Vol.3, No.9, 561-569 (2013)
http://dx.doi.org/10.4236/ojpm.2013.39075

Open Journal of Preventive Medicine

Are *Umezu* polyphenols in the Japanese plum (*Prunus mume*) protective against mild hypertension and oxidation? Evidence from a double-blind randomized placebo-controlled trial

Shigeki Takemura[1]*, Kouichi Yoshimasu[1], Kanae Mure[2], Jin Fukumoto[1], Nobuhiro Nishio[2], Naomi Kitano[2], Kunihiro Kishida[3], Fumiko Yano[3], Takahiko Mitani[3,4], Tatsuya Takeshita[2], Kazuhisa Miyashita[1]

[1]Department of Hygiene, School of Medicine, Wakayama Medical University, Wakayama, Japan;
[*]Corresponding Author: steakmmi@wakayama-med.ac.jp
[2]Department of Public Health, School of Medicine, Wakayama Medical University, Wakayama, Japan
[3]Faculty of Biology-Oriented Science and Technology, Kinki University, Wakayama, Japan
[4]Wakayama Industry Promotion Foundation, Wakayama, Japan

Received 8 October 2013; revised 9 November 2013; accepted 2 December 2013

ABSTRACT

Medications or lifestyle changes to prevent or improve hypertension often press considerable efforts on patients suffering from mild hypertension. Beverages including *Umezu* polyphenols (UP), polyphenols in Japanese plums, may help them to control their blood pressure. Healthy medical students, workers, and community dwellers who had some concerns about their blood pressure were randomized in a double-blind manner into UP ingesting and placebo groups. The first two samples (medical students and workers) were placed in a preliminary study, and based on the results from that study, and the dose of UP for the community dwellers was determined. All three groups were followed for 5 weeks, and blood pressure, as well as biochemical markers related to hypertension and atherosclerosis and self-perceived quality-of-life outcomes, was monitored during that period. Group × time interactions on blood pressure changes were examined. For the community dwellers, blood pressure changes did not significantly differ between the UP ingesting and placebo groups. And although studies of students and workers showed subtle differences in blood pressure among the UP and placebo groups, a dose-dependent effect of UP on decreasing blood pressure could not be confirmed. On the other hand, anti-oxidative effects of UP were confirmed especially among male drinkers who were community dwellers. For the community dwellers, self-perceived physical health significantly improved in those who ingested UP. In conclusion, UP might prevent oxidation. A longer monitoring period as well as a higher dose of UP might enable us to confirm effects of UP against hypertension.

Keywords: Hypertension; Prevention; Antioxidants; Japanese Plum; Polyphenol

1. INTRODUCTION

Hypertension is one of the major risk factors associated with cardio- and cerebrovascular diseases, and has a strong impact on global health. In Japan, the mortality rates from those diseases have declined since the second half of 1960s, which is considered to be partially due to the decline in blood pressure levels and the lower prevalence of hypertension during the years 1965-1990 [1]. However, it is estimated that there are still 40 million people with hypertension in Japan [2]. It is a serious concern that many of those with hypertension do not receive appropriate treatments for hypertension, especially among young and middle-aged people. When hypertension is defined as 140 mm Hg or more of systolic blood pressure and/or 90 mm Hg or more of diastolic blood pressure, almost 80% - 90% of Japanese in their twenties and thirties with hypertension are considered

not to have received any treatment [2]. Since most of the existing hypertension drugs often show adverse effects, their applications are limited, especially for those with mild hypertension who are approaching clinically dangerous levels. Therefore, those people should lower their blood pressures, at least by changing their lifestyles to healthier ones. Although low salt diets and exercise are proven to be effective against hypertension, following through with them is often burdensome for those people. Regarding dietary factors, high consumption of fruits and vegetables has been correlated with a decrease in cardiovascular disease [3-5]. However, intake of fruits and vegetables cannot be strongly recommended for patients with serious renal failure, since such diets may induce hyperkalemia in them [2]. Furthermore, excessive intake of fruits with high concentrations of glucose should be avoided by people with diabetes mellitus.

On the other hand, significant protective effects of antioxidants included in vegetables and fruits have been noted [6], while interventional trials of antioxidants have provided mixed results, some showing deleterious ones [6,7]. Among those antioxidants, polyphenols from tea, wine, grapes, berries and other plants have been shown to activate endothelial cells and to increase the formation of potent vasoprotective factors, including nitric oxide (NO) and endothelium-derived hyperpolarizing factors. In addition, polyphenols interfere with mechanisms that lead to inflammation, platelet aggregation, and endothelial apoptosis, and contribute to the prevention of endothelial dysfunction, which is known to play a central role in the pathogenesis of cardiovascular diseases [8-11].

Polyphenols are found abundantly in fruits such as plums or prunes. Interestingly, the prune was reported to have protective effects against cardiovascular diseases, inducing significant reductions of blood pressure and reducing serum total cholesterol and LDL cholesterol [12]. This effect was considered to be due to the antioxidant constituents of prunes [12]. If such constituents were added to common soft drinks, people with mild hypertension might successfully reduce their blood pressure without having to make considerable efforts. In this regard, Japanese plums, especially the well-known products of Wakayama Prefecture in Japan, have been reported to be effective for improving human health including cardiovascular conditions [13,14].

Based on the above-mentioned background, we conducted an interventional study with community dwellers using *Umezu* polyphenols (UP), *i.e.*, polyphenols extracted from Japanese plums (Japanese name, *Ume*; botanical name, *Prunus mume*), with assessments of blood pressure as well as some biochemical factors related to the progression of atherosclerosis. Furthermore, self-perceived physical and mental health conditions that are considered to affect hypertension were also evaluated.

2. METHODS

2.1. Subjects

(Preliminary Study)

Before initiating the primary study using a community sample, we conducted a preliminary study using a sample of workers (clerical workers, university faculty members and comedicals) and medical students in our principal study center from May to July in 2011. We recruited participants interested in prevention or control of their hypertension. Those who fulfilled the following exclusive criteria could not participate in the study: 1) those who ate 2 or more pickled *Ume* per day, 2) those under medication for hypertension, 3) those with serious somatic disorders including cerebrovascular disease, ischemic heart disease, cancer, and diabetes mellitus, 4) those who were pregnant or within 1 year after delivery, 5) those with ingestion difficulty, 6) those having night-shift work with night duty, and 7) those who could not participate in the periodical physical measurement.

Thus, a total of 48 workers (36 men and 12 women) and 20 students (10 men and 10 women) participated in the study. Workers and students were analyzed separately since age distribution between those 2 groups greatly differed. Workers were randomly divided into 4 groups whose sex and age distributions were adjusted to be as equal as possible. Thus, each group included 9 men and 3 women. Four kinds of beverages that contained 4 degrees of UP doses (0 (placebo), 50, 100, and 200 mg) were distributed to those 4 groups, respectively. These dosages were determined based on the study results of rats conducted in advance (data not shown).

A total of 20 students were divided into 2 groups, each of which included 5 men and 5 women. Two kinds of beverages (0 (placebo) or 200 mg of UP) were distributed to each of those groups, respectively. Workers and students were instructed to drink a can of beverage (190 ml) that contained a variety of UP concentrations every day for 5 weeks. Since 3 workers and 1 student were excluded for failing to meet the criteria, 45 workers (34 men and 11 women) and 19 students (10 men and 9 women) remained in the analysis. Mean ages of those participants were 43.5 (SD 11.4) years and 22.7 (SD 1.9) years, respectively.

(Community-Based Study)

After the safety of UP was confirmed among students in the preliminary study, a community-based study was conducted from October to November 2011 in Minabe Town, Wakayama Prefecture, Japan (population as of November 30, 2011: 14,150). The method for recruiting participants was the same one used in the preliminary study. That is, the participants in that town had some concerns about their hypertension but did not receive any medications for it. We invited the participants through an

announcement by the town office, and a total of 122 dwellers (67 males and 55 females) were registered. Due to the exclusion criteria and declination to participate after registration, 89 (47 men and 42 women) finally remained in the statistical analysis. The mean age of the participants was 52.1 (SD 8.1) years. This exclusion procedure is shown in **Figure 1**.

In the preliminary study, systolic blood pressure decreased by an average of 0.5 mm Hg with a standard deviation of 5.9 mm Hg in the placebo group while it decreased by 3.3 mm Hg in the 200 mg group. Based on these findings, we calculated the sample size in the community study as follows: to detect a difference of 3 mm Hg in the decrease of SBP between the placebo group and the 200 mg group, provided that the standard deviation of the SBP decrease in the placebo group is 3 mm Hg, the sample size for each group should be

$$2\times 32/3\times\left(1.6449+0.8416\right)^2=37.09\cdots\fallingdotseq 38,$$

where alpha is 0.05 (two sides) and beta is 0.20.

As shown above, we successfully included a sufficient number of subjects for analysis.

The participants were randomly divided into 2 groups whose sex and age distributions were adjusted to be as equal as possible. Two kinds of beverages containing 0 (placebo) or 200 mg of UP were distributed to each of those groups, respectively. This UP content was determined based on the results of the preliminary study. All of the community dwellers were instructed to drink a can of the beverage (190 ml) that contained either UP or a placebo every day for 5 weeks.

In all cases described above, neither the examiners nor subjects knew which kind of beverages were being drunk (*i.e.*, double-blind design) throughout the study period. The current study was approved by the Institutional Review Board of Wakayama Medical University.

2.2. Extraction of Polyphenols

Fruit samples of *Prunus mume* cv. "Nanko" were randomly collected from one fixed tree grown at the experimental orchard of the Laboratory of Japanese Plum, Fruit Tree Experiment Station, Wakayama Research Center of Agriculture, Minabe Town, from 2006 to 2008, and stored in polyethylene bags at −20˚C until analysis.

Since details surrounding the determination of total polyphenols and the preparation of polyphenol fractions through a biochemical experimental system are beyond the current study's scope, they will be described elsewhere. In brief, the Folin-Ciocalteu method with gallic acid as a standard was used for the determination of total polyphenols, and a batch method was adopted for the preparation of polyphenol fractions.

UP was found to show many chromatographically isolated peaks. Our experimental analysis clarified that the UP was chemically composed of hydroxycinnamic acid derivatives. Four aglycones were identified as cis-*p*-coumaric acid, trans-*p*-coumaric acid, caffeic acid, and ferulic acid. Those aglycones are bound to various kinds of organic acids or sugars, and exist as the ingredients of UP. The extracted UP was sent to a beverage company, and added into each can of the beverage.

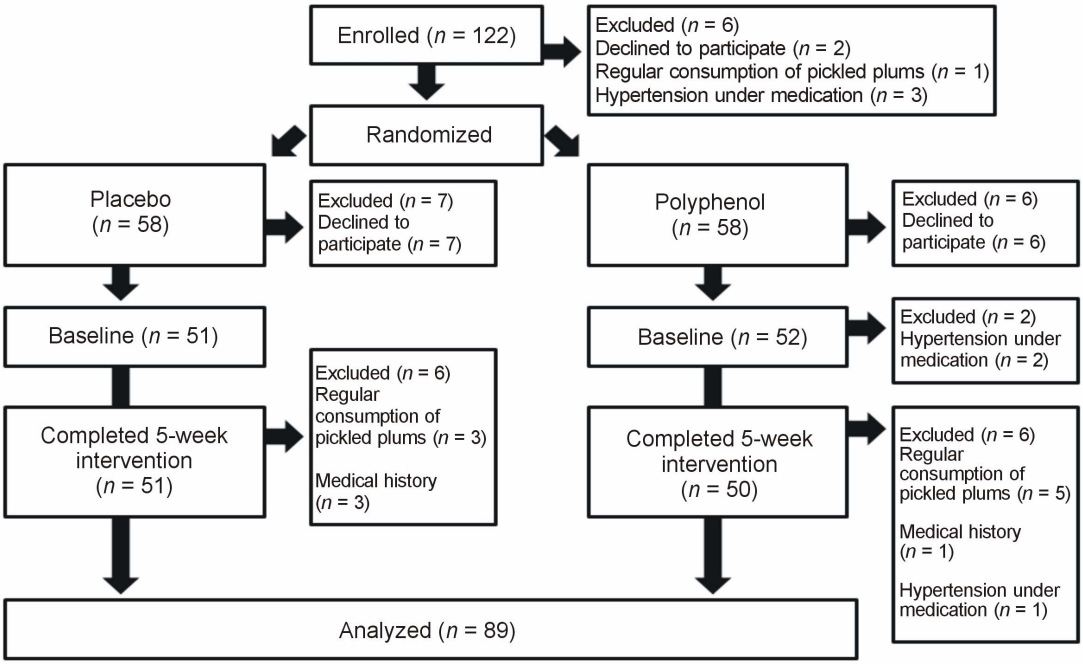

Figure 1. Flowchart of subject enrollment, intervention and analysis.

2.3. Blood Pressure, Physical Measurements, Lifestyle Factors, and Biochemical Profiles

Workers and students as well as community dwellers were asked to measure their blood pressure (systolic/diastolic) early in the morning every day; after urinating and/or defecating, and before eating breakfast, systolic and diastolic blood pressure of the right arm were measured with an automatic sphygmomanometer (HEM-747IC or HEM-7080IT, Omron, Kyoto, Japan), twice in a sitting position with arms supported at the right atrium level. When the difference of systolic blood pressure between the first measure and the second measure was 10 mm Hg or greater, blood pressure was measured one more time (a total of three times). Blood pressure as well as body weight and waist circumference were measured at the study center in the morning (8:30 a.m. to 0:00 p.m.) once a week during the study period for both workers and students, and at the community office at baseline and 1, 3 and 5 weeks later for community dwellers. Height was measured at baseline only, and hip circumference was measured at baseline and 5 weeks later. At the study center, systolic and diastolic pressure were measured with an autonomic sphygmomanometer (HEM-907, Omron, Kyoto Japan) by research staff (a trained physician or nurse), twice in a sitting position with arms supported at the right atrium level. When the difference of systolic blood pressure between the first measure and the second measure was 10 mm Hg or greater, blood pressure was measured one more time (a total of three times).

Lifestyle factors such as smoking, drinking, sleep condition, work, physical exercise and medical history were confirmed with a questionnaire including relevant items on the first day of the study period for both workers/students and community dwellers. Biochemical profiles related to atherosclerosis such as LDL/HDL cholesterol or triglyceride were measured by blood sample on the first and last day of the study period. In the community dwellers, urine 8-isoprostane as a marker of oxidative stress [15,16] was assessed 3 times (1, 3 and 5 weeks after beginning the study) during the period with adjustments for creatinine from urine samples by using urine 8-isoprostane ELISA kit (Oxford Biomedical Research, MI, USA). A higher concentration of 8-isoprostane indicates more severe oxidative stress. To evaluate changes in subjective health conditions, subjects' perceived mental and physical QOL was assessed by SF-8 [17]. A higher SF-8 score indicates a better mental/physical condition. In Japanese people, the average score and the standard deviation are 50 points and 10 points, respectively.

2.4. Statistical Analysis

We calculated the mean value of subject-measured blood pressure for each day, including all measured values, then we defined the blood pressure at the beginning of the study (week 0) as the mean value of the blood pressure measured on the first day, and the blood pressure at each week (weeks 1-5) as the mean value of all seven days of the week, excluding the data for week 0. We also calculated the mean value of staff-measured blood pressure, for each week (weeks 1-5, if available) as well as the beginning of the study period (week 0). These blood pressure values were chosen as outcome variables. Two-way analysis of variance (ANOVA) was conducted for comparing the variation of blood pressure between/among the groups during the study period. In each group, time-dependent repeated-measure analyses and Dunnett's test were performed (vs data at baseline (week 0)).

To adjust the skewed distribution of 8-isoprostane to a normal one, 8-isoprostane values were translated into logarithms. The following community dwellers were excluded from the analyses for 8-isoprostane: current smokers, those who received any medications, and those whose urine was not gathered at the examination site in the community office. Analyses of 8-isoprostane were also conducted on the community dwellers restricted to male drinkers, since alcohol consumption is considered to be high from autumn to winter. Paired *t*-test was conducted for comparisons of the volume of 8-isoprostane before and after ingesting UP or placebo. *p*-values (two-sided) less than 0.05 were considered statistically significant. All analyses were conducted using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC, USA), or SPSS Statistics software 20 (IBM, Chicago, IL, USA).

3. RESULTS

Figure 2 shows changes in systolic/diastolic blood pressure of the students measured by research staff. Diastolic blood pressure of the students who ingested 200 mg of UP daily showed a significantly decreased pattern, compared to the placebo group, during the first half of the study period (time × group interaction, $p = 0.02$). During the second half, however, those who ingested UP showed somewhat higher systolic and diastolic blood pressures than the placebo group.

Changes of blood pressure in workers measured by research staff are presented in **Figure 3**. Magnitudes of decrease in blood pressure seem most apparent in those who ingested 50 mg of UP per day but did not reach statistical significance.

Figure 4 shows changes in blood pressure of the community dwellers measured by research staff. A decrease in systolic and diastolic blood pressure was observed in both groups who ingested UP or placebo, with almost the same degree of decrease, and the differences were far from being statistically significant (time × group

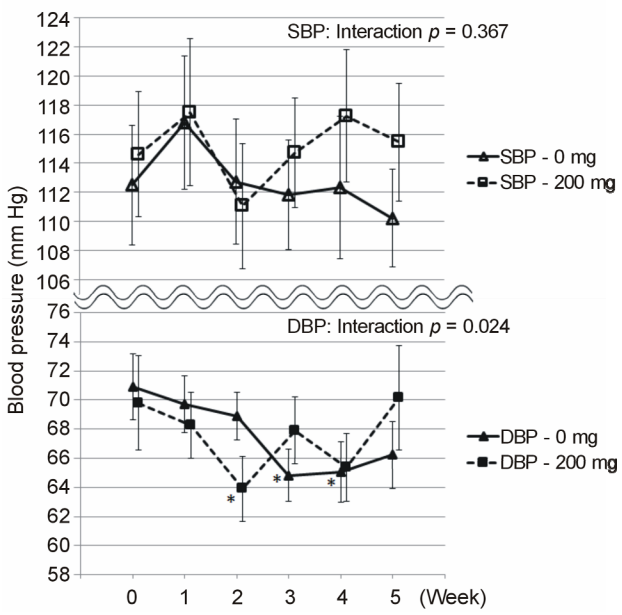

Figure 2. Effect of *Umezu* polyphenols on blood pressure measured at examination site among students. Shown are the mean values of SBP and DBP among subjects between week 0 and week 12. Error bars denote one standard error for corresponding mean values. Time-dependent repeated-measure analyses and Dunnett's test were performed (vs data in week 0). The interaction between intervention and time was also evaluated ($^{*}p < 0.05$; $^{\#}p < 0.10$). SBP, systolic blood pressure; DBP, diastolic blood pressure.

interaction, $p = 0.75$ for systolic blood pressure, and 0.53 for diastolic blood pressure). Changes in the self-measured blood pressure of students, workers, and community dwellers, and between the UP group and the placebo group, showed almost the same pattern as those measured by the research staff (data not shown).

Table 1 shows biochemical markers related to atherosclerosis among community dwellers at the start and end points of the study period. Platelet counts, plasma glucose levels and hemoglobin A1c significantly increased in those who ingested placebos, while serum triglyceride as well as leukocyte and erythrocyte counts significantly increased in those who ingested UP, with significant or marginally significant interactions. Similar results were observed when the analysis was limited to those whose systolic blood pressure was 140 mm Hg or higher, and/or diastolic blood pressure was 90 mm Hg or higher (data not shown).

Table 2 shows self-perceived mental and physical health among the community dwellers, assessed at the start and end points of the study period with SF-8. Self-perceived physical health was significantly improved in those who ingested UP, while those who ingested placebo reported significantly improved mental health at the end of the study.

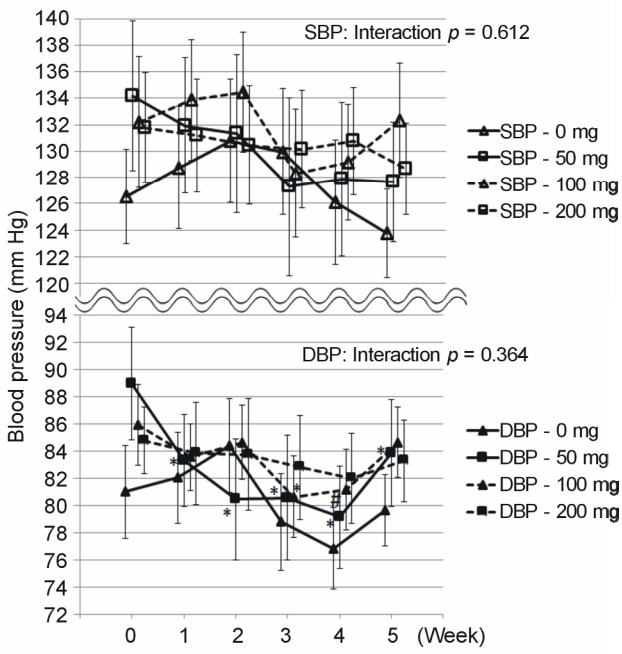

Figure 3. Dose-response effect of *Umezu* polyphenols on blood pressure measured at examination site among office workers. Shown are the mean values of SBP and DBP among subjects between week 0 and week 12. Error bars denote one standard error for corresponding mean values. Time-dependent repeated-measure analyses and Dunnett's test were performed (vs data in week 0). The interaction between intervention and time was also evaluated ($^{*}p < 0.05$; $^{\#}p < 0.10$). SBP, systolic blood pressure; DBP, diastolic blood pressure.

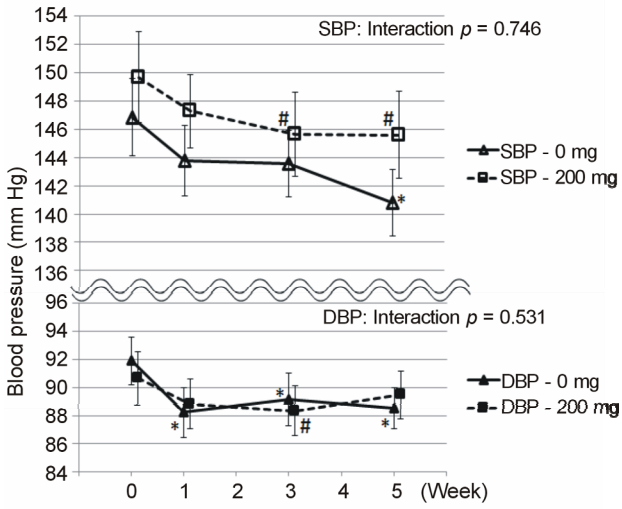

Figure 4. Effect of *Umezu* polyphenols on blood pressure measured at examination site among community-dwelling people. Shown are the mean values of SBP and DBP among subjects between week 0 and week 12. Error bars denote one standard error for corresponding mean values. Time-dependent repeated-measure analyses and Dunnett's test were performed (vs data in week 0). The interaction between intervention and time was also evaluated ($^{*}p < 0.05$; $^{\#}p < 0.10$). SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 1. Changes in hematology and biochemistry among community-dwelling people.

	Placebo (n = 45)					Polyphenols (n = 44)					Interaction $p^{\dagger}$
	Week 0		Week 5		p^{*}	Week 0		Week 5		p^{*}	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		
Hematology											
Leukocyte ($10^{9}\cdot l^{-1}$)	5.90	1.84	5.94	1.71	0.776	5.63	1.54	6.04	1.99	0.005	0.085
Erythrocyte ($10^{12}\cdot l^{-1}$)	4.81	0.45	4.80	0.43	0.702	4.67	0.38	4.75	0.41	0.018	0.053
Hemoglobin ($g\cdot l^{-1}$)	144.4	18.3	143.7	18.3	0.440	143.0	13.4	145.1	14.2	0.028	0.039
Hematocrit (%)	43.0	4.3	43.1	4.5	0.655	42.5	3.3	43.2	3.7	0.004	0.117
Platelet ($10^{9}\cdot l^{-1}$)	230.7	53.4	245.2	61.5	0.000	246.4	59.0	247.8	58.7	0.641	0.005
Biochemistry											
AST ($IU\cdot l^{-1}$)	26.9	10.0	25.6	10.5	0.069	26.4	10.4	28.0	17.3	0.529	0.238
ALT ($IU\cdot l^{-1}$)	27.1	18.6	25.5	19.4	0.127	28.1	22.2	27.3	25.9	0.631	0.769
GGT ($IU\cdot l^{-1}$)	43.5	45.8	42.8	53.9	0.805	36.9	37.3	33.0	27.4	0.156	0.443
Creatinine ($mg\cdot dl^{-1}$)	0.706	0.155	0.718	0.147	0.126	0.700	0.128	0.711	0.125	0.156	0.852
Uric acid ($mg\cdot dl^{-1}$)	5.3	1.5	5.0	1.4	0.000	5.2	1.5	5.0	1.4	0.033	0.138
Triglyceride ($mg\cdot dl^{-1}$)	102.1	55.2	105.1	54.0	0.724	101.1	44.8	129.8	104.0	0.035	0.1004
HDL cholesterol ($mg\cdot dl^{-1}$)	62.2	13.6	61.9	13.9	0.674	60.3	16.0	59.7	16.2	0.535	0.807
LDL cholesterol ($mg\cdot dl^{-1}$)	123.6	30.1	123.0	29.5	0.786	132.8	28.5	133.1	25.6	0.712	0.948
LDL/HDL ratio	2.1	0.8	2.1	0.8	0.960	2.3	0.7	2.4	0.7	0.920	0.947
Immunoreactive insulin ($\mu U\cdot ml^{-1}$)	5.6	3.8	6.4	4.1	0.134	7.2	5.9	8.1	7.2	0.208	0.868
Fasting glucose ($mg\cdot dl^{-1}$)	95.8	28.7	98.1	29.8	0.029	96.2	16.6	96.5	15.4	0.796	0.197
Hemoglobin A1c (%)	5.37	0.91	5.45	0.94	0.000	5.34	0.47	5.38	0.45	0.019	0.055

AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *Paired *t*-test was conducted to compare the date between Week 0 and Week 5 by intervention. †Two-way analysis of variance was conducted to evaluate the interaction between intervention and time.

Table 2. Effects of *Umezu* polyphenols on physical and mental component summaries in SF-8 among community-dwelling people.

	Placebo (n = 45)					Polyphenols (n = 44)					Interaction $p^{\dagger}$
	Week 0		Week 5		p^{*}	Week 0		Week 5		p^{*}	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		
PCS	50.49	5.92	50.58	5.05	0.911	47.34	7.42	50.29	5.29	0.011	0.035
MCS	47.90	6.38	49.84	6.06	0.040	49.42	7.35	48.94	6.23	0.683	0.105

PCS, physical component summary; MCS, mental component summary. *Paired *t*-test was conducted (Week 5 vs Week 0). †The interaction between intervention and time was evaluated.

Changes of 8-isoprostane during the study period among community dwellers are shown in **Table 3**. The concentration of 8-isoprostane significantly increased at the end of the study in the male placebo group, indicating the increase of oxidative stress, while that of those who ingested UP did not show any substantial change. Analyses restricted to male drinkers showed almost the same pattern as described above (data not shown). Women showed no material differences between UP and placebo groups, in both of which 8-isoporstane concentrations did not significantly change.

4. DISCUSSION

The current double-blind randomized controlled trial (RCT) provided preliminary evidence on the association between ingestion of UP with a decreased risk of hypertension. The findings regarding the beneficial effects of UP on hypertension are limited. That is, dose-effect relationships were not observed in the preliminary study. Furthermore, the RCT conducted in the community dwellers did not provide any significant differences in blood pressure changes between those who ingested UP and those who did not, while the systolic blood pressure

Table 3. Changes of 8-isoprostane before and after ingesting *Umezu* polyphenols between placebo and polyphenols ingesting groups.

Men						Women					
Placebo (n = 19)		p^*	Polyphenols (n = 15)		p^*	Placebo (n = 18)		p^*	Polyphenols (n = 14)		p^*
Before	After		Before	After		Before	After		Before	After	
2.8 (0.8)	3.4 (0.9)	0.04	2.8 (0.9)	3.0 (0.6)	0.49	2.3 (0.6)	2.5 (1.2)	0.53	2.3 (1.0)	2.7 (0.6)	0.29

Figures are means (SDs) transformed into logarithms. *Paired *t*-test was conducted (After vs Before).

of the both groups after the RCT had either statistically or marginally significant decreases compared to their values at baseline. In addition, biochemical changes between UP and placebo groups during the study period were somewhat puzzling.

However, the antioxidant capacity of those who ingested UP did not change, whereas that of the placebo group significantly decreased in men. Basu *et al*. [18] showed that cranberry juice, rich in polyphenols, increased plasma antioxidant capacity in women with metabolic syndrome, supporting our findings. In that study [18], 8 weeks of cranberry juice consumption reportedly caused no significant improvements in blood pressure. Another double-blind placebo-controlled trial [19] showed that red wine polyphenols did not lower peripheral or central blood pressure in patients with borderline hypertension. Negative reports of polyphenols showing no decrease in blood pressure were consistent with our findings.

As for the antioxidant capacity of polyphenols, measurement of 8-isoplantane, an abundant F(2)-isoprostane formed *in vivo*, may provide sensitive biochemical endpoints for the assessment of the oxidant status of patients and the efficacy of antioxidant therapies [20,21]. This biomarker has been shown to be significantly reduced by polyphenols [22,23]. Nemzer *et al*. [23] demonstrated that serum values for 8-iso-PGF2-alpha and advanced oxidation protein products decreased significantly at 1 hour post-intake of polyphenol-rich beverage, implying the need to test the cumulative effects of repeated intakes of a polyphenol-rich beverages. Our findings support the antioxidant effects of UP with repeated intakes of such beverages. Among male subjects, the placebo group showed a nearly significant change with an increase in the oxidative status, while those who ingested UP managed to maintain their baseline level of 8-isoplantane. These findings suggest that UP might have effects that prevent oxidation although it remains unclear why the oxidant level of the placebo group increased during the study period. If this change in the placebo group was due to the seasonal change, we could explain that the change which could have manifested without UP ingestion was prevented by UP antioxidative effects in the UP group. Interestingly, self-perceived physical health significantly improved in the UP ingesting group, which would suggest that the actual physical effects of UP are associated with decreased levels of oxidant status. On the other hand, improvement in self-perceived mental health in the placebo group may suggest some placebo effects.

Regarding the biochemical markers related to atherosclerosis, platelet counts and plasma glucose levels did not increase in those who ingested UP, and lipid profiles (HDL cholesterol, LDL cholesterol and triglyceride) did not show significant decreases. Those factors are considered to promote blood aggregation that is strongly associated with atherosclerosis. Thus, UP might have effects that prevent the progress of atherosclerosis through those biochemical factors, in people with mild hypertension.

Several reasons may explain why the effects of UP against blood pressure were limited. First, it has been suggested that the beneficial effects of polyphenols in the prevention of hypertension result from their complex influence on the NO balance in the cardiovascular system [24]. However, it may take more time for those effects to emerge compared to the antioxidant effects. It could be speculated that the preventive effects against mild hypertension can be observed if the observation period is prolonged for more than 5 weeks. Hence, the authors are now conducting a 12-week RCT to reveal long-term effects of UP. Second, the dose of UP may not be sufficient to decrease blood pressure. Since UP's ability to decrease blood pressure was observed even in those who ingested 50 mg of UP in the preliminary study, the volume of UP used in the community-based study was determined as 200 mg. However, dose-response effects were observed among rats (data not shown). Administering higher doses of UP to humans should be considered in order to achieve hypotensive effects. Therefore, the authors are administering 800 mg of UP or placebo daily to study subject in the above-mentioned 12-week RCT. Third, it should be noted that while self-perceived mental health significantly improved in the placebo group, it did not in those who ingested UP. Although it is unclear why this occurred, psychological factors might have been involved in the decrease of blood pressure in the placebo group, diminishing the differences between UP and placebo groups. Fourth, involvement of type 2 error should be taken into account. Although the analysis of blood pressure was conducted with a sufficient sample size, valid urine samples were

not collected from all subjects, which might have reduced the power to detect antioxidative effects of UP.

Based on the current findings and taking our study limitations into consideration, a further investigation with a longer period and a higher dose of UP is in progress now.

5. CONCLUSION

In conclusion, UP's ability to decrease blood pressure is no more remarkable than that of a placebo. However, preventive effects against oxidative stress were observed in those who ingested UP, especially in male drinkers. Those findings suggest that a longer period as well as a higher dose of UP are needed to confirm the UP effects on decreasing blood pressure.

6. ACKNOWLEDGEMENTS

This project was supported by research grants from by the City Area Program (Ministry of Education, Culture, Sports, Science and Technology, Japan) and Sapporo Beverage Co., Ltd., Tokyo, Japan.

A part of this study was presented in the 71st Annual Meeting of the Japanese Society of Public Health on October 24-26, 2012.

REFERENCES

[1] Ueshima, H. (2007) Explanation for the Japanese paradox: Prevention of increase in coronary heart disease and reduction in stroke. *Journal of Atherosclerosis and Thrombosis*, **14**, 278-286. http://dx.doi.org/10.5551/jat.E529

[2] Japanese Society of Hypertension (2011) Guidebook for medical specialists of hypertension. 2nd Revision, Shindantochiryo Press, Tokyo. (in Japanese)

[3] Ness, A.R. and Powles J.W. (1997) Fruit and vegetables, and cardiovascular disease: A review. *International Journal of Epidemiology* **26**, 1-13. http://dx.doi.org/10.1093/ije/26.1.1

[4] Ness, A., Egger, M. and Powles, J. (1999) Fruit and vegetables and ischemic heart disease: Systematic review or misleading meta-analysis? *European Journal of Clinical Nutrition*, **53**, 900-904. http://dx.doi.org/10.1038/sj.ejcn.1600856

[5] Sasazuki, S., Fukuoka Heart Study Group (2001) Case-control study of nonfatal myocardial infarction in relation to selected foods in Japanese men and women. *Japanese Circulation Journal*, **65**, 200-206. http://dx.doi.org/10.1253/jcj.65.200

[6] Schiffrin, E.L. (2010) Antioxidants in hypertension and cardiovascular disease. *Molecular Interventions*, **10**, 354-362. http://dx.doi.org/10.1124/mi.10.6.4

[7] Rapola, J.M., Virtamo, J., Ripatti, S., Huttunen, J.K., Albanes, D., Taylor, P.R., *et al.* (1997) Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet*, **349**, 1715-1720. http://dx.doi.org/10.1016/S0140-6736(97)01234-8

[8] Curin, Y. and Andriantsitohaina, R. (2005) Polyphenols as potential therapeutical agents against cardiovascular diseases. *Pharmacological Reports*, **57**, 97-107.

[9] Schini-Kerth, V.B., Etienne-Selloum, N., Chataigneau, T. and Auger, C. (2011) Vascular protection by natural product-derived polyphenols: *In vitro* and *in vivo* evidence. *Planta Medica*, **77**, 1161-1167. http://dx.doi.org/10.1055/s-0030-1250737

[10] Galleano, M., Pechanova, O. and Fraga, C.G. (2010) Hypertension, nitric oxide, oxidants, and dietary plant polyphenols. *Current Pharmaceutical Biotechnology*, **11**, 837-848. http://dx.doi.org/10.2174/138920110793262114

[11] Michalska, M., Gluba, A., Mikhailidis, D.P., Nowak, P., Bielecka-Dabrowa, A., Rysz, J., *et al.* (2010) The role of polyphenols in cardiovascular disease. *Medical Science Monitor*, **16**, 110-119.

[12] Ahmed, T., Sadia, H., Batool, S., Janjua, A. and Shuja, F. (2010) Use of prunes as a control of hypertension. *Journal of Ayub Medical College Abbottabad*, **22**, 28-31.

[13] Enomoto, S., Yanaoka, K., Utsunomiya, H., Niwa, T., Inada, K., Deguchi, H., *et al.* (2010) Inhibitory effects of Japanese apricot (*Prunus mume* Siebold et Zucc.; *Ume*) on *Helicobacter pylori*-related chronic gastritis. *European Journal of Clinical Nutrition*, **64**, 714-719. http://dx.doi.org/10.1038/ejcn.2010.70

[14] Utsunomiya, H., Takekoshi, S., Gato, N., Utatsu, H., Motley, E.D., Eguchi, K., *et al.* (2002) Fruit-juice concentrate of Asian plum inhibits growth signals of vascular smooth muscle cells induced by angiotensin II. *Life Sciences*, **72**, 659-667. http://dx.doi.org/10.1016/S0024-3205(02)02300-7

[15] Comporti, M., Arezzini, B., Signorini, C., Vecchio, D. and Gardi, C. (2009) Oxidative stress, isoprostanes and hepatic fibrosis. *Histology and Histopathology*, **24**, 893-900.

[16] Ohashi, N., Urushihara, M. and Kobori, H. (2009) Activated intrarenal reactive oxygen species and renin angiotensin system in IgA nephropathy. *Minerva Urologica e Nefrologica*, **61**, 55-66.

[17] Fukuhara, S. and Suzukamo, Y. (2004) Manual of the SF-8 Japanese version. Institute for Health Outcomes & Process Evaluation Research, Kyoto. (in Japanese)

[18] Basu, A., Betts, N.M., Ortiz, J., Simmons, B., Wu, M. and Lyons, T.J. (2011) Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. *Nutrition Research*, **31**, 190-196. http://dx.doi.org/10.1016/j.nutres.2011.02.003

[19] Botden, I.P., Draijer, R., Westerhof, B.E., Rutten, J.H., Langendonk, J.G., Sijbrands, E.J., *et al.* (2012) Red wine polyphenols do not lower peripheral or central blood pressure in high normal blood pressure and hypertension. *American Journal of Hypertension*, **25**, 718-723. http://dx.doi.org/10.1038/ajh.2012.25

[20] Morrow, J.D. (2006) The isoprostanes—Unique products of arachidonate peroxidation: Their role as mediators of oxidant stress. *Current Pharmaceutical Design*, **12**, 895-902. http://dx.doi.org/10.2174/138161206776055985

[21] Patrignani, P. and Tacconelli, S. (2005) Isoprostanes and other markers of peroxidation in atherosclerosis. *Biomarkers*, **10**, S24-S29. http://dx.doi.org/10.1080/13547500500215084

[22] Wendeburg, L., de Oliveira, A.C., Bhatia, H.S., Candelario-Jalil, E. and Fiebich, B.L. (2009) Resveratrol inhibits prostaglandin formation in IL-1beta-stimulated SK-N-SH neuronal cells. *Journal of Neuroinflammation*, **14**, 26. http://dx.doi.org/10.1186/1742-2094-6-26

[23] Nemzer, B.V., Rodriguez, L.C., Hammond, L., Disilvestro, R., Hunter, J.M. and Pietrzkowski, Z. (2011) Acute reduction of serum 8-iso-PGF2-alpha and advanced oxidation protein products *in vivo* by a polyphenol-rich beverage; a pilot clinical study with phytochemical and *in vitro* antioxidant characterization. *Nutrition Journal*, **15**, 67. http://dx.doi.org/10.1186/1475-2891-10-67

[24] Pechanova, O., Bernatova, I.I., Babal, P., Martinez, M.C., Kysela, S., Stvrtina, S., *et al*. (2004) Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. *Journal of Hypertension*s, **22**, 1551-1559. http://dx.doi.org/10.1097/01.hjh.0000133734.32125.c7

ABBREVIATIONS AND ACRONYMS

UP, *Umezu* polyphenol.
SBP, systolic blood pressure.
DBP, diastolic blood pressure.
AST, aspartate transaminase.
ALT, alanine transaminase.
GGT, gamma-glutamyltransferase.
HDL, high-density lipoprotein.
LDL, low-density lipoprotein.
SD, standard deviation.
PCS, physical component summary.
MCS, mental component summary.
NO, nitric oxide.
ANOVA, analysis of variance.
RCT, randomized controlled trial.

