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

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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, 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 *Ume* 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
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.
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 day of 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. Blood pressure measured on the first day, and the 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 interaction, \( p = 0.83 \)).
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 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.

**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.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 45)</th>
<th>Polyphenols (n = 44)</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 5</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocyte (10^9·l^{-1})</td>
<td>5.90</td>
<td>1.84</td>
<td>5.94</td>
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<tr>
<td>Erythrocyte (10^{12}·l^{-1})</td>
<td>4.81</td>
<td>0.45</td>
<td>4.80</td>
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<tr>
<td>Hemoglobin (g·l^{-1})</td>
<td>144.4</td>
<td>18.3</td>
<td>143.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.0</td>
<td>4.3</td>
<td>43.1</td>
</tr>
<tr>
<td>Platelet (10^9·l^{-1})</td>
<td>230.7</td>
<td>53.4</td>
<td>245.2</td>
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<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU·l^{-1})</td>
<td>26.9</td>
<td>10.0</td>
<td>25.6</td>
</tr>
<tr>
<td>ALT (IU·l^{-1})</td>
<td>27.1</td>
<td>18.6</td>
<td>25.5</td>
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<tr>
<td>GGT (IU·l^{-1})</td>
<td>43.5</td>
<td>45.8</td>
<td>42.8</td>
</tr>
<tr>
<td>Creatinine (mg·dl^{-1})</td>
<td>0.706</td>
<td>0.155</td>
<td>0.718</td>
</tr>
<tr>
<td>Uric acid (mg·dl^{-1})</td>
<td>5.3</td>
<td>1.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Triglyceride (mg·dl^{-1})</td>
<td>102.1</td>
<td>55.2</td>
<td>101.5</td>
</tr>
<tr>
<td>HDL cholesterol (mg·dl^{-1})</td>
<td>62.2</td>
<td>13.6</td>
<td>61.9</td>
</tr>
<tr>
<td>LDL cholesterol (mg·dl^{-1})</td>
<td>123.6</td>
<td>30.1</td>
<td>123.0</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>2.1</td>
<td>0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Immunoreactive insulin (µU·ml^{-1})</td>
<td>5.6</td>
<td>3.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Fasting glucose (mg·dl^{-1})</td>
<td>95.8</td>
<td>28.7</td>
<td>98.1</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>5.37</td>
<td>0.91</td>
<td>5.45</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 45)</th>
<th>Polyphenols (n = 44)</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 5</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>PCS</strong></td>
<td>50.49</td>
<td>5.92</td>
<td>50.58</td>
</tr>
<tr>
<td><strong>MCS</strong></td>
<td>47.90</td>
<td>6.38</td>
<td>49.84</td>
</tr>
</tbody>
</table>

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-isoprostane 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...
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 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 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

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

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n = 19)</td>
<td>Polyphenols (n = 15)</td>
<td>Placebo (n = 18)</td>
<td>Polyphenols (n = 14)</td>
</tr>
<tr>
<td></td>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
</tr>
<tr>
<td><strong>Before</strong></td>
<td>2.8</td>
<td>3.4</td>
<td>2.8</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>(0.8)</strong></td>
<td>(0.9)</td>
<td>(0.9)</td>
<td><strong>0.04</strong></td>
<td>0.49</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td>2.8</td>
<td>3.0</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td><strong>(0.9)</strong></td>
<td>(0.6)</td>
<td>(1.2)</td>
<td><strong>0.53</strong></td>
<td>0.29</td>
</tr>
</tbody>
</table>

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

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REFERENCES


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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.