

# A Randomized, Double-Blind Comparison Study of Royal Jelly to Augment Vascular Endothelial Function in Healthy Volunteers

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**Aims:** Royal jelly, a creamy substance secreted by honeybees, has been reported to have beneficial effects against dyslipidemia and metabolic syndrome. However, the effects of royal jelly on atherogenesis remain unknown. Hence, we prospectively evaluated whether royal jelly augments vascular endothelial function, which can reflect early atherogenesis, in healthy volunteers.

**Methods:** This was a single-center, double-blind, 1:1 randomized placebo-controlled study conducted from October 2018 to December 2019. A total of 100 healthy volunteers were randomly assigned to receive either royal jelly 690 mg or placebo daily for 4 weeks. The primary endpoint was augmentation in vascular endothelial function as assessed using the change in the reactive hyperemia peripheral arterial tonometry index (RH-PAT) index, and the secondary endpoints were the changes in liver function and lipid profiles between baseline and 4 weeks after enrollment.

**Results:** The mean age of the participants was  $35.0 \pm 9.3$  years in the placebo group and  $36.1 \pm 9.1$  years in the royal jelly groups; 45% and 50% of the placebo and the royal jelly groups, respectively, were male. The percentage relative change in the RH-PAT index was significantly higher in the royal jelly group than in the placebo group ( $21.4\% \pm 53.1\%$  vs.  $0.05\% \pm 40.9\%$ ,  $P=0.037$ ). The percentage relative changes in alanine aminotransferase and  $\gamma$ -glutamyl transpeptidase were significantly lower in the royal jelly group than in the placebo group (alanine aminotransferase:  $-6.06\% \pm 22.2\%$  vs.  $11.6\% \pm 46.5\%$ ,  $P=0.02$ ;  $\gamma$ -glutamyl transpeptidase:  $-3.45\% \pm 17.8\%$  vs.  $4.62\% \pm 19.4\%$ ,  $P=0.045$ ). Lipid profiles were not significantly different between the two groups.

**Conclusions:** Royal jelly might have antiatherogenic property by improving vascular endothelial function. It also augmented liver functions in healthy volunteers.

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**Key words:** Royal jelly, Vascular endothelial function, Atherosclerosis, Healthy volunteer

## Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the main cause of death, hospitalization, and nursing care worldwide<sup>1</sup>. Because atherosclerosis and

its risk factors, such as diabetes mellitus, hypertension, and dyslipidemia, are often already advanced at the onset of ASCVD, primary prevention is key to mitigating the occurrence of atherosclerosis-related cardiovascular events and death. Although many

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researchers have investigated the preventive strategies for atherosclerosis, development of novel methods to prevent the progression of atherosclerosis is of great clinical importance.

Vascular endothelial dysfunction plays a crucial role in the pathogenesis of ASCVD. We reported that peripheral vascular endothelial dysfunction evaluated using reactive hyperemia peripheral arterial tonometry (RH-PAT) is associated with the pathophysiology of atherosclerosis and the prognosis of patients with various cardiovascular diseases<sup>2-6</sup>. Vascular endothelial dysfunction is now considered to be not only an integrative clinical indicator for ASCVD but also a novel therapeutic target in ASCVD. We previously demonstrated that a dipeptidyl peptidase-4 inhibitor improved endothelial dysfunction in ASCVD patients with uncontrolled diabetes<sup>7</sup>. Previous studies have also reported that antihypertensive and lipid-lowering drugs improve vascular endothelial function<sup>8-12</sup>. However, these drugs are used for treating “diseases” and are not approved for prevention of vascular endothelial dysfunction or atherosclerosis in healthy or borderline populations due to the poor evidence of their effects on vascular endothelial function in healthy or borderline populations.

Royal jelly, a creamy substance secreted by the hypopharyngeal gland of honeybees to feed young larvae and the adult queen bee, consists of proteins, sugar, and fatty acids, such as 10-hydroxy-2-decenoic acid<sup>13</sup>. It is widely consumed as a health food and herbal/health supplement. The use of this product as medicine has not yet been approved by the Food and Drug Administration because it is currently uncertain whether it is effective in treating any medical condition. Previous basic studies reported some effects of royal jelly against hypertension, dyslipidemia, and diabetes mellitus<sup>14, 15</sup>. Royal jelly is expected to have potential clinical effects on vascular condition and atherosclerosis. However, there are no studies that investigated the clinical benefit of royal jelly in terms of vascular function. Hence, in this study, we investigated the effects of royal jelly on vascular endothelial function as an indicator of early changes in atherosclerosis in healthy volunteers.

## Methods

### Study Design

This was a single-center, 1:1 randomized, double-blind, placebo-controlled study conducted to evaluate the effect of royal jelly on vascular endothelial function in healthy volunteers. The participants were randomly divided into two groups: one group receiving royal jelly 690 mg (equivalent to 2040 mg of

fresh royal jelly) and the other group receiving placebo daily both for 4 weeks. Comparison of vascular endothelial function assessed on the basis of RH-PAT and blood chemistry between baseline and 4 weeks after enrollment was performed. Although there is no clear evidence of the period to augment the RH-PAT index (RHI) in healthy subjects, a previous study reported that the RHI improved 2 weeks after exercise training in patients with heart failure<sup>16</sup>. Therefore, we estimated the study period to be at least 4 weeks to enable royal jelly administration to augment the RHI. The primary endpoint was augmentation in vascular endothelial function as assessed using the change in the RHI. The secondary endpoints were the change in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and  $\gamma$ -glutamyl transpeptidase ( $\gamma$ GT) as markers of liver function and high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglycerides. Written informed consent was obtained from all participants. The data of all participants were recorded in structured electronic form for this study at the Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University. This study was conducted at the Department of Cardiovascular Medicine, Graduate School of Medical Sciences and Center for Metabolic Regulation of Healthy Aging, Kumamoto University. The study protocol conformed to the principles of the Declaration of Helsinki and was approved by the Human Ethics Review Committee of Kumamoto University. This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000032482). This study follows the Consolidated Standards of Reporting Trials reporting guideline (the checklist and flowchart are presented in the Supplementary Material).

### Study Participants

We recruited 100 healthy volunteers aged 20–60 years from October 2018 to December 2019. The participants who did not meet the following criteria were enrolled on the basis of the results of a medical interview: 1) participants who have been diagnosed with diabetes, hypertension, or dyslipidemia in a previous physical examination or health consultation; 2) patients undergoing treatment for diabetes, hypertension, and/or dyslipidemia; and 3) current smokers or those who have smoked within the past 1 year. The exclusion criteria were 1) patients with renal disease, gastrointestinal disease, heart disease, lung disease, neurological disease, endocrine disease, collagen disease, blood disease under medical

treatment, or receiving any regular medications; 2) patients with serious complications, such as malignancy, or poor life expectancy; 3) pregnant or potentially pregnant women; and 4) patients allergic to honey. The participants were prohibited from taking any other medications or supplements during the study period. Those who newly developed one or more of the aforementioned diseases or were suspected of meeting the above criteria during the study period were also excluded from the analyses in this study.

### Randomization

The minimization method was employed to allocate participants to the placebo group or the royal jelly group to ensure that the factors such as sex, age, and body mass index (BMI) were balanced between the two groups. After confirmation of eligibility of participants, an independent investigator performed randomization using computer-generated random number. Allocation concealment was ensured through sequentially numbered, opaque, sealed envelopes. The study participants, investigators, sponsor, and other staff were blinded to the allocated study group of participants until completion of study analyses.

### Royal Jelly and Placebo

The dosage of royal jelly was determined based on previous clinical trial dosages (1000–3000 mg/day)<sup>17–20</sup>. Royal jelly was orally administered as a tablet. The royal jelly tablet is a tasteless and odorless product made from dried royal jelly and excipient with hard waterproof sugarcoating that cannot be easily crushed. The placebo was made from the same ingredients (reduced maltose syrup, cellulose, and sucrose fatty acid ester), excipients, and coating so it can have the same weight as the royal jelly tablet. The placebo has similar form and color to the royal jelly tablet; it is also tasteless and odorless. Royal jelly has 6.3 kcal per 2040 mg of fresh royal jelly (daily study dose). The compositions of fresh royal jelly were reported as follows: moisture, 66.9%; protein, 11.4%; ether extract, 6.2%; sugar, 9.1%; and ash, 0.94%<sup>21</sup>. The other compositions are presented in [Supplementary Table 1](#).

### RH-PAT

The principle of RH-PAT has been described previously<sup>2, 3, 22</sup>. Briefly, this approach noninvasively measures blood volume changes that accompany pulse waves in the distal finger. A cuff for blood pressure was placed on one upper arm, whereas the contralateral arm acted as a control. PAT probes were placed on one finger of each hand. After a 5-min rest period, the cuff was inflated to 60 mmHg greater than

the systolic pressure or 200 mmHg for 5 min and then deflated to induce reactive hyperemia. The RHI indicates the extent of reactive hyperemia and was automatically calculated using an online computer based on the ratio of the mean amplitude of the PAT signal over 1 min starting 1.5 min after cuff deflation (control arm, A; occluded arm, C) divided by the mean amplitude of the PAT signal during a 2.5-min time period before cuff inflation (baseline) (control arm, B; occluded arm, D). The RHI was calculated as the value based on the ratio of (C/D)/(A/B). Data were automatically analyzed using a computer software in an operator-independent manner (Endo-PAT2000; Itamar Medical, Caesarea, Israel, software versions 3.0.4 and 3.4.4). Because the RHI exhibits a skewed distribution, the values of the natural logarithmically scaled RHI (Ln-RHI) were used for analysis. The variation of RH-PAT has been verified in previous studies<sup>23–26</sup>, and the reproducibility of RH-PAT examination has also been demonstrated in previous studies<sup>2, 22, 27</sup>. Reproducibility in our institutes was confirmed in previous studies<sup>2, 4</sup>.

### Measurements of Biochemistries

After acquisition of written informed consent, fasting blood samples were collected from the antecubital vein using standard phlebotomy techniques in the early morning at baseline and 4 weeks after enrollment. The biomarkers levels were measured by SRL Corp., Ltd. (Tokyo, Japan).

### Statistical Analysis

Power analysis was conducted to estimate the required number of patients based on previous study. No studies have evaluated the effect of royal jelly on endothelial function. Therefore, the minimum sample size was calculated to show a difference in the trend of the effect between two groups in exploratory analysis. For the primary hypothesis, the sample size estimation was based on the detection of a difference of 10% in the levels of Ln-RHI change between the royal jelly and the placebo groups. There are no reports of the standard deviation (SD) of the percentage change in Ln-RHI after the administration of royal jelly. Therefore, if the SD was assumed to be 10%, the required number of patients was calculated to be 44 for each group for a significance level (two-sided) of  $\alpha = 0.05$  and power  $(1 - \beta) = 0.9$ . We estimated that 10% of the participants would drop out of this study. Therefore, we decided that a target sample size of 100 participants (50 participants for each group) was required to satisfy the principal study hypotheses that royal jelly is superior to placebo in terms of the average rate of change in Ln-RHI from baseline. Data

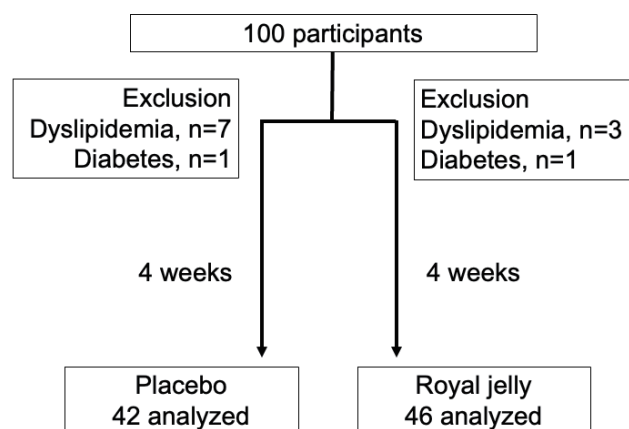


Fig. 1. Study flow chart

are expressed as mean  $\pm$  SD for normally distributed variables according to the Shapiro–Wilk test, and those with nonnormally skewed distributions are expressed as the median value with interquartile range (IQR). Categorical data are expressed as frequencies and percentages. Differences between the groups were determined using Fisher's exact test for the categorical variables and unpaired *t*-test or the Mann–Whitney *U* test for the continuous variables, as appropriate. A multivariate linear regression model was used to test for an independent association between royal jelly use and Ln-RHI by using the forced-inclusion model with significant variables from the univariate linear regression analysis. A value of  $P < 0.05$  was considered statistically significant. Statistical analyses were conducted using SPSS version 25 for Macintosh (SPSS Inc., Tokyo, Japan).

## Results

For this clinical trial, we recruited 100 participants from October 2018 to December 2019. The detailed flow chart is presented in Fig. 1. During the study period, eight participants in the placebo group and four in the royal jelly group had or were suspected of having changes in their diagnosis for the following diseases: in the placebo group, seven participants were suspected of having dyslipidemia, and one was suspected of having diabetes; in the royal jelly group, three participants were suspected of having dyslipidemia, and one participant was suspected of having diabetes (Fig. 1). There were no cases in which the intervention was discontinued. Finally, 42 participants in the placebo groups and 46 in the royal jelly group completed the 4-week intervention and were analyzed in this study. Serious adverse events were not observed in any participants. We conducted

the discrimination test to assess blind allocation in participants. The hit ratio was not significantly different between the two study groups (placebo, 65.2% vs. royal jelly, 48.8%,  $P = 0.12$ ).

The baseline characteristics are presented in Table 1. The mean age of the participants was  $35.0 \pm 9.3$  years in the placebo group and  $36.1 \pm 9.1$  years in the royal jelly group; 45% and 50% in the placebo and the royal jelly groups, respectively, were male. The other clinical characteristics, including Ln-RHI, were comparable between the two study groups. The baseline levels of fasting blood glucose and hemoglobin A1c were not significantly different between the two study groups (placebo vs. royal jelly: fasting blood glucose,  $91.9 \pm 10.0$  mg/dL vs.  $91.2 \pm 6.7$  mg/dL,  $P = 0.70$ ; hemoglobin A1c,  $5.4\% \pm 0.3\%$  vs.  $5.3\% \pm 0.2\%$ ,  $P = 0.09$ ).

Follow-up data are presented in Table 2. The BMI, blood pressure, and laboratory data except for high-sensitivity C-reactive protein (hsCRP), were not significantly different between the two study groups. Moreover, the follow-up levels of fasting blood glucose and hemoglobin A1c were not significantly different between the two study groups (placebo vs. royal jelly: fasting blood glucose,  $91.2 \pm 6.4$  mg/dL vs.  $90.9 \pm 6.3$  mg/dL,  $P = 0.80$ ; hemoglobin A1c,  $5.4\% \pm 0.3\%$  vs.  $5.3\% \pm 0.2\%$ ,  $P = 0.09$ ). hsCRP was significantly lower in the royal jelly group than in the placebo group ( $0.03$  [0.02–0.04] vs.  $0.04$  [0.02–0.10],  $P = 0.03$ ). Ln-RHI was not significantly different between the two study groups. However, the percentage relative change in Ln-RHI was significantly higher in the royal jelly group than in the placebo group ( $21.4\% \pm 53.1\%$  vs.  $0.05\% \pm 40.9\%$ ,  $P = 0.037$ ) (Table 2 and Fig. 2). The percentage relative changes in ALT and  $\gamma$ GT were significantly lower in the royal jelly group than in the placebo group (ALT:  $-6.06\% \pm 22.2\%$  vs.

**Table 1.** Baseline characteristics of enrolled participants

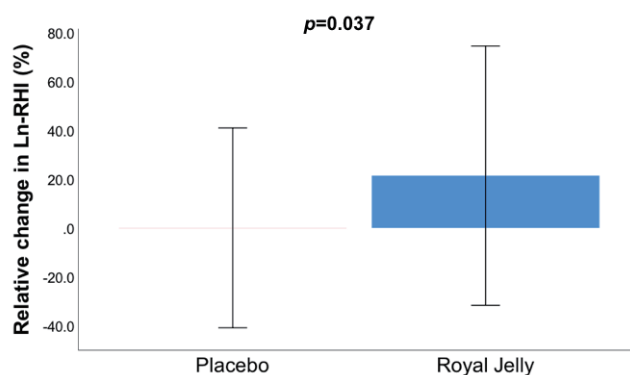
	Placebo <i>n</i> =42	Royal jelly <i>n</i> =46	<i>P</i> value
Age, yr	35.0 ± 9.3	36.1 ± 9.1	0.59
Male, <i>n</i> (%)	19 (45)	23 (50)	0.68
BMI, kg/m <sup>2</sup>	22.4 ± 3.0	22.3 ± 2.4	0.88
Systolic blood pressure, mmHg	107.9 ± 12.9	106.7 ± 7.9	0.61
Diastolic blood pressure, mmHg	64.3 ± 10.0	64.7 ± 7.3	0.83
Pulse rate, per min	61.6 ± 8.8	62.0 ± 8.6	0.87
HDL-cholesterol, mg/dL	59.0 ± 12.4	61.5 ± 13.4	0.37
LDL-cholesterol, mg/dL	97.0 ± 20.4	100.4 ± 19.1	0.41
Triglycerides, mg/dL	60.5 (39.0 to 92.0)	54.5 (38.0 to 80.0)	0.68
Creatinine, mg/dL	0.72 ± 0.14	0.73 ± 0.12	0.83
AST, U/L	18.0 (15.0 to 21.0)	18.0 (15.0 to 23.0)	0.98
ALT, U/L	14.0 (11.0 to 19.0)	15.0 (11.0 to 21.0)	0.59
ALP, U/L	155.0 (131.0 to 192.0)	158.5 (133.0 to 194.0)	0.98
γGT, U/L	15.0 (13.0 to 25.0)	17.0 (12.0 to 22.0)	0.73
Hemoglobin, g/dL	13.4 ± 1.6	13.8 ± 1.2	0.20
Fasting blood glucose, mg/dL	91.9 ± 10.0	91.2 ± 6.7	0.70
HemoglobinA1c, %	5.4 ± 0.3	5.3 ± 0.2	0.09
hsCRP, mg/dL	0.05 (0.02 to 0.10)	0.03 (0.02 to 0.04)	0.11
Ln-RHI	0.72 ± 0.25	0.65 ± 0.22	0.17

Data are shown as *n* (%), mean ± SD, or median (IQR). BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γGT, γ-glutamyl transpeptidase; hsCRP, high-sensitive C-reactive protein; and RHI, reactive hyperemia peripheral arterial tonometry index.

**Table 2.** Follow-up data at 4 weeks after enrollment and percentage relative change from baseline

	Follow-up data			% relative change from baseline		
	Placebo <i>n</i> = 42	Royal jelly <i>n</i> = 46	<i>P</i> value	Placebo <i>n</i> = 42	Royal jelly <i>n</i> = 46	<i>P</i> value
BMI, kg/m <sup>2</sup>	22.5 ± 3.1	22.4 ± 2.4	0.90	0.06 ± 1.85	0.15 ± 1.92	0.83
Systolic blood pressure, mmHg	106.3 ± 10.2	106.4 ± 9.2	0.96	-0.91 ± 8.13	-1.62 ± 7.10	0.65
Diastolic blood pressure, mmHg	63.9 ± 9.8	61.9 ± 6.9	0.27	0.28 ± 13.1	-3.79 ± 10.6	0.11
Pulse rate, per min	61.9 ± 7.0	60.2 ± 8.3	0.30	1.30 ± 10.8	-2.50 ± 8.05	0.06
HDL-cholesterol, mg/dL	57.5 ± 10.4	60.8 ± 12.4	0.18	-1.64 ± 9.45	-0.41 ± 9.97	0.56
LDL-cholesterol, mg/dL	96.6 ± 24.7	98.9 ± 21.1	0.64	-0.66 ± 12.4	-1.10 ± 13.6	0.87
Triglycerides, mg/dL	62.5 (40.0 to 93.0)	57.5 (44.0 to 86.0)	0.61	12.1 ± 50.9	4.99 ± 33.7	0.44
Creatine, mg/dL	0.72 ± 0.15	0.72 ± 0.13	0.92	0.22 ± 6.84	-0.23 ± 7.10	0.76
AST, U/L	17.5 (16.0 to 23.0)	17.0 (15.0 to 21.0)	0.56	7.42 ± 46.0	-6.06 ± 22.2	0.13
ALT, U/L	14.5 (11.0 to 25.0)	14.0 (10.0 to 21.0)	0.66	11.6 ± 46.5	-6.06 ± 22.2	0.02
ALP, U/L	151.5 (126.0 to 190.0)	156.5 (126.0 to 194.0)	0.91	-1.09 ± 14.1	1.38 ± 12.0	0.38
γGT, U/L	15.0 (12.0 to 29.0)	16.0 (13.0 to 21.0)	0.95	4.62 ± 19.4	-3.45 ± 17.8	0.045
Hemoglobin, g/dL	13.3 ± 1.8	13.7 ± 1.2	0.19	-0.85 ± 3.16	-0.31 ± 3.29	0.44
Fasting blood glucose, mg/dL	91.2 ± 6.4	90.9 ± 6.3	0.80	-0.17 ± 7.9	-0.08 ± 7.6	0.95
HemoglobinA1c, %	5.4 ± 0.3	5.3 ± 0.2	0.09	0.15 ± 2.0	0.09 ± 2.3	0.91
hsCRP, mg/dL	0.04 (0.02 to 0.10)	0.03 (0.02 to 0.04)	0.03	0 (-20.0 to 50.0)	0 (-33.3 to 50.0)	0.69
Ln-RHI	0.68 ± 0.27	0.71 ± 0.18	0.50	0.05 ± 40.9	21.4 ± 53.1	0.037

See Table 1 for abbreviations.



**Fig. 2.** Comparison of the relative change in reactive hyperemia peripheral arterial tonometry index between the placebo control and royal jelly groups  
RHI, reactive hyperemia peripheral arterial tonometry index.

**Table 3.** Univariate and multivariable linear regression analysis between percentage relative change in Ln-RHI, clinical parameters, and royal jelly use

Variable	Univariate analysis					Multivariable analysis						
	Unstandardized coefficients		Standardized coefficients	95% CI for B		P value	Unstandardized coefficients		Standardized coefficients	95% CI for B		P value
	B	SEM	Beta	Lower Bound	Upper Bound		B	SEM	Beta	Lower Bound	Upper Bound	
% relative change												
BMI	3.88	2.76	0.15	-1.61	9.37	0.16	–	–	–	–	–	–
Systolic blood pressure	-0.30	0.69	-0.046	-1.67	1.08	0.67	–	–	–	–	–	–
Diastolic blood pressure	-0.51	0.43	-0.13	-1.37	0.36	0.25	–	–	–	–	–	–
Pulse rate	-0.29	0.54	-0.058	-1.38	0.79	0.59	–	–	–	–	–	–
HDL-cholesterol	-0.56	0.54	-0.11	-1.63	0.51	0.18	–	–	–	–	–	–
LDL-cholesterol	0.057	0.40	0.015	-0.75	0.86	0.89	–	–	–	–	–	–
Triglycerides	0.081	0.12	0.071	-0.16	0.32	0.51	–	–	–	–	–	–
Creatine	-0.61	0.75	-0.087	-2.11	0.88	0.42	–	–	–	–	–	–
AST	0.057	0.15	0.040	-0.25	0.36	0.71	–	–	–	–	–	–
ALT	0.13	0.14	0.095	-0.16	0.41	0.38	–	–	–	–	–	–
ALP	0.25	0.40	0.066	-0.55	1.05	0.54	–	–	–	–	–	–
$\gamma$ GT	0.21	0.28	0.083	-0.34	0.76	0.44	–	–	–	–	–	–
Hemoglobin	2.30	1.61	0.15	-0.90	5.50	0.16	–	–	–	–	–	–
HemoglobinA1c	0.38	2.44	0.017	-4.47	5.23	0.88	–	–	–	–	–	–
hsCRP	0.089	0.027	0.33	0.034	0.14	0.002	0.088	0.027	0.33	0.034	0.14	0.002
Royal jelly use	21.36	10.18	0.22	1.13	41.59	0.039	20.82	9.65	0.22	1.64	40.00	0.034

See Table 1 for abbreviations.

11.6%  $\pm$  46.5%,  $P=0.02$ ;  $\gamma$ GT: -3.45%  $\pm$  17.8% vs. 4.62%  $\pm$  19.4%,  $P=0.045$ ) (Table 2). The percentage relative changes in fasting blood glucose and hemoglobin A1c were not significantly different between the two study groups (placebo vs. royal jelly: fasting blood glucose, -0.17%  $\pm$  7.9% vs. -0.08%  $\pm$  7.6%,  $P=0.95$ ; hemoglobin A1c, -0.15%  $\pm$  2.0% vs. -0.09%  $\pm$  2.3%,  $P=0.91$ ). The percentage relative changes in LDL-C, HDL-C, and triglycerides were

not significantly different between the two study groups (Table 2).

Table 3 presents the results of univariate and multivariable linear regression analyses between the percentage relative changes in Ln-RHI, clinical parameters, and royal jelly use. The univariate linear regression analysis revealed that the percentage relative changes in hsCRP and royal jelly use were significantly correlated with the percentage relative change in

Ln-RHI (hsCRP,  $\beta=0.33$ ,  $P=0.002$ ; royal jelly use,  $\beta=0.22$ ,  $P=0.039$ ) (Table 3). Multivariable linear regression analysis with the forced-inclusion model using significant factors in the univariate analysis revealed that hsCRP and royal jelly use were identified as independent determinants of Ln-RHI augmentation (hsCRP,  $\beta=0.33$ ,  $P=0.002$ ; royal jelly use,  $\beta=0.22$ ,  $P=0.034$ ) (Table 3). The percentage relative changes in AST, ALT, ALP, and  $\gamma$ GT were not correlated with the percentage relative change in Ln-RHI (Table 3).

## Discussion

This study clearly indicated that royal jelly augmented vascular endothelial function as assessed using RH-PAT in healthy volunteers. The multivariable linear regression analysis revealed that royal jelly use and hsCRP were significantly and independently associated with Ln-RHI. In addition, royal jelly augmented the levels of ALT and  $\gamma$ GT, suggesting its beneficial effects on liver function. Moreover, adverse events were not observed in either study group.

Royal jelly is reported to have a vasorelaxant property and to contain acetylcholine<sup>28, 29</sup>. Liang *et al.* examined the vasorelaxation effects of royal jelly using Wistar rats in basic research. Oral administration of royal jelly induced the elevation of tail blood flow and vasorelaxation in the isolated aorta and superior mesenteric arteries of rats. Furthermore, the vasorelaxation was canceled by N<sup>G</sup>-nitro-L-arginine methyl ester (a nitric oxide synthase inhibitor) and atropine (a muscarinic receptor antagonist), indicating that royal jelly acts *via* muscarinic receptor agonism and nitric oxide (NO) production from the vascular endothelium<sup>30</sup>. Moreover, we speculate that another possible mechanism of the vasorelaxation effects of royal jelly is through insulin signaling. The trans-10-hydroxy-2-decenoic acid in royal jelly has been reported to have a similar effect to insulin<sup>13</sup>. Insulin acts on the vascular endothelium and results in endothelium-dependent vasodilator responses through the activation of endothelial NO synthase and subsequent production of NO.

Furthermore, some peptides in royal jelly were reported to inhibit angiotensin-converting enzyme (ACE) activity<sup>14, 31</sup>. Sultana *et al.* reported that pancreatic peptide tyrosine tyrosine (peptide YY), an isolated peptide from royal jelly, inhibits renin activity *in vitro*<sup>31</sup>. In this study, single oral administration of peptide YY significantly reduced systolic blood pressure in spontaneously hypertensive rats (SHR)<sup>31</sup>. Tokunaga *et al.* also reported that peptides (Ile-Tyr, Val-Tyr, and Ile-Val-Tyr) had an antihypertensive

effect in SHR through the inhibition of ACE activity<sup>14</sup>. However, no clinical studies have clearly shown the blood pressure-lowering effects of royal jelly. In the present study, a change in blood pressure was not observed in the royal jelly group. One possible reason is that this study did not include individuals with hypertension. Further investigation is needed regarding the effect of royal jelly on blood pressure in normotensive individuals. Although the present study did not measure the ACE activity, royal jelly could augment vascular endothelial function within a range not affecting blood pressure through the inhibition of ACE activity. Taken together, we consider that Ln-RHI is augmented by the effects of various components of royal jelly through the aforementioned antiatherosclerotic and vasorelaxant mechanisms.

This study also demonstrated that royal jelly may benefit liver function, as reflected by the ALT and  $\gamma$ GT levels, in addition to vascular endothelial function. Previous experimental studies have reported that royal jelly has a liver protective property<sup>32</sup>. Kanbur *et al.* experimentally demonstrated that royal jelly inhibited liver oxidative stress and serum biochemical markers, such as AST and ALT, in a paracetamol-induced acute hepatotoxicity mouse model<sup>32</sup>. Recently, nonalcoholic fatty liver disease (NAFLD) was highlighted as an aggravating factor for insulin resistance and chronic inflammation<sup>33, 34</sup>, leading to the development of atherosclerosis<sup>35, 36</sup>. Therefore, royal jelly might also be useful in preventing atherosclerosis in healthy individuals by preventing NAFLD.

There were several limitations in this study. First, this was a single-center study with a relatively small sample size. Further multicenter clinical studies with larger cohorts are required to validate our results. Second, this study targeted healthy volunteers. Hence, the results of this study may not apply to patients with advanced atherosclerosis. Third, a recent systematic review reported dose-dependent effects of royal jelly on the improvement of glycemic control and insulin resistance in patients with DM<sup>37</sup>. This study could not assess the dose-dependent effects of royal jelly on RHI. However, royal jelly may be beneficial to healthy individuals in preventing the development of atherosclerosis.

## Conclusions

Royal jelly might have antiatherogenic property by improving vascular endothelial function. It may also augment liver functions in healthy volunteers.

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## Clinical Trial Registration

The University Hospital Medical Information Network Clinical Trials Registry ([https://upload.umin.ac.jp/cgi-bin/ctr/ctr\\_view\\_reg.cgi?recptno=R000032482](https://upload.umin.ac.jp/cgi-bin/ctr/ctr_view_reg.cgi?recptno=R000032482)) (UMIN000032482)

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## Conflict of Interest

K.K. has received significant research grant support from Bayer, Yakuhin Ltd., Daiichi Sankyo Co. Ltd., Novartis Pharma AG., and SBI, Pharma Co. Ltd., and has received honoraria from Bayer Yakuhin Ltd. and Daiichi Sankyo Co. Ltd. outside the submitted work. K.T. has received significant research grant support from Astra Zeneca Kabushiki Kaisha, Sugi Bee Garden Company Ltd., and Nihon Iryo Kiki Giken Company Ltd., and has received honoraria from Kowa Company Ltd., Sanofi Kabushiki Kaisha, Daiichi Sankyo Company Ltd., Takeda Pharmaceutical Company Ltd., Bayer Yakuhin Ltd., and MSD Kabushiki Kaisha outside the submitted work. All other authors report that they have no relationships relevant to the contents of this paper to disclose.

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**Supplementary Table 1.** The components of fresh royal jelly

Main components of flesh royal jelly		Amino acid (%)		Whole	Free
Moisture	66.9%	Lysine		8.9	21.3
Protein	11.4%	Histidine		2.8	5.2
Sugar	9.1%	Arginine		5.2	5.4
Ether extract	6.2%	Aspartic acid		17.1	1.0
Ash	0.94%	Threonine		4.8	0.4
		Serine		8.9	1.3
Sugar components (g/flesh royal jelly 100g)		Glutamic acid		8.6	10.0
Glucose	3.1	Proline		4.8	50.6
Fructose	3.6	Glycine		6.0	0.9
Sucrose	1.0	Alanine		5.0	0.4
Maltose	1.0	Valine		6.2	0.4
Trehalose	1.0	Isoleucine		4.0	0.6
		Leucine		7.9	0.6
Vitamin ( $\mu$ g/flesh royal jelly 1g)		Tyrosine		3.0	0.6
Thiamin	1.2-1.8	Phenylalanine		3.5	1.3
Riboflavin	6-28				
Pyridoxine	2.2-5.0	Ether extract (g/acid components 100g)*			
Nicotinic acid	48-125	10-hydroxy 2-decenoic acid			50
Pantothenic acid	110-220	10-hydroxydecanoic acid			30
Biotin	1.6-4.1	10-hydroxy-2-decenoic acid			5
Folic acid	0.16-0.5	Sebacic acid			3
Inositol	78-150	* acid components 80g/ether extract 100g			
Minerals (mg/flesh royal jelly 1g)					
Sodium	529	Copper			2.6
Potassium	107	Ferrum			3.8
Calcium	27	Manganese			0.7
Magnesium	59	Zinc			19.6