Polyphenols prevent lipid abnormalities and arterial dysfunction in hamsters on a high-fat diet: a comparative study of red grape and white persimmon wines

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Polyphenols prevent lipid abnormalities and arterial dysfunction in hamsters on a high-fat diet: a comparative study of red grape and white persimmon wines

Jin-Hyang Suh,a Anne Virsolvy,b Aurélie Goux,a Cécile Cassan,b Sylvain Richard,b Jean-Paul Cristol,a Pierre-Louis Teissédrea and Jean-Max Rouaneta,a

Scope: We compared the effects of two dealcoholized wines, persimmon (P) and Merlot (M), in hypercholesterolemic hamsters. Methods and results: Four groups of hamsters received a standard (ST) or an atherogenic diet (AT) for 12 weeks. AT animals received either dealcoholized persimmon wine (AT + P) or Merlot wine (AT + M) by gavage, while controls received water (AT and ST). Plasma cholesterol, triglycerides and glucose and paraoxonase activity were measured. Oxidative stress was assessed by aortic $\text{O}_2^-$ production, and vascular function was evaluated in aortic rings. The atherogenic diet led to higher plasma triglycerides (246%), total cholesterol (142%), LDL-cholesterol (91%) and HDL-cholesterol (49%). Aortic production of $\text{O}_2^-$ also increased (207%) and vascular reactivity was modified with altered endothelial function as assessed by acetylcholine-dependent vasorelaxation. The two wines partially prevented these alterations, reducing $\text{O}_2^-$ production and improving vascular reactivity without altering endothelial function. There was no difference between the P and M groups, although the procyanidin composition of the two dealcoholized fractions differed significantly, and only dimer concentrations were similar. Conclusion: These findings indicate that polyphenols are responsible, at least in part, for the antiatherogenic/antioxidant effects of wines.

1 Introduction

Epidemiological studies demonstrate that the consumption of red wine is beneficial in the prevention of cardiovascular diseases, a concept embodied by the term “French Paradox.”1 The cardioprotective effects of red wine have been attributed mainly to the antioxidant properties of a broad range of polyphenols,2 especially those of the procyanidin group,3 which are supposed to affect lipid metabolism and to have beneficial effects on cardiovascular function4 by reducing cholesterolemia and triglyceridemia. They are widely present in vegetables and fruits, including grapes and persimmons, and consist of polymeric catechin and epicatechin dimers, trimers, tetramers and oligomers of up to 8 units. They are abundant in many types of grape wine and their biological properties have been extensively reviewed.5 Several studies have shown the beneficial effects of procyanidins in the prevention of atherosclerosis both in humans and in animal models (see ref. 4 and 5). However, there is no correlation between antioxidant capacity and total phenolic content, in particular procyanidin composition and the degree of polymerization of procyanidin compounds.6 Oligomeric procyanidin dimers (group B) are thought to possess the best antioxidant properties7 and have been shown to reproduce the bioactivity of wine extracts with respect to glucose and lipid metabolism.8 However, despite the large number of reports on this subject, it is difficult to draw clear conclusions regarding the effectiveness or the most active forms of procyanidins.

The persimmon (Diospyros kaki) is a widely available fruit in Korea, Japan and China, and is marketed throughout Europe. This fruit contains the richest source of condensed tannins, mainly those belonging to the proanthocyanidin B group,9 which exhibit high antioxidant potential.10 Studies using fresh and dry persimmons have revealed a decrease in plasma lipid levels and an increase in plasma antioxidant activity in rats.11 In addition, persimmon tannins increase life expectancy and reduce the incidence of stroke in hypertensive rats,12 an effect attributed to the fact that persimmon tannins are 20 times more potent than the antioxidant vitamin E. Gorinstein et al.13 have reported that the whole ripe fruit exhibits hypolipidemic properties in rats subjected to a cholesterol-supplemented diet, but that the antioxidant effect of this fruit is associated mainly with persimmon
phenols. However, the effects of persimmon wine on the anti-
oxidant status of hamsters administered a high fat diet have not
been examined.

The process of vinification is known to prevent polyphenol
alterations and to increase their extraction rate from fruits. It has
also been established that oenological practices and ageing affect
phenolic content, composition and antioxidant activity, which
also depend on the variety of grape and the vintage. Among
French wines, the highest polyphenol concentrations are found
in Cabernet-Sauvignon, Merlot and Pinot Noir wines.

Here, we investigated the protective and antioxidant properties
of two dealcoholized wines made from persimmons and Merlot
grapes, respectively, in correlation to their polyphenol composi-
tion, in a well-characterized model of early atherosclerosis.

2 Results
2.1 Wines and diets

As shown in Table 1, the polyphenol content and composition of
P and M are fundamentally different. The level of total poly-
phenols in M (4427 ± 57 mg L⁻¹) was 6.2 times as high as that in
P (714 ± 13 mg L⁻¹). Monomers, trimers and non determined
polyphenol fractions differed significantly between the two
wines. Monomers were about 10 times more concentrated in M
than in P. Trimers were not detected in P whereas they were
found at low concentrations in M (11.8 ± 0.3 mg L⁻¹). Dimers
were found at high levels in both M and P (313.4 ± 1.2 mg L⁻¹
and 410.8 ± 1.4 mg L⁻¹, respectively). The dimer fraction rep-
resented 57.5% of total polyphenol content in P and 7.2% in M.
The determination of the dimer composition of the two wines
revealed that in P, only the procyanidin dimer B2 was present,
whereas the four procyanidins dimers, B1, B2, B3, and B4, were
detected in M at similar concentrations, with B4 occurring at
a slightly higher level.

2.2 Effects of wines on body weight

The average energy intake was 65.3, 81.2, 75.8 and 74.9 kJ per
day (data not shown) for ST, AT, AT + P, AT + M animals,
respectively. Nevertheless, weight gain was 4.3 times greater in
ST than in AT animals, while no difference was seen between the
AT and the two wine groups. This can be attributed to the
unbalanced AT diet. Abdominal adipose tissue weight was
significantly greater (24%) in AT than in ST animals. The
consumption of P and M reduced this effect by 22% and 9%,
respectively (Table 2).

2.3 Effects of wines on total-, HDL- and LDL-cholesterol and
triglycerides

At the end of the experimental period, plasma levels of TC,
HDL-C, LDL-C and TG were significantly higher in the AT
group than in ST animals (Table 2). Animals receiving either P or
M had significantly lower cholesterolemia (5.58 ± 0.12 and
5.80 ± 0.17mmol L⁻¹, respectively) than AT animals (7.32 ±
0.45mmol L⁻¹). Wine treatment did not modify LDL-C levels.
However, both wines led to higher HDL-C/TC ratios; HDL-C
values represented 50% and 35% of TC in the P and M group,
respectively. Administration of P or M also triggered a reduction
in the increase of LDL-C. While the LDL-C level in AT animals
was 200% of that in the ST group, it was only 126% and 118%
in the AT + P and AT + M groups, respectively. The same was true
of plasma TG values, where levels after P and M treatment were
154% and 190% of ST values, compared to AT animals in which
they increased to 346%.

2.4 Effect of wines on oxidative stress

The AT diet significantly lowered plasma paraoxonase activity
(32%) compared to the ST diet. However, the consumption of P
and M reduced the decrease in PON activity (to 19% and 6%,
respectively; Fig. 1A). As shown in Fig. 2B, the aortic production
of O₂⁻⁻ displayed a 210% increase in AT animals when compared
to the ST group. However, hamsters consuming P or M wines
exhibited a lower increase in O₂⁻⁻ production (55% and 21%
respectively), not significantly different from levels seen in ST.

2.5 Wines and endothelial function

The characterization of the vascular reactivity of the aorta
showed that the maximal contraction induced by the depolariz-
ing agent KCl was lower in AT animals (7.32 ± 0.2 g) than in the
ST group (2.1 ± 0.1 g, p < 0.05), corresponding to a 43%

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Polyphenol and procyanidin composition of dealcoholized winesa,d</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg L⁻¹)</td>
<td>Persimmon wine</td>
</tr>
<tr>
<td>Total polyphenolsb</td>
<td>714.2 ± 35.9</td>
</tr>
<tr>
<td>Monomersc</td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>n.d.</td>
</tr>
<tr>
<td>Epicatechine-3-O-gallate</td>
<td>3.11 ± 0.08***</td>
</tr>
<tr>
<td>Epigallocatechin-4-gallate</td>
<td>n.d.</td>
</tr>
<tr>
<td>Dimerse</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>29.44 ± 0.11</td>
</tr>
<tr>
<td>B2</td>
<td>32.55 ± 0.19</td>
</tr>
<tr>
<td>B3</td>
<td>n.d.</td>
</tr>
<tr>
<td>B4</td>
<td>410.82 ± 3.89</td>
</tr>
<tr>
<td>Trimersd</td>
<td></td>
</tr>
<tr>
<td>n.d.</td>
<td>36.89 ± 0.63</td>
</tr>
<tr>
<td>n.d.</td>
<td>152.10 ± 0.98</td>
</tr>
<tr>
<td>n.d.</td>
<td>31.12 ± 0.54</td>
</tr>
<tr>
<td>n.d.</td>
<td>11.77 ± 0.08</td>
</tr>
</tbody>
</table>

a Values are means ± SEM from three measurements. ***: p < 0.001. b Total polyphenol content determined according to Singleton and Rossi. c Procyanidin composition (mg L⁻¹) measured by high-performance liquid chromatography using UV detection at 280 nm, according to Chira et al. d Not detected.
This reduction was not observed in AT animals receiving either P or M. The maximal responses, 2.0±0.3 g and 1.6±0.1 g, respectively, for these two groups, were different from those in animals on an AT diet (p<0.05) but not from those in ST animals (Fig. 2A, Table 3). Additionally, KCl induced a concentration-dependent reduction in the contractile response. This reduction was not observed in AT animals receiving either P or M. The maximal responses, 2.0±0.3 g and 1.6±0.1 g, respectively, for these two groups, were different from those in animals on an AT diet (p<0.05) but not from those in ST animals (Fig. 2A, Table 3). Additionally, KCl induced a concentration-dependent

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**Table 2** Body weight gain, food intake and metabolic parameters in hamsters fed a standard diet (ST), a high-cholesterol diet (atherogenic diet, AT), or a high-cholesterol diet plus either white persimmon wine or red Merlot grape wine for 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>ST</th>
<th>AT diet</th>
<th>AT diet + Persimmon</th>
<th>AT diet + Merlot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g)</td>
<td>29.42 ± 2.03</td>
<td>6.80 ± 3.34 ***</td>
<td>3.30 ± 1.54 ***</td>
<td>2.54 ± 3.57 ***</td>
</tr>
<tr>
<td>Fat/weight (%)</td>
<td>1.57 ± 0.06</td>
<td>1.95 ± 0.07 **</td>
<td>1.52 ± 0.09 8</td>
<td>1.78 ± 0.08 8</td>
</tr>
<tr>
<td>Glycemia (mmol L⁻¹)</td>
<td>8.35 ± 0.31</td>
<td>8.44 ± 0.72</td>
<td>6.98 ± 0.44</td>
<td>8.39 ± 0.43</td>
</tr>
<tr>
<td>Total cholesterol (mmol L⁻¹)</td>
<td>3.02 ± 0.09</td>
<td>7.32 ± 0.45 ***</td>
<td>5.58 ± 0.12 ***(8)</td>
<td>5.80 ± 0.17 ***(8)</td>
</tr>
<tr>
<td>HDL-C (mmol L⁻¹)</td>
<td>1.86 ± 0.05</td>
<td>2.77 ± 0.15 ***</td>
<td>2.77 ± 0.14 ***</td>
<td>3.05 ± 0.09 ***</td>
</tr>
<tr>
<td>Triglycerides (mmol L⁻¹)</td>
<td>0.89 ± 0.05</td>
<td>3.62 ± 0.28 ***</td>
<td>2.39 ± 0.11 ***(8)</td>
<td>2.23 ± 0.13 ***(8)</td>
</tr>
</tbody>
</table>

* Circulating levels of glucose, total cholesterol, HDL-C, LDL-C and triglycerides were measured in fasting plasma of hamsters. Superscripts refer to statistical comparisons vs. ST (*) and vs. AT groups (†). 8: p<0.05; ** and §§: p<0.01; *** and §§§: p<0.001.
contraction of aortic rings with endothelium from all groups (Fig. 2A). The sensitivity to KCl was modified by the AT diet. The EC_{50} values, determined from dose-response curves (Fig. 2A), decreased from 21.9 ± 0.4 mM in ST hamsters to 11.5 ± 1.2 mM in the AT group. This decrease was prevented to a similar extent by polyphenol supplementation with P (25.8 ± 0.3 mM) and with M (23.9 ± 0.4 mM).

Endothelial dysfunction is a key early factor in the development of atherosclerosis. We therefore evaluated endothelial function in all groups by analyzing the response to acetylcholine (ACh) and using sodium nitroprusside (SNP) as a control for the contribution of smooth muscle contraction. The cumulative addition of ACh (1 nM to 10 μM) and SNP (1 mM to 10 μM) resulted in a concentration-dependent relaxation of hamster aortic rings with endothelium that had been previously contracted with 1 μM phenylephrine (PE) (Fig. 2B–2C). AT animals had reduced endothelium-dependent vasodilator responses to ACh as compared to ST animals. The maximal relaxation obtained was 34.8 ± 3.4% of PE-induced contraction in ST hamsters versus 23.8 ± 1.9% in AT animals (p = 0.01; n = 10), which corresponds to a 32% decrease in the response to ACh in AT animals. With P and M administration, endothelial function was conserved and no difference was observed in the vasodilator response to ACh as compared to ST animals; relaxation represented 31.1 ± 2.8% of PE-induced contraction for P (p = 0.04; n = 10) and 32.9 ± 3.8% for M animals (p = 0.04; n = 10). Unlike ACh, the relaxant response elicited by SNP was identical in all groups (Fig. 2C).

### 3 Discussion

Many in vivo studies have demonstrated the antioxidant properties of wine polyphenols, but so far, none has identified the phenolic compounds potentially involved. In the present study, we correlated polyphenol composition to antioxidant properties in the dealkoholized fraction of two different wines, a red grape (Merlot) wine and a white persimmon wine. For the first time, we show that an atherogenic diet impairs vascular reactivity and that these alterations are prevented by wine polyphenols in a well-characterized animal model of atherosclerosis.46 We have previously shown that this atherogenic diet induces the development of atherosclerosis in association with hypercholesterolemia and O_2^- overproduction in hamsters.14,45,19 Paraoxonase activity and reactive oxygen species (ROS), including O_2^-, are markers of oxidative stress. While low paraoxonase activity promotes LDL oxidation and has been linked to the progression of arteriosclerosis,17 the overproduction of O_2^- could be responsible for the generation and/or maintenance of oxidative stress conditions.18 These events contribute to the initiation and progression of vascular and endothelial dysfunction. In our study, hamsters given the AT diet had increased plasma TC, HDL-C, LDL-C and TG levels, signifying dyslipidemia. Oxidative stress was induced in this group, as shown by the decrease in PON activity and the increased production of aortic O_2^-, and there were corresponding alterations in contractile function, as demonstrated by the vascular reactivity of aortic rings. The fact that there was a decrease in the vasodilator response to acetylcholine but not to SNP suggests that these alterations are related to endothelial dysfunction. Together, these results substantiate the effectiveness of the atherogenic diet.

We have previously shown that the consumption of polyphenol-rich foods, polyphenol extracts and polyphenol-enriched white wine or sparkling red Pinot Noir wine prevents the development of atherosclerosis in hamsters through a mechanism related to increased antioxidant availability.15,17 and an improved serum lipid profile.14,15,19 Here, we observed the same beneficial effects with both P and M wines. They significantly prevented the alteration of the serum lipid profile and the overproduction of O_2^- via the decreased activity of NADPH oxidase in animals given an atherogenic diet. Paraoxonase activity was maintained at levels identical to those seen in controls given a standard diet. PON has also been shown to attenuate the postprandial oxidative stress response, possibly due to its lipase-like activity on chylomicron triacylglycerols17 and reflected by reduced triglyceridemia. Accordingly, in hamsters that were given wine, triglyceridemia was significantly reduced as compared to AT animals. Additionally, the atherogenic index, reflected by the HDL-C/TC ratio, increased from 1.62 in ST to 2.64 in AT animals, but improved in the P (2.01) and M (1.90) groups. Consequently, the vascular contractile response and endothelial function were not impaired. Surprisingly, while no difference was observed between the effects of P and M wines in experimental animals, the analysis of their polyphenol composition revealed substantial differences both at the qualitative and quantitative levels. Total polyphenol content was 6 times lower in P wine than in M wine. The concentrations of procyanidin monomers and trimers varied significantly and were at least 10 times lower in P wine. Only the levels of procyanidin dimers were comparable between the two. However, while all four procyanidin dimers (B1 to B4) were present in M wine, only B2 was found in P wine. Therefore, taking into account the improvement in atherosclerotic markers in AT hamsters with the two wines, our analysis suggests that the antioxidant properties of both P and M wines are related to the presence of procyanidin dimers. Consistent

<table>
<thead>
<tr>
<th></th>
<th>ST</th>
<th>AT diet</th>
<th>AT diet+ Persimmon</th>
<th>AT diet+ Merlot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max contraction (g)</td>
<td>2.1 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>KCl EC_{50} (mM)</td>
<td>21.9 ± 0.4</td>
<td>11.5 ± 1.2</td>
<td>25.8 ± 0.3</td>
<td>23.9 ± 0.4</td>
</tr>
<tr>
<td>ACh relaxation (%)</td>
<td>34.8 ± 3.4</td>
<td>23.8 ± 1.9</td>
<td>31.1 ± 2.8</td>
<td>32.9 ± 3.8</td>
</tr>
</tbody>
</table>

* For each group, values represent the maximal contraction induced by a maximally active concentration of KCl (80 mM) expressed in grams, and the EC_{50} for KCl expressed in mM and determined from dose-response curves after fitting with a non-linear function using GraphPad Prism® software. Superscripts refer to statistical comparisons vs. ST (*) and vs. AT(i) groups. * and §§: p < 0.05; *** and §§§: p < 0.001.
with these findings, it has been shown that antioxidant activity is not correlated to total polyphenol content but is instead dependent on polyphenol composition.\textsuperscript{21} Particularly the composition of procyanidin compounds. Procyanidin dimers and trimers are the most powerful molecules when it comes to mimicking the effects of whole grape seed procyanidin extracts.\textsuperscript{22} Furthermore, procyanidin B2 might have beneficial properties, such as anti-atherosclerotic, anti-inflammatory and antihypertensive activity, \textit{in vitro} and \textit{in vivo}.\textsuperscript{23} Procyanidin B2 has also been shown to be a major contributor to the antioxidant activity of cocoa\textsuperscript{24} and apples.\textsuperscript{25} Even if dimer B2 was found as the major dimer in persimmon wine composition, and that in such a case it should have an essential activity, in Merlot wine, a synergistic effect of all the dimers appears imaginable and in conjunction with anthocyanins (4427 ± 57 mg L\textsuperscript{-1}, not shown in Table 1). In persimmon wine, epigallocatechin-gallate (29.4 mg L\textsuperscript{-1}) could act synergistically with dimer B2.

In addition, the inhibition of lipid absorption by procyanidins is another mechanism that cannot be ruled out.\textsuperscript{26} However, in a previous study\textsuperscript{27} we used wine polyphenol extracts ExGrape Seed (EGS) that contained 46% procyanidins and 54% monomeric flavanols, and ExGrape Total (EGT) that contained 34% anthocyanins, 35% procyanidins, and 27% monomeric flavanols. EGS and EGT respectively induced 77% and 84% endothelium-dependent relaxation. In an older study,\textsuperscript{28} we demonstrated that a red wine phenolic extract, shown by others to induce an endo- thelium-dependent relaxation via an enhancement of endothelial NO synthesis\textsuperscript{29} and containing 42% procyanidins, 20% monomeric flavanols, 14% anthocyanins and 24% phenolic acids, prevented early atherosclerosis in hamsters. Moreover, it has been recently shown that both procyanidins and anthocyanins in red wine are able to stimulate the endothelial NO synthase.\textsuperscript{30} Thus, it is likely that, alongside procyanidins, anthocyanins also contribute to the beneficial effect observed in the present study. These findings indicate that polyphenols are responsible, at least in part, for the antiatherogenic/antioxidant effects of wines.

4 Experimental

4.1 Wines

The Merlot wine (M) (2008 vintage) was produced in Bordeaux (France), under the Appellation Pessac-Léognan (Château Seguin). The Persimmon wine (P) (2008 vintage) was produced in Gyeongbuk Province (Korea) (Cheongdo Wine Co. Ltd., Korea). Ethanol was removed by vacuum evaporation at 18°C for 20 min under reduced pressure and an equivalent volume of distilled water was subsequently added to reconstitute the ethanol-free fraction.

4.2 HPLC analysis

Total phenolic content was determined for each wine according to the method of Singleton and Rossi,\textsuperscript{31} and expressed in mg L\textsuperscript{-1} as gallic acid equivalents.

For each wine, the separation of polyphenol compounds was performed by high-performance liquid chromatography (HPLC) using UV detection at 280 nm. The amounts of the monomers C, EC, ECG and EGCG, the dimers B1, B2, B3 and B4, and trimers were determined. A Finnigan ternary pump coupled to an Xcalibur data treatment system and a Finnigan UV-vis detector (UV-vis 200), and a Finnigan autosampler were used for solvent and sample delivery and detection. Separations were performed on reversed-phase Agilent Nucleosil C18 columns (250 mm × 4 mm, 5 µm particle size) eluted at a flow rate of 1 mL min\textsuperscript{-1}. The solvents used were as follows: solvent A, 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B, 20% A with 80% acetonitrile; solvent C, 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5.\textsuperscript{32} Calibration curves were constructed with standards of each compound and data were expressed as mg per liter of wine.

4.3 Animals, diets, and experimental design

Forty male golden Syrian hamsters (Janvier, Le Genest-St-Isle, France) weighing 90–100 g were randomly assigned to four groups (n = 12/group) with free access to food and water. Animals were handled according to the guidelines of the committee for Animal Care at the University of Montpellier (France) and NIH guidelines no 85–123 (Washington, DC, 1985). Hamsters were fed with either a standard diet (ST) or an atherogenic diet (AT) for a 12-wk period, and AT animals were administered tap water (AT), dealcoholized P (AT + P) or dealcoholized M (AT + M) daily by gavage during this period. The volume of the liquid administered was established by extrapolating from two glasses of wine per meal per day for a 70 kg human. This represents a daily volume of 7.14 mL kg\textsuperscript{-1} body weight. ST animals received tap water by gavage. The composition of the diet was identical to that previously described by Décondé \textit{et al.}\textsuperscript{33} To be precise, the atherogenic diet contained 15% lard and 0.5% cholesterol, and the mineral and vitamin mixes did not contain selenium, vitamin C or vitamin E.

4.4 Analytical procedures

At the end of the 12-wk period hamsters were deprived of food overnight. Then, animals were anesthetized with an intraperito- neal injection of pentobarbital (150 mg kg\textsuperscript{-1}) and fasting blood was collected by cardiac puncture. Plasma concentrations of total cholesterol (TC), HDL- and LDL-cholesterol were determined using commercially available kits (CH 200 and CH 203 respectively, Randox Laboratories LTD, Crumlin, UK) on a Pentra 400 automated analyzer (HORIBA ABX Montpellier, France). Plasma glucose and triglyceride (TG) levels were measured enzymatically (KonePro, KoneLab, Evry-Les-Lys, France) using reagents from the Thermo Electron Corporation (Cergy Pontoise, France). The thoracic aorta was excised and immersed in ice-cold buffer as required for the following procedures (superoxide anion determination and vascular reactivity).

Paraoxonase (PON) activity was determined according to the method of Jaouad \textit{et al.}\textsuperscript{34}

NADPH oxidase activity was measured as superoxide anion production and was evaluated in the thoracic aorta immersed and equilibrated in Krebs buffer containing lucigenin (10 µM). The intensity of luminescence was measured on a luminometer (Perkin Elmer Wallac, Victor, Turku, Finland). Results were expressed as relative light units (RLU)/µg tissue.
4.5 Aortic preparation and mounting for vasorelaxation studies

The thoracic aorta, immersed in phosphate buffered saline pH 7.4 containing 140 mmol L\(^{-1}\) NaCl, 5 mmol L\(^{-1}\) KCl, 1 mmol L\(^{-1}\) MgCl\(_2\), 0.5 mmol L\(^{-1}\) KH\(_2\)PO\(_4\), 0.5 mmol L\(^{-1}\) Na\(_2\)HPO\(_4\), 2.5 mmol L\(^{-1}\) CaCl\(_2\), 10 mmol L\(^{-1}\) HEPE and 10 mmol L\(^{-1}\) glucose, was cleaned of fat and connective tissue and cut into 2–3 mm-wide rings. Aortic rings were mounted between two stainless steel hooks placed in a conventional organ bath chamber filled with 5 mL of Physiological Saline Solution (PSS), maintained at 37°C and continuously bubbled with O\(_2\). Changes in isometric tension were recorded as previously described\(^{[32-36]}\) using an ITI-25 force transducer and an IOX computerized system (EMKA Technologies, Paris, France). Each arterial (aortic) segment was subjected to a 60 min equilibration period at the predetermined optimal point of its active length–tension curve established at 1 g by measuring the response to 30 mM KCl at different levels of stretch. The contractile function of each arterial segment was assessed with 1 μM phenylephrine (PE). Acetylcholine causes endothelium-dependent vasorelaxation. Thus the presence of endothelium was confirmed by the application of acetylcholine (ACh, 1 μM), and vasorelaxation was assessed after PE-induced contraction. After wash-out and a 20–30 min period of stabilization, dose responses to the depolarizing agent KCl were evaluated by cumulative increases in the concentration of KCl (1–80 mM range). Endothelial function was assessed by studying the relaxing effects of ACh in arteries contracted using a maximally active concentration of PE (10 μM) inducing similar submaximal contractile level in all groups. Dose-response relaxation curves were then generated by cumulative increases in the concentration of ACh (1 nM–10 μM), and vasorelaxation was assessed by studying the relaxing effects of ACh in arteries contracted using a maximally active concentration of PE (10 μM) inducing similar submaximal contractile level in all groups. Dose-response relaxation curves were then generated by cumulative increases in the concentration of ACh (1 nM–10 μM) range. Each protocol was performed in quadruplicate in tissue from 4 different animals per group.

4.6 Statistical analysis

Data are shown as means ± SEM. Statistical analysis was carried out using StatView IV software (Abacus Concepts, Berkeley, CA), by one-way ANOVA followed by Fisher’s protected least significant difference test. Differences were considered significant at a P < 0.05.

5 Conclusion

Our findings regarding oxidative stress, plasma lipoprotein distribution, vascular reactivity and endothelial function and their correlation to the phenolic composition of wines highlights the contribution of procyanidins to the antioxidant biological activity of wine extracts. To confirm these results, a similar study using fractions extracted from different wines is in progress.

6 References

23. J. Yamakoshi, S. Kataoka, T. Koga and T. Ariga, Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic


