Original Article

Evaluation of effects of cassis (*Ribes nigrum L.*) juice on human vascular function and gene expression using a microarray system

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Abstract

Objective: Vascular function testing and microarray analysis were performed to evaluate the physical effects of cassis (*Ribes nigrum L.*) juice in women.

Methods: In healthy women (Study I: n=21, age 53.6±3.6 years, BMI 24.2±3.9; Study II: n=40, age 47.4±8.8 years, BMI 22.4±3.9) assigned to one of four cassis groups (polysaccharide (PS) content: 0, 50, 125, and 250 mg) or a water control group (duration of intake: 2 to 8 weeks), the following parameters were assessed: for vascular function, blood pressure, thermographically measured body surface temperature, CAVI (cardio-ankle vascular index), ABI (ankle-brachial pressure index), accelerated plethysmography, and FMD (flow-mediated dilation) were determined. In the PS 250 mg (n=3) and water (n=4) groups, total RNA was extracted from blood collected before and 2 weeks after the study to perform gene expression analysis using Human Whole Genome 4 × 44K kit (Agilent).

Results: Cassis juice (PS content: 125 to 250 mg) (n=16) increased body fat percentage (+2.5%, p=0.011), decreased systolic (121.3±15.8 mmHg at baseline, -4.8%, p=0.001) and diastolic blood pressure (76.9±11.2 mmHg at baseline, -5.9%, p=0.001), and increased FMD blood flow (p=0.004), upper limb temperature (+0.23±0.47°C, p=0.011), and lower limb temperature (+0.55±0.50°C, p<0.001). The percentage increase in lower limb temperature was dependent on PS (p<0.05) and polyphenol (p<0.01) contents. In gene analysis, inhibition of expression of α -adrenoceptor ADRA1D and thromboxane A2 receptor TBXA2R was prominent. Pathway analysis revealed significant accumulation of less frequently expressed genes (CACNA1B, GJB3, etc.) in the "Ca regulation in the cardiac cell" pathway (p=0.006). No serious adverse events occurred during the study.

Conclusion: Cassis juice induced limb peripheral vasodilatation, increase in blood flow, and decrease in blood pressure. The microarray analysis showed inhibited expression of genes of α -adrenoceptor, thromboxane A2 receptor and Ca channel, and an inhibited pathway of Ca influx into vascular smooth muscle. Coupled with the fact that vascular endothelium plays a role in vasodilatation associated with various mRNA expressions, it is suggested that cassis juice may improve the function of endothelial cells.

KEY WORDS: black currant, polysaccharide, blood pressure, vascular endothelium, accelerated plethysmography, microarray, thermography, gene expression, pathway analysis

Introduction

Cassis (*Ribes nigrum L.*) fruit, which contains various bioactive components, for example antioxidant polyphenols (PP)¹⁾ such as anthocyanin and flavonol, polysaccharides (PS) involved in immune activation,²⁾ and hydroxycinnamic acid, is a functional food that has been studied for its effects on skin in recent years. We have clinically evaluated the effects of cassis juice on physical and mental health as well as skin/vascular system, and reported that it improved a number of subjective symptoms related to quality of life (QOL), significantly decreased diastolic blood pressure,³⁾ and improved the accelerated plethysmogram index and peripheral blood flow with an increase in body surface temperature.⁴⁾

This study, which focused on the effects of cassis-derived polysaccharides on the human vascular system, was conducted to comprehensively analyze previous data^{3,4)} and evaluate the effects on gene expression using microarray technology.

Methods

Subjects

A total of 21 (age: 53.6 ± 3.6 years old, BMI: 24.2 ± 3.9) and 40 (age: 47.4 ± 8.8 years old, BMI: 22.4 ± 3.9) healthy Japanese female volunteers who met all of the following inclusion criteria were included in Studies 1 and 2, respectively: aged between 30 and 60 years; systolic blood pressure 110 to 160 mmHg (excluding antihypertensive users); and written consent to participate in the study. The results of individual studies have been reported previously.^{3,4)} In the present study, the combined results were comprehensively analyzed, including gene analysis.

In Study I, subjects consumed one bottle (50 mL) of cassis juice within 30 minutes after breakfast once daily for 8 weeks. The test product was provided by Mercian Corporation (Tokyo). The test product was identical to that for the PS250 group in Study II (Table 1).

In Study II, subjects were randomized to one of five groups (8 subjects per group) which were classified according to the PS and PP contents as follows: water group (PS: 0 mg, PP: 0 mg), PS0 group (PS: 0 mg, PP: 130 mg), PS50 group (PS: 50 mg, PP: 28 mg), PS125 group (PS: 125 mg, PP: 70 mg), and PS250 group (PS: 250 mg, PP: 140 mg). The subjects consumed the test product once daily for 2 weeks, as did those in Study I.

Methods

Subjects were instructed to keep regular hours during the study, and maintain the same quantity and quality of sleep, diet, exercise, and alcohol intake as in daily life prior to the study, but to refrain from alcohol one day before each observation day.

This was a five-group open-label comparative study comparing each parameter before and after use of the test product. The study protocol was approved by the Institutional Review Board, and the study was conducted in compliance with the protocol as well as the ethical principles specified in the “Declaration of Helsinki” and “Ethical Guidelines for Epidemiological Research” by the Ministry of Health, Labour and Welfare/Ministry of Education, Culture, Sports, Science and Technology (<http://www.mhlw.go.jp/general/seido/kousei/i-kenkyu/ekigaku/0504sisin.html>).

In accordance with the protocol, clinical assessment was performed twice, immediately before and 2 weeks after the start of intake of the test product. The study was conducted under the direction of Toshio Mochizuki at Medical Urban Clinic (Nishinomiya, Hyogo) from March 1 to June 30, 2008.

Assessment of subjective symptoms

In assessment of subjective symptoms, “physical symptoms” and “mental symptoms” were assessed on a five-grade scale (1 to

5 points) using the Anti-Aging QOL Common Questionnaire (AAQol), as reported previously.⁵⁻⁷⁾ This questionnaire was downloaded from the homepage of the Japanese Society of Anti-Aging Medicine (<http://www.anti-aging.gr.jp/anti/clinical.phtml>).

Anthropomorphic and laboratory tests

Anthropomorphy

The following anthropomorphic parameters were determined: body weight (kg), total body muscle mass (kg), fat-free mass (kg), body fat percentage (%) (measured using an InBody (Biospace Co., Ltd., Seoul, Korea) segmental multi-frequency bioimpedance analysis system),⁸⁾ BMI, blood pressure (systolic/diastolic: mmHg), pulse rate (/min), and body surface temperature. Blood pressure was measured in duplicate and expressed as the mean. Height (cm) was measured only before use. Body surface temperature was measured at a total of 11 sites including the neck (posterior), shoulder (right, left), upper limbs (bilateral forearms and upper arms), and lower limbs (bilateral thighs and legs) before and 30 minutes after intake of the test product on the first study day and Day 14 (2 weeks after the start of the study) using infrared thermography (TH9100 MV/WV, NEC Avio, Tokyo).

Vascular examination

The following arteriosclerosis indexes were determined using a VaSeraVS-100 (Fukuda Denshi, Tokyo) vascular screening system: cardio-ankle vascular index (CAVI) and ankle-brachial pressure index (ABI).^{9,10)} While CAVI reflects vascular stiffness/tone, ABI is useful in assessing arterial stenosis/occlusion in the lower limbs. In addition, as a finger pulse wave analysis, the second derivative of the finger photoplethysmogram (SDPTG) was determined using an acceleration plethysmogram (Dyna Pulse SDP-100, Fukuda Denshi, Tokyo) to calculate vascular age.¹¹⁾

Vascular endothelial function was assessed by determining flow-mediated dilation (FMD)¹²⁻¹⁵⁾ of the right brachial artery during reactive hyperemia following ischemia by high-resolution ultrasonography.

Gene analysis

Blood samples collected before (A1) and after (A2) intake in the cassis juice PS250 group as well as before (A3) and after (A4) intake in the water group were used to perform microarray analysis using a Human Whole Genome 4 × 44K kit (Agilent, Santa Clara, CA, USA). Blood was collected from 3 and 4 subjects randomly selected in the PS250 and water groups, respectively, using a blood collection tube (PAXgene Blood RNA Tube, Becton, Dickinson and Company, Tokyo), and the peripheral blood (whole blood) was treated with a RNA stabilizer to extract total RNA using a PAXgene Blood RNA Kit (Qiagen, Tokyo). For each group, the extracted total RNA was pooled to prepare a sample of total RNA mix. A total of 4 samples were used for analysis.

Table 1 Contents of test products

	Number of subjects	Volume	Calorie	Water	Cassis juice	Sugar content	Polysaccharide (PS)	Polyphenol (PP)
Subjects		ml	kcal	ml	ml	%	mg	mg
Water	8	50	0	100	0	0	0	0
Cassis								
PS0	8	100	18	85	15	5.6	0	130
PS50	8	50	8	45	5	3.7	50	28
PS125	8	50	20	37.5	12.5	9.2	125	70
PS250*	21+8	50	41	25	25	18.4	250	140

* Number of subjects represents the total number of subjects in Studies I and II.

RNA in each sample was labeled by the one-color method (Cy3) to adjust the dynamic range among the samples by comparing the fluorescence intensity. If the fluorescence intensity was near zero (including intensity values indicative of no fluorescence), “0.01” was applied to avoid analytical problems. To minimize experimental errors resulting from RNA extraction or those among the samples, the dynamic range was adjusted so that the median was equal for 4 samples. For each gene, the median was calculated for each sample and divided by the sample median. In addition, the median was calculated for all 4 samples. For each gene, the number of genes expressed was defined as Log_2 (number of genes expressed / median for all samples). Software GeneSpring V7.3.1 (Agilent) was used for these formula manipulations.

Of all 41,000 probes on the chip, 22,569, 24,216, 23,617, and 22,546 probes were considered effective in the A1, A2, A3, and A4 groups, respectively. To compile matrices without any missing values (100% filling), data on probes missing in at least 1 of the 4 samples were excluded, leaving 21,439 effective probes in all samples. Subsequently, probe ID was converted to Human Entrez Gene ID, identifying a total of 19,637 genes in the population. For an Entrez Gene ID corresponding to at least 2 probes, the number of genes expressed was defined as the median of these probes.

To eliminate individual variation, moreover, genes expressed at least 2 times more frequently or less frequently before intake of PS250 (A1) than before intake of water (A3) were excluded, leaving 12,136 genes for final analysis. In contrast to genes differentially expressed, defined as genes expressed at least 1.4 times more frequently or less frequently (Log_2 : $\geq +0.485$ or ≤ -0.485), a less stringent exclusion criterion (at least 2 times more frequently or less frequently) was employed for individual variation in order to include as many genes as possible in analysis. Gene Ontology (GO) (<http://www.geneontology.org/>), a database for information about gene functions, and GO data mining software GO::TermFinder¹⁶⁾ were used for functional analysis of genes differentially expressed.

Statistical analysis

Statistical analysis was performed using statistical analysis software Dr. SPSSII (SPSS Japan Inc.) and Excel Statistics (Social Survey Research Information Co., Ltd.). For comparison between users and non-users of the test product, paired *t*-test was performed at a two-sided significance level of 5%, in principle. One-way analysis of variance followed by Tukey's or Dunnett's multiple comparison test was performed for parametric functions, and Kruskal-Wallis test followed by Steel-Dwass multiple comparison test was performed for nonparametric functions.

For each GO term on the differentially expressed gene list, a test was performed to determine whether the percentage relative to all genes (population) with data available was significantly high or not. In addition to the *p*-value, the false discovery rate (FDR) was used as a measure for this multiple test involving simultaneous massive tests. For pathway analysis, GenMAPP (<http://www.GenMAPP.org>) and MAPPFinder, developed by Gladstone Institute, University of California at San Francisco (CA, USA) were used.¹⁷⁾ Analysis was performed using human gene database Hs-Std_20060526.gdb and human MAPP pathway database Hs_Contributed_20070308.

Results

Physical findings

Of the body components, body fat percentage (+2.5%), fat mass (+2.3%), fat-free mass (−1.1%), total body water (−4.0%), and basal metabolism (−5.2%) significantly changed 2 to 4 weeks after the start of the study in subjects provided with 25 mL or more of cassis juice (PS125 and PS250 groups, *n*=37) (Table 2). Systolic and diastolic blood pressure significantly decreased from 121.3±15.8 to 115.5±13.1 mmHg (−4.8%, *p*=0.001) and from 76.9±11.2 to 72.4±10.9 mmHg (−5.9%, *p*=0.001), respectively.

Thermographically measured skin surface temperature is shown as pre- and post-consumption temperature difference (Fig. 1). The mean was calculated for the shoulder (mean of the right and left shoulders), upper limbs (mean of bilateral forearms and upper arms), and lower limbs (bilateral thighs and legs) before and 2 weeks after the start of the study. In all groups, lower limb surface temperature significantly increased 15 minutes after intake of the test product, namely from 32.46±0.46°C to 32.91±0.70°C (+1.9%, *p*=0.005) in the water group, 32.22±0.44°C to 32.74±0.54°C (+1.7%, *p*<0.001) in the PS0 group, 32.66±0.48°C to 32.94±0.49°C (+0.9%, *p*=0.003) in the PS50 group, 32.32±0.73°C to 32.66±0.78°C (+1.1%, *p*=0.001) in the PS125 group, and 32.16±0.54°C to 32.92±0.39°C (+2.4%, *p*<0.001) in the PS250 group.

In stratified analysis, skin surface temperature significantly increased at the following sites 15 minutes after intake in the PS125 and PS250 groups (*n*=16): +0.23±0.47°C (*p*=0.011) in the upper limbs and +0.55±0.50°C (*p*<0.001) in the lower limbs. Skin surface temperature did not change significantly in the neck or shoulder. Possible correlation with the PS content in the PS0, PS50, PS125, and PS250 groups (0, 50, 125, and 250 mg, respectively) was analyzed, showing that lower limb surface temperature increased dose-dependently (*p*<0.05). Analysis of possible correlation with the PP content (130, 28, 70, and 140 mg, respectively) revealed that lower limb surface temperature increased dose-dependently (*p*<0.01).

Mental and physical subjective symptoms assessed by Anti-Aging QOL Common Questionnaire

Changes in “physical symptoms” and “mental symptoms” observed 2 to 4 weeks after the start of the study in the cassis group (PS125 and PS250 groups, *n*=37) are shown in Tables 3 and 4.

Of 33 physical symptoms listed in the common questionnaire, 7 symptoms significantly improved in the cassis group, including “tired eyes,” “stiff shoulders,” “muscular pain/stiffness,” “tendency to gain weight,” “skin problems,” “constipation,” and “headache” (Table 3). Intergroup analysis showed that the 3 symptoms of “tendency to gain weight,” “lethargy,” and “edematous” significantly improved in the cassis group as compared with the water group and no symptom improved in the water group as compared with the cassis group.

Of 21 mental symptoms, no symptom significantly improved in the cassis or water group (Table 4).

Vascular examination (Table 5)

Cardio-ankle vascular index (CAVI), an index of arteriosclerosis, tended to increase in the left side in the cassis group (PS125 and PS250 groups, *n*=16) with a significant intergroup difference, but did not change significantly in the right side or bilaterally. Ankle-brachial pressure index (ABI) did not change significantly.

In accelerated plethysmography, vascular age tended to decrease from 57.53±14.12 to 53.73±15.35 years in the cassis group (one-sided test: *p*=0.030).

Table 2 Anthropomorphic measurements

		Cassis PS125+250			Water			Between groups
		Before	After	p value	Before	After	p value	p value
Height	cm	157.3 ± 6.4			159.1 ± 7.9			
Body weight	kg	58.4 ± 11.6	58.4 ± 11.6	0.799	54.8 ± 6.3	54.6 ± 6.3	0.435	0.401
Body fat percentage	%	32.3 ± 6.7	33.1 ± 6.4	0.003	29.7 ± 7.1	29.3 ± 7.3	0.249	0.011
Body fat mass	kg	19.5 ± 8.9	19.9 ± 8.6	0.012	16.5 ± 5.4	16.3 ± 5.6	0.257	0.015
Fat-free mass	kg	38.9 ± 4.0	38.5 ± 4.0	0.014	38.3 ± 3.8	38.4 ± 3.2	0.773	0.122
Total body water	kg	28.9 ± 3.4	27.7 ± 2.9	0.000	28.0 ± 2.7	28.1 ± 2.4	0.686	0.001
Basal metabolism	kcal	1122 ± 140	1064 ± 120	0.000	1078 ± 103	1079 ± 89	0.881	0.000
BMI		23.6 ± 4.3	23.6 ± 4.3	0.816	21.8 ± 3.4	21.7 ± 3.5	0.420	0.329
Systolic blood pressure	mmHg	121.3 ± 15.8	115.5 ± 13.1	0.001	118.5 ± 12.9	113.5 ± 12.8	0.176	0.825
Diastolic blood pressure	mmHg	76.9 ± 11.2	72.4 ± 10.9	0.001	77.8 ± 13.2	76.3 ± 10.7	0.625	0.274
Pulse rate	/min.	74.9 ± 9.3	75.8 ± 9.5	0.550	72.5 ± 7.2	75.0 ± 11.9	0.463	0.650

Cassis PS125+250: n=37, Water: n=8. BMI:body mass index.

Fig. 1. Changes in skin surface temperature at 15 minutes after intake of water or cassis juice. Temperature was thermographically measured. 8 subjects per group. Mean±standard deviation. Unit: °C * p<0.05, ** p<0.01 (compared with pre-consumption value)**Table 3** Physical symptoms

		Cassis PS125+250			Water			Between groups
		Before	After	p value	Before	After	p value	p value
Tired eyes		2.7 ± 0.9	2.4 ± 1.0	0.041	2.6 ± 1.2	2.5 ± 1.2	0.598	0.432
Blurry eyes		2.2 ± 1.0	2.2 ± 1.1	0.701	1.8 ± 1.0	1.9 ± 0.8	0.685	0.364
Eye pain		1.8 ± 0.9	1.6 ± 0.9	0.304	1.9 ± 1.0	1.9 ± 0.8	1.000	0.640
Stiff shoulders		3.3 ± 1.0	3.0 ± 1.1	0.048	2.9 ± 1.2	2.8 ± 1.2	0.351	0.500
Muscular pain/stiffness		2.7 ± 0.9	2.4 ± 0.8	0.011	2.5 ± 1.4	2.5 ± 1.3	1.000	0.129
Palpitations		1.8 ± 0.9	1.6 ± 0.8	0.281	1.4 ± 0.7	1.1 ± 0.4	0.170	0.529
Shortness of breath		1.9 ± 0.9	1.8 ± 0.9	0.291	1.4 ± 0.7	1.1 ± 0.4	0.170	0.449
Tendency to gain weight		3.6 ± 1.1	3.2 ± 1.2	0.029	3.0 ± 1.3	3.3 ± 1.4	0.351	0.041
Weight loss; thin		1.3 ± 0.5	1.3 ± 0.5	0.768	1.4 ± 0.5	1.6 ± 0.5	0.170	0.257
Lethargy		2.1 ± 0.9	2.2 ± 0.8	0.812	2.3 ± 1.0	3.0 ± 1.4	0.080	0.018
Lack of sense of wellness		2.0 ± 0.8	2.0 ± 0.8	0.845	1.9 ± 1.0	1.9 ± 1.4	1.000	0.791
Thirst		2.0 ± 0.8	1.8 ± 0.8	0.147	1.5 ± 0.8	1.4 ± 0.7	0.351	0.722
Skin problems		2.6 ± 1.2	2.2 ± 1.0	0.014	2.5 ± 0.8	2.4 ± 0.7	0.598	0.364
Anorexia		1.4 ± 0.6	1.4 ± 0.5	0.800	1.4 ± 0.7	1.3 ± 0.5	0.685	0.713
Early satiety		1.7 ± 0.7	1.6 ± 0.6	0.304	1.9 ± 1.0	1.5 ± 0.8	0.285	0.452
Epigastralgia		1.5 ± 0.7	1.4 ± 0.5	0.201	1.5 ± 0.8	1.3 ± 0.5	0.351	0.650
Liable to catch colds		1.8 ± 0.7	1.7 ± 0.7	0.499	1.6 ± 0.5	1.4 ± 0.5	0.170	0.315
Coughing and sputum		1.7 ± 0.7	1.6 ± 0.6	0.401	1.4 ± 0.7	1.1 ± 0.4	0.351	0.423
Diarrhea		1.6 ± 0.8	1.6 ± 0.8	0.822	1.3 ± 0.7	1.8 ± 0.9	0.227	0.064
Constipation		2.0 ± 0.8	1.7 ± 0.7	0.014	2.1 ± 1.4	2.0 ± 1.2	0.351	0.323
Hair loss		2.2 ± 0.9	2.3 ± 0.8	0.324	2.3 ± 1.5	1.9 ± 1.0	0.197	0.072
Gray hair		3.5 ± 0.9	3.3 ± 1.1	0.051	2.4 ± 1.3	2.1 ± 1.2	0.170	0.637
Headache		1.8 ± 0.8	1.6 ± 0.8	0.033	1.4 ± 0.5	1.5 ± 0.5	0.351	0.050
Dizziness		1.5 ± 0.6	1.4 ± 0.7	0.711	1.3 ± 0.5	1.1 ± 0.4	0.351	0.494
Tinnitus		1.3 ± 0.6	1.3 ± 0.7	0.768	1.3 ± 0.5	1.1 ± 0.4	0.351	0.506
Lumbago		2.3 ± 1.1	2.2 ± 1.0	0.281	2.1 ± 1.1	2.3 ± 1.5	0.732	0.406
Arthralgia		1.8 ± 0.9	1.6 ± 0.8	0.343	1.6 ± 1.1	1.9 ± 1.1	0.351	0.204
Edematous		1.8 ± 0.8	1.7 ± 0.7	0.676	1.6 ± 1.1	2.4 ± 1.4	0.048	0.013
Easily breaking into a sweat		2.6 ± 1.1	2.5 ± 1.3	0.391	1.8 ± 0.7	1.4 ± 0.7	0.197	0.309
Frequent urination		1.9 ± 0.9	1.7 ± 0.8	0.051	1.8 ± 0.7	1.5 ± 0.8	0.170	0.583
Hot flashes		1.9 ± 0.9	1.9 ± 1.0	1.000	1.1 ± 0.4	1.3 ± 0.5	0.351	0.482
Cold skin		2.1 ± 1.0	2.0 ± 0.9	0.474	1.9 ± 1.4	1.9 ± 1.4	1.000	0.718

Cassis PS125+250: n=37, Water: n=8.

Table 4 *Mental symptoms*

	Cassis PS125+250			Water			Between groups p value
	Before	After	p value	Before	After	p value	
Irritability	2.2 ± 0.9	2.2 ± 1.0	0.600	2.1 ± 1.2	2.0 ± 1.3	0.685	0.784
Easily angered	2.1 ± 0.8	2.0 ± 0.9	0.413	1.6 ± 0.7	1.9 ± 1.4	0.351	0.174
Loss of motivation	1.9 ± 0.8	2.1 ± 0.9	0.128	1.6 ± 1.1	1.4 ± 0.5	0.451	0.148
No feeling of happiness	1.9 ± 0.8	1.9 ± 0.8	0.534	1.8 ± 1.2	1.5 ± 0.8	0.351	0.169
Nothing to look forward to in life	1.7 ± 0.8	1.9 ± 0.7	0.051	1.3 ± 0.5	1.3 ± 0.5	1.000	0.051
Daily life is not enjoyable	1.8 ± 0.7	1.8 ± 0.8	0.600	1.4 ± 0.7	1.3 ± 0.5	0.351	0.282
Lose confidence	1.9 ± 0.7	2.0 ± 0.7	0.291	1.6 ± 1.1	1.3 ± 0.5	0.197	0.058
Reluctance to talk with others	1.8 ± 0.8	1.7 ± 0.7	0.160	1.5 ± 0.5	1.3 ± 0.5	0.170	0.306
Depressed	1.8 ± 0.7	1.7 ± 0.7	0.487	1.3 ± 0.5	1.3 ± 0.5	1.000	0.487
Feeling of uselessness	1.7 ± 0.7	1.6 ± 0.6	0.257	1.3 ± 0.5	1.3 ± 0.5	1.000	0.257
Shallow sleep	2.0 ± 0.9	1.9 ± 0.9	0.413	2.0 ± 1.6	2.0 ± 1.6	1.000	0.671
Difficulty in falling asleep	1.6 ± 0.8	1.8 ± 0.7	0.096	1.5 ± 1.1	1.4 ± 0.7	0.598	0.198
Pessimism	2.1 ± 0.9	2.0 ± 0.7	0.600	1.6 ± 0.7	1.6 ± 1.1	1.000	0.554
Lapse of memory	2.7 ± 0.8	2.6 ± 0.6	0.571	2.4 ± 1.1	1.9 ± 0.6	0.104	0.067
Inability to concentrate	2.1 ± 0.8	2.1 ± 0.6	0.413	1.6 ± 0.7	1.4 ± 0.5	0.170	0.378
Inability to solve problems	2.0 ± 0.8	1.9 ± 0.8	0.134	1.4 ± 0.5	1.3 ± 0.5	0.351	0.807
Inability to make judgments readily	2.0 ± 0.8	1.9 ± 0.8	0.413	1.6 ± 1.1	1.4 ± 0.5	0.351	0.485
Inability to sleep because of worries	1.7 ± 0.7	1.6 ± 0.6	0.446	1.6 ± 0.9	1.5 ± 0.8	0.351	0.631
A sense of tension	2.1 ± 0.8	2.0 ± 0.6	0.257	1.8 ± 1.2	1.4 ± 0.5	0.351	0.435
Feeling of anxiety for no special reason	1.7 ± 0.8	1.8 ± 0.8	0.571	1.4 ± 0.7	1.3 ± 0.5	0.351	0.270
Vague feeling of fear	1.6 ± 0.8	1.5 ± 0.7	0.446	1.3 ± 0.5	1.3 ± 0.5	1.000	0.446

Cassis PS125+250: n=37, Water: n=8.

Table 5 *Blood vessel function test*

		Cassis PS125+250			Water			Between groups p value
		Before	After	p value	Before	After	p value	
CAVI	right	6.85 ± 1.81	6.79 ± 0.40	0.896	6.43 ± 0.54	6.26 ± 0.79	0.335	0.382
	left	6.59 ± 0.98	6.79 ± 0.48	0.296	6.63 ± 0.61	6.31 ± 0.82	0.093	
	both sides	6.72 ± 1.14	6.79 ± 0.44	0.797	6.53 ± 0.56	6.29 ± 0.80	0.161	
ABI	right	1.16 ± 0.10	1.14 ± 0.06	0.383	1.13 ± 0.07	1.16 ± 0.06	0.234	0.134
	left	1.12 ± 0.07	1.13 ± 0.09	0.694	1.13 ± 0.05	1.12 ± 0.06	0.660	
	both sides	1.14 ± 0.08	1.13 ± 0.07	0.850	1.13 ± 0.06	1.14 ± 0.06	0.625	
Accelerated plethysmography	years old	56.9 ± 13.9	52.9 ± 15.2	0.061	45.6 ± 9.7	43.8 ± 6.2	0.512	0.000
Vascular endothelial function test (FMD)								
Vascular diameter at rest	mm	3.37 ± 0.34	3.42 ± 0.40	0.315	3.41 ± 0.40	3.40 ± 0.45	0.885	0.387
Vascular diameter at maximum dilation	mm	3.61 ± 0.36	3.64 ± 0.42	0.560	3.68 ± 0.41	3.68 ± 0.41	0.976	0.621
Dilation	mm	0.24 ± 0.13	0.22 ± 0.12	0.613	0.27 ± 0.12	0.27 ± 0.13	0.881	0.497
Percentage increase in blood flow	%	2.43 ± 1.31	4.43 ± 1.72	0.004	4.86 ± 4.89	4.50 ± 3.65	0.701	0.035
Endothelium-dependent vasodilatation	%	7.13 ± 4.21	6.48 ± 3.75	0.563	7.99 ± 3.76	8.38 ± 4.75	0.731	0.510
Time to peak vascular diameter	sec.	48.4 ± 11.6	48.2 ± 16.4	0.956	51.7 ± 16.8	40.6 ± 12.3	0.042	0.062
Vascular diameter following release	mm	3.38 ± 0.32	3.45 ± 0.40	0.283	3.44 ± 0.44	3.43 ± 0.50	0.921	0.488
L-FMC	mm	0.00 ± 0.07	-0.02 ± 0.09	0.517	-0.06 ± 0.17	-0.01 ± 0.06	0.562	0.143

Cassis PS125+250: n=16, Water: n=8.

CAVI: cardio ankle vascular index. ABI: ankle brachial pressure index.

FMD: flow-mediated dilation.

L-FMC: low-flow-mediated constriction.

In FMD examination, which reflects vascular endothelial function, the percentage increase in blood flow significantly increased in the cassis group, showing a 2.43±1.31- to 4.43±1.72-fold increase ($p=0.004$), with a significant intergroup difference ($p=0.035$). Time to the peak vascular diameter did not change in the cassis group, but significantly decreased in the water group. None of the other parameters changed significantly. No serious adverse event occurred during the study.

Gene analysis test

Of 12,136 probes included in the final analysis, the number of genes significantly differentially expressed (converted to Entrez Gene ID) ($p < 0.05$) was as follows:

Cassis PS250 group (A1 vs. A2)

expressed at least 1.4 times more frequently 288 (1.5%, 2.37%)

expressed at least 1.4 times less frequently 587 (3.0%, 4.84%)

Water group (A3 vs. A4)

expressed at least 1.4 times more frequently 439 (2.2%, 3.62%)

expressed at least 1.4 times less frequently 462 (2.4%, 3.81%)

Figures in parentheses represent the percentage relative to the number of all genes on the chip (19,637) and effective genes (12,136), respectively. The percentage of genes differentially expressed relative to effective genes was lower than 5%.

Genes differentially expressed 2 weeks after the start of intake in the cassis PS250 (A2) and control water (A4) groups were compared using a Venn diagram (Fig. 2). In the A2 group, expression of 234 and 516 genes were characteristically increased (more frequently expressed) and inhibited (less frequently expressed), respectively. In the control A4 group, expression of 385 and 396 genes were characteristically increased (more frequently expressed) and inhibited (less frequently expressed), respectively. In both groups, 54 genes were expressed more frequently, and 71 genes were expressed less frequently.

Fig. 2. Number of genes expressed differentially in cassis and water groups (Venn diagram).

In the A2 group, 104 genes were expressed more frequently than in the A1 group and expressed at least 1.4 times more frequently than in the A4 group. In the A2 group, 160 genes were expressed less frequently than in the A1 group and expressed at least 1.4 times less frequently than in the A4 group.

Pathway analysis was performed to detect significantly affected pathways (Table 6). Precise results related to the blood vessels are as follows.

In the “oxidative stress” pathway (Table 7), we observed accumulation of genes expressed more frequently meeting the analytical criterion of $0.3 < \text{Log ratio} \leq 0.5$ in the A2 group ($p = 0.015$). Expression of glutathione reductase GSR, transcription factor Sp1 (SP1) involved in adaptive response of transcriptional activation, and proto-oncogene protein c-fos (FOS) (Cellular oncogene fos) (G0/G1 switch regulatory protein 7) representing immediate-early gene induction was prominent.

Marked accumulation of genes expressed less frequently meeting the analytical criterion of $-0.5 < \text{Log ratio} \leq -0.3$ in the A2 group

Table 6 Pathway analysis

	Cassis PS250		Water	
	Before A1	After A2	Before A3	After A4
Criteria: $1.0 < \text{Log ratio}$				
Cell cycle	1.000	<0.001	0.149	1.000
Ovarian Infertility Genes	1.000	0.032	1.000	1.000
Peptide GPCRs	1.000	0.054	1.000	1.000
Criteria: $0.5 < \text{Log ratio} \leq 1.0$				
Irinotecan Pathway (Homo Sapiens)	0.083	0.032	1.000	1.000
Criteria: $0.3 < \text{Log ratio} \leq 0.5$				
Oxidative Stress	1.000	0.015	1.000	1.000
Pentose Phosphate Pathway	1.000	0.021	0.160	0.661
Blood Clotting Cascade	0.315	0.026	1.000	0.647
Criteria: $-0.5 < \text{Log ratio} \leq -0.3$				
Matrix Metalloproteinases	0.375	<0.001	1.000	0.276
GPCRs, Class A Rhodopsin-like	0.520	<0.001	0.403	1.000
Ca Regulation in the Cardiac Cell	0.538	0.006	1.000	0.081
Heme Biosynthesis	0.035	0.010	1.000	0.641
Peptide GPCRs	1.000	0.017	1.000	0.428
GPCRs, Other	0.101	0.017	0.641	0.715
Criteria: $-1.0 < \text{Log ratio} \leq -0.5$				
GPCRs, Class A Rhodopsin-like	0.044	0.001	1.000	0.438
Prostaglandin Synthesis and Regulation	1.000	0.044	1.000	1.000
Criteria: $\text{Log ratio} \leq -1.0$				
Wnt Signaling Pathway	1.000	<0.001	1.000	1.000

Cassis PS250: n=3, Water: n=4.

Table 7 “Oxidative stress” pathway

Name	Description	Log ratio			
		Cassis PS250		Water	
		Before A1	After A2	Before A3	After A4
GSR	glutathione reductase	0.13	0.37	-0.17	-0.15
SP1	Sp1 transcription factor (Probe ID:P53397)	0.11	0.32	-0.16	-0.11
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	0.07	0.32	-0.36	-0.07
SP1	Sp1 transcription factor (Probe ID:P366312)	0.09	0.30	-0.10	-0.34

was observed in the “Ca regulation in the cardiac cell” ($p = 0.006$) and “G-protein coupled receptor” (class A: $p < 0.001$, other: $p = 0.017$) pathways. In the “G-protein coupled receptor” pathway, marked accumulation of genes expressed less frequently and meeting the analytical criterion of $-1.0 < \text{Log ratio} \leq -0.5$ was also observed.

In the “Ca regulation in the cardiac cell” pathway involved in contraction/dilatation of vascular smooth muscle (Table 8), expression of CACNA1B, an N type voltage-dependent Ca channel α -1B subunit, and GJB3, a Gap junction β -3 protein (Connexin 31:C $\times$ 31), was markedly inhibited. While expression of α -adrenoceptor ADRA1D tended to be inhibited, expression of β -adrenoceptor ADRB1, ADRB2, or ADRB3 was apparently not affected. In addition, the protein kinase A system PRKAR2A, a cAMP-dependent protein kinase type II- α regulatory subunit, was inhibited.

In the “G-protein coupled receptor” pathway, expression of PGD2 receptor gene PTGDR and thromboxane A2 (TXA2)

Table 8 “Ca regulation in the cardiac cell” pathway

Name	Description	Log ratio			
		Cassis PS250		Water	
		Before A1	After A2	Before A3	After A4
CACNA1B	Ca channel, voltage-dependent, N type, α 1B subunit (Probe ID:P159622)	0.12	-0.60	0.06	-0.06
CACNB3	Ca channel, voltage-dependent, β 3 subunit	0.12	-0.56	0.17	-0.13
GJB3	gap junction protein, β 3, 31kDa	-0.20	-0.56	0.37	0.18
ATP1B1	ATPase, Na^+/K^+ transporting, β 1 polypeptide	-0.08	-0.46	0.08	0.08
GNG13	guanine nucleotide binding protein (G protein), γ 13	0.05	-0.43	0.22	-0.06
RGS6	regulator of G-protein signaling 6	-0.02	-0.39	0.26	0.02
CACNA1E	Ca channel, voltage-dependent, R type, α 1E subunit	0.01	-0.39	0.07	-0.01
RGS9	regulator of G-protein signaling 9	-0.20	-0.38	0.18	0.30
CAMK2D	Ca/calmodulin-dependent protein kinase (CaM kinase) II δ	0.31	-0.36	0.08	-0.08
PRKAR2A	protein kinase, cAMP-dependent, regulatory, type II, α	-0.03	-0.36	0.03	1.83
ADRA1D	adrenergic, α -1D-, receptor	0.41	-0.36	0.07	-0.07
CACNA1B	Ca channel, voltage-dependent, N type, α 1B subunit (Probe ID:P84849)	0.10	-0.35	0.01	-0.01
FXYP2	FXYP domain containing ion transport regulator 2	-0.12	-0.31	0.14	0.11
GNG8	guanine nucleotide binding protein (G protein), γ 8	-0.14	-0.31	0.12	0.43

receptor gene TBXA2R was specifically inhibited in the A2 group (Table 9). Expression of peripheral tissue receptor NMUR1 gene, a neuromedin U (NMU), was inhibited in the A2 group, but also in the A1 group.

Significant accumulation of genes expressed less frequently and meeting the analytical criterion of $-1.0 < \text{Log ratio} \leq -0.5$ in the A2 group was observed in the “prostaglandin synthesis and regulation” pathway ($p=0.044$). Expression of PGD2 receptor gene PTGDR, prostaglandin D2 synthase PTGDS, and PGI2 receptor gene PTGIR was inhibited (Table 10).

Table 9 “G-protein coupled receptor” pathway

Name	Description	Log ratio			
		Cassis PS250		Water	
		Before A1	After A2	Before A3	After A4
Class A: Rhodopsin-like					
PTGDR	prostaglandin D2 receptor (DP)	-0.48	-0.79	0.36	0.43
OR5V1	olfactory receptor, family 5, subfamily V, member 1	0.25	-0.74	0.25	-0.30
HRH3	histamine receptor H3	0.18	-0.65	0.15	-0.17
OR10H2	olfactory receptor, family 10, subfamily H, member 2	-0.07	-0.60	0.23	0.07
OR11A1	olfactory receptor, family 11, subfamily A, member 1	-0.04	-0.57	0.24	0.04
NMUR1	neuromedin U receptor 1	-0.59	-0.54	0.39	0.44
GHSR	growth hormone secretagogue receptor	-0.15	-0.53	0.31	0.14
OR10A4	olfactory receptor, family 10, subfamily A, member 4	0.54	-0.50	0.09	-0.09
PTGIR	prostaglandin I2 (prostacyclin) receptor (IP) (Probe ID: 322426)	-0.07	-0.48	0.14	0.07
SSTR2	somatostatin receptor 2	0.17	-0.43	0.25	-0.20
GPR37	G protein-coupled receptor 37 (endothelin receptor type B-like)	0.00	-0.39	0.00	0.30
CCR10	chemokine (C-C motif) receptor 10	0.55	-0.39	0.03	-0.04
GPR42	G protein-coupled receptor 42	0.25	-0.37	0.18	-0.21
GPR12	G protein-coupled receptor 12	0.36	-0.37	0.13	-0.14
PTGIR	prostaglandin I2 (prostacyclin) receptor (IP) (Probe ID: 340848)	-0.17	-0.37	0.15	0.22
ADRA1D	adrenergic, alpha-1D-, receptor	0.41	-0.36	0.07	-0.07
MC1R	melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	-0.24	-0.35	0.20	0.23
CMKLR1	chemokine-like receptor 1	-0.13	-0.34	0.13	0.12
GPR25	G protein-coupled receptor 25	0.15	-0.33	0.08	-0.09
OPN3	opsin 3 (encephalopsin, panopsin)	-0.01	-0.32	0.07	0.01
GRPR	gastrin-releasing peptide receptor	0.38	-0.31	0.09	-0.10
TBXA2R	thromboxane A2 receptor	0.23	-0.30	0.09	-0.09
Others					
UTS2R	urotensin 2 receptor	0.12	-0.56	0.12	-0.13
LGR6	leucine-rich repeat-containing G protein-coupled receptor 6	0.27	-0.45	0.11	-0.12
SSTR2	somatostatin receptor 2	0.17	-0.43	0.25	-0.20
LTB4R2	leukotriene B4 receptor 2	0.22	-0.40	0.04	-0.05
ADRA1D	adrenergic, α-1D-, receptor	0.41	-0.36	0.07	-0.07
GPR56	G protein-coupled receptor 56 (Probe ID:P206280)	-0.26	-0.34	0.30	0.22
GRPR	gastrin-releasing peptide receptor	0.38	-0.31	0.09	-0.10
GPR56	G protein-coupled receptor 56 (Probe ID:P206284)	-0.38	-0.31	0.29	0.26

Table 10 “Prostaglandin synthesis and regulation” pathway

Name	Description	Log ratio			
		Cassis PS250		Water	
		Before A1	After A2	Before A3	After A4
PTGDR	prostaglandin D2 receptor (DP)	-0.48	-0.79	0.36	0.43
SCGB1A1	secretoglobin, family 1A, member 1 (uteroglobulin)	-0.01	-0.55	0.02	0.01
PTGDS	prostaglandin D2 synthase 21kDa (brain)	-0.17	-0.48	0.15	0.19
PTGIR	prostaglandin I2 (prostacyclin) receptor (IP) (Probe ID: P322426)	-0.07	-0.48	0.14	0.07
PTGIR	prostaglandin I2 (prostacyclin) receptor (IP) (Probe ID: P340848)	-0.17	-0.37	0.15	0.22

Discussion

Effects on body surface temperature

Changes in body surface temperature are regulated by changes in arteriolar diameter in microvascular networks. Arteriolar vasomotion, periodic variation in arteriolar diameter, affects microvascular mechanisms in the regulation of interstitial fluid formation. Effects of 36% bilberry (*Vaccinium myrtillus*) extract on arteriolar vasomotion in animal models [cheek pouch in anesthetized hamsters and skin fold window preparation (muscle) in unanesthetized hamsters] have been reported.¹⁸⁾ After intravenous administration, 36% bilberry extract (5 to 10 mg/kg) induced anesthetic-inhibited vasomotion in cheek pouch arterioles and terminal arterioles and increased vasomotion frequency in the skeletal muscle arteriolar networks. These findings indicate that bilberry extract can play a role in redistribution of microvascular blood flow by preventing or controlling interstitial fluid formation.

In the present study, body surface temperature was precisely measured by skin thermography before and after intake of the test product cassis juice, generating interesting results. In the cassis PS125 and PS250 groups (PS $\geq$ 125 mg), temperatures on the trunk (neck and shoulder) did not change significantly, but temperatures on the limbs (upper and lower limbs) significantly increased. Lower limb skin temperature was also increased in the PS250 group, with the percentage increase depending on the PS content.

Increase in limb skin temperature, which is affected by peripheral circulation, reflects peripheral vasodilatation and increase in peripheral blood flow. It is reasonable to consider that the increase in limb skin temperature in this study can be attributed to the effect of PS in cassis juice on the vascular system. Of course, since cassis juice contains various components, and this change was also correlated with the PP content, the possible involvement of non-PS components should also be considered.

Effects on the vascular system

There have been several reports on the vascular effects of cassis juice. In a study using rat thoracic aorta, endothelial cell-dependent vascular dilatation was induced after incubation with cassis concentrate.¹⁹⁾ This effect was inhibited by removal of endothelial cells, NO (nitric oxide) scavenger oxyhemoglobin, guanylate cyclase inhibitor, and NO synthase inhibitor L-NAME (NG-nitro-L-arginine methyl ester), indicating that it may be mediated by NO formation in the vascular endothelium. Delphinidin-3-rutinoside, an anthocyanin with smooth muscle relaxant effects, potentially relaxes the smooth muscles.²⁰⁾

Our previous clinical studies showed that blood pressure was mildly decreased after administration of antioxidants.²¹⁾ In the present review, cassis juice containing 125 to 250 mg of PS decreased systolic blood pressure by 4.8% and diastolic blood pressure by 5.9% after 2- to 4-week consumption, supporting the previous results.

In the present study, CAVI (cardio-ankle vascular index), goo, accelerated plethysmography, and FMD (flow-mediated dilation) were simultaneously assessed as measures of vascular function. CAVI, which reflects arteriosclerosis from the heart to the ankle, increases as sclerosis progresses. Unlike the traditional pulse wave velocity, which depends on blood pressure, CAVI is said to reflect blood vessel-specific stiffness, irrespective of blood pressure. ABI, which is useful in assessing arterial stenosis/occlusion in lower limbs, is used for differential diagnosis of intermittent claudication in the orthopedic field and for postoperative assessment following PTA (percutaneous transluminal angioplasty) or assessment of thromboangiitis obliterans (Buerger's disease) in the circulatory

field. An accelerated plethysmography is designed to determine the plethysmographic inflection point by the second derivative of photoplethysmogram (SDPTG). Vascular age is estimated based on data from approximately 600 Japanese people.¹¹⁾ In the cassis PS125 and PS250 groups, neither CAVI nor ABI changed significantly between pre- and post-consumption, whereas acceleration pulse waves tended to change significantly ($p=0.030$ in one-sided test), suggesting that acceleration pulse waves may be a more sensitive measure to assess vascular tone. Fingertip acceleration pulse waves reflect "organic arteriosclerosis" such as vascular wall calcification as well as "vascular wall tension." Short-term changes over 2 weeks are primarily attributed to changes in vascular wall tension.

FMD is a measure designed to assess endothelial function using the percentage change in ultrasonic vasodilatation caused by blood flow increase following the release of forearm arterial occlusion. FMD is negatively correlated with PWV (pulse wave velocity) and carotid IMT (intima media thickness) and positively correlated with ABI.²²⁾ In diabetic patients, hyperglycemia may result in vascular endothelial dysfunction at the arteriolar level. In an FMD study involving 71 patients with type 2 diabetes mellitus, FMD decreased in diabetic patients, with the decrease significantly correlated with progression of diabetic retinopathy.²³⁾ In the present study, the percentage increase in blood flow was significantly higher in the cassis groups than in the water group, indicating improvement of the endothelial function. "Time to the peak vascular diameter," which tends to be lower in young people and increase in the elderly,²⁴⁾ did not change significantly in the PS125 or PS250 group and improved in the water group, indicating that hydration is important for health promotion in those who are not adequately hydrated. "Change in vascular diameter following release," which is also called low-flow-mediated constriction (L-FMC), reflects vascular tone at rest.²⁵⁾

In microarray-based gene analysis, significant accumulation of less frequently expressed genes was observed in the "Ca regulation in the cardiac cell" pathway. Vascular smooth muscle is known to contract upon influx of Ca ions and dilate upon blockade of influx.²⁶⁾ In the cassis PS250 A2 group, overall inhibition, including L-type Ca channel and protein kinase A (PKA), was observed, compared with the water A4 group. In particular, Ca channel genes, which are involved in the influx of Ca ions and targeted by Ca antagonists,²⁷⁾ tended to be inhibited, primarily suggesting that PS or other components in cassis juice may act like Ca antagonists to dilate blood vessels and improve blood flow.

Secondarily, it is possible that vascular smooth muscle receptors may be involved. In the cassis A2 group, expression of PTGDR and TBXA2R, receptor genes for prostaglandin D2 (PGD2)²⁸⁾ and thromboxane A2 (TXA2),²⁹⁾ respectively, which have smooth muscle contracting and platelet-aggregating effects, was relatively strongly inhibited. These contribute to vasodilatation. Of adrenoceptors, β -receptors were hardly affected, but expression of α -receptor-related constitutive genes ($\alpha 1D$) tended to be inhibited, suggesting that some components in cassis juice may act like α -blockers to dilate blood vessels and thereby decrease blood pressure.

A third possible mechanism is involvement of the "oxidative stress" pathway. Endothelial cell-dependent vascular dilation may be mediated by NO formation in the vascular endothelium, including NO scavenger oxyhemoglobin, guanylate cyclase inhibitor, and NO synthase.¹⁹⁾ Among others, expression of SP1, which is involved in adaptive response and is a transcription factor for antioxidant enzyme production,³⁰⁾ and FOS³¹⁾ tended to be increased in the A2 group, suggesting that cassis juice may contribute to vasodilatation by enhancing oxidative stress defense and thereby playing a role in protection from oxidation by NO in the vascular endothelium.

The vascular endothelium plays an important role in vasoconstriction and vasodilatation, and in this reaction various kinds of signals are involved associated with mRNA expressions. It is easy to explain this phenomenon if we hypothesize that cassis juice may improve the function of vascular endothelial cells.

G-protein coupled receptor pathway

The G-protein coupled receptors (GPCR) include receptors related to vascular constriction and dilatation. Expression of prostaglandin (PG) receptor tended to be inhibited in the cassis A2 group as compared with the water A4 group. While PG receptor-mediated signals are involved in the control of vascular smooth muscle cell proliferation, maintenance of blood flow and pressure, and platelet aggregation, PG effectively controls vascular remodeling. Cassis juice tended to inhibit PG receptor-mediated signals, suggesting that it may be effective in controlling vascular remodeling, which is deeply involved in pathogenesis of lifestyle-related diseases such as arteriosclerosis.

In this study, the PTGDR gene, a PGD2 receptor, was inhibited. PGD2, which is produced by mast cells, plays an important role in late response via eosinotaxis and cytokine production. Decreased expression of PGD2 receptors, which are classified into CRTH2 (expressed in Th2, eosinophils, and basophils) and D-type prostanoid (DP), results in inhibition of eosinotaxis, although the eosinotaxis activity of PGD2 is relatively weak and between that of eotaxin3 and PAF.³²⁾ Cassis juice inhibited PGD2 receptor PTGDR gene, suggesting that it may be effective in alleviating bronchial asthma and allergic dermatitis by relaxing the vascular smooth muscle and improving the increase in vascular permeability.

Thromboxane A2 (TXA2) is a potent vasoconstrictor that has platelet-aggregating effects.²⁹⁾ TXA2 is reported to be related with circulatory diseases such as arteriosclerosis, myocardial infarction, and cerebral infarction. It may aggravate arteriosclerosis by stimulating the proliferation of vascular smooth muscle cells, as demonstrated by the fact that a TXA2 antagonist effectively shrank plaques, which are involved in arteriosclerosis, in an *in vivo* animal model.³³⁾ TXA2 signaling leads to secretory production of cell growth stimulants FGF (fibroblast growth factor), EDN1 (endothelin 1), and IRS2 (insulin receptor substrate 2) and secretion of cell adhesion factors in vascular smooth muscle cells, promoting the accumulation of leukocytes such as monocytes and T-cells at arteriosclerotic lesions. It also results in cytokine production (IL-6, IL-11), accelerating the cycle of inflammatory responses [check] in plaques and thus promoting arteriosclerosis. TXA2 antagonists may act as antiarteriosclerotics by inhibiting the increased expression of these proarteriosclerotic genes.

Neuromedin U (NMU) is a smooth muscle-contracting peptide isolated from the swine spinal cord. Its receptors NMU1R and NMU2R are primarily found in peripheral tissues and the central nervous system, especially the paraventricular nucleus (PVN) and hippocampal CA1 area, respectively.³⁴⁾ After intracerebroventricular administration in rats, NMU decreases food consumption, inhibits gastric acid secretion, and increases the activity level, oxygen consumption, blood pressure/heart rate, body temperature, and endocrine stress response (blood ACTH and corticosterone). In the cassis juice A2 group, NMU receptor-mediated signaling was inhibited, suggesting that cassis juice may be involved in inhibition of vascular smooth muscle contraction. However, since inhibition of this signaling was also observed before intake of cassis juice (A1), no definite conclusion can be drawn from this study alone.

Conclusion

In this study, the effects of cassis juice (2- to 8-week consumption) on the circulatory system and gene expression were evaluated in healthy women by assessing vascular function and microarray analysis. Cassis juice induced limb peripheral vasodilatation, increase in blood flow, and decrease in blood pressure. The microarray analysis showed inhibited expression of genes of α -adrenoceptor, thromboxane A2 receptor and Ca channel, and an inhibited pathway of Ca influx into vascular smooth muscle. Vascular endothelium plays a role in vasodilatation mediated by various gene expressions. It is suggested that cassis juice may improve the function of vascular endothelium.

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