Extract of Wine Phenolics Improves Aortic Biomechanical Properties in Stroke-Prone Spontaneously Hypertensive Rats (SHRSP)

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(Received August 24, 1998)

Summary We studied the effect of the extract of wine phenolics (EWP) on blood pressure, vasorelaxing activity and aortic biomechanical properties in stroke-prone hypertensive rats (SHRSP). Thirty-six 4-week-old male SHRSP/Izm rats were divided into 6 equal groups fed one of the following 6 diets: A control diet (plain laboratory diet), the control diet substituted with 0.5 or 1.0% polyphenolic compounds derived from the extract of apple phenolics (EAP), the control diet substituted with 0.5 or 1.0% polyphenolic compounds derived from the extract of tea phenolics (ETP), or the control diet along with drinking water containing 1.0% polyphenolic compounds derived from EWP. Systolic blood pressure (SBP) and body weight (BW) were checked once a week. At the end of the 8th week of feeding, all of the rats were sacrificed and the heart weight and aortic biomechanical properties were measured. The relaxation effect of the addition of EWP on endothelium-intact aortic rings precontracted with prostaglandin (PG) F2\(\alpha\) was also measured. Only EWP, not EAP or ETP, significantly lowered the SBP values as compared with the control group at the 4th, 7th and 8th weeks of feeding (p<0.05). The heart weight and ventricular weight, expressed as the percentage of BW, were significantly lower in the EWP group than in the control group (p<0.05). The aortic maximum stress was significantly increased (p<0.05), and the aortic incremental elastic modulus was significantly reduced (meaning higher elasticity) (p<0.001) in the EWP group as compared with the control group. The aortic rings showed concentration-dependent relaxation induced by EWP, and the relaxation was significantly greater than that induced by a commercial red wine preparation. In

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conclusion, EWP attenuated the elevation of blood pressure in SHRSP possibly by increasing the vasorelaxation activity. The aortic fragility and elasticity were also improved in EWP-fed SHRSP.

**Key Words** stroke-prone spontaneously hypertensive rats, wine polyphenol, aorta, biomechanics, elasticity

Coronary heart disease (CHD) and stroke are the leading causes of death in many countries. The major risk factors of these diseases, such as hypertension and hypercholesterolemia, are influenced by lifestyle, especially dietary factors.

The “French paradox” concerns interesting yet confusing statistics regarding the CHD mortality rate and the lifestyle of French people. French people eat 3.8 times as much butter and 2.8 times as much lard as Americans, and they have higher serum cholesterol levels and higher blood pressure than Americans (1). Other risk factors for cardiovascular diseases, such as body mass index and cigarette smoking, are comparable between the two countries, yet Americans have a 2.5-fold greater rate of death due to CHD than the French (2). A number of hypotheses have been proposed to account for this paradox.

Some findings were reported that regular wine drinkers had a lower CHD mortality rate than those that consumed mostly other alcoholic beverages (3, 4). Reports on red wine’s beneficial effects on health are increasing (5, 6). The benefits of red wine ingestion may be partly due to the moderate consumption of alcohol involved, but are largely due to the abundant phenolics, catechin, epicatechin, quercetin and resveratrol found in red wine. In the present study, we examined the effect of the extract of wine phenolics (EWP) on blood pressure and aortic biomechanical properties in stroke-prone spontaneously hypertensive rats (SHRSP).

**MATERIALS AND METHODS**

**Materials.** Thirty-six 4-week-old male SHRSP/Izm rats were obtained from the Disease Model Co-operative Research Association (Kyoto, Japan). They were divided into 6 equal groups, which were fed one of the following 6 diets: a control diet (plain laboratory diet) (Funahashi SP diet, Funahashi Farm, Chiba, Japan); the control diet substituted with 0.5 or 1.0% polyphenolic compounds derived from extract of apple phenolics (EAP), which is composed mainly of procyanidin (47.2%), chlorogenic acid (27.0%), phrolidin (9.7%), (−)-epicatechin (8.9%) and (+)-catechin (4.1%) extracted from unripe apples (polyphenol content=50%); the control diet substituted with 0.5 or 1.0% polyphenolic compounds derived from extract of tea phenolics (ETP), which is composed of tannins including (−)-epigallocatechin gallate (59.1%), (−)-epigallocatechin (19.3%), (−)-epicatechin gallate (13.7%), (−)-epicatechin (6.4%) and (−)-gallocatechin (15.1%) extracted from green tea (polyphenol content=70%); or the control diet along with drinking water containing 1.0% polyphenolic compounds derived from EWP.

**J Nutr Sci Vitaminol**
compounds prepared as follows. The pressed juice grounds formed in the process of making rosé wine (starting grape species: *Vitis vinifera*) were stored and fermented for 7 d and then mixed with water, followed by 10 h extraction. The extract thus obtained was subjected to solid/liquid separation by filter pressing, and was then concentrated under reduced pressure to give a soluble solid content of 55%, while eliminating alcohol (the alcohol content in this extract was less than 0.01%) to give EWP (polyphenol content = 50%). The polyphenolic compound intake was adjusted among groups to 0.5 or 1.0%.

All rats were housed in cages in groups of six. Body weight (BW) and blood pressure were checked before the assignment to groups to ensure weight and blood pressure homogeneity in each group. The environment was controlled at 23 ± 1°C with a 12 h light-dark cycle. Systolic blood pressure (SBP) values were measured without anesthesia by a photoelectric oscillometric tail-cuff method (UR-1000, UEDA, Tokyo, Japan), and BW was checked once a week. At the end of the 8th week of the feeding period, all of the rats were sacrificed under diethyl ether anesthesia. Their hearts and thoracic aortae from the point of the 5th ramus to that of the 14th ramus were carefully removed without stretching. The heart weights and ventricular weights were checked and aortic biomechanical properties were analyzed as described below.

**Biomechanical analysis.** Each aorta was stripped of all loosely adhering adventitial materials and then rinsed in physiological salt solution. The determination of the biomechanical properties of the thoracic aorta was conducted immediately, and cross-sectional areas of the specimen were recorded by a computer image-processing software program (NIH image). To obtain a static stress-strain curve, we analyzed the samples of the thoracic aorta by means of a tensile testing machine (EZ-test, Shimadzu, Kyoto, Japan). Each specimen was stretched at a constant tensile speed of 2 mm/min until it broke, while the load and deformation were continuously recorded. The stress was calculated by dividing each tensile load by the initial load-free cross-sectional area of the specimen. The strain was defined as the extension ratio, which was the ratio of the specimen length under each tensile load to the initial stress-free length. The load-deformation curves were read into a computer, using an analytical software program (WinAGS Lite, Shimadzu), and the load value for each strain increment of 1% was transformed to stress-strain curves. The maximum tensile stress (N/mm²) and maximum tensile strain (%) were obtained from the stress-strain curves as the values at the breaking point of each specimen. The failure energy (N·mm/mm²) was measured as the area between the stress-strain curves and the x-axis to the point of breaking. The incremental elastic modulus (N/mm²) of the aorta at specific values of strain (percentage strain of 10%) was defined as the ratio of incremental strain to incremental stress of 1.0% from the stress-strain data. When the breaking of a sample was induced by the load stress, not the tensile stress, the data was excluded.

**Vasorelaxing activity.** The thoracic aorta of four 13-week-old male SHRSP were rapidly isolated and placed in a physiological salt solution of the following
composition (mm): NaCl, 120; KCl, 4.7; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25; and glucose, 10. After the removal of fat and connective tissue, the aorta were cut into 3-4 mm rings, and each ring was mounted in a 10 mL organ bath containing physiological salt solution maintained at 37°C and bubbled with 95% O₂-5% CO₂ throughout the experiments. The tension of the tissue was measured by attaching it to a force-displacement transducer connected to an input board, and changes in tension were recorded. The rings were stretched to 1 g tension and allowed to equilibrate for at least 90 min before the initiation of the experiment. During this period, the bath solution was replaced every 20 min. The testing tension was removed by rubbing the intimal surface. The presence of endothelium was first confirmed by the ability of acetylcholine (ACh) to cause relaxation during a contraction induced by prostaglandin (PG) F2α. Any preparation that did not relax in response to ACh was discarded.

PG F2α (10⁻⁵ m) was prepared in deionized distilled water and used to precontract each aortic ring. The sample solution of EWP and a commercial red wine (Chianti) were prepared in distilled water, and 0.2, 2 and 20 μL samples were added directly to the bath fluid.

**Determination of resveratrol glucosides and isomers.** A method described by Goldberg et al (7) was used to determine the content of resveratrol in EWP and some commercial wines. Trans-resveratrol was purchased from Sigma (St. Louis, MO, USA) and dissolved in 96% ethanol in a stock solution of 5 mmol/L. The working solution was prepared in 0.2 M phosphoric acid–acetonitrile (4:1 (v/v)). For cis-resveratrol calibration, aliquots of the trans-resveratrol stock were diluted in 0.2 M phosphoric acid–acetonitrile (4:1 (v/v)). A portion of each standard was placed in a UV box (UVG-54, UVP, Osaka, Japan) and irradiated for 30 min at 254 nm with the intensity value of 580 μW/cm². The peak of trans-resveratrol was diminished upon irradiation and was preceded by an earlier peak which was shown to be cis-resveratrol in an amount identical to the decrease in the trans-isomer. The values for the cis-resveratrol standards were therefore assigned on the basis of the decrease in trans-resveratrol following irradiation.

In accordance with a previous publication (7), β-glucosidase (Sigma) was added at a concentration of 2 mg/mL of untreated EWP or the commercial wines were adjusted to pH 6.0. The mixtures were then incubated at room temperature for 20 h.

A high-performance liquid chromatography (HPLC) system coupled to a pump (L-7100, Hitachi, Tokyo, Japan), autosampler (L-7200), column oven (L-7300) and interface (D-7000) was used with a Lichrosphere 100 CN column (Hewlett-Packard, Mississauga, Ont., Canada) in the normal-phase mode. The mobile phase was water–acetonitrile–methanol (90:5:5 (v/v)), and the flow rate was 1 mL/min. The samples were directly injected, and the absorbance was modified at 306 nm using a UV detector (L-7400).

**Statistics.** Results are presented as the mean ± SD. Differences between the means were calculated using one-way analysis of variance (ANOVA) and Fisher’s PLSD. Probability values less than 0.05 were considered significant.

*J Nutr Sci Vitaminol*
RESULTS

Feed intake and body weight

The feed intake in the 1.0% ETP group and EWP group was lower than that in the control group. However, there was no significant difference in polyphenolic compound intake shown among groups on 0.5 or 1.0% (0.4–0.5 or 0.8–1.0 g/kg/d). The body weight gain in the 1.0% ETP group and EWP group was significantly lower than that in the control group at the end of the 8th week of the feeding period (Fig. 1).

Blood pressure and heart weight

Figure 2 shows the SBP values of each group over the 8-week feeding period. The 1.0% EWP group, but not the EAP or ETP groups, showed significantly lower SBP as compared with the control group at the 4th, 7th and 8th weeks (p < 0.05). The heart weight and ventricular weight, expressed as the percentage of BW (Table 1), were significantly lower in the EWP group than in the control group (p < 0.05).

Aortic biomechanical properties

The biomechanical properties of the thoracic aorta specimens at the breaking point are given in Table 2. The maximum stress was significantly increased in the EWP group as compared with the control group (p < 0.05). Increased biomechanical
Fig. 2. Changes in systolic blood pressure of SHRSP fed different polyphenolic diets. EWP but not EAP or ETP significantly lowered the systolic blood pressure as compared with the control group at the 4th, 7th and 8th weeks of the feeding period \( (p < 0.05) \). \( n = 6 \) rats in each group. Mean values with SD are given \( (n = 6) \). *EWP group is significantly different from control group, \( p < 0.05 \). **EWP group is significantly different from 0.5% ETP group, \( p < 0.05 \). ***EWP group is significantly different from 0.5% EAP group, \( p < 0.01 \).

Table 1. Heart weight and ventricle weight to body weight ratios in SHRSP fed different polyphenolic diets.

| Group  | Number | Heart weight as percent body weight (%) | Ventricle weight as percent body weight (%) |
|--------|--------|----------------------------------------|--------------------------------------------|
| Control| 6      | 0.447 ± 0.026                          | 0.422 ± 0.015                              |
| 0.5% EAP| 6     | 0.436 ± 0.009                          | 0.412 ± 0.020                              |
| 1.0% EAP| 6     | 0.428 ± 0.023                          | 0.411 ± 0.009                              |
| 0.5% ETP| 6     | 0.429 ± 0.020                          | 0.412 ± 0.019                              |
| 1.0% ETP| 6     | 0.442 ± 0.033                          | 0.404 ± 0.017                              |
| EWP    | 6      | 0.417 ± 0.010*                         | 0.401 ± 0.008*                             |

Mean values with SD are given.
*Significantly different from the control group, \( p < 0.05 \).

Properties in the EWP group were observed not only in maximum stress but also in failure energy, which however did not reach significance. The maximum strain was increased in the EWP group, but not significantly. The incremental elastic

\textit{J Nutr Sci Vitaminol}
Wine Phenolics Improves Aortic Properties in SHRSP

Table 2. Biomechanical properties of the thoracic aorta in SHRSP fed different polyphenolic diets.

| Number | Maximum stress (N/mm²) | Maximum strain (%) | Failure energy (N·mm/mm²) |
|--------|------------------------|--------------------|--------------------------|
| Control | 3.93 ± 0.21            | 58.23 ± 24.93      | 17.91 ± 5.91             |
| 0.5% EAP | 4.43 ± 0.51            | 55.85 ± 12.07      | 17.47 ± 3.13             |
| 1.0% EAP | 4.12 ± 0.49            | 61.49 ± 17.51      | 17.50 ± 6.96             |
| 0.5% ETP | 4.34 ± 0.58            | 57.24 ± 5.48       | 14.40 ± 2.69             |
| 1.0% ETP | 4.70 ± 0.85            | 59.65 ± 10.74      | 16.23 ± 5.23             |
| EWP     | 4.57 ± 0.39*           | 69.64 ± 26.16      | 18.34 ± 4.73             |

Mean values with SD are given.
*Significantly different from the control and 0.5% ETP groups, $p < 0.05$.

Table 3. Comparison of passive incremental elastic modulus values in SHRSP fed different polyphenolic diets.

| Number | Einc (10) N/mm² |
|--------|-----------------|
| Control | 4.55 ± 0.46     |
| 0.5% EAP | 4.62 ± 0.35    |
| 1.0% EAP | 4.22 ± 0.39    |
| 0.5% ETP | 4.53 ± 0.55    |
| 1.0% ETP | 4.77 ± 0.40    |
| EWP     | 3.85 ± 0.44*    |

Mean values with SD are given.
Einc (10): incremental elastic modulus at the extension ratio of 10.
*Significantly different from the control, 0.5% EAP and 0.5% ETP groups, $p < 0.001$, and 1.0% ETP groups, $p < 0.0001$.

modulus values were significantly reduced (meaning higher elasticity) in the EWP group as compared to the control group ($p < 0.001$) at a strain of 10% (Table 3), whereas no significant differences as compared to the control values were observed in the EAP and ETP groups.

Vasorelaxing activity of the EWP and commercial red wine

In endothelium-intact rat aortic rings precontracted with PG F2α, EWP and a commercial red wine caused concentration-dependent relaxation (Fig. 3). The relaxing effect of the EWP was significantly greater as compared to the commercial red wine.

The content of trans- and cis-resveratrol in the EWP and commercial wines

The trans- and cis-resveratrol contents of three commercial red wines, one
Fig. 3. Relaxation effects of the addition of EWP and commercial red wine to endothelium-intact aortic rings precontracted with 1.0 x 10^{-5} M PG F2α. EWP and the commercial red wine both caused concentration-dependent relaxation. The relaxing effect of EWP was significantly greater than that of the commercial red wine (p < 0.0001). Mean values with SD are given (n = 4). ■, EWP; ◦, commercial red wine (Chianti).

Table 4. Concentration of resveratrol in EWP and some commercial wines.

| Grape products                  | Trans-resveratrol (mm) | Cis-resveratrol (mm) | Total (mm) |
|---------------------------------|------------------------|----------------------|------------|
| EWP                             | 1.610                  | 1.203                | 2.813      |
| Chianti (red wine)              | 0.023                  | 0.015                | 0.038      |
| Balolo (red wine)               | 0.026                  | 0.016                | 0.042      |
| Gevrey-Chambertin (red wine)    | 0.045                  | 0.018                | 0.063      |
| Tabel (rose wine)               | 0.021                  | 0.008                | 0.029      |
| Chianti (white wine)            | 0.009                  | ND                   | 0.009      |

ND: not detected.

commercial rosé wine, one commercial white wine and EWP were analyzed (Table 4). The red wines, rosé wine and EWP contained measurable and high concentrations of both glucosides and two free isomers of resveratrol, although the concentrations were low or below the limit of detection in the white wine. The highest concentrations of resveratrol were observed in EWP.

DISCUSSION

Our previous studies clearly demonstrated that hypertension and stroke were
Wine Phenolics Improves Aortic Properties in SHRSP

prevented by dietary conditions (8, 9) even in SHRSP strongly predisposed to hypertension.

The "French paradox" is the apparent discrepancy between a high-fat diet and a low incidence of CHD, which has been attributed to the regular drinking of red wine. In the present study, the extracts of wine, tea and apple polyphenolic compounds were examined for their effects on aortic biomechanical properties and hypertension in SHRSP. The SBP in the SHRSP fed EWP was significantly lower than in that of the control rats. Heart weights were also reduced in the EWP-fed SHRSP, confirming the lowering of blood pressure. These results clearly indicate that the diets containing EWP attenuated hypertension in SHRSP.

Increases in the vasorelaxing activity are considered to be a mechanism underlying the attenuation of blood pressure elevation by EWP feeding. This observation is in agreement with the findings of a previous study suggesting that an extract of red wine promotes vascular relaxation (10). The active component responsible for the endothelium-dependent relaxing activity of wine and grape juice is apparently the phenolic compounds derived from grape skin. It is known that wine includes some polyphenolic compounds that promote vascular relaxation. Chen and Pace-Asciak (11) indicated that resveratrol and quercetin exert both indirect and direct vasodilator effects on the blood vessels by nitric oxide (NO)-mediated and non-NO-mediated mechanisms, respectively. Andriambeloson et al (12) indicated that red wine polyphenolic compounds, but not the structurally closely related polyphenol catechin, induced endothelium-dependent relaxation in the rat aorta. On the other hand, epicatechin derivatives have been shown to relax rat mesenteric arteries probably by inhibiting Ca\(^{2+}\) influx (13). In the present quantitative analysis of resveratrol, commercial red wine was found to have 20–60 \(\mu\)mol/L resveratrol, and this observation was consistent with some previous studies (14, 15). EWP has high contents of resveratrol, quercetin and epicatechin (resveratrol, 2.813 mm; quercetin, 1.142 mm; epicatechin, 74.966 mm), 40–90 times higher than those of commercial wines, because this wine extract is highly condensed and is long-fermented in the extraction process compared with commercial wines. Furthermore, recently we confirmed that the SBP of ovariectomized-female SHRSP fed a diet containing resveratrol remained lower than that in the control group, possibly by increasing the vasorelaxing activity (data not shown). Thus, we believe that the attenuated SBP in EWP-fed SHRSP but not the EAP- or ETP-fed SHRSP, by increasing the vasorelaxing activity observed in the present study are due to high-concentrated, wine-derived specific polyphenolic compounds, including resveratrol, quercetin and epicatechin, in the diet.

SHRSP have been shown to be susceptible to the early development of cerebral vascular lesions (16) and are widely considered a good model for stroke in humans (17). Although severe hypertension and its rapid onset have been shown to be major determinants for the development of stroke in SHRSP (18, 19), other factors such as those related to renal vascular changes (20), a genetically determined increased cerebrovascular permeability (21), and increased vascular fragility (22, 23) may also
be implicated. In our previous study, increased fragility and decreased distensibility of the thoracic aorta were observed in 4- and 12-week-old SHRSP when compared with age-matched WKY (data not shown). In the present study, the altered aortic biomechanical properties of SHRSP were also improved in the EWP-fed group; the maximum stress was significantly increased (meaning decreased aortic fragility), and the elastic modulus was significantly reduced (meaning increased aortic elasticity). Wine polyphenolic compounds have been reported to exhibit a wide range of biological effects. Resveratrol is attracting the attention of many researchers because it is one of the compounds present in wines that could be responsible for the lower prevalence of CHD observed among wine drinkers. The effect could be due to the ability of resveratrol to inhibit low-density-lipoprotein (LDL) oxidation (24), its anti-platelet aggregation activity (25–27), and its inhibition of eicosanoid synthesis (28). In addition, Gehm et al (29) indicated that resveratrol is a phytoestrogen and that it exhibits variable degrees of estrogen receptor agonism. Nestel et al (30) reported that the administration of soy isoflavones, which are phytoestrogens, significantly improved arterial compliance in perimenopausal and menopausal women. Therefore, the improvements in aortic biomechanical properties of EWP-fed SHRSP, i.e., decreased aortic fragility and increased aortic elasticity, may be partly related to the estrogenic effect of resveratrol in EWP.

In conclusion, the extract of wine polyphenolic compounds attenuated the elevation of blood pressure in SHRSP, possibly by increasing the vasorelaxing activity. Improvements in the biomechanical properties of the aorta were also observed in EWP-fed SHRSP. The present study is the first demonstration of the effect of wine phenolics on aortic biomechanical properties. These observations may explain in part the low rate of CHD and the decreased risk of stroke in the French population.

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J Nutr Sci Vitaminol
Wine Phenolics Improves Aortic Properties in SHRSP

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