THE EFFECT OF NATURAL DIETARY ANTIOXIDANTS ON LOW DENSITY LIPOPROTEIN OXIDATION AND ATHEROSCLEROSIS

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ABSTRACT

The objectives of the present studies were to investigate the *in vitro* antioxidant properties of red wine containing polyphenols and the isoflavone genistein. Subsequently, the effect of red wine on LDL oxidation and fatty streak lesion development in cholesterol-fed rabbits was examined. Since LDL oxidation is generally thought to promote the development of atherosclerosis, the hypothesis of the present study was that dietary intervention with antioxidants could inhibit LDL oxidation and slow atheroma formation.

In vitro experiments demonstrated that both red wine and genistein were effective inhibitors of copper and peroxy radical-catalysed LDL oxidation. Red wine containing 0.025 to 20 mg/L gallic acid equivalents (GAE) and genistein at concentrations of 0.2 to 200 μmol/L increased the lag time of conjugated diene formation, inhibited the generation of thiobarbituric acid reactive substances and decreased the relative electrophoretic mobility of LDL in a concentration-dependent manner. These changes were not apparent in LDL incubated with ethanol or red wine stripped of phenols. Red wine polyphenols have the ability to be incorporated into LDL during an *in vitro* incubation with plasma, as evident by the 60% increase in lag time following copper-mediated oxidation of isolated LDL which also resulted in 3-fold lower uptake of this LDL by macrophages compared to control LDL. Genistein did not prevent the oxidation of LDL in this model due to its poor incorporation into LDL following plasma incubations.

Red wine was fractionated into phenolic acids (fraction 1), catechins and monomeric anthocyanidins (fraction 2), flavonols (fraction 3) and polymeric anthocyanidins (fraction 4) and the antioxidant properties of each fraction was determined. All red wine fractions increased the lag time of copper-mediated LDL oxidation compared to control with the order of potency being fraction 2 (92% increase in lag time) > fraction 1 (65%) > fraction 4 (42%) > fraction 3 (37%). Similarly, malondialdehyde concentrations following azo-initiated oxidation of LDL were inhibited by 12 to 40% in the presence of red wine fractions and followed the same order of potency.

In a dietary intervention study, male New Zealand White rabbits were grouped into one of four treatments. One group were fed a normal commerically available rabbit diet (n=6). The remaining 18 rabbits were fed a diet containing cholesterol (0.25 to 0.5% wgt/wgt) alone (n=6), or in combination with red wine (n=6) or ethanol (n=6). Rabbits consumed approximately 22.5 ml of red wine/d for 12 weeks which was equivalent to 36.3 mg GAE/d. Due to the unpalatability of ethanol, rabbits consumed less ethanol, approximately 0.9 g ethanol/d in this treatment compared to red wine-treated rabbits which consumed 1.7 g ethanol/d.

Plasma cholesterol, VLDL + IDL, LDL and HDL-cholesterol levels were significantly elevated in cholesterol-fed rabbits. There were no differences in plasma lipids or lipid and protein compositions in any lipoprotein fractions following dietary intervention with red wine or ethanol. The oxidisability of LDL was determined as the lag time for conjugated diene formation following copper-mediated oxidation. LDL isolated from cholesterol + red wine-treated rabbits displayed a significantly shorter lag time (112.1 ± 1.1 min, $P<0.05$) compared to rabbits fed cholesterol alone (160.8 ± 16.8 min). The lag time of LDL oxidation in ethanol-treated rabbits (124.4 ± 9.6 min) was not significantly different to that measured in rabbits fed cholesterol alone or in combination with red wine. There were no significant differences in oxidation rate, maximum conjugated diene concentration and malondialdehyde formation between rabbit treatment groups. Despite the difference in lag time there were no differences in α-tocopherol or the fatty acid composition in LDL isolated from rabbit plasma.

At completion of the dietary intervention anaesthetised rabbits were killed by exsanguination from the abdominal aorta. Atherosclerosis in aortic arch segments from rabbits was assessed by lipophilic staining using oil red O and quantified using image analysis. The % lipophilic stain in the aortic arches from rabbits were not different in cholesterol-fed rabbits 19.3 ± 7.0, cholesterol + red wine rabbits 21.1 ± 4.1 and cholesterol + ethanol rabbits 15.9 ± 6.1, although the fatty lesions in these rabbits were significantly greater than in control rabbits 0.06 ± 0.04. Rabbits fed cholesterol in combination with red wine displayed significantly

greater cholesterol deposition in segments of the descending thoracic aorta 1.13 ± 0.16 μg cholesterol/mg wt wgt compared to rabbits fed cholesterol alone (0.65 ± 0.03, $P<0.01$), but this was not different to rabbits consuming cholesterol + ethanol (1.11 ± 0.2).

For experiments investigating vasorelaxation, thoracic aortic rings with intact endothelium were mounted in organ bath chambers and smooth muscle contractions to potassium and phenylephrine were measured. Cumulative dose-response curves to acetylcholine, calcium ionophore (A23187) and sodium nitroprusside were performed in precontracted aorta. Although there was an impaired aortic relaxation to acetylcholine in cholesterol-fed rabbits compared to normocholesterolemic rabbits, the response of aorta to acetylcholine was the same in rabbits fed cholesterol alone or in combination with red wine or ethanol.

Catechins (140 mg/L) and procyanidins (polymeric catechins, 400 mg/L) are abundant polyphenols in red wine and were used as markers for red wine bioavailability in this rabbit study. Monomeric catechins could not be detected by high performance liquid chromatography with electrochemical detection in rabbit plasma nor could we detect levels of monomeric and polymeric catechins using a sensitive colorimetric assay.

In summary, red wine was an effective antioxidant *in vitro* protecting against LDL oxidation. In a dietary intervention study red wine and ethanol had no effect on plasma lipids and lipoprotein levels nor endothelium-dependent relaxation of aortic preparations in cholesterol-fed rabbits. Red wine supplementation in conjunction with cholesterol feeding increased LDL oxidisability and thoracic aorta cholesterol concentrations compared to rabbits fed cholesterol alone. There were no significant differences between red wine and ethanol supplemented rabbits. The low plasma levels of red wine polyphenols and the increased ethanol consumption in red wine treated rabbits could contribute to the apparent proatherogenic effects of red wine observed in cholesterol-fed rabbits.