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Original article

Effects of hot water extract of mangosteen pericarp on vascular function: Re-analysis focusing on factors affecting vascular function.

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Abstract

***Purpose*:** Advanced glycation endproducts (AGEs) are involved in age-related deterioration of vascular function. In our clinical study, we evaluated the vascular stiffness of mangosteen pericarp (water extract of mangosteen: WEM), which has an inhibitory effect on the formation of AGEs, and found a trend toward improvement in vascular stiffness, however the improvement was not significant. Factors that affect vascular function include age, blood glucose, blood pressure, and low-density-lipoprotein-cholesterol (LDL-C) or triglycerides (TG). In the present study, we examined the effect of WEM on vascular flexibility by performing a stratified analysis focusing on factors that affect vascular function. In the present study, we performed a stratified analysis focusing on factors affecting vascular function and examined the effect of WEM on vascular flexibility.

***Methods*:** In a previous report, 38 women (19 in the WEM group and 19 in the placebo group) aged 25 to 59 years were administered WEM (200 mg) or a placebo for 12 weeks, and blood tests and vascular function tests were performed before, 4, 8, and 12 weeks after intake in a placebo-controlled, double-blind, randomized, parallel-group comparison study. Whereas, in the present study, we reanalyzed the results of the above studies, focusing on factors affecting vascular function (age, blood glucose, blood pressure, and LDL-C or TG). First, we excluded subjects who exceeded the scope indicated in Attachment 2, "Points to Consider in Preparing Application Forms for Foods for Specified Health Use" of "Permission for Labeling of Foods for Specified Health Use." (Notice No. 109 of April 1, 2020, by the Deputy Director-General of the Consumer Affairs Agency). Next, we reanalyzed the effect of WEM on vascular stiffness by selecting subjects who were at least 30 years of age, had borderline blood glucose, normal hypertension and I-degree hypertension, borderline and mild LDL-C, and normal high and slightly high TG, respectively. Vascular stiffness was assessed by PASESA AVE-1500 (Shisei Datum, Japan), API (Arterial Pressure Index) and AVI (Arterial Velocity Pulse Index) in the right upper arm.

***Results*:** Subjects with blood LDL-C levels above (≥ 160 mg/dL) those in the mild disease range were excluded (2 subjects in the WEM group and 4 subjects in the placebo group). Subjects with blood glucose levels of 110～125 mg/dL, systolic blood pressure of 130～159 mmHg or diastolic blood pressure of 85～99 mmHg, and TG of 120-199 mg/dL were not extracted for analysis. There were 7 subjects in the WEM group and 9 subjects in the placebo group with an LDL-C of 120～ 159 mg/dL, but no significant difference in API and AVI. 14 subjects in the WEM intake group and 13 subjects in the placebo group aged 30 years or older could be extracted, and WEM intake significantly reduced API after 8 and 12 weeks of intake. However, there was no difference in AVI.

***Conclusion*:** The results of this reanalysis results showed that WEM intake, which inhibits glycation, reduced API in healthy women aged 30 years or older. This suggests that WEM intake alleviates age-related vascular stiffening and maintains flexibility.

***KEY WORDS*:** mangosteen (*Garcinia mangostana*), glycation, advanced glycation endproducts (AGEs), vascular flexibility

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Introduction

Glycation is a non-enzymatic reaction between proteins and carbohydrates, also known as the Maillard reaction. AGEs are known to accumulate in the human body proceeds in an age-dependent manner [1, 2]. AGEs are believed to cross-link proteins such as collagen in the vascular wall, thereby causing functional impairment of the vascular wall [3], and bind to the receptor for AGEs (RAGE) expressed on vascular endothelial cells, thereby causing atherosclerosis [4].

To prevent the accumulation of AGEs, daily exercise and dietary habits are important, concurrently it is important to consume food components that prevent the production of AGEs. It has been reported that polyphenols, which are antioxidants found in fruits and other foods, inhibit the production of AGEs by inhibiting the Maillard reaction [5-7]. Water-soluble components of mangosteen pericarp have been reported to have strong antioxidant properties [8], and Parengkuan *et al*. reported that mangosteen pericarp extract has a strong Maillard reaction inhibitory effect [9].

We have previously reported that women aged 25 to 59 years who intook a hot water extract of mangosteen pericarp (WEM) for 12 weeks showed significantly lower Arterial Pressure Index (API) measured by the PASESA AVE-1500 (Shisei Datum, Tokyo, Japan) than those in the placebo group after 8 weeks of intake, whereas there was no significant difference between the groups after 12 weeks of intake [10].

Factors that decrease vascular function include aging, blood glucose, blood pressure, and increases in blood LDL-cholesterol (LDL-C) or triglycerides (TG) [11, 12]. The analysis of subjects in the previous clinical trial did not focus on these factors. This resulted in a mixture of subjects who did and did not benefit from WEM intake, and no significant differences were found overall. We thought that analysis by these factors would clarify the effect of WEM intake on vascular stiffness.

In this study, we reanalyzed the subjects in the previous study [10] with a focus on factors affecting vascular function (age, fasting plasma glucose [FPG], blood pressure [BP], and LDL-C or TG) to determine the impact of WEM on vascular flexibility. The above reanalysis was performed after excluding subjects who exceeded the indicated ranges according to the "Permission for Labeling of Foods for Specified Health Use" as shown in Attachment 2 "Points in concern when preparing forms for applications for food for specified health use" (Notice of the Deputy Director-General of the Consumer Affairs Agency No. 109, dated April 1, 2020) [13].

Methods

1) Study methods of prior studies

Study design

The study was a placebo-controlled, double-blind, randomized, parallel-group comparative study. Forty healthy Japanese women in their 20s to 50s were randomly assigned to the WEM or placebo group. One person in each group dropped out during the study period, ultimately there were 19 in the WEM group and 19 in the placebo group. The test food was ingested in 2 capsules (200 mg as WEM containing 0.078% rhodanthenone B) per day, and endpoints were measured before (0 week) and 4, 8, and 12 weeks after intake. This study was registered and conducted in the University Hospital Medical Information Network Clinical Trial System (UMIN-CTR, Registration number UMIN#000032310).

Endpoints: vascular function

PASESA AVE-1500 (Shisei Datum, Machida, Tokyo, Japan) was used as an index of vascular function, and API (Arterial Pressure Index) and AVI (Arterial Velocity Pulse Index) [14] were measured on the right upper arm before and 4, 8 and 12 weeks after intake. The results are shown in ***Table 1***. API is a measure of local vascular flexibility in the upper arm, while AVI is a measure of systemic hemodynamics, including the aorta.

2) Analysis in this study

Exclusion of subjects in the disease range

Subjects were initially excluded from the study if they met the following criteria for factors affecting vascular function (FPG, BP, LDL-C, or TG): FPG ≥ 126 mg/dL, systolic BP (sBP) ≥ 160 mmHg or diastolic BP (dBP) ≥ 100 mmHg, LDL-C ≥ 160 mg/dL, and subjects with TG of 200 mg/dL or higher [13].

	LDL-C (120 ~ 159 mg/dL)		Age (≥ 30 years old)	
	WEM (n = 7)	Placebo (n = 9)	WEM (n = 14)	Placebo (n = 13)
Age	45.4 ± 10.7	48.4 ± 5.5	45.0 ± 7.8	45.2 ± 7.6
Weight (kg)	51.5 ± 8.7	52.3 ± 7.5	52.3 ± 7.4	52.6 ± 6.5
Height (cm)	156.5 ± 3.9	156.0 ± 5.5	157.9 ± 5.0	156.3 ± 6.1

Results are expressed as means ± SD. WEM, water extract of mangosteen; LDL-C, low-density-lipoprotein-cholesterol; SD, standard deviation.

Selection of subjects for analysis

Subjects with elevated factors affecting vascular function (age, FPG, BP, LDL-C or TG) were included in the analysis. Criteria were as follows: age ≥ 30 years, subjects with borderline FPG (110 ~ 125 mg/dL), normotensive (sBP 130-139 mmHg or dBP 85-89 mmHg) or grade I hypertension (sBP 140-159 mmHg or dBP 90 ~ 99 mmHg), LDL-C in the borderline (120 ~ 139 mg/dL) or mild (140 ~ 159 mg/dL) range, or TG in the normal high (120 ~ 149 mg/dL) or slightly elevated (150 ~ 199 mg/dL) range were included in the analysis.

Statistical analysis

Results are presented as mean ± standard deviation. Pre- and post-intake comparisons were performed using the Sidak method, and between-group comparisons were performed with an unpaired t-test. p-values less than 5% were considered statistically significant differences.

Result

Exclusion of subjects in the disease range

There were no subjects with FPG, BP, or TG in the diseased range, LDL-C >160mg/dL in 2 subjects in the WEM group (161 mg/dL, 207 mg/dL) and 4 subjects in the placebo group (192 mg/dL, 167 mg/dL, 214 mg/dL, 174 mg/dL); 17 subjects in the WEM group and 15 patients in the placebo group were included in the sampling.

Extraction of subjects

After excluding the above subjects, when subjects were extracted byFPG (subjects with 110 ~ 125 mg/dL), the number of subjects was 0 in the WEM group and 0 in the placebo group. Then, when subjects were selected by BP (sBP: 130 ~ 159 mmHg or dBP: 85 ~ 99 mmHg), there was one subject in the WEM group and 2 subjects in the placebo group; when subjects were selected by TG (120 ~ 199 mg/dL), there were 3 subjects in the WEM group and 2 subjects in the placebo group; both were not reanalyzed due to small numbers.

Whereas, when subjects were selected for LDL-C (120 ~ 159 mg/dL), there were 7 subjects in the WEM group and 9 subjects in the placebo group; when subjects were over 30 years of age or older, there were 14 subjects in the WEM group and 13 subjects in the placebo group, both of which could be re-analyzed (***Table 1***).

API and AVI

Reanalysis of API and AVI results was limited to subjects with LDL-C in the 120 ~ 159 mg/dL range or subjects over 30 years of age or older; subjects with LDL-C in the borderline and mild range showed no significant differences before and after API and AVI intake or between groups (***Fig. 1-a, b***). Alternatively, for subjects over 30 years of age or older, API was -9.1%, -7.0%, and -13.9% in the WEM group and +8.3%, +11.2%, and +6.1% in the placebo group after 4, 8, and 12 weeks of intake, respectively, compared to before intake and after 8 and 12 weeks of intake compared to the placebo group, a significant decrease was observed in the WEM group ($p < 0.05$, ***Fig. 1-c***). With regard to AVI, there was a significant decrease in the WEM group after 4 and 8 weeks of intake compared to before intake ($p < 0.05$), while no significant difference from the placebo group (***Fig. 1-d***).

Discussion

This study attempted a stratified analysis of previous studies focusing on factors affecting vascular function: age, FPG, BP, LDL-C or TG. The number of cases for blood glucose, blood pressure, and TG was small (0 to 3) and could not be analyzed. The number of subjects with LDL-C in the borderline and mild disease range was considered insufficient to detect differences due to the small number of subjects extracted. It was not possible to verify in this study whether WEM intake improves the decline in vascular function caused by hyperglycemia, hypertension, and increases in LDL-C and TG in the blood, excluding aging, among the factors that reduce vascular function.

The decline of estrogen in the blood begins after age 30, and this decline decreases vascular function[15]. In a previous study[10], WEM intake decreased API and AVI[14], indices of vascular stiffness, however, not significantly. In an analysis limited to healthy subjects aged 30 years or older, a significant decrease in API was observed in the WEM intake group compared to the placebo group after 8 and 12 weeks (***Fig. 1-c***), and vascular flexibility was improved. This suggested that vascular function declines with age, but the effects of WEM intake on vascular function are likely to become apparent. Regarding the fact that no apparent effect was observed for AVI in this study, we speculate that WEM intake may have affected API earlier than AVI. WEM has been reported to reduce fluorescence levels, which reflect the amount of AGEs in the skin, in human ingestion studies, as well as pentosidine levels in the blood[16]. In addition, skin fluorescence and blood pentosidine levels correlate with atherosclerosis[17, 18], and there is a report that vascular function was improved by intake of alagebrium, an AGEs cross-link scission agent[19]. Based on the above, the decrease in API with WEM intake is thought to be due to the glycation inhibitory effect of WEM.

In addition to the vascular endothelial protective effect of estrogen[20], it also inhibits AGE generation[21]. Considering that estrogen levels begin to decline over the age of 30[15], it is likely that the ability to inhibit AGE generation begins to decrease over the age of 30. The fact that WEM intake had an effect on API items in an analysis limited to those aged 30 years or older, in which age group the blood estrogen and AGE-formation inhibiting ability begins to decrease, also supports the possibility that WEM's inhibitory effect on glycation is responsible for the improvement in API with WEM.

In the previous study[10], there was no clear significant difference in API with WEM intake, while, in the present report, a significant decrease in API with WEM intake was observed in subjects aged 30 years or older, when vascular

function begins to decline. Thus, the state of vascular stiffness varies even among healthy subjects, and it was considered important to consider the state of vascular stiffness of subjects prior to intake in order to verify the effects of WEM.

Conclusion

In healthy women aged 30 years or older, WEM intake improved API, suggesting that WEM improves age-related vascular stiffening and maintains vascular flexibility. Consumption of WEM with its anti-glycation properties is highly expected as a food that reduces the risk of atherosclerosis in middle-aged and older adults.

Conflict of interest declaration

The clinical trial for the previous study was funded by Nippon Shinyaku Co., Ltd. and conducted at TES Holdings (Tokyo, Japan).

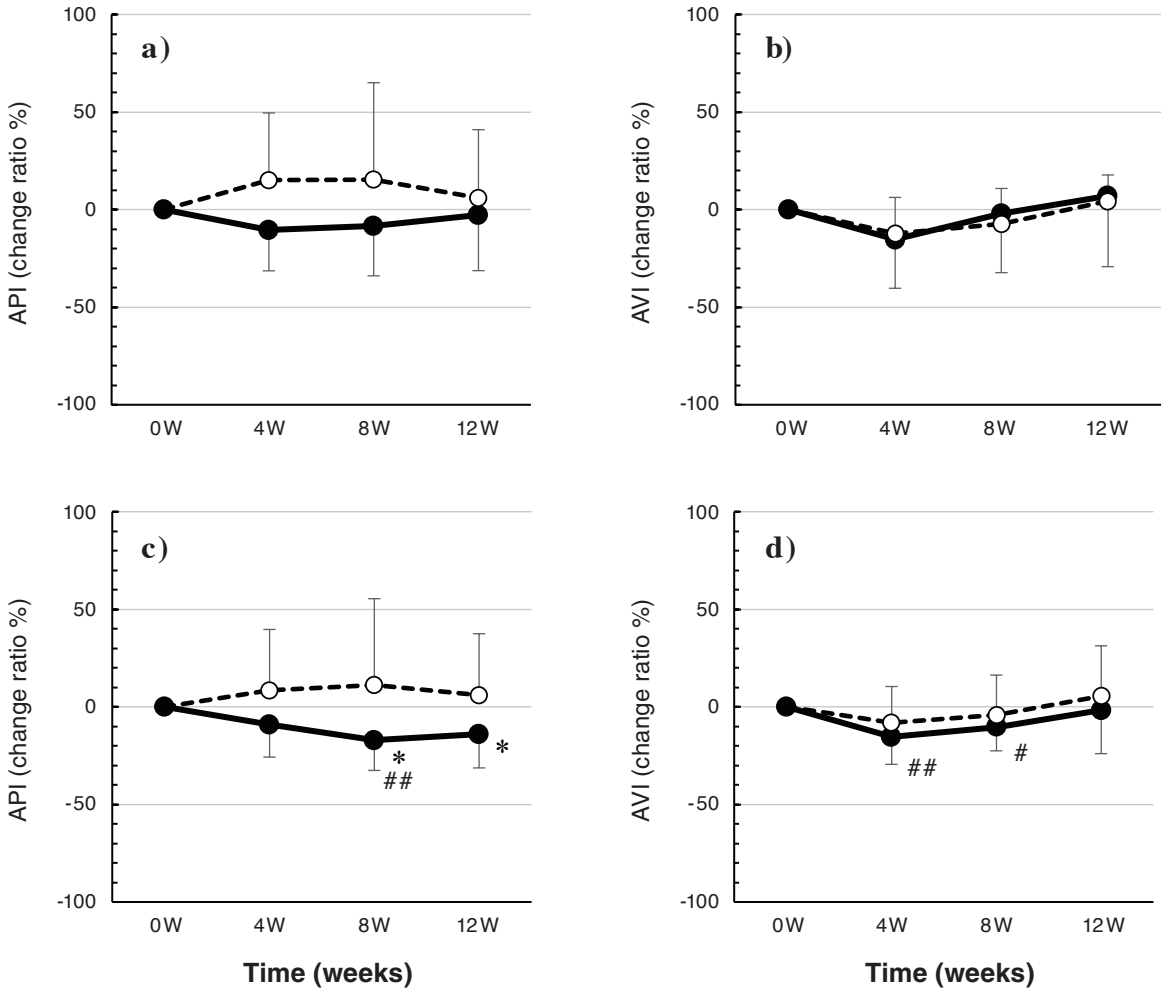

Fig. 1. **Values of API and AVI**

Change ratio (%) of WEM group (●) and Placebo group (○) are expressed as means ± SD. **a**) and **b**) indicate API and AVI of subjects with LDL-C between 120-159 mg/dL, respectively. WEM group includes 7 subjects and Placebo group includes 9 subjects. **c**) and **d**) indicate API and AVI of subjects aged 30 years or older, respectively. WEM group includes 14 subjects and Placebo group includes 13 subjects. * $p < 0.05$, compared with the Placebo group by t-test., # $p < 0.05$, ## $p < 0.01$, comparison with before intake by Sidak test. WEM, water extract of mangosteen; API, Arterial Pressure Index; AVI, Arterial Velocity Pulse Index; LDL-C, low-density-lipoprotein-cholesterol; SD, standard deviation.

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