manufactured by enzymatic glycosylation of rutin and hesperidin, respectively. This process increased the water solubility of the flavonoids by approximately 30,000-fold for rutin [8] and approximately 10,000-fold for hesperidin [9], translating to improved bioavailability [10,11]. Both MR and MH have been used in food and beverage products for their antioxidative functions and stability. It is suggested that increased water solubility facilitates their decomposition of aglycons by digestive enzymes and intestinal bacteria.

Flow-mediated dilation (FMD) is an indicator of change in artery diameter in response to reactive hyperemia [12] and has been shown to have a borderline range of endothelial dysfunction ranging from 4–7% [13]. It is evaluated by measuring vasodilation associated with relaxation of vascular smooth muscles via endothelium-derived nitric oxide (NO due to the stimulation of shear

stress between the vascular endothelium and blood produced by increased blood flow following released avascularization. There are several human studies on the effects of hesperidin on FMD [14], peripheral blood flow, and blood pressure [15]. In one of those studies, a dose of 159.5 mg of hesperidin and 21.5 mg of narirutin per day was found to improve FMD. Moreover, MH, reported to be three times more bioavailable than hesperidin [9], improved capillary flow in a clinical trial [16]. As for MR, no research was found on its intake's effect on FMD.

FMD has been used to evaluate vascular endothelial function. As for brachial-ankle pulse wave velocity (baPWV), it has been used to quantify vascular arterial stiffness and predict the risk of cardiovascular events. baPWV has been shown to have a borderline value of 14 to 18 m/sec for predicting cardiovascular events [17]. baPWV increases with aging and is higher with risk factors, such as hypertension and diabetes [18]. Notably, a baPWV value of 1220 cm/s or lower indicates the absence of vascular stiffness upon evaluation [22]

Rutin and hesperidin are reported to act on vascular endothelial cells to produce nitric oxide (NO) [15,20]. Rutin is known to have a higher DPPH radical scavenging activity than hesperidin [21]. Therefore, we believe that MH produces NO by improving capillary flow, while MR protects endothelial cells from stress and maintains the function of nitric oxide production through its antioxidant effect.

In this 8-week study, we examined the effects of the intake of MH alone (70 mg per day), or in combination with MR (140 mg per day), on vascular flexibility.

METHODS

Study design: A randomized, placebo-controlled, double-blind, parallel-group study was approved by the independent ethical committee of Takara Clinic, Medical Corporation Seishinkai (Tokyo, Japan) on October 27, 2021 (Approval number: 2110-00334-0033-14-TC). This study was conducted in accordance with the Declaration of Helsinki (2013) and strictly followed the Ethical Guidelines for Medical and Biological Research Involving Human Subjects of the Ministry of Education, Culture, Sports, Science, and Technology; the Ministry of Health, Labor, and Welfare; and the Ministry of Economy, Trade, and Industry of Japan. The protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000046054).

Study participants: The inclusion criteria were as follows: (a) healthy men and women aged ≥20 years; (b) ≥18.5 kg/m^2 body mass index (BMI), (c) low FMD value at screening (before consumption; Scr), and (d) agreement to participate in the study and meeting the eligibility criteria. The exclusion criteria were as follows: (a) any medical history of a malignant tumor, heart failure, or myocardial infarction; (b) current treatment for a chronic disease (cardiac arrhythmia, liver failure, kidney failure, cerebrovascular disorder, rheumatism, diabetes mellitus, dyslipidemia, hypertension, hypotension, hemorrhagic disease, or other chronic disease); (c) daily intake of "Foods for Specified Health Uses," "Foods with Function Claims," or other functional foods/beverages; (d) regular use of medications, including herbal medicines and supplements; (e) allergic reactions to medications and/or products associated with the study substances, particularly citrus fruits; (f) expected/planned surgery; (g) COVID-19 history, pregnancy, lactation, or an expected/planned pregnancy during the study period; (h) participation in another clinical study within the last 28 days prior to signing the study's informed consent document; and (i) identification as ineligible to participate in this study by the principal investigator.

All participants signed an informed consent document at the office of ORTHOMEDICO Inc. (Tokyo, Japan) prior to their participation in the study. The examinations were conducted at Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan). No members of sponsors participated in the study.

No study has yet evaluated the effects of an 8-week intervention using only MH or MHMR on FMD in healthy people. We assumed a significant difference in FMD values between the MH or MHMR group and the placebo

group. The effect size (d) was set as 1.00 [22]. Therefore, the sample size was calculated with an assumed effect size (d) of 1.00, significance level (α) of 0.05, and statistical power (1−β) of 0.80, thus requiring 17 participants per group. To maximize the statistical power (1−β) as much as possible, the sample size was set to 20 participants per group, and statistical power (1−β) was recalculated to be 87.9%. Eventually, considering dropout or deviation from the protocol during this trial, the number of participants was set to 22 per group.

A total of 66 participants signed the informed consent form and participated in the study. The intervention foods were provided by Toyo Sugar Refining Co., Ltd. (Tokyo, Japan) to the contract research organization (CRO), ORTHOMEDICO Inc. Due to indistinguishability between the intervention foods, the person in charge of shipping from the CRO provided the intervention food code to an allocation controller who was not directly involved in the study. Participants were randomly allocated to either the MR group, MHMR group, or placebo group (each group, n = 22; assigned ratio, 1:1:1) based on stratified randomized allocation of FMD, age, and gender. The allocation was performed according to a computer-generated randomization list. The allocation table with the coded intervention foods was only provided to the person in charge of shipping, and this person sent the intervention foods to each of the participants. The allocation controller sealed the allocation table until the key-opening day. No individual related to the study was aware of the group assignments or involved in allocation.

Intervention: αG Rutin PS™ (glycosylated rutin) and αG Hesperidin PA-T™ (glycosylated hesperidin) were manufactured by Toyo Sugar Refining Co., Ltd. (Tokyo, Japan). These materials were used to prepare tablets containing MH and MHMR as test foods. The participants were divided into the following three groups according to the intervention received: MH group (MH alone), MHMR group (combination of MH and MR), and placebo group (placebo). Participants took 2 tablets with water before breakfast or on fasting (2 tablets/day). Based on preclinical date (unpublished), The daily intakes of MH and MR were equivalent to 70 mg and 140 mg, respectively. The intervention period was 8 weeks. MR tablets, MHMR tablets, and placebo tablets could not be distinguished by color, smell, or flavor. αG Rutin PS™ and αG Hesperidin PA-T™ are considered functional foods defined by the Functional Food Center [23,24].

Outcomes: Efficacy and safety assessments were conducted at Scr, at 4 weeks (4w) and 8 weeks (8w) of intervention.

Primary outcome: The measured value of FMD at 8w

FMD was measured in the right upper brachial artery using the ultrasound device UNEXEF18VG (UNEX Co., Aichi, Japan). The procedure for FMD measurement followed the report of Mućka *et al*. [12] and the guidelines by the Japanese Circulation Society [25]. In brief, the ultrasound terminal was applied to the upper right arm region while the participant was in the supine position, and the resting artery diameter (rAD) was first measured. After 5 minutes of avascularization, the change in vessel diameter in the subsequent 2 minutes was observed, and the maximum post-avascularization artery diameter (maxAD) was measured. FMD was calculated using the following formula:

$$FMD(\%) = \frac{maxAD(mm) - rAD(mm)}{rAD(mm)} \times 100 \cdots (1)$$

Secondary outcomes:

(a) Vascular endothelial function: In addition to the primary outcome, we measured FMD values at Scr and 4w, and maxAD and rAD values at Scr, 4w, and 8w.

As with the primary outcome, FMD, rAD, and maxAD were measured using the UNEXEF18VG system (UNEX Co.).

(b) baPWV and blood flow: baPWV was measured with a volume-plethysmographic apparatus using FORM-5 (FUKUDA COLIN Co., Ltd., Tokyo, Japan) at Scr, 4w, and 8w. Pulse wave velocity (PWV) is considered the gold

standard for arterial stiffness assessment, and this parameter is determined from the time elapsed between two pressure waveforms in the supine position from two different anatomic sites [28,29]. In this study, we obtained measurements in the radial and posterior tibial arteries.

Capillary blood flow was measured using the 2D laser blood flow imager OMEGAZONE (Omegawave Inc., Tokyo, Japan) at Scr, 4w, and 8w. The measurement principle is based on the report by Kashima *et al*. [26] . The procedure for measuring blood flow was based on previous studies using the same device [27,28]. The palm of the dominant hand was projected by the camera dedicated to the device, and blood flow was measured according to the instruction manual of the device.

(c) High-sensitivity C-reactive protein (h-CRP), soluble vascular cell adhesion molecule 1 (sVCAM-1), and E-selectin: h-CRP, sVCAM-1, and E-selectin in the blood were measured by LSI Medience Corporation (Tokyo, Japan) at Scr, 4w, and 8w.

To confirm the health status of the participants, interviews and a dietary survey using the Calorie and Nutrition Diary (CAND) [29] were conducted on each examination day. Additionally, all participants were asked to record in a diary their daily living conditions, such as consumption of test foods, changes in physical condition, and use of medications.

Statistical method: All statistical analyses in this study were two-sided, and the significance level was set at 5%, with no adjustment for multiple comparisons. Data analyses were performed using Windows SPSS version 23.0 (IBM Japan, Ltd., Tokyo, Japan).

The participants' characteristics were demographically aggregated according to the analyzed participants. The participants' age, height, body weight, BMI, body fat percentage, blood pressure, and pulse rate in each group were compared using a general linear model with group as a factor. Gender in each group was compared using the chi-square test.

The primary and secondary outcomes have been presented as mean ± standard deviation (SD) at Scr and as estimated marginal mean (EMM) ± standard deviation after the intervention. Group differences have been presented as the difference values of mean or EMM between groups (MH group or MHMR group minus placebo group) and its 95% confidence interval (CI). The baseline values of the primary and secondary outcomes were compared between groups using the general linear model with group as a factor. The measured values and changed values from the baseline of the primary and secondary outcomes at 4w and 8w were analyzed using a linear mixed model, with the baseline values used as covariates and with time, group, group–time interaction, baseline value–time interaction, and participants used as factors.

For the safety endpoints, the occurrence of side effects and adverse events was shown as the number and percentage of affected participants in the group, and between-group comparisons were performed using the chi-square test. Intergroup comparisons were indicated by percentage difference (MH group or MHMR group minus placebo group) and the 95% CI of the difference. The safety assessment items, and blood and urine test results were coded "1" for within reference values and "0" for outside reference values, and the numbers for each group were tabulated. These items were compared using the chi-square test. Furthermore, the principal investigator evaluated and checked the data case-by-case to confirm that there were no medical problems associated with the consumption of the test foods.

A subgroup analysis was also conducted in this study. The analysis methods were the same as for the per-protocol set (PPS).

The analysis dataset was constructed based on the following definitions. The intention-to-treat set involved all participants enrolled in the present study. Next, a full analysis set (FAS) defined when cases were excluded, that is they did not receive the allocated intervention, did not meet eligibility criteria, never received an intervention after allocation, and had no post-allocation data. In addition, among the FAS, the data set was the PPS when individuals meeting the following criteria were excluded: (1) cases whose intake rate of the test food was less than 80%; (2) cases with behavior that affected the reliability of the test results, such as missing diary records; (3) cases

found to meet the exclusion criteria after enrollment; (4) cases found to have violated compliance rules during the study period; (5) cases who took foods or medications that could be expected to significantly affect the test results; (6) cases engaged in activities that were significantly different from their lifestyle at the time of enrollment; and (7) other cases in which there were clear reasons why exclusion was deemed appropriate.

Table 1-1. Demographic characteristics (Intention to treat; ITT).

		Placebo group (n = 22)	MH group (n = 22)	MHMR group (n = 22)
Gender	Male	7 (31.8%)	7 (31.8%)	7 (31.8%)
	Female	15 (68.2%)	15 (68.2%)	15 (68.2%)
Age (year)	Mean (SD)	45.7 (13.4)	45.4 (13.0)	45.5 (11.6)
	Medium	50.0	43.0	45.0
	Minimum–Maximum	24–66	28–78	26–63
	P value	-	0.924	0.943
Height (cm)	Mean (SD)	163.5 (10.2)	162.7 (10.7)	164.0 (9.8)
	Medium	160.50	160.00	163.00
	Minimum–Maximum	147.0–183.0	145.0–186.1	147.0–188.8
	P value	-	0.791	0.871
Body weight (kg)	Mean (SD)	69.9 (14.3)	68.7 (11.4)	65.5 (14.1)
	Medium	69.80	67.40	66.35
	Minimum–Maximum	43.9–102.8	51.9–94.8	48.5–106.8
	P value	-	0.758	0.275
BMI (kg/m^2)	Mean (SD)	25.9 (3.0)	25.8 (2.0)	24.1 (3.1)
	Medium	26.9	25.7	23.6
	Minimum–Maximum	20.3–30.7	21.9–29.6	18.9–30.0
	P value	-	0.908	0.036*
Body fat percentage (%)	Mean (SD)	29.3 (4.1)	28.7 (6.1)	26.2 (5.0)
	Medium	29.6	29.8	25.7
	Minimum–Maximum	22.9–35.9	12.9–38.0	18.8–36.7
	P value	-	0.694	0.050*
Systolic blood pressure (mmHg)	Mean (SD)	119.3 (12.5)	119.4 (10.4)	125.4 (17.1)
	Medium	122.5	118.5	123.0
	Minimum–Maximum	94–142	101–139	101–163
	P value	-	0.991	0.146
Diastolic blood pressure (mmHg)	Mean (SD)	75.9 (8.4)	77.9 (7.9)	81.5 (11.5)
	Medium	76.5	78.0	80.5
	Minimum–Maximum	58–91	63–93	62–103
	P value	-	0.492	0.051
Pulse rate (bpm)	Mean (SD)	71.4 (9.4)	71.4 (8.0)	66.5 (5.7)
	Medium	70.0	73.0	67.0
	Minimum–Maximum	55–91	54–83	54–75
	P value	-	0.985	0.045*

Data other than gender are presented as mean, standard deviation (SD), median, minimum, and maximum. The data in the gender row indicate the corresponding number of relevant subjects. *$P < 0.05$

Table 1-2. Demographic characteristics (Per-protocol set; PPS).

		Placebo group (n = 20)	MH group (n = 22)	MHMR group (n = 22)
Gender	Male	7 (35.0%)	7 (31.8%)	7 (31.8%)
	Female	13 (65.0%)	15 (68.2%)	15 (68.2%)
Age (year)	Mean (SD)	44.7 (13.6)	45.4 (13.0)	45.5 (11.6)
	Medium	48.0	43.0	45.0
	Minimum–Maximum	24–66	28–78	26–63
	P value	-	0.856	0.838
Height (cm)	Mean (SD)	164.4 (10.3)	162.7 (10.7)	164.0 (9.8)
	Medium	161.00	160.00	163.00
	Minimum–Maximum	147.0–183.0	145.0–186.1	147.0–188.8
	P value	-	0.588	0.901
Body weight (kg)	Mean (SD)	70.9 (14.6)	68.7 (11.4)	65.5 (14.1)
	Medium	71.50	67.40	66.35
	Minimum–Maximum	43.9–102.8	51.9–94.8	48.5–106.8
	P value	-	0.590	0.195
BMI (kg/m²)	Mean (SD)	26.0 (3.0)	25.8 (2.0)	24.1 (3.1)
	Medium	26.90	25.65	23.55
	Minimum–Maximum	20.3–30.7	21.9–29.6	18.9–30.0
	P value	-	0.841	0.034*
Body fat percentage (%)	Mean (SD)	29.0 (4.1)	28.7 (6.1)	26.2 (5.0)
	Medium	28.9	29.8	25.7
	Minimum–Maximum	22.9–35.8	12.9–38.0	18.8–36.7
	P value	-	0.877	0.091
Systolic blood pressure (mmHg)	Mean (SD)	118.5 (12.8)	119.4 (10.4)	125.4 (17.1)
	Medium	121.5	118.5	123.0
	Minimum–Maximum	94–142	101–139	101–163
	P value	-	0.830	0.109
Diastolic blood pressure (mmHg)	Mean (SD)	75.5 (8.6)	77.9 (7.9)	81.5 (11.5)
	Medium	76.0	78.0	80.5
	Minimum–Maximum	58–91	63–93	62–103
	P value	-	0.414	0.042*
Pulse rate (bpm)	Mean (SD)	72.3 (9.4)	71.4 (8.0)	66.5 (5.7)
	Medium	71.5	73.0	67.0
	Minimum–Maximum	55–91	54–83	54–75
	P value	-	0.712	0.019*

Data other than gender are presented as mean, standard deviation (SD), median, minimum, and maximum. The data in the gender row indicate the corresponding number of relevant subjects. *$P < 0.05$

Table 1-3. Demographic characteristics (Sub-group).

		Placebo group (n = 9)	MH group (n = 9)	MHMR group (n = 12)
Gender	Male	2 (22.2%)	3 (33.3%)	3 (25.0%)
	Female	7 (77.8%)	6 (66.7%)	9 (75.0%)
Age (year)	Mean (SD)	53.2 (9.3)	56.2 (11.1)	51.3 (8.8)
	Medium	56.0	57.0	53.5
	Minimum–Maximum	37–66	36–78	33–63
	P value	-	0.516	0.648
Height (cm)	Mean (SD)	162.1 (10.2)	162.6 (8.3)	162.6 (7.3)
	Medium	160.00	160.00	161.00
	Minimum–Maximum	147.0–183.0	148.0–176.0	150.0–174.5
	P value	-	0.917	0.897
Body weight (kg)	Mean (SD)	67.7 (16.1)	68.1 (8.0)	62.9 (11.0)
	Medium	68.20	68.80	66.35
	Minimum–Maximum	43.9–102.8	55.3–78.5	48.5–82.9
	P value	-	0.946	0.371
BMI (kg/m^2)	Mean (SD)	25.4 (3.1)	25.7 (1.6)	23.7 (3.3)
	Medium	24.50	25.30	22.95
	Minimum–Maximum	20.3–30.7	23.3–27.9	18.9–28.9
	P value	-	0.823	0.183
Body fat percentage (%)	Mean (SD)	30.0 (3.8)	30.2 (3.8)	26.6 (6.0)
	Medium	30.4	29.7	26.4
	Minimum–Maximum	23.9–35.8	24.1–35.5	18.8–36.7
	P value	-	0.927	0.126
Systolic blood pressure (mmHg)	Mean (SD)	127.3 (8.3)	125.2 (10.7)	134.3 (16.3)
	Medium	125.0	128.0	138.5
	Minimum–Maximum	117–142	108–139	108–163
	P value	-	0.728	0.229
Diastolic blood pressure (mmHg)	Mean (SD)	79.2 (7.7)	83.4 (7.4)	88.3 (9.8)
	Medium	80.0	85.0	89.0
	Minimum–Maximum	68–91	72–93	72–103
	P value	-	0.305	0.024*
Pulse rate (bpm)	Mean (SD)	72.2 (12.1)	73.2 (6.1)	66.6 (6.0)
	Medium	68.0	74.0	68.0
	Minimum–Maximum	55–91	63–80	54–75
	P value	-	0.800	0.135

Data other than gender are presented as mean, standard deviation (SD), median, minimum, and maximum. The data in the gender row indicate the corresponding number of relevant subjects. *$P < 0.05$

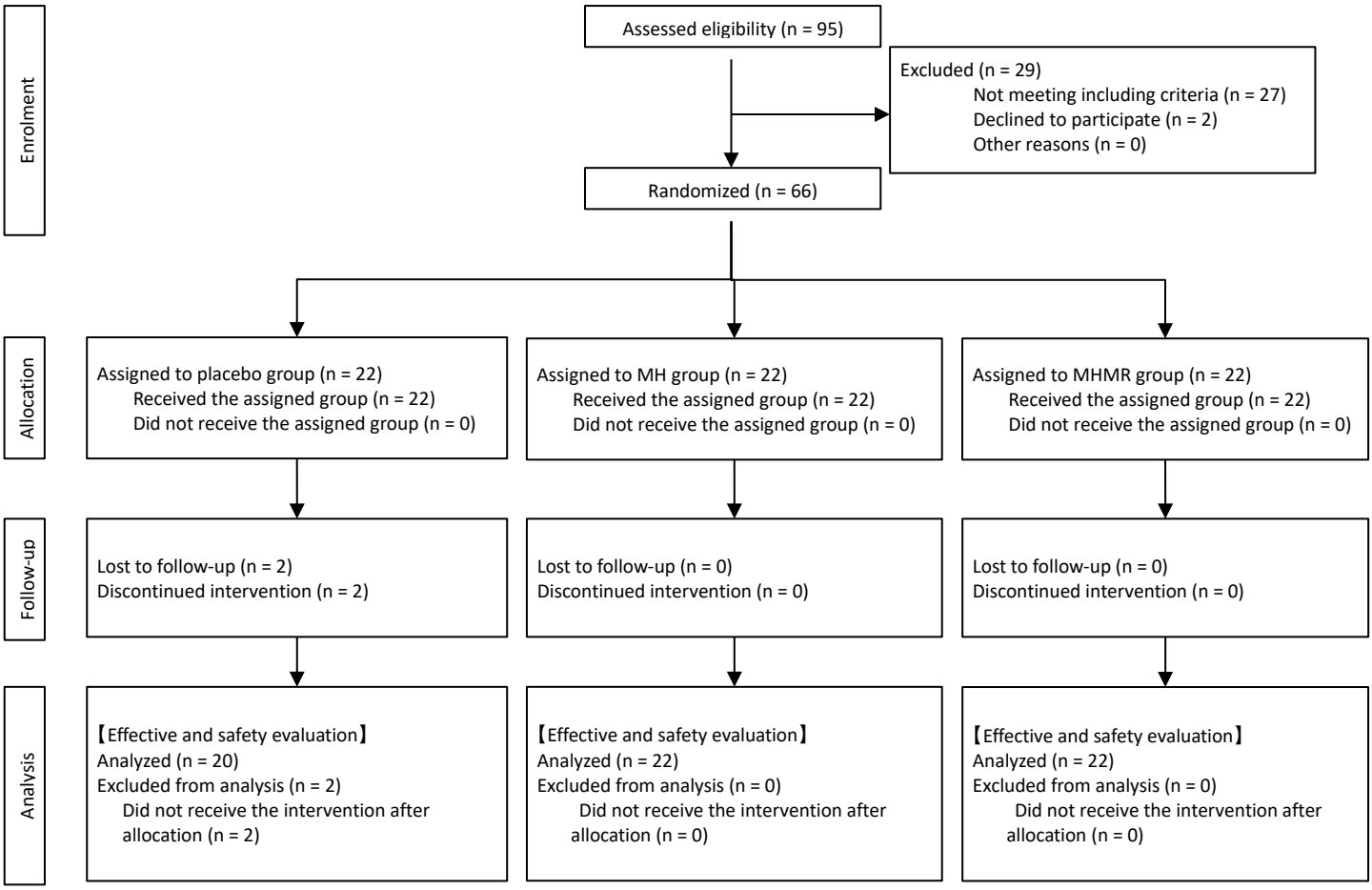


Figure 1. Flowchart of participants in this study

Table 2. Variations in vascular function indicators in PPS.

		Scr			4w			8w		
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
FMD (%)	Placebo group	20	6.0 (1.8)	-	20	5.0 (0.6)	-	20	5.2 (0.6)	-
	MH group	22	6.1 (1.9)	-	22	6.3 (0.6)	-	22	6.0 (0.6)	-
	MHMR group	22	6.1 (1.9)	-	21	5.8 (0.6)	-	22	6.1 (0.6)	-
	MH group vs. Placebo group	-	0.1 (–1.0, 1.3)	0.814	-	1.3 (–0.4, 3.1)	0.141	-	0.8 (–1.0, 2.5)	0.371
	MHMR group vs. Placebo group	-	0.1 (–1.0, 1.2)	0.876	-	0.8 (–1.0, 2.6)	0.375	-	0.9 (–0.9, 2.6)	0.323
Maximum expansion width of the blood vessel (mm)	Placebo group	20	0.23 (0.07)	-	20	0.21 (0.02)	-	20	0.20 (0.02)	-
	MH group	20	0.23 (0.07)	-	22	0.23 (0.02)	-	22	0.23 (0.02)	-
	MHMR group	20	0.22 (0.07)	-	21	0.20 (0.02)	-	22	0.23 (0.02)	-
	MH group vs. Placebo group	-	0.00 (–0.04, 0.05)	0.820	-	0.03 (–0.03, 0.08)	0.370	-	0.02 (–0.03, 0.08)	0.440
	MHMR group vs. Placebo group	-	0.00 (–0.05, 0.04)	0.866	-	–0.01 (–0.06, 0.05)	0.818	-	0.02 (–0.04, 0.08)	0.455
Resting vessel diameter (mm)	Placebo group	20	3.8 (0.6)	-	20	4.1 (0.1)	-	20	4.0 (0.1)	-
	MH group	20	3.9 (0.8)	-	22	3.8 (0.1)	-	22	3.9 (0.1)	-
	MHMR group	20	3.7 (0.6)	-	21	3.7 (0.1)	-	22	3.8 (0.1)	-
	MH group vs. Placebo group	-	0.1 (–0.4, 0.5)	0.798	-	–0.3 (–0.7, 0.0)	0.077	-	–0.1 (–0.4, 0.3)	0.776
	MHMR group vs. Placebo group	-	–0.1 (–0.5, 0.3)	0.629	-	–0.5 (–0.8, –0.1)	0.015*	-	–0.1 (–0.5, 0.2)	0.470
Blood flow volume (mL/min)	Placebo group	20	12.0 (5.6)	-	20	10.8 (1.8)	-	20	8.8 (1.8)	-
	MH group	20	10.1 (3.3)	-	22	14.9 (1.7)	-	22	9.9 (1.7)	-
	MHMR group	20	10.3 (3.0)	-	21	14.4 (1.7)	-	22	8.9 (1.7)	-
	MH group vs. Placebo group	-	–1.9 (–4.4, 0.6)	0.133	-	4.2 (–0.8, 9.1)	0.100	-	1.1 (–3.9, 6.0)	0.671
	MHMR group vs. Placebo group	-	–1.7 (–4.3, 0.8)	0.172	-	3.6 (–1.4, 8.6)	0.155	-	0.1 (–4.9, 5.0)	0.978

Data at time point "Scr" are presented as mean and standard deviation (SD). Data after the intervention (at "4w" and "8w") are presented as estimated marginal mean (EMM) and standard error (SE). *$P < 0.05$

Table 2. Variations in vascular function indicators in PPS (Continued).

		Scr			4w			8w		
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
Average of right and left in baPWV (cm/s)	Placebo group	20	1186.3 (208.0)	-	20	1292.0 (25.0)	-	20	1270.9 (25.0)	-
	MH group	20	1231.0 (299.8)	-	22	1308.1 (23.7)	-	22	1284.2 (23.7)	-
	MHMR group	20	1273.9 (271.5)	-	21	1220.4 (24.4)	-	22	1222.7 (23.8)	-
	MH group vs. Placebo group	-	44.7 (–118.5, 207.9)	0.586	-	16.1 (–52.3, 84.5)	0.641	-	13.3 (–55.1, 81.7)	0.701
	MHMR group vs. Placebo group	-	87.6 (–75.6, 250.8)	0.287	-	–71.6 (–141.3, –1.9)	0.044*	-	–48.3 (–117.1, 20.6)	0.168
Right in baPWV (cm/s)	Placebo group	20	1194.2 (210.0)	-	20	1280.6 (24.2)	-	20	1264.2 (24.2)	-
	MH group	20	1224.7 (297.9)	-	22	1296.4 (23.0)	-	22	1271.0 (23.0)	-
	MHMR group	20	1269.4 (263.5)	-	21	1218.1 (23.6)	-	22	1222.0 (23.0)	-
	MH group vs. Placebo group	-	30.5 (–130.8, 191.9)	0.706	-	15.7 (–50.3, 81.8)	0.638	-	6.8 (–59.3, 72.8)	0.839
	MHMR group vs. Placebo group	-	75.2 (–86.2, 236.5)	0.355	-	–62.5 (–129.8, 4.7)	0.068	-	–42.2 (–108.6, 24.2)	0.211
Left in baPWV (cm/s)	Placebo group	20	1177.7 (213.4)	-	20	1301.6 (28.7)	-	20	1276.0 (28.7)	-
	MH group	20	1236.9 (305.2)	-	22	1319.5 (27.2)	-	22	1297.0 (27.2)	-
	MHMR group	20	1277.9 (281.6)	-	21	1223.4 (27.9)	-	22	1223.7 (27.3)	-
	MH group vs. Placebo group	-	59.2 (–108.3, 226.8)	0.482	-	17.9 (–60.5, 96.3)	0.652	-	21.0 (–57.4, 99.4)	0.596
	MHMR group vs. Placebo group	-	100.2 (–67.3, 267.8)	0.236	-	–78.2 (–158.1, 1.7)	0.055	-	–52.4 (–131.4, 26.7)	0.192
h-CRP (mg/dL)	Placebo group	20	0.09 (0.15)	-	20	0.20 (0.12)	-	20	0.14 (0.12)	-
	MH group	20	0.09 (0.10)	-	22	0.36 (0.12)	-	22	0.09 (0.12)	-
	MHMR group	20	0.06 (0.08)	-	21	0.03 (0.12)	-	22	0.05 (0.12)	-
	MH group vs. Placebo group	-	0.00 (–0.07, 0.07)	0.959	-	0.16 (–0.17, 0.50)	0.334	-	–0.05 (–0.38, 0.29)	0.780
	MHMR group vs. Placebo group	-	–0.03 (–0.10, 0.04)	0.382	-	–0.17 (–0.51, 0.17)	0.336	-	–0.09 (–0.42, 0.25)	0.614

Data at time point "Scr" are presented as mean and standard deviation (SD). Data after the intervention (at "4w" and "8w") are presented as estimated marginal mean (EMM) and standard error (SE). *$P < 0.05$

Table 2. Variations in vascular function indicators in PPS (Continued).

		Scr			4w			8w		
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
sVCAM-1 (ng/mL)	Placebo group	20	525.7 (77.5)	-	20	563.3 (28.1)	-	20	525.7 (28.1)	-
	MH group	20	577.3 (112.2)	-	22	584.2 (26.3)	-	22	548.4 (26.3)	-
	MHMR group	20	579.2 (111.4)	-	21	541.5 (26.7)	-	22	527.7 (26.3)	-
	MH group vs. Placebo group	-	51.6 (–11.6, 114.9)	0.108	-	20.9 (–56.3, 98.2)	0.591	-	22.7 (–54.4, 99.9)	0.559
	MHMR group vs. Placebo group	-	53.6 (–9.7, 116.8)	0.095	-	–21.7 (–99.8, 56.4)	0.581	-	2.0 (–75.3, 79.3)	0.958
E-selectin (ng/mL)	Placebo group	20	25.3 (9.1)	-	20	24.0 (1.1)	-	20	23.5 (1.1)	-
	MH group	20	26.4 (11.1)	-	22	24.9 (1.0)	-	22	24.0 (1.0)	-
	MHMR group	20	27.6 (13.6)	-	21	24.4 (1.0)	-	22	22.8 (1.0)	-
	MH group vs. Placebo group	-	1.1 (–6.0, 8.2)	0.757	-	0.8 (–2.1, 3.7)	0.585	-	0.5 (–2.4, 3.5)	0.710
	MHMR group vs. Placebo group	-	2.3 (–4.8, 9.4)	0.513	-	0.3 (–2.6, 3.3)	0.837	-	–0.7 (–3.6, 2.2)	0.644

Data at time point "Scr" are presented as mean and standard deviation (SD). Data after the intervention (at "4w" and "8w") are presented as estimated marginal mean (EMM) and standard error (SE). *$P < 0.05$

RESULTS

Analysis Set: Figure 1 shows the flowchart of this study. Two participants in the placebo group failed to show up for the examination at 4w and hence were excluded from the analysis dataset. The 64 participants in the final efficacy and safety analysis datasets were included in the PPS (22 participants in the MR group, 22 in the MHMR group, and 20 in the placebo group).

Furthermore, we exploratorily selected and analyzed a subgroup of participants whose left baPWV was ≥1232.5 cm/s at baseline in the PPS. The baPWV of 1232.5 cm/s was the average value for the entire PPS.

Table 1 provides a summary of the participants' background factors. BMI, diastolic blood pressure, and pulse rate were significantly different between groups.

PPS: We did not detect group differences in vascular endothelial function, blood flow, h-CRP, sVCAM-1, and E-selectin. However, the average of the right and left baPWV at 4w was significantly lower in the MHMR group than in the placebo group (Placebo group, 1292.0 ± 25.0 cm/s; MHMR group, 1220.4 ± 24.4 cm/s; Group difference and 95%CI, –71.6 cm/s [–141.3, – 1.9]; P = 0.044; Table 2). This finding suggested that MHMR partially improved vascular function.

Subgroup (baPMW ≥1232.5 cm/s in PPS): Results in the subgroup showed that MHMR group significantly improved FMD (8w: Placebo group, 4.0 ± 0.7%; MHMR group, 5.9 ± 0.6%; Group difference and 95% CI, 1.9% [0.1, 3.6]; P = 0.041), maxAD (8w: Placebo group, 0.15

±0.02 mm; MHMR group, 0.22 ± 0.02 mm; Group difference and 95% CI, 0.07 mm [0.00, 0.13]; *P* = 0.040), baPWV (Average of both legs at 4w: Placebo group, 1529.3 ± 40.2 cm/s; MHMR group, 1405.0 ± 34.2 cm/s; Group difference and 95% CI, –124.2 cm/s [–230.8, –17.7]; *P* = 0.023; Average of both legs at 8w: Placebo group, 1528.7 ± 40.2 cm/s; MHMR group, 1391.1 ± 34.2 cm/s; Group difference and 95% CI, –137.6 cm/s [–244.1, –31.1]; *P* = 0.012; Right leg at 8w: Placebo group, 1525.5 ± 39.9 cm/s; MHMR group, 1399.2 ± 34.0 cm/s; Group difference and 95% CI, –126.3 cm/s [–232.0, –20.7]; *P* = 0.020; Left leg at 4w: Placebo group, 1543.7 ± 45.9 cm/s; MHMR group, 1400.9 ± 39.0 cm/s; Group difference and 95% CI, –142.7 cm/s [–264.5, –21.0]; *P* = 0.023; Left leg at 8w: Placebo group, 1531.6 ± 45.9 cm/s; MHMR group, 1382.4 ± 39.0 cm/s; Group difference and 95% CI, –149.1 cm/s [–270.9, –27.4]; *P* = 0.017) and E-selectin (8w: Placebo group, 27.1 ± 1.4 ng/mL; MHMR group, 23.2 ± 1.2 ng/mL; Group difference and 95% CI, –3.8 ng/mL [–7.5, –0.1]; *P* = 0.043) compared with the placebo group (Table 3).

The subgroup analysis results suggested that MHMR had a more beneficial effect on vascular function than MH alone.

Safety Assessment: The continuous ingestion of the test foods did not result in any side effects or adverse effects during the study.

Table 3. Variations in vascular function indicators in the subgroup (baPMW≥1232.5 cm/s in PPS).

		Scr			4w			8w		
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
FMD (%)	Placebo group	9	6.0 (1.0)	-	9	5.3 (0.7)	-	9	4.0 (0.7)	-
	MH group	9	6.0 (2.5)	-	9	7.1 (0.7)	-	9	4.9 (0.7)	-
	MHMR group	12	6.1 (2.2)	-	12	5.2 (0.6)	-	12	5.9 (0.6)	-
	MH group vs. Placebo group	-	0.0 (–1.9, 2.0)	0.982	-	1.8 (–0.1, 3.7)	0.060	-	0.9 (–1.0, 2.8)	0.369
	MHMR group vs. Placebo group	-	0.2 (–1.7, 2.0)	0.864	-	–0.1 (–1.9, 1.7)	0.928	-	1.9 (0.1, 3.6)	0.041*
Maximum expansion width of the blood vessel (mm)	Placebo group	9	0.22 (0.06)	-	9	0.21 (0.02)	-	9	0.15 (0.02)	-
	MH group	9	0.24 (0.08)	-	9	0.25 (0.02)	-	9	0.19 (0.02)	-
	MHMR group	12	0.22 (0.08)	-	12	0.20 (0.02)	-	12	0.22 (0.02)	-
	MH group vs. Placebo group	-	0.01 (–0.06, 0.09)	0.679	-	0.04 (–0.03, 0.11)	0.276	-	0.04 (–0.03, 0.11)	0.284

		Scr			4w			8w	
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
	MHMR group vs. Placebo group	-	0.00 (–0.07, 0.06)	0.898	-	–0.01 (–0.07, 0.06)	0.764	-	0.07 (0.00, 0.13)	0.040*
Resting vessel diameter (mm)	Placebo group	9	3.7 (0.6)	-	9	4.1 (0.2)	-	9	4.0 (0.2)	-
	MH group	9	4.1 (0.6)	-	9	3.8 (0.2)	-	9	4.1 (0.2)	-
	MHMR group	12	3.6 (0.6)	-	12	3.9 (0.2)	-	12	3.9 (0.2)	-
	MH group vs. Placebo group	-	0.3 (–0.2, 0.9)	0.227	-	–0.3 (–0.9, 0.4)	0.405	-	0.1 (–0.5, 0.8)	0.692
	MHMR group vs. Placebo group	-	–0.1 (–0.7, 0.4)	0.681	-	–0.2 (–0.8, 0.4)	0.459	-	–0.1 (–0.7, 0.5)	0.685
Blood flow volume (mL/min)	Placebo group	9	12.0 (3.0)	-	9	10.3 (3.1)	-	9	9.1 (3.1)	-
	MH group	9	8.8 (2.9)	-	9	16.4 (3.1)	-	9	9.8 (3.1)	-
	MHMR group	12	10.6 (2.9)	-	12	13.9 (2.6)	-	12	8.6 (2.6)	-
	MH group vs. Placebo group	-	–3.2 (–6.1, –0.4)	0.028*	-	6.2 (–3.0, 15.4)	0.184	-	0.7 (–8.5, 9.9)	0.882
	MHMR group vs. Placebo group	-	–1.4 (–4.1, 1.3)	0.290	-	3.7 (–4.4, 11.7)	0.364	-	–0.4 (–8.5, 7.6)	0.918

Data at time point "Scr" are presented as mean and standard deviation (SD). Data after the intervention (at "4w" and "8w") are presented as estimated marginal mean (EMM) and standard error (SE). *$P < 0.05$

Table 3. Variations in vascular function indicators in the subgroup (baPMW≥1232.5 cm/s in PPS (Continued).

		Scr			4w			8w	
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
Average of right and left in	Placebo group	9	1,365.9 (135.0)	-	9	1,529.3 (40.2)	-	9	1,528.7 (40.2)	-
	MH group	9	1,497.1 (292.1)	-	9	1,521.5 (39.9)	-	9	1,496.6 (39.9)	-

		Scr			4w			8w	
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
baPWV (cm/s)	MHMR group	12	1,456.3 (226.9)	-	12	1,405.0 (34.2)	-	12	1,391.1 (34.2)	-
	MH group vs. Placebo group	-	131.2 (–88.6, 351.0)	0.231	-	–7.7 (–123.0, 107.6)	0.893	-	–32.1 (–147.4, 83.2)	0.578
	MHMR group vs. Placebo group	-	90.4 (–115.2, 296.1)	0.375	-	–124.2 (–230.8, –17.7)	0.023*	-	–137.6 (–244.1, –31.1)	0.012*
Right in baPWV (cm/s)	Placebo group	9	1,366.2 (154.8)	-	9	1,512.6 (39.9)	-	9	1,525.5 (39.9)	-
	MH group	9	1,490.9 (289.7)	-	9	1,507.9 (39.7)	-	9	1,477.4 (39.7)	-
	MHMR group	12	1,444.8 (216.3)	-	12	1,409.4 (34.0)	-	12	1,399.2 (34.0)	-
	MH group vs. Placebo group	-	124.7 (–93.8, 343.1)	0.252	-	–4.7 (–119.1, 109.8)	0.935	-	–48.0 (–162.5, 66.4)	0.403
	MHMR group vs. Placebo group	-	78.6 (–125.8, 283.0)	0.437	-	–103.2 (–208.9, 2.4)	0.055	-	–126.3 (–232.0, –20.7)	0.020*
Left in baPWV (cm/s)	Placebo group	9	1364.8 (123.1)	-	9	1543.7 (45.9)	-	9	1531.6 (45.9)	-
	MH group	9	1502.9 (295.6)	-	9	1535.9 (45.4)	-	9	1515.4 (45.4)	-
	MHMR group	12	1467.4 (239.5)	-	12	1400.9 (39.0)	-	12	1382.4 (39.0)	-
	MH group vs. Placebo group	-	138.1 (–86.1, 362.4)	0.217	-	–7.8 (–139.3, 123.7)	0.905	-	–16.2 (–147.7, 115.3)	0.806
	MHMR group vs. Placebo group	-	102.6 (–107.1, 312.4)	0.324	-	–142.7 (–264.5, –21.0)	0.023*	-	–149.1 (–270.9, –27.4)	0.017*
h-CRP (mg/dL)	Placebo group	9	0.07 (0.07)	-	9	0.25 (0.26)	-	9	0.10 (0.26)	-
	MH group	9	0.11 (0.09)	-	9	0.82 (0.26)	-	9	0.11 (0.26)	-
	MHMR group	12	0.07 (0.09)	-	12	0.02 (0.22)	-	12	0.05 (0.22)	-
	MH group vs. Placebo group	-	0.04 (–0.04, 0.12)	0.301	-	0.57 (–0.17, 1.32)	0.127	-	0.00 (–0.74, 0.75)	0.996
	MHMR group vs.	-	0.00 (–0.08, 0.07)	0.987	-	–0.23 (–0.92, 0.45)	0.495	-	–0.06 (–0.74, 0.63)	0.872

		Scr			4w			8w	
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
	Placebo group									

Data at time point "Scr" are presented as mean and standard deviation (SD). Data after the intervention (at "4w" and "8w") are presented as estimated marginal mean (EMM) and standard error (SE). *$P < 0.05$

Table 3. Variations in vascular function indicators in the subgroup (baPMW≥1232.5 cm/s in PPS (Continued).

		Scr			4w			8w	
		n	Mean (SD) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value	n	EMM (SE) Difference (95% CI)	P value
sVCAM-1 (ng/mL)	Placebo group	9	558.2 (74.5)	-	9	575.3 (53.2)	-	9	510.2 (53.2)	-
	MH group	9	535.3 (75.6)	-	9	633.8 (53.9)	-	9	577.9 (53.9)	-
	MHMR group	12	576.7 (86.1)	-	12	433.5 (623.1)	-	12	422.9 (612.5)	-
	MH group vs. Placebo group	-	–22.9 (–100.0, 54.2)	0.548	-	58.5 (–95.6, 212.6)	0.445	-	67.8 (–86.4, 221.9)	0.377
	MHMR group vs. Placebo group	-	18.4 (–53.7, 90.6)	0.604	-	–47.0 (–190.9, 96.9)	0.511	-	7.5 (–136.4, 151.5)	0.916
E-selectin (ng/mL)	Placebo group	9	26.8 (9.1)	-	9	26.7 (1.4)	-	9	27.1 (1.4)	-
	MH group	9	32.6 (10.1)	-	9	25.2 (1.4)	-	9	25.5 (1.4)	-
	MHMR group	12	28.2 (9.7)	-	12	24.3 (1.2)	-	12	23.2 (1.2)	-
	MH group vs. Placebo group	-	5.7 (–3.6, 15.0)	0.218	-	–1.5 (–5.5, 2.6)	0.466	-	–1.6 (–5.6, 2.5)	0.436
	MHMR group vs. Placebo group	-	1.4 (–7.3, 10.1)	0.747	-	–2.3 (–6.0, 1.3)	0.205	-	–3.8 (–7.5, –0.1)	0.043*

Data at time point "Scr" are presented as mean and standard deviation (SD). Data after the intervention (at "4w" and" 8w") are presented as estimated marginal mean (EMM) and standard error (SE). *$P < 0.05$

DISCUSSION

In this study, we examined the effect of MH alone, or in combination with MR, on FMD. The PPS analysis showed that post-intervention FMD was consistently higher in both the MHMR and MH groups than in the placebo group. In addition, the left and right baPWV averages at 4w were significantly lower in the MHMR group than in

the placebo group. The left and right baPWV at 4w also tended to be lower in the MHMR group than in the placebo group.

PWV is a highly reproducible measure for evaluating vascular endothelial dysfunction and arterial stiffness. A pulsatile wave is transmitted to the periphery by the arterial pulsation produced by the ejection of blood from the heart. Arteries with superior extensibility buffer the pulsatile component. As a marker for arterial stiffness, PWV rises with loss of elasticity and intimal thickening of arteries caused by age-related changes such as (1) rupture of elastin in the arterial tunica media, (2) increase in extracellular matrix in the endothelium, and (3) hypertrophy and proliferation of vascular smooth muscles [17]. Although PWV has the disadvantage of including the effects of changes in blood pressure, it is a widely used clinical indicator because of its simplicity [17]. A 100 cm/s increase in baPWV has been reported to increase the risk of developing vascular disease by 12% [30].

Compared with the placebo group, the MHMR group had a lower baPWV (−71.6 cm/s), suggesting an improvement in vascular function. Although some studies suggest a baPWV borderline of 14 to 18 m/s [31,32], others recommend a cut-off value of about 12 m/s [33–36]. Since the mean value of baPWV in this study was 1232.5 cm/s, we performed an additional analysis in participants with baPWV values higher than that mark. FMD values at 8w were significantly higher in the MHMR group than in the placebo group. No difference was observed in the MH group. In this subgroup, placebo increased baPWV; it may be due to aging and cardiovascular risk factors [17]. Conversely, MHMR intake significantly decreased baPWV.

The normal endothelial function cutoff value of FMD for the brachial arteries was considered to be 7.1% by Maruhashi *et al*. [37], and Kajikawa and Higashi [38] assessed vascular dysfunction and the border zone according to FMD as follows: abnormal, <4.0%; borderline, between ≥4.0% and <7.0%; and normal, ≥7.0%. Although our data in the subgroup analysis showed that the mean FMD of both the MHMR and placebo groups was at the borderline level, the value of the placebo group was close to the abnormal level, and the value of the MHMR group was within the borderline range. A meta-analysis by Inada *et al*. [39] reported that a 1% increase in FMD was associated with a 13% reduction in the risk of developing cardiovascular events. The between-group difference (MHMR group minus placebo group) in FMD at 8w was 1.9%; hence, we considered that the significant difference in FMD between the MHMR group and the placebo group was medically meaningful.

Possible mechanisms for the effects on FMD include antioxidant and anti-inflammatory effects, stimulation of NO production in vascular endothelial cells, sympathomimetic effects, and suppression of damage involving vascular endothelial cells by MH and/or MR. Oxidative stress and inflammatory cytokines are known factors that impair vasodilatory function [40]. Hesperidin has been shown to exhibit antioxidant and anti-inflammatory effects in various cell and animal studies [15]. MH, a derivative of hesperidin, has been found to enhance the gene expression of endothelial NO synthase (eNOS), a NOS characteristic of the vascular endothelium, and promote NO production in cellular studies using human umbilical vein endothelial cells (HUVECs) [41]. Furthermore, in animal studies, MH has been reported to inhibit sympathetic nerves that control the perfusion of skin surface capillaries [42]. Another cell study using bovine aortic endothelial cells suggested that hesperidin inhibits vascular cell adhesion of monocytes and damage to the vascular endothelium via suppression of vascular cell adhesion molecule expression induced by inflammatory cytokines [43]. An inhibitory effect on vascular cell adhesion molecules was also confirmed in

this study with E-selectin, a type of vascular cell adhesion molecule, being significantly lower in the MHMR group than in the placebo group at 8w. Rutin and rutin glycosides (MR included) have exhibited radical scavenging activity in *in vitro* studies and been shown to inhibit the production of inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6, in macrophage-like cell lines [44]. In HUVECs, Ugusman *et al*. [20] reported that rutin promoted the expression and activity of eNOS. Moreover, in a model of contraction stimulation of the aortic rings in rats, rutin was found to relax the aortic rings in a concentration-dependent manner [45].

One of the limitations of this study was that the improvement in FMD by MH in combination with MR was observed in the subgroup only. Further studies on vascular function targeting other biomarkers may elucidate the beneficial effects of MH in combination with MR.

CONCLUSION

This study examined the effects of 8 weeks continuous intake of MH alone, or in combination with MR, on vascular function in healthy individuals. Although the results showed that there was no effect on FMD in the PPS, the left-right average of baPWV after the 4-week intervention was significantly lower in the group that consumed MH in combination with MR (MHMR), suggesting an improvement in vascular function. When the effects of MHMR were examined in the PPS with participants having baPWV ≥1232.5 cm/s, FMD was significantly higher, and baPWV and E-selectin, an inflammatory marker, were significantly lower after the 8-week intervention. These results indicate that consumption of MHMR improves vascular flexibility and may help reduce cardiovascular risks.

List of Abbreviations: MR, monoglucosyl rutin; MH, monoglucosyl hesperidin; FMD, flow-mediated dilation; NO, nitric oxide; baPWV, brachial-ankle pulse wave velocity; BMI, body mass index; Scr, screening; CRO, contract research organization; 4w, 4 weeks; 8w, 8 weeks; rAD, resting artery diameter; maxAD, maximum post-avascularization artery diameter, h-CRP, high-sensitivity C-reactive protein; sVCAM-1, soluble vascular cell adhesion molecule 1; SD, standard deviation; EMM, estimated marginal mean; CI, confidence interval; eNOS, endothelial nitric oxide synthase; HUVECs, human umbilical vein endothelial cells

Authors' Contributions: Author1 drafted the manuscript. Author1 and Author2 designed the study and interpreted the results.

Competing Interests: The sponsor of this study, Toyo Sugar Refining Co., Ltd., entrusted ORTHOMEDICO Inc., with conducting this study. Author1 and Author2 are part of Toyo Sugar Refining Co., Ltd.

REFERENCES:

1. Suzuki T, Kurokoh R, Murakami S, Takahashi N, Matsuura A, Katsu K, Murata K: Rutin Concentration and Characterization of Rutinosidase in Perennial Buckwheat (Fagopyrum cymosum) and Its Application in Foods. Foods 2023, 12 (7): 1417. DOI: https://doi.org/10.3390/foods12071417
2. Iriti M, Varoni EM, Vitalini S: Bioactive Compounds in Health and Disease – Focus on Rutin. Bioact Compd Health Dis 2023, 6 (10): 235. DOI: https://doi.org/10.31989/bchd.v6i10.1145
3. Chitrakar B, Hou Y, Devahastin S, Zhang M, Sang Y: Protocols for extraction and purification of rutin from leafy by-products of asparagus (Asparagus officinalis) and characterization of the purified product. Food Chem 2023, 418 136014. DOI: https://doi.org/10.1016/j.foodchem.2023.136014
4. Altunayar-Unsalan C, Unsalan O, Mavromoustakos T: Insights into molecular mechanism of action of citrus flavonoids hesperidin and naringin on lipid bilayers using spectroscopic, calorimetric, microscopic and theoretical studies. J Mol Liq 2022, 347 118411.

DOI: https://doi.org/10.1016/j.molliq.2021.118411

5. Goyal J, Verma PK: An Overview of Biosynthetic Pathway and Therapeutic Potential of Rutin. Mini-Reviews in Medicinal Chemistry 2023, 23 (14): 1451–1460. DOI: https://doi.org/10.2174/1389557523666230125104101
6. Ahmad A, Afzaal M, Saeed F, Ali SW, Imran A, Zaidi SYR, Saleem MA, et al.: A comprehensive review of the therapeutic potential of citrus bioflavonoid hesperidin against lifestyle-related disorders. Cogent Food & Agriculture, 2023, 9(1). DOI: https://doi.org/10.1080/23311932.2023.2226427
7. Visvanathan R, Williamson G: Review of factors affecting citrus polyphenol bioavailability and their importance in designing in vitro, animal, and intervention studies. Compr Rev Food Sci Food Saf 2022, 21 (6): 4509–4545. DOI: https://doi.org/10.1111/1541-4337.13057
8. Suzuki Y, Suzuki K: Enzymatic formation of 4G-.ALPHA.-D-glucopyranosyl-rutin. Agric Biol Chem 1991, 55 (1): 181–187. DOI: https://doi.org/10.1271/bbb1961.55.181
9. Nagayama S, Aoki K, Komine S, Arai N, Endo S, Ohmori H: Improvement of low-intensity long-time running performance in rats by intake of glucosyl hesperidin. Physiol Rep. 2023, 11(2) DOI: https://doi.org/10.14814/phy2.15413
10. Yamada M, Tanabe F, Arai N, Mitsuzumi H, Miwa Y, Kubota M, Chaen H, Kibata M: Bioavailability of glucosyl hesperidin in rats. Biosci Biotechnol Biochem 2006, 70 (6): 1386–1394. DOI: https://doi.org/10.1271/bbb.50657
11. Shimoi K, Yoshizumi K, Kido T, Usui Y, Yumoto T: Absorption and Urinary Excretion of Quercetin, Rutin, and αG-Rutin, a Water-Soluble Flavonoid, in Rats. J Agric Food Chem 2003, 51 (9): 2785–2789. DOI: https://doi.org/10.1021/jf026108a
12. Mućka S, Miodońska M, Jakubiak GK, Starzak M, Cieślar G, Stanek A: Endothelial Function Assessment by Flow-Mediated Dilation Method: A Valuable Tool in the Evaluation of the Cardiovascular System. Int J Environ Res Public Health 2022, 19 (18): 11242. DOI: https://doi.org/10.3390/ijerph191811242
13. Hokimoto S, Kaikita K, Yasuda S, Tsujita K,Ishihara M, Matoba T, Matsuzawa Y, et al.: JCS/CVIT/JCC 2023 guideline focused update on diagnosis and treatment of vasospastic angina (coronary spastic angina) and coronary microvascular dysfunction. Circ J. 2023;87(6):879-936. DOI: https://doi.org/10.1253/circj.CJ-22-0779
14. Jalili F, Moradi S, Talebi S, Mehrabani, Ghoreishy SM, Wong A, Jalalvand AR, et al.: The effects of citrus flavonoids supplementation on endothelial function: A systematic review and dose–response meta-analysis of randomized clinical trials. Phytotherapy Research 2024, DOI: https://doi.org/10.1002/ptr.8190
15. Imperatrice M, Cuijpers I, Troost FJ, Sthijns MMJPE: Hesperidin Functions as an Ergogenic Aid by Increasing Endothelial Function and Decreasing Exercise-Induced Oxidative Stress and Inflammation, Thereby Contributing to Improved Exercise Performance. Nutrients 2022, 14 (14): 2955. DOI: https://doi.org/10.3390/nu14142955
16. Morishita N, Ogihara S, Endo S, Mitsuzumi H, Ushio S: Effects of Glucosyl Hesperidin on Skin Blood Flow and Temperature: A Randomized, Double-blind, Placebo-controlled, Crossover Study. Medical Consultation and New Remedies 2020, 57 (2): 129–134.
17. Stone K, Veerasingam D, Meyer ML, Heffernan KS, Higgins S, Maria Bruno R, Bueno CA, et al.: Reimagining the Value of Brachial-Ankle Pulse Wave Velocity as a Biomarker of Cardiovascular Disease Risk—A Call to Action on Behalf of VascAgeNet. Hypertension 2023, 80 (10): 1980–1992. DOI: https://doi.org/10.1161/HYPERTENSIONAHA.123.21314
18. Lin C-C, Li C-I, Liu C-S, Lin C-H, Yang S-Y, Li T C: Prediction of all-cause and cardiovascular mortality using ankle-brachial index and brachial-ankle pulse wave velocity in patients with type 2 diabetes. Sci Rep 2022, 12 (1): 11053. DOI: https://doi.org/10.1038/s41598-022-15346-9
19. Maeda Y, Oya T: Evaluation of the Standard Value of ABI・baPWVin Subjects Undergoing Health Screening in Our Hospita (in Japanese). Tokushima Red Cross Hosp Med J 2003, 8 160–163.
20. Ugusman A, Zakaria Z, Chua KH, Megat Mohd Nordin NA, Abdullah Mahdy Z: Role of Rutin on Nitric Oxide Synthesis in Human Umbilical Vein Endothelial Cells. Sci World J 2014, 2014 1–9. DOI: https://doi.org/10.1155/2014/169370
21. Wu S, Tachibana S, Tanimoto S, Inoue T, Murai Y, Watanabe Y, Okada Y, Nomura M: Physiological Activities of Several Different Types of Flavonoids. Res Reports Fac Eng Kinki Univ 2012, 46 7–13.
22. Cohen J: A power primer. Psychol Bull 1992,112 (1): 155–159.
23. Martirosyan D, Kanya H, Nadalet C: Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. Functional Foods in Health and Disease 2021, 11 (5): 213. DOI: https://doi.org/10.31989/ffhd.v11i5.788
24. Martirosyan D, Stratton S: Advancing functional food regulation. Bioact Compd Health Dis 2023, 6 (7): 166. DOI: https://doi.org/10.31989/bchd.v6i7.1178
25. Fukazawa R, Kobayashi J, Ayusawa M, Hamada H, Miura M, Mitani Y, Tsuda E, et al.: JCS/JSCS 2020 Guideline on Diagnosis and Management of Cardiovascular Sequelae in Kawasaki Disease. Circulation Journal 2020, 84 (8): 1348–1407. DOI: https://doi.org/10.1253/circj.CJ-19-1094
26. Kashima S, Sohda A, Hiroki Takeuchi HT, Toshihiko Ohsawa TO: Study of Measuring the Velocity of Erythrocytes in Tissue by the

Dynamic Light Scattering Method. Jpn J Appl Phys 1993, 32 (5R): 2177.

27. Ikawa M, Karita K: Relation between blood flow and tissue blood oxygenation in human fingertip skin. Microvasc Res 2015, 101 135–142. DOI: https://doi.org/10.1016/j.mvr.2015.07.006
28. Uchida Y, Atsumi K, Takamata A, Morimoto K: The effect of menstrual cycle phase on foot skin temperature during mild local cooling in young women. J Physiol Sci 2019, 69 (1): 151–157. DOI: https://doi.org/10.1007/s12576-018-0629-1
29. Suzuki N, Baba A, Kakinuma T, Sano Y, Tanaka M, Ouchi S, Watanabe T, Yamamoto K: A novel dietary questionnaire: The Calorie and Nutrition Diary (CAND). New Food Ind 2019, 61 (10): 721–732.
30. Vlachopoulos C, Aznaouridis K, Terentes Printzios D, Ioakeimidis N, Stefanadis C: Prediction of Cardiovascular Events and All-Cause Mortality With Brachial-Ankle Elasticity Index. Hypertension 2012, 60 (2): 556–562. DOI: https://doi.org/10.1161/HYPERTENSIONAHA.112.194779
31. Mori R, Miyata M, Kubozono T, Inadome N, Kawasoe S, Ojima S, Kawabata T, et al.: Cutoff Values of Brachial-Ankle Pulse Wave Velocity for Atherosclerotic Risks by Age and Sex in the Japanese General Population. J Atheroscler Thromb 2023, 30 (5): 63557. DOI: https://doi.org/10.5551/jat.63557
32. Song W, Song Z, Zhang N, Zhang M, Tse G, Chou OHI, Li G, et al.: Association between brachial-ankle pulse wave velocity and the risk of new-onset atrial fibrillation: A report from Kailuan prospective cohort study. npj Cardiovasc Health 2024, 1(1). DOI: https://doi.org/10.1038/s44325-023-00001-7
33. Kinoshita Y, Nakamura T, Ishii K, Iwase H: The relationship between lifestyle habits and brachial-ankle pulse wave velocity (baPWV) in men college students. Japanese J Heal Promot Phys Ther 2022, 12 (1): 7–12. DOI: https://doi.org/10.9759/hppt.12.7
34. Jiang Y, Fan F, Jia J, He D, Sun P, Wu Z, Huo Y, Zhang Y: Brachial–ankle pulse wave velocity is independently associated with urine albumin-to-creatinine ratio in a Chinese community-based cohort. Int Urol Nephrol 2020, 52 (4): 713–720. DOI: https://doi.org/10.1007/s11255-020-02404-2
35. Orlova IA, Kuz'mina AE, Barinova I V, Iarovaia EB, Ageev FT: [Assessment of major artery stiffness: new perspectives of noninvasive diagnosis of coronary atherosclerosis]. Ter Arkh 2009, 81 (4): 8–13.
36. Kim H-L, Im M-S, Seo J-B, Chung W-Y, Kim S H, Kim M-A, Zo J-H: The association between arterial stiffness and left ventricular filling pressure in an apparently healthy Korean population. Cardiovasc Ultrasound 2013, 11 (1): 2. DOI: https://doi.org/10.1186/1476-7120-11-2
37. Maruhashi T, Kajikawa M, Kishimoto S, Hashimoto H, Takaeko Y, Yamaji T, Harada T, et al.: Diagnostic Criteria of Flow-Mediated Vasodilation for Normal Endothelial Function and Nitroglycerin-Induced Vasodilation for Normal Vascular Smooth Muscle Function of the Brachial Artery. J Am Heart Assoc 2020, 9 (2): DOI: https://doi.org/10.1161/JAHA.119.013915
38. Kajikawa M, Higashi Y: Promising Assessment of Vascular Function for Future Cardiovascular Events. J Atheroscler Thromb 2021, 28 (11): ED154. DOI: https://doi.org/10.5551/jat.ED154
39. Inaba Y, Chen JA, Bergmann SR: Prediction of future cardiovascular outcomes by flow-mediated vasodilation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging 2010, 26 (6): 631–640. DOI: https://doi.org/10.1007/s10554-010-9616-1
40. Higashi Y: Roles of Oxidative Stress and Inflammation in Vascular Endothelial Dysfunction-Related Disease. Antioxidants 2022, 11 (10): 1958. DOI: https://doi.org/10.3390/antiox11101958
41. Yoshikawa K, Nakamura Y, Hoshino T, Yamashita S, Nakamura T: Effects of "αG hesperidin PA-LE" on Human Umbilical Vein Endothelial Cells and Proximal Convoluted Tubule Epithelial Cells (in Japanese). Japanese Pharmacology & Therapeutics 2018, 46 (10): 1665–1674.
42. Shen J, Nakamura H, Fujisaki Y, Tanida M, Horii Y, Fuyuki R, Takumi H, et al.: Effect of 4G-α-glucopyranosyl hesperidin on brown fat adipose tissue- and cutaneous-sympathetic nerve activity and peripheral body temperature. Neurosci Lett 2009, 461 (1): 30–35. DOI: https://doi.org/10.1016/j.neulet.2009.05.067
43. Ebrahimi F, Ghazimodadi MM, Fatima G, Bahramsoltani R: Citrus flavonoids and adhesion molecules: Potential role in the management of atherosclerosis. Heliyon. 2023;9(11) DOI: https://doi.org/10.1016/j.heliyon.2023.e21849
44. Choi S-S, Park H-R, Lee K-A: A Comparative Study of Rutin and Rutin Glycoside: Antioxidant Activity, Anti-Inflammatory Effect, Effect on Platelet Aggregation and Blood Coagulation. Antioxidants 2021, 10 (11): 1696. DOI: https://doi.org/10.3390/antiox10111696
45. David SR, Lai PPN, Chellian J, Chakravarthi S, Rajabalaya R: Influence of rutin and its combination with metformin on vascular functions in type 1 diabetes. Scientific reports, 13(1), 12423. DOI: https://doi.org/10.1038/s41598-023-39442-6