

Wine polyphenols and promotion of cardiac health

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Wine polyphenols are considered to have beneficial effects on CHD and atherosclerosis. The consumption of red wine is high in Italy and France, approximately four times greater than that in the UK. This disparity in red wine consumption is thought to be the reason for the 'French paradox', where France was shown to have a coronary mortality rate close to that of China or Japan despite saturated fat intakes and cholesterol levels similar to the UK and USA. In the present review, we discuss the effects of wine and some of its polyphenol constituents on early pathological indicators of CHD such as plasma lipids, the endothelium and vasculature, platelets and serum antioxidant activity. The review also examines whether the polyphenols or the alcohol in wine is responsible for the effects on markers of heart disease. The present review concludes that red wine polyphenols have little effect on plasma lipid concentrations but wine consumption appears to reduce the susceptibility of LDL to oxidation and increase serum antioxidant capacity. However, these effects do depend on the amount of wine and period of supplementation. Authors who have examined specific polyphenols suggest that some phenolics appear to have endothelium-dependent vaso-relaxing abilities and some a positive effect on NO concentrations. Red wine phenolics also have an inhibitory effect on platelet aggregation, and individual phenolics also have a similar effect *in vitro*, although it should be noted that there are often discrepancies as large as ten-fold between the concentrations of polyphenolics tested *in vitro* and their measured levels *in vivo*. Evidence suggests that alcohol has a positive synergistic effect with wine polyphenols on some atherosclerotic risk factors. Thus evidence that wine drinking is beneficial for cardiac health continues to accumulate but more research is required to understand fully and exactly the functions of red wine polyphenols.

Wine polyphenols: Coronary heart disease: Alcohol: Antioxidants

Introduction

'Wine is a biochemical challenge. It is a daunting task to probe the alchemy of this elixir and to determine what lies 'at the heart of the matter'.'

(Goldberg *et al.* 1995a)

Wine has been a part of human culture for 6000 years, with the first documented vineyard being planted by Noah after the Great Flood (Jackson, 1994; Soleas *et al.* 1997a). Since that time, wine consumption has been recorded in various countries as a part of their rich culture and diet with the Mediterranean areas appearing to enjoy better health as a consequence of their indulgence (Kushi *et al.* 1995; Trichopoulou *et al.* 1995; Willett *et al.* 1995). The Mediterranean diet is characterised by high intakes of fruit, fish, cooked vegetables, salad, wines and the use of olive

oil (Tjønneland *et al.* 1999) and has long been considered one of the healthier European diets (Nestle, 1995; Trichopoulou *et al.* 1995).

In 1997, Luxembourg was recorded as having the highest annual wine consumption per capita in the world at 63.3 litres (The Wine Institute, 1999) (Table 1). Wine consumption in France and Italy was only slightly lower than in Luxembourg although it has fallen almost by half since 1970. Annual consumption levels in the UK were 14 litres per capita while in the USA and Canada, the levels were only half that and Oriental countries tend to have the lowest consumption. In Denmark and Australia more wine is drunk than in the UK and consumption has increased three- to five-fold since 1970. In the UK there was a very low wine consumption in 1970 (2.2 litres per capita) but in 1980 it apparently rose steeply to 30 litres and fell to 14 litres by 1997. However these amounts pale in significance

Abbreviations: CETP, cholesteryl ester transfer protein; oxLDL, oxidised LDL.

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Table 1. Per capita wine consumption by country over the past three decades

Country	Per capita annual wine consumption (litres)*		
	1997	1980	1970
Luxembourg	63.30	/	/
France	60.13	90.87	108.22
Italy	58.05	79.87	110.83
Spain	36.87	59.91	61.43
Denmark	29.14	12.78	5.90
Australia	18.95	17.38	8.50
UK	13.99	30.13	2.87
Chile	13.57	50.24	43.85
South Africa	8.92	9.07	11.19
Canada	7.39	8.35	2.19
USA	7.38	7.98	4.95
Ireland	5.32	3.21	3.29
Japan	1.69	0.38	0.30
China	0.36	/	/
Egypt	0.05	/	/

*The Wine Institute (1999).

in comparison with the volumes consumed in the top three countries of the world, i.e. Luxembourg, France and Italy. Furthermore, despite the known associations between excessive alcohol consumption and mortality from liver diseases (Singh & Hoyert, 2000), there is a general trend

for lower mortality from IHD in countries with a higher wine consumption per capita (Figs. 1 (a) and (b)). However, there are exceptions and in other countries with extremely low IHD mortality rates, such as Japan and China, wine consumption is unlikely to be exerting any benefits.

The beneficial properties that wine specifically contributes to the diet are far from clear. Furthermore, different wines have different compositions and therefore benefits may be due to different components. Wines vary depending on the grape cultivar used, the winemaking procedure and conditions of storage. The development of new technology has facilitated the identification of many of the compounds in wine that were previously unknown. At the moment, over 500 different compounds have been documented in different types of wines including water (74–87 %, w/w), sugars (0.05–10 %), ethanol (10–14 %), acids (0.05–0.7 %), and phenols (0.01–0.2 %) (Soleas *et al.* 1997a). Polyphenols have long been considered to be the ingredients in wine that may be beneficial for health because of their antioxidant properties. Hence much research has been directed into this area with several reviews on wine reporting its potential cardioprotective benefits (Cook & Samman, 1996; Chung *et al.* 1998; Cao & Prior, 2000; Estruch, 2000).

The present paper will review the epidemiology of wine consumption and CHD, polyphenols and their structure–function relationships, the pathology of CHD and the

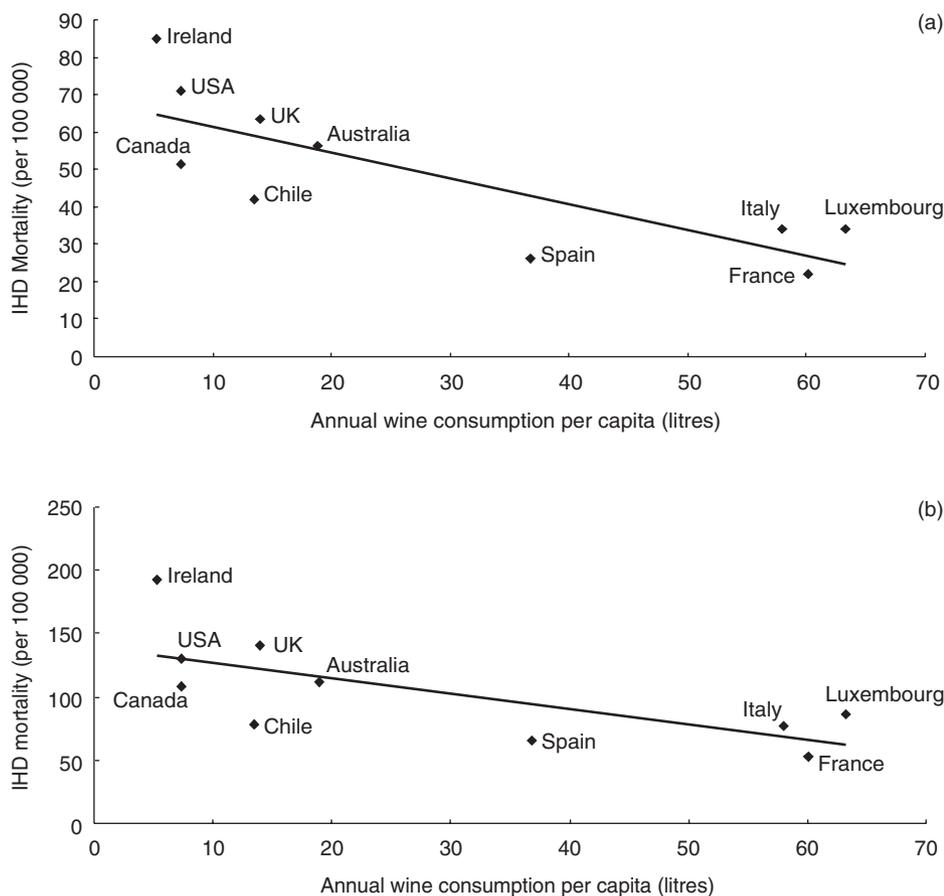


Fig. 1. IHD mortality and wine consumption in the 1990s by country in females (a) (r 0.6469) and males (b) (r 0.4877), not including Japan, Egypt or China.

effects of wine and its polyphenols on different aspects of the disease. The overall aim is to describe some of the more recent studies and examine possible mechanisms involved to indicate the direction of current research in this area.

Epidemiology of wine intake and coronary heart disease

The 'French paradox' was the term that became better known after an unexpected finding of the Monitoring Trends in Cardiovascular Diseases (MONICA) Project (World Health Organization, 1989) and further investigated by Renaud & de Lorgeril (1992). France was shown to have a coronary mortality rate close to that of China or Japan despite saturated fat intakes and plasma cholesterol concentrations similar to the UK and USA. Wine consumption was suggested as a possible protective factor and a significant inverse correlation was found between the country's wine consumption and mortality from CHD (Renaud & de Lorgeril, 1992). However, the time-lag hypothesis cast doubt on this explanation of the French paradox (Law & Wald, 1999). The new hypothesis observed that high animal fat consumption and serum cholesterol levels have only been similar between France and Britain since the mid 1980s. Before this time, the levels of animal fat consumption (about 21 % of total energy consumption in France *v.* about 31 % in Britain) and mean serum cholesterol (5.7 *v.* 6.3 mmol/l) in men aged 50–70 years were lower in France than the UK (World Health Organization, 1989; Crique & Ringel, 1994). Therefore Law & Wald (1999) have suggested that changes will occur in the pattern of CHD in France with increases in atheroma and deaths from CHD within the next 25 to 35 years. However, the criticisms have not been widely accepted and although it may indeed be a factor influencing the pattern of world CHD, human intervention studies with wine polyphenols have indicated effects that should be investigated further.

There is also the possibility that other aspects of diet and lifestyle in France and other Mediterranean countries may be the more important determinants of CHD risk. In a Danish study (Tjønneland *et al.* 1999), wine drinking in comparison with other alcoholic drinks was associated with a more healthy diet; for example, higher intakes of fruit, fish, cooked vegetables, salad, and the use of olive oil for cooking. However, a similar study in Italy that compared the diets of wine drinkers, mixed drinkers and abstainers found no such correlation between wine drinking and consumption of other 'healthy' foods (Chatenoud *et al.* 2000). It should be noted, however, that the consumption of wine in Italy and Denmark is not comparable. Italians consume approximately twice as much per capita as Danes (Table 1) and IHD mortality is almost 3.5 times higher in Denmark than in Italy (Figs. 1 (a) and (b)). Recently, Di Bari *et al.* (2003) conducted a study on an elderly Italian population in whom the mean daily consumption of wine was 176 ml (equivalent to 64 litres annually and comparable with the 1997 values in Table 1 of 60 litres annually). It was found that the daily wine intake was significantly inversely associated with a marker of CHD, namely antibodies for oxidised LDL (oxLDL) (Di Bari *et al.* 2003). Therefore evidence continues to appear suggesting that wine con-

sumption is a separate dietary factor protecting against heart disease.

One question, which is still not answered, is whether the apparent cardioprotective effects associated with wine consumption stem from the alcohol, other wine components or a mixture of the two. Alcohol intake in general is inversely correlated to the risk of CHD but the link with wine alcohol specifically is stronger than beer alcohol and more consistent (Crique & Ringel, 1994). In an early paper by St Leger *et al.* (1979), a strong negative correlation between IHD mortality and alcohol consumption was reported. However, after categorising the alcohol consumption into wines, beers and spirits, it appeared that wine was the most strongly associated with the cardioprotective effect. Such effects may be due to trace compounds present in wine but the authors precluded further work to isolate these constituents by suggesting it was 'almost a sacrilege, as the medicine is already in a highly palatable form'! However, as some societies do not consume alcohol for cultural reasons, further research to isolate and identify the factors responsible for the beneficial effects of wine is needed. A meta-analysis in 1996 of ecological, case-control and cohort studies comparing wine, beer and spirits and their associations with CHD risk found that there was strong evidence to suggest that all alcoholic drinks in moderation were linked to lower risk. The authors suggested that any stronger association of wine with a lower CHD risk was probably due to patterns of drinking and lifestyle factors that were not fully controlled for in other studies (Rimm *et al.* 1996), although if there were not enough wine drinkers in the country, this may explain why a stronger association was not made. Drinking patterns such as wine drinking with meals, or daily *v.* weekend binge drinking are important aspects to be documented when reporting epidemiological links between alcohol consumption and CHD risk. For example, it has been found that coronary event risk is lowest among men who report one to four drinks (and women one to two drinks) daily on 5 or 6 d of the week (McElduff & Dobson, 1997). It should be noted that alcoholic patterns of drinking habits are continually on the change; for example, France has a beer industry worth £5 billion that is on the increase, whilst the wine industry at £8 billion is shrinking (UK Trade and Investment, 2004).

Polyphenols in wine

Structure and classification

Polyphenols can be split into two groups; flavonoids and non-flavonoids. Flavonoids are the most common and widely distributed group of phenolics (Bravo, 1998). Fig. 2 shows the basic structure of flavonoids, which comprise two phenol moieties connected via a pyran ring and possessing several hydroxyl and/or ketone groups. They can exist in the free form (*i.e.* aglycone), polymerised, linked to a sugar moiety (*i.e.* glycoside) or linked to other non-flavonoid phenols (Ursini *et al.* 1999). The most common sugar residue is glucose but others such as galactose, rhamnose, xylose and arabinose are found (Bravo, 1998). Over 4000 individual flavonoid compounds are known to occur in nature (Harbourne, 1994). The structure of flavonoids

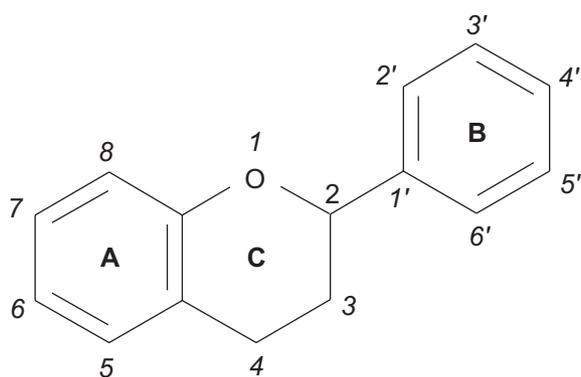


Fig. 2. The general structure of flavonoids. The three phenolic (pyrane) rings are referred to as A, B and C and the numbering systems are used to allow easier identification of the position of substitutions in the rings.

allows many patterns of substitution into the benzene rings to create the wide variety of compounds found in nature (Hollman, 1997). Most flavonoids are soluble in aqueous media but the degree of solubility depends on their polarity and chemical structure (Bravo, 1998). Extensive information on flavonoids and advances in research has been reviewed by Middleton & Kandaswami (1995).

Flavonoids can be classified by the oxidation level of their central C-ring, i.e. by the number and position of hydroxyl groups. The flavonoid classes include anthocyanidins, flavones, flavonols, flavandiols (leucoanthocyanidins), aurones, biflavonoids, chalcones, dihydrochalcones, flavanones, dihydroflavonols, flavans and isoflavonoids (Bravo, 1998). In addition, there are polymeric compounds called tannins (Cook & Samman, 1996). These classes can further be subdivided by the number and type of substituents present.

Flavonols, flavanols and flavones

The flavonol class is an important group of compounds in the diet and includes such examples as quercetin, myricetin (Fig. 3), kaempferol and isorhamnetin. Flavonols are found in a wide variety of foods, e.g. leeks, broccoli, onion, lettuce, cranberries, apples, olives and beverages such as tea and red wine. Red wine contains approximately 4–16 mg quercetin/l and 7–9 mg myricetin/l (Hertog *et al.* 1993). Flavones differ from flavonols by a lack of hydroxylation on the C₃ on the C-ring (Fig. 2) (Harbourne, 2000). The most abundant flavones are luteolin and apigenin. Flavonoids and other similar types such as catechins are generally bound to sugars to increase their solubility. The flavanol group consists of optically active isomers such as (+)-catechin, (–)-epicatechin (Fig. 3) and their gallates. The flavanols are also known as the catechins or flavan-3-ols. Flavanols have a feature that is only shared by anthocyanidins, namely they lack the ketone group at the 4-position of the heterocyclic C-ring (Hollman & Arts, 2000). Flavanols are popularly associated with tea, rather than wine, but there are higher concentrations of catechins in red wine than all the other flavonoid compounds (Goldberg *et al.* 1998). A detailed review on flavonols,

flavones and flavanols has been published recently that covers the nature, occurrence and dietary amounts of these compounds (Hollman & Arts, 2000).

Tannins

Tannins are polymeric structures comprising from a few to over 100 flavonoid units giving a high molecular weight (Santos-Buelga & Scalbert, 2000). Tannins can form insoluble complexes with carbohydrates and proteins such as the salivary proteins *in vivo* (Bravo, 1998). Some tannins are hydrolysable, meaning that gallic acid or a similar compound is present, bonded to a carbohydrate (for example, tannic acid). Other tannins can be formed by the polymerisation of flavonoids through condensation reactions, for example, proanthocyanidins (Cook & Samman, 1996). Proanthocyanidins are so called because they yield one or more anthocyanidins in the presence of strong acids. Specific proanthocyanidins give distinct anthocyanidins; for example, procyanidins give cyanidin on hydrolysis and prodelphinidins give delphinidin.

Proanthocyanidins possess a polymerised flavan-3-ol structure with basic units of catechin (dihydroxylated in the B-ring) or gallo catechin (trihydroxylated in the B-ring) (de Pascual-Teresa *et al.* 2000). Red wine can have a great range of proanthocyanidin concentrations, for example, 1–200 mg type B1/l (a dimer of epicatechin and catechin) (Santos-Buelga & Scalbert, 2000). Proanthocyanidins can act as antioxidants by complexing with metallic ions or radical scavengers (Virgili *et al.* 1998; Plumb *et al.* 2000; Santos-Buelga & Scalbert, 2000). Other properties include providing astringency in food, possibly protecting against some human diseases, for example, CHD and cancer (Bravo, 1998), interacting with anthocyanins to affect the colour of red wine (Francia-Aricha *et al.* 1997; Saucier *et al.* 1997) and complexing with metal ions to inhibit non-haem Fe absorption. It is suggested that proanthocyanidins can aid the prevention of cardiovascular disease by protecting LDL against oxidation and inhibiting platelet aggregation (Santos-Buelga & Scalbert, 2000). The anthocyanins are important pigments with over 300 occurring in nature and the water-soluble and acyl-glycosidic forms of anthocyanidins are now widely used in the food industry as food colourants (Harbourne, 1994).

Resveratrol

Resveratrol (Fig. 3) is a molecule (3,5,4'-trihydroxystilbene) that has been the subject of much speculation and is renowned for its possibly beneficial effects on atherosclerosis and cancer (Jeandet *et al.* 1993). Resveratrol and piceid (3 β -glucoside of resveratrol) are both sensitive to light and air oxidation, which makes their extraction from grapes and wines difficult (Pezet *et al.* 1994). It can be found in grape products where it is primarily sourced from the skin of the grape but virtually absent in the flesh (Lamuella-Raventós & Waterhouse, 1999); however, it has been found at relatively high concentrations in seeds of the Muscadine grape (Ector *et al.* 1996). Resveratrol is a phytoalexin, which is part of the natural plant defence system against fungal infection (Soleas *et al.* 1995) and is reported to be an indicator of

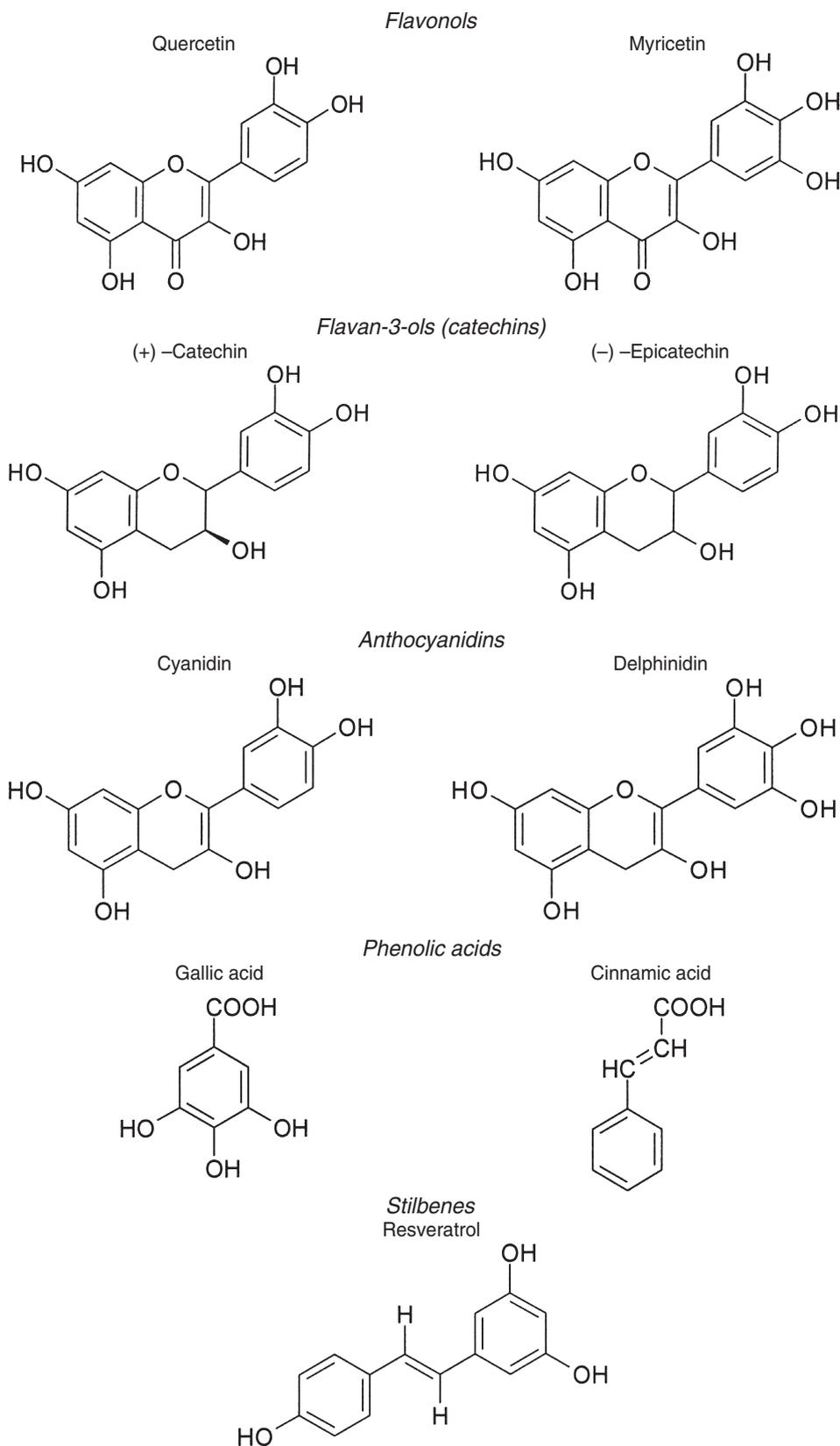


Fig. 3. Examples of structures of five major phenolic groups. A flavonol has oxygen double-bonded at the 4-position, an –OH at position 3 and a double bond between carbons 2 and 3. A flavan-3-ol does not have the two double bonds but does have the –OH group at position 3. An anthocyanin has a double bond between carbons 2 and 3, and the –OH group at carbon 3, but not the double-bonded oxygen at position 4. Phenolic acids have the simple phenol as their basic structure. Resveratrol is not a flavonoid as it has an incomplete C-ring.

disease resistance in different vine varieties (Pezet *et al.* 1994). However, it has been found that extensive infection before harvest can lower the resveratrol content of the resultant wine by rapidly spreading lesions from which laccase, an enzyme from the *Botrytis* pathogen, is released and degrades the resveratrol (Jeandet *et al.* 1995). Burgundy Pinot Noir red wines have been found to contain relatively high concentrations of resveratrol from 1.6 to 8.7 $\mu\text{mol/l}$, compared with white Chardonnay wines that contained only 0.004 to 0.4 $\mu\text{mol/l}$ (Jeandet *et al.* 1993). Another study also found consistently high resveratrol levels in Pinot Noir wines (Goldberg *et al.* 1995b). Varying concentrations have been measured in Swiss (Pezet *et al.* 1994), Californian (Lamuela-Raventos & Waterhouse, 1993) and Japanese (Okuda & Yokotsuka, 1996) wines of different grape types. Recently the occurrence of a resveratrol dihydrodimer at a concentration range of 0.44 to 1.32 $\mu\text{mol/l}$ was reported for red and rosé wines (Adrian *et al.* 2000). Ingestion of resveratrol and its derivatives may be as high as 3.1 to 12.3 $\mu\text{mol/d}$ for moderate consumers of Spanish red wines (200 ml/d) (Lamuela-Raventos *et al.* 1995). However, this suggests a maximum resveratrol concentration of approximately 60 $\mu\text{mol/l}$, which is much higher than that found in most of the wines tested by Lamuela-Raventos *et al.* (1995).

Non-flavonoids

Non-flavonoid components of wine are simple abundant phenols with a variety of different functional groups present. They can be grouped as benzoic-based compounds (for example, vanillic, gallic and protocatechuic acids), benzaldehydes (for example, vanillin, syringaldehyde), cinnamic acids (for example, *p*-coumaric, ferulic, chlorogenic and caffeic acids) and cinnamaldehydes (for example, coniferaldehyde, sinapaldehyde). Wine astringency is attributed to the presence of the larger polyphenols and the smaller phenolic acids account for bitterness. Volatile phenols can contribute towards effects such as smokiness, vanilla character, pungency and bitterness but these components are usually only present in tiny amounts and especially so in young wines (Singleton, 1982). Polyphenols in wine originate mainly from the skin and seeds of the grape but can also be derived from the vine stems, the wood used for barrels and from yeast metabolism (Soleas *et al.* 1997a).

In summary, there are many different groups of phenolics present in red wine, with flavonols being considered to be the most biologically important. However, flavanols and resveratrol have also received much attention recently. Non-flavonoids appear to not be considered as being important although they are generally present in abundance. The concentrations of total phenolics in commercially available red wines are rarely over 2.5 g/l as measured by the Folin–Ciocalteu method (Singleton, 1982).

Structure and antioxidant-function relationships

For a flavonoid to be an effective antioxidant, it requires three characteristics. The first requirement is a hydroxyl group (–OH) on the unsaturated C-ring at position 3 (Fig.

2), which confers higher stability of the radical due to delocalisation of electrons. The second requirement is a 2,3-double bond in conjugation with the oxo group (=O) at position 4 in the C-ring with the presence of an –OH group at positions 3 and 5 with the oxo function in the A- and C-rings increasing the radical-scavenging potential of the compound. Third, to be effective, a flavonoid requires an *o*-dihydroxy structure in the B-ring (Bors *et al.* 1990). Quercetin is a flavonol that has all three of these characteristics and is one of the most powerful natural antioxidants (Bravo, 1998). If any of these requirements are not met, the antioxidant activity of the molecule is reduced. The number of hydroxyl groups the flavonoid has can determine its radical-scavenging ability; for example, myricetin has six hydroxyl groups and has a greater antioxidant capacity than kaempferol, which has four hydroxyl groups when tested under the same conditions (Husain *et al.* 1987; Cook & Samman, 1996). If one of these –OH groups is at position 3, then this greatly enhances its antioxidant capacity compared with another flavonoid with the same number of hydroxyl groups. The pattern of hydroxylation is important, mainly positions 3 (C-ring), 5 and 7 (A-ring), and 3' and 4' (B-ring) appear to enhance the inhibition of lipoprotein oxidation (Cook & Samman, 1996). If the flavonoid is linked to a sugar moiety, it hinders the scavenging ability of the remaining hydroxyl groups. Thus, *in vitro*, aglycones are reported to show a higher scavenging potency than their comparable glycosides (Cook & Samman, 1996). A flavonoid with both a ketone group at position 4 and a hydroxyl group at either position 3 or 5 (for example, rutin, quercetin) will have the ability to chelate Fe ions, which prevents the metal ions being used in the Fenton system to generate free radicals. Then, once formed, the flavonoid–Fe complex still has the ability to scavenge free radicals.

Antioxidants are thought to lower the risk of CHD by lowering the concentration of free radicals in the body and so reduce the likelihood that one of the radicals will cause oxidative damage (Parr & Bolwell, 2000). Vitamin E is one of the most important antioxidants present in the plasma and LDL, and additional dietary antioxidants can either spare vitamin E from oxidation or assist in its regeneration after oxidation. Testing the antioxidant potential of a compound *in vitro* on plasma or LDL can be performed by either the compound being added at the start of the reaction, i.e. to prevent vitamin E utilisation, or being added at different time points after initiation, i.e. to assist the regeneration of vitamin E. A flavonoid-rich extract has been shown to prevent LDL oxidation *in vitro* in a concentration-dependent manner and to delay the consumption of vitamin E, but not to terminate or delay LDL oxidation once the vitamin E in the LDL was consumed (Viana *et al.* 1996). Specific flavonoids have also been tested in a similar manner (Zhu *et al.* 2000). When present at the start of oxidation, the flavonoids quercetin, myricetin, kaempferol and morin exerted dose-dependent protective activity against the depletion of α -tocopherol in LDL over the concentration range 1–20 $\mu\text{mol/l}$. Kaempferol (–OH group at position 4') and morin (–OH groups at positions 2' and 4') were less effective at preventing α -tocopherol depletion than quercetin (–OH groups at positions 3' and 4') and myricetin (–OH groups at positions 3', 4' and 5'). When the

flavonoids were added 5 min after initiation, significant regeneration of α -tocopherol was observed, with activity related to the number and location of hydroxyl groups in the B-ring (Zhu *et al.* 2000). These experiments show that flavonoids are active, though not all to the same extent, in both protecting and regenerating α -tocopherol *in vitro*.

In vivo studies may not show the same effect as *in vitro* work. An animal study found that there were multiple effects from supplementing Fe-loaded rats (impaired antioxidant status) with catechin; more specifically an enhanced platelet function was seen with an increase in total antioxidant status and vitamin E sparing (Blache *et al.* 2002). However, these effects were only seen in animals with impaired antioxidant status and not in control animals, indicating that if the individual has a normal antioxidant status, then supplementation may have no effect. An *in vivo* study, in which human volunteers were supplemented with either red wine or quercetin to determine if there is an increase in the oxidative resistance of LDL, did not find any changes in plasma or LDL α -tocopherol concentrations (Chopra *et al.* 2000). Where no effect of flavonoids on vitamin E concentrations was observed, it is possible the concentrations of flavonoids were lower *in vivo* than in studies performed *in vitro*, or that the flavonoids were metabolised into a form where the active functional groups were blocked (Day *et al.* 2000). However, it is more probable that no change in α -tocopherol was seen as the concentrations were already optimal and were not under oxidation pressure, and therefore not susceptible to alteration. This is especially so *in vivo* as α -tocopherol is believed to be regenerated by vitamin C and therefore no changes would be expected in α -tocopherol concentrations except under extreme or sustained oxidative stress.

One of the ways of comparing the antioxidant properties of different wines is by measuring their total antioxidant activity by one of the standard methods available, for example, Trolox-equivalent antioxidant activity (Rice-Evans & Miller, 1996) or total phenolic acid content (Singleton & Rossi, 1965). As expected, a relationship exists between Trolox-equivalent antioxidant activity and the phenolic acid content and the ratio of the two measurements is approximately the same over a range of different wines. Likewise, white wine shows a similar relationship between its total phenolic acid content and Trolox-equivalent antioxidant activity as red wine (Rice-Evans & Miller, 1996). Although it is considered important to characterise the antioxidant power of wine *in vitro*, it should be stated that the results do not predict the *in vivo* situation as the relative absorption of active compounds and efficacy of the resulting metabolites are yet to be established. It should be mentioned that the measurement of the antioxidant power of crude wines should be considered informative only as it cannot represent the real activity that could potentially be obtained *in vivo* due to the deglycosylation that occurs before absorption, changing the phenolic from conjugate to aglycone and therefore its antioxidant activity.

Soleas *et al.* (1997b) made an interesting observation with regard to the antioxidant capacity of wine and the concentration of certain phenolics. Using a multivariate analysis, they determined the relative contributions of different polyphenolic constituents to the antioxidant capacity of

thirty-two different Ontario wines. They found that 96 % of total antioxidant capacity as measured by the Randox assay (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK) could be predicted from the sum of the micromolar concentrations of catechin, *m*-coumaric acid, epicatechin, *cis*-polydatin, *trans*-polydatin, quercetin, *trans*-resveratrol and vanillic acid in the wine (concentrations of individual polyphenol values not given). The remaining polyphenols (caffeic acid, *p*-coumaric acid, ferulic acid, gentisic acid, isoquercitrin, myricetin, *cis*-resveratrol, gallic acid, syringic acid) did not contribute significantly to the antioxidant protection. Thus knowledge of the concentrations of certain polyphenols in wines can be used to predict total antioxidant capacity.

In summary, the data available suggest that the mechanisms by which polyphenols exert their antioxidant properties *in vivo* are: (1) reduction of free radical formation; (2) protection of α -tocopherol by preferential oxidation; (3) regeneration of oxidised α -tocopherol; (4) chelation of metal ions (Cook & Samman, 1996).

Coronary heart disease pathology

Wine polyphenols are believed to have beneficial effects on CHD and atherosclerosis. The term CHD is generally used to refer to cardiac disease specifically due to atherosclerosis of the coronary arteries (Mangiapanne & Salter, 1999). Atherosclerosis is characterised by an accumulation of lipids in the intima of the large arteries and can begin in early childhood. Atherogenesis results from a combination of many pathological factors that are all closely linked and interact to form a lesion. These include endothelial dysfunction, platelet activation, lipoprotein retention, lipoprotein oxidation, lipoprotein aggregation, macrophage foam cell formation, inflammation and thrombosis (Aviram, 1996). Plasma lipoproteins are the major transporters of lipid and lipid-soluble antioxidant compounds and vitamins in serum. LDL is the principal carrier of cholesterol in the blood and HDL particles remove excess or unused cholesterol from cells.

Elevated plasma concentrations of LDL are considered as a risk factor for atherosclerosis, and it is now evident that it is a cause of atherosclerosis as well as a consequence of disease. The permeability of the arterial endothelium to oxLDL may be increased by factors such as high blood pressure, genetic factors, infection and smoking and thereby decreasing the amount of oxLDL in the plasma. Erythrocyte membrane fluidity measurements can be used to indirectly assess endothelial cell membrane fluidity. The latter is a measure of membrane cell flexibility (Sharpe *et al.* 1995) and low flexibility is associated with an increased risk of atherosclerosis. Modification of LDL via oxidation, nitration, glycation or alkylation can also increase the risk of atherosclerosis. Normally, LDL particles are removed from the circulation via native receptors on macrophages, which recognise the apo B part of LDL. Native receptors are down regulated by elevated cellular cholesterol levels. Modified LDL can be removed by several different scavenger receptors and since these receptors are not down regulated by the cellular cholesterol content, entry of LDL particles through these receptors can lead to increased accu-

mulation in the cells. It should also be noted that at least one of these receptors (CD36; macrophage type B scavenger receptor) is even up regulated by cellular cholesterol in a lipid-driven self-regulated cycle (Han *et al.* 1999). Free radicals generated by endothelial cells, monocyte-derived macrophages and smooth muscle cells can oxidise LDL (Cook & Samman, 1996). OxLDL is found in atherosclerotic lesions and oxLDL can be produced by peroxidation of the PUFA of surface phospholipids in the LDL particle (Mangiapane & Salter, 1999). Serum HDL concentrations are inversely associated with the risk of CHD (Mangiapane & Salter, 1999) and may also prevent lipid oxidation by aiding the removal of excess cholesterol from cells within the artery wall. Both LDL and HDL are important factors in atherosclerosis and CHD, and monitoring of these lipoproteins is important in studies investigating potential cardio-protective dietary components.

Atherosclerosis is a chronic inflammatory process that involves a complex interplay of circulating cellular and blood elements with the cells of the artery wall (Witztum & Steinberg, 2001). OxLDL is now well accepted as an important factor in the development of atherosclerotic plaques and inhibition of LDL oxidation is suggested to inhibit atherosclerosis independent of lowering plasma cholesterol levels (Witztum & Steinberg, 2001). Various pro-atherogenic effects of oxLDL have been suggested. These include the promotion of cholesterol accumulation in subendothelial space, macrophage and smooth muscle cell proliferation (Martens *et al.* 1999; Chai *et al.* 2002), immunogenic effects leading to autoantibody formation (Tsimikas *et al.* 2001; Witztum & Steinberg, 2001), and the induction of NO synthase and apoptosis (Cromheeke *et al.* 1999). Other pro-atherogenic effects of oxLDL that have been suggested are the activation of signal transducers (Maziere *et al.* 1999) and transcription factors such as NF- κ B (Parhami *et al.* 1993). The latter is reported to colonise in early and advanced atherosclerotic plaques (Brand *et al.* 1996).

Some of these mechanisms, i.e. the inhibition of smooth muscle cell proliferation by resveratrol (Liu & Liu, 2004) and the inhibition of oxLDL cytotoxicity by red wine and quercetin (Carrero *et al.* 1998), have been shown in *in vitro* experiments. However, further studies investigating the effect of wine flavonoids on the transcription of NF- κ B are warranted. The latter is important due to its effects on the pro-inflammatory response and apoptosis, both of which play an important role in the development of atherosclerosis.

Effects of wine on risk factors for coronary heart disease

Lipid content and oxidative modification: *in vitro* studies. Many *in vitro* studies use lipoproteins and plasma obtained from healthy individuals to measure oxidation potential and also to test the effect of added antioxidants. Recently, it was shown that whole red wine could inhibit macrophage-mediated oxidation of lipoproteins when exposed *in vitro* and that white wine (of similar alcohol content) was not as effective (Rifici *et al.* 1999). It was found that alcohol alone had no effect; thus the effects of red wine did not appear to be wholly due to the alcohol content. However, as the study

did not test de-alcoholised red wine, the possibility of synergistic effects between the alcohol and other wine constituents cannot be excluded.

Instead of using whole wine, some workers have used extracts from red wine and tested these *in vitro* for antioxidant properties. Frankel *et al.* (1993) were the first to show the beneficial effects of the non-alcoholic components of red wine on the oxidation of human LDL *in vitro*. They reported that lipid peroxidation of LDL was inhibited by 60 and 98 % in the presence of 3.8 and 10 μ mol red wine polyphenols/l respectively. Also, polyphenols from a Cabernet Sauvignon wine prevented cholesterol oxide formation *in vitro* and displayed α -tocopherol-sparing activity (Deckert *et al.* 2002). Red wine polyphenols have also been shown to have a protective effect on erythrocytes *in vitro* against H₂O₂-induced oxidation (Tedesco *et al.* 2000). A pure Concord red grape extract rather than wine also inhibited lipid peroxidation *in vitro* (Lanningham-Foster *et al.* 1995). These experiments have shown that components of both red grapes and wine, other than alcohol, have inhibitory effects on lipid peroxidation *in vitro*, but at this point in time, no single component has been identified as the active agent.

Polyphenols in red wine can be fractionated into phenolic groups, allowing comparison between groups for effectiveness of inhibition of LDL oxidation *in vitro*. One group fractionated a concentrated ($\times 10$) Syrah wine sample (25 μ l) using HPLC, collecting ten fractions of 3 ml each in methanol (Teissedre *et al.* 1996). Three fractions, (1) catechin and procyanidins, (2) epicatechin and procyanidin B2, and (3) procyanidin C1, inhibited Cu-catalysed LDL oxidation *in vitro* by 71, 65 and 61 % respectively. Fractions containing mainly benzoic acids, *p*-hydroxybenzoic acid and cinnamic acids only inhibited 43 % of the LDL oxidation. The inhibitory effects of fractions containing rutin, myricetin, quercetin and the anthocyanins ranged from 31 to 45 %. Studies performed with some increasing equimolar concentrations of simple phenolic acids found the order of effectiveness on reducing LDL oxidation was caffeic acid > sinapic acid > ferulic acid (Carbonneau *et al.* 1997). These experiments indicate that not all components of red wine are equally effective in inhibiting LDL oxidation, and considering that wines have dissimilar compositions, this might indicate why red wine supplementation trials using different red wines show contradictory results (see p. 121).

As each group of phenolic compounds appears to differ in its effectiveness to inhibit LDL oxidation, it may well be that only a few of the polyphenols present in red wine are responsible for the differences between white and red wine on cardiovascular risk factors. Sharpe *et al.* (1995) made the suggestion that resveratrol may be one of the protective factors in red wine as it tends to be present in much lower concentrations in white wine than red. However, this is also true for the flavonoids and the more complex polyphenols such as anthocyanins. Still, it is thought that resveratrol could potentially help modulate lipoprotein metabolism, eicosanoid synthesis, oxidation and coagulation in mechanisms currently being explored through *in vitro* cell culture (Goldberg *et al.* 1995a). This is particularly of interest as resveratrol and piceid (the β -glucoside of resveratrol) are known to be the primary active components in the Oriental

folk medicine for atherosclerosis; 'Kojikon' or 'Itadorikon' made with the roots of *Polygonum cuspidatum* (Lamuela-Raventós & Waterhouse, 1999). Thus, *in vitro* studies are useful indicators of the ranges of effectiveness of the different components in red wine. They indicate which components have the greatest potential as cardioprotective substances and therefore should be further investigated with *in vivo* human studies.

Lipid content and oxidative modification: in vivo animal studies. Klurfeld & Kritchevsky (1981) were the first to show the protective effects of red wine using animal studies, with the lowering of the incidence of atherosclerotic lesions. Rabbits were fed diets that approximated that of the US energy consumption with added cholesterol to induce atherosclerosis within a short period and provided red wine, white wine, bourbon whisky, beer (4.9 % ethanol), ethanol in water or 12.5 % glucose in water as drinking fluids. All beverages excluding beer and the glucose drink were diluted to 9.5 % ethanol. Red wine consumption significantly reduced the incidence of aortic atherosclerosis compared with controls, whilst ethanol, white wine and whisky provided only slight protection. Beer showed no effect at all. All the alcoholic beverages except beer also raised HDL. A more recent study using a hamster model was even more positive and found that diluted (beverage–water, 1:1) red wine and grape juice elicited a decrease in atherosclerosis and lipoprotein concentrations (even HDL), and when controlled for polyphenol content, grape juice was found to be more effective than red wine, either with or without alcohol (Vinson *et al.* 2001). Therefore phenolic constituents appear to be more effective than alcohol in reducing the incidence of experimental atherosclerosis, but most alcoholic beverages can increase HDL. However the greater efficiency of grape juice compared with wine, when controlled for polyphenol content, could highlight differential absorption parameters of the various polyphenols and addresses the problem of what are the truly active compounds *in vivo*.

Other animal studies appear to contradict some of these findings. Hayek *et al.* (1997) fed either catechin (50 µg/d in 1.1 % alcohol solution), quercetin (50 µg/d in 1.1 % alcohol solution) or red wine (1.1 % alcohol, 0.5 ml/d, i.e. 50 µg catechin equivalent) to atherosclerotic, apo E-deficient mice for 6 weeks. They found no effect of any of the alcohol treatments on plasma lipids when compared with placebo. Yet when the lesion areas were investigated in the mice, the areas were smaller in the alcohol-treated groups than those in the control group by 39, 46 and 48 % respectively for each treatment. In addition these authors showed that lesion size was directly associated with the *in vitro* uptake of LDL by macrophage cells and a reduced susceptibility of isolated LDL to oxidation and aggregation. A similar lack of effect on the plasma lipid profile was observed with experimental studies using Sprague–Dawley rats receiving one of four beverages (35 ml/d), i.e. red wine, de-alcoholised red wine, 12 % alcohol, and water with sucrose (Cestaro *et al.* 1996). However, in this study there was also no effect observed on the indices of peroxidation in plasma or erythrocytes. Neither of these studies showed the expected effect on HDL in rats or mice and only with mice

was there any effect on LDL oxidation, which questions the usefulness of comparing results of animal studies (using different species) with the situation in human studies.

Hepatic damage (defined as degree of abnormal histological structure) in rats caused by 2 months of alcohol consumption was corrected by supplementing with grape polyphenols over the same period (Sun *et al.* 1999). The rats were fed daily diets that contained either: (1) no ethanol and no grape polyphenols, (2) 50 g ethanol/l and no grape polyphenols, (3) no ethanol and 50 mg grape polyphenols/l or (4) ethanol and grape polyphenols. Less hepatic damage was observed in the rats fed the diet with both alcohol and grape polyphenols than in the rats fed the diet with alcohol alone. The study suggests a protective role for grape polyphenols against alcohol damage but only when the two are consumed together.

In reality, animal studies are useful only up to a point. Lipoprotein metabolism is different in man compared with other species, especially rats (Chopra & Thurnham, 1999), and this may explain the lack of effect of different alcoholic beverages on LDL oxidation and HDL concentrations reported by Cestaro *et al.* (1996). In contrast, man and rabbits have a similar fat metabolism where cholesteryl ester transfer protein (CETP) activity is unaffected by vitamin E supplementation (Liu *et al.* 1997), but in rats the CETP activity is suppressed (Shen *et al.* 1996). This is relevant to lipoprotein metabolism in atherosclerosis as an increased CETP level in the plasma is associated with decreased plasma HDL; therefore, increases in HDL seen with vitamin E supplementation in rats could be related to decreased CETP activity. Thus any extrapolation from experimental work to man should be considered with care. The effects of alcohol on hepatic morphology are also most probably subject to the same difficulties in interpretation, as alcohol metabolism may well differ between species and possibly within species as well. In man, there are different mutations of the gene for the enzyme alcohol dehydrogenase, the first-step enzyme involved in metabolising alcohol (Li *et al.* 2001). Previously it was observed that there were large individual and racial differences in blood and breath acetaldehyde (a product of the alcohol dehydrogenase reaction) after alcohol consumption, with individuals of Oriental origin having extremely high levels, and Caucasians low levels (Von Wartburg *et al.* 1983). This is due to the fact that a large percentage of those of Oriental origin have very low levels of aldehyde dehydrogenase, blocking alcohol metabolism at the stage of acetaldehyde formation. In all, it is wise to consider the limitations of extrapolating animal studies to man due to the large variation in genetic and metabolic reactions.

Lipid content and oxidative modification: in vivo human studies. Studies have been performed to investigate whether alcohol or polyphenols in wine confer protection against atherosclerosis. Evidence has been found to suggest that long-term and regular consumption of red wine, but not alcohol, inhibits LDL oxidation *ex vivo* (Kondo *et al.* 1994). Generally, supplementation trials of red and white wine are designed to allow observation of short-term changes that may be indicative of long-term protection against CHD, for example, of plasma lipids. Typically the trials supplement

with 200–500 ml/d for approximately 2 weeks, but some for as long as 4 weeks. Longer trials are not generally performed as effects are seen within weeks on plasma lipids, and the ethics of supplementing alcoholic beverages for long periods of time must be considered. Trials that only supplement with single doses may not show effects within the monitoring period. For example, Cacetta *et al.* (2000) conducted a study involving a range of the beverages consumed on random and separate visits 1 week apart. Blood samples were taken at 0, 1, 2 and 4 h after consumption. No effect was reported from the consumption of red wine, de-alcoholised red wine or phenol-stripped red wine on serum or LDL oxidation. However these results might not be totally unexpected since LDL has a half-life of 2–3 d; thus beneficial effects of feeding polyphenols would take some time to appear before they might influence the composition of LDL.

Table 2 shows the results of five wine supplementation trials on changes in plasma lipid concentrations. The studies supplemented with a range of volumes of beverages, from 200 to 500 ml/d, and ranged from 10 to 30 d duration. In the two studies where the effects of red wine on plasma LDL concentrations were measured, the effects were not consistent, with only one study indicating a reduction in LDL (Sharpe *et al.* 1995). Four out of the five studies showed an increase in HDL after supplementation with red wine. The fifth that did not show an increase was shorter and used less wine than the other four (Sharpe *et al.* 1995). Overall, the studies indicate that red wine consumption is effective in increasing HDL. Some of the studies also showed a similar effect on HDL with white wine, and a non-phenolic alcoholic beverage. In one study, grape juice (both with and without resveratrol enrichment) was used as a comparative supplement but no effect was seen on any lipid parameters measured (Goldberg *et al.* 1996). The lack of effect from the grape juices and the positive results from the white wines and non-phenolic alcoholic beverage suggest that alcohol in these cases was the most probable factor causing the effects on HDL. The mechanism by which this occurs is not known although there is some evidence to

support a stimulation of the reverse cholesterol-transport pathway. One study has shown that the first two steps of this pathway (cellular cholesterol efflux and plasma cholesterol esterification) are stimulated by alcohol consumption (van Der Gaag *et al.* 2001).

The results from studies to determine the effects of wine supplements on susceptibility of LDL to peroxidation are mixed (Table 3). Five studies found no effect of either red or white wine or powdered red wine on LDL oxidation and four studies observed a decrease in the propensity for LDL to oxidise following the consumption of red wine. Positive results were seen with supplementation of at least 375 ml red wine/d (or equivalent) for at least 2 weeks. Thus the lack of effect of red wine could be explained by the low volume of wine consumed over only a short period of time (Sharpe *et al.* 1995; Cacetta *et al.* 2000). Another study still had a negative outcome despite supplementing with 550 ml/d for 4 weeks. However, low-alcohol wines were used and this may explain the lack of effect, as the alcohol may be needed to act synergistically with the wine phenolics or potentially aid absorption into the bloodstream (de Rijke *et al.* 1996). Quercetin supplementation alone has also been shown to protect LDL from oxidation, indicating that a phenolic component of red wine can be effective taken without alcohol (Chopra *et al.* 2000). Comparisons have been made between the modes of administering the red wine polyphenols (Nigdikar *et al.* 1998). The study showed a protective effect of red wine and its polyphenols against LDL peroxidation but the effect of the red wine extract seemed to depend on whether it is presented in an alcoholic medium. There is a commercial red wine extract known as Nutrivine™ (marketed by the Howard Foundation, Cambridge, UK), which can be taken either as a capsule or as a formulation to which water is added for drinking; both forms are equivalent to 375 ml red wine. Studies on this extract have shown it has the ability to protect LDL from oxidation after supplementation for 2 weeks (Nigdikar & Howard, 1997a).

There is a possibility that the protective effects of wine polyphenols may only be observed in hyperlipidaemic sub-

Table 2. Human supplementation studies: effects of wines and polyphenols on plasma lipid concentrations

Study	Duration of study	Supplementation groups	Outcome
Seigneur <i>et al.</i> (1990)	15 d	(1) Red wine (500 ml/d) (2) White wine (500 ml/d)	White wine consumption was associated with increases in total cholesterol, LDL and HDL, but red wine only induced an increase in HDL
Lavy <i>et al.</i> (1994)	2 weeks	(1) Red wine (400 ml/d) (2) White wine (400 ml/d)	Increased plasma HDL, apo A-I and triacylglycerol with red wine consumption, not white wine
Sharpe <i>et al.</i> (1995)	10 d cross-over trial	(1) Red wine (200 ml/d) (2) White wine (200 ml/d)	No differences between treatments for total cholesterol, triacylglycerol and HDL. Red wine reduced LDL cholesterol and lipoprotein A. Both wines reduced LDL apo B and increased LDL:apo B ratio
Goldberg <i>et al.</i> (1996)	4 weeks cross-over trial	(1) Red wine (375 ml/d) (2) White wine (375 ml/d) (3) Grape juice (500 ml/d) (4) Grape juice plus resveratrol (500 ml/d)	No effect of grape juice or that enriched with resveratrol. Both wines raised plasma HDL, apo A-I and apo A-II concentrations and the apo A-I:apo B ratio
Estruch (2000)	30 d cross-over trial	(1) Red wine (equivalent to 30 g ethanol/d) (2) Alcoholic beverage low in polyphenols (equivalent to 30 g ethanol/d)	Both red wine and the alcoholic beverage increased plasma HDL

Table 3. Human supplementation studies: effects of wines and polyphenols on lipid and serum oxidation

Study	Duration of study	Supplementation groups	Outcome
Sharpe <i>et al.</i> (1995)	10 d cross-over trial	(1) Red wine (200 ml/d) (2) White wine (200 ml/d)	No effect with either treatment on LDL oxidation Red wine increased erythrocyte membrane fluidity
Fuhrman <i>et al.</i> (1995)	2 weeks	(1) Red wine (400 ml/d) (2) White wine (400 ml/d)	Red wine consumption reduced while white wine consumption increased susceptibility of plasma to lipid peroxidation. White wine consumption also increased susceptibility of LDL to lipid peroxidation
de Rijke <i>et al.</i> (1996)	4 weeks	(1) Low alcohol red wine (550 ml/d) (2) Low alcohol white wine (550 ml/d)	No effect of either wine on Cu mediated oxidation of LDL
Carbonneau <i>et al.</i> (1997)	2 weeks	(1) Powdered red wine supplement (0.33 g/capsule; six times daily)	No change in LDL resistance to oxidation
Nigidikar <i>et al.</i> (1998)	2 weeks	(1) Red wine (375 ml/d) (2) White wine (375 ml/d) (3) Red wine polyphenol powder capsules (1 g/d) (4) Same powder dissolved in white wine (5) Control alcoholic drink (40 g ethanol/d)	The lag time for Cu mediated oxidation of LDL was increased by: the red wine powder (14.2 min, 27 %) the red wine powder in white wine (11.7 min, 21 %) the red wine (17.8 min, 31 %)
Chopra <i>et al.</i> (2000)	2 weeks	(1) Red wine extract equivalent to 375 ml/d (2) Quercetin supplementation (30 mg/d)	Both treatments increased the lag time for the Cu mediated oxidation of LDL
Estruch (2000)	30 d cross-over trial	(1) Red wine (equivalent to 30 g ethanol/d; about 250 ml/d) (2) Alcoholic beverage low in polyphenols (equivalent to 30 g ethanol/d)	Red wine decreased parameters of serum oxidation and the propensity for LDL to undergo lipid peroxidation
Cacetta <i>et al.</i> (2000)	Random and separate visits 1 week apart; twelve subjects	(1) Red wine (2) Dealcoholised red wine (3) Phenol-stripped red wine (5 ml/kg body weight over 30 min)	No effect on serum or LDL oxidation with any treatment
Cacetta <i>et al.</i> (2001)	2 weeks	(1) Red wine (2) White wine (3) Dealcoholised red wine	No reduction in lipid peroxidation (as measured with F2-isoprostane) with red or white wine consumption, but a reduction with dealcoholised red wine was seen

jects (Chopra & Thurnham, 1999). Hypercholesterolaemia is believed to be associated with elevated plasma vitamin E, as a positive correlation between concentrations of these two components is normally found in plasma (Thurnham *et al.* 1986). However, the amount of vitamin E may still not be adequate. A high plasma lipid level allows more lipid to be available for oxidative modification by free radical species; therefore, hyperlipidaemia can lead to increased oxidative damage (Prasad & Kalra, 1993). Most studies reported in the literature were done with normolipidaemic subjects and so potential benefits from wines may have been marginal and not significant. Subjects with elevated lipid status or in a disease state where oxidative damage is being caused may have a more pronounced response to polyphenol supplementation.

Serum antioxidant capacity

In an attempt to determine whether wine consumption increases serum antioxidant capacity, four different methods have been used to measure plasma antioxidant activity following supplementation. Five studies have reported

results; two using red wine, one using red grape juice, one using a powdered red wine supplement and one using alcohol-free red wine (Table 4). In all cases there was an increased antioxidant activity following consumption of the treatment. Comparable white wine supplementation, both with and without alcohol, gave no significant increases in antioxidant activity. Single doses of red wine (113 ml alcohol-free and 300 ml alcohol-containing wine) were effective within 1 h of consumption (Whitehead *et al.* 1995; Serafini *et al.* 1998). Consumption of 300 ml alcoholised red wine was still effective after 4 h (Cao *et al.* 1998). Grape juice was also effective within 1 h of consumption, and this was maintained and increased after 7 d of supplementation (Day *et al.* 1997). Serafini *et al.* (1998) observed parallel increases in plasma phenolic content to increases in total plasma antioxidant capacity with alcohol-free red wine consumption, but not white wine. So polyphenols supplemented *in vivo* in different forms, i.e. non-alcoholic wine, red wine powder and grape juice, all had a positive effect on serum antioxidant capacity despite differences in their methods of measuring the change. These studies

Table 4. Studies showing effects of wines and polyphenols on plasma antioxidant capacity in man

Study	Study design	Supplementation groups	Outcome
Whitehead <i>et al.</i> (1995)	Single dose; nine subjects	(1) 300 ml red wine (2) 300 ml white wine	SAOC increased significantly ($P<0.005$) compared with baseline with red wine (18 and 11 % after 1 and 2 h respectively). White wine gave no significant increases (4 and 7 % after 1 and 2 h respectively)
Day <i>et al.</i> (1997)	7 d; seven subjects	(1) 125 ml concentrated red grape juice/d	SAOC increased by 8.4 % after 60 min on day 1 ($P<0.005$) and by 11.3 % ($P<0.05$) on day 8
Carbonneau <i>et al.</i> (1997)	2-week supplementation; twenty subjects	(1) Powdered red wine supplement (0.33 g/capsule; six times daily)	Significant increase (8.7 %, $P=0.01$) in total plasma antioxidant capacity (measured with a chemiluminescence reaction) but no change in LDL resistance to oxidation
Serafini <i>et al.</i> (1998)	Single dose; ten subjects	(1) Alcohol-free red wine (113 ml) (2) Alcohol-free white wine (113 ml) (3) Water (control) (113 ml)	Alcohol-free red wine increased total plasma antioxidant capacity by 14 % after 50 min (measured by TRAP) with parallel increases in plasma polyphenolic content. No response from the other two treatments
Cao <i>et al.</i> (1998)	Single dose; eight subjects	(1) 300 ml red wine	SAOC increased by 11 and 7 % after 4 h when measured by FRAP and ORAC respectively

SAOC, serum antioxidant capacity; TRAP, total radical-trapping antioxidant parameter (μmol peroxy radicals trapped/litre of plasma); FRAP, ferric-reducing ability of plasma; ORAC, oxygen radical absorbance capacity.

appear to indicate the effective component in this case might not be alcohol, but rather polyphenol concentration.

The effects of grape juice may not be completely analogous to the non-alcoholic content of red wine, as grape juice does not contain flavonoids from the skin and seeds of the grape whereas red wine does. The difference in the two preparations is particularly true for resveratrol as it is typically found in the grape skin. It should also be noted that phenolics are labile compounds, and so the de-alcoholisation process used to remove alcohol from the wine may cause chemical alterations that makes comparison with the native wine potentially biased. Thus using grape juice as a substitute for de-alcoholised red wine may not be appropriate (Waterhouse & Walzem, 1998). It should also be noted that red wine powder may not be consistent in its composition because each powder can come from a different wine and therefore can affect supplementation study results. For example, Carbonneau *et al.* (1997) used a red wine powder low in catechins and proanthocyanidins compared with standard red wine and found no effect on LDL oxidation. In contrast a similar study from our group did show an inhibitory effect on LDL oxidation by the non-alcoholic red wine extract NutrivineTM (Chopra *et al.* 2000).

Vasculature, smooth muscle cells and nitric oxide production

Endothelial dysfunction is linked to many of the risk factors for CHD including hypercholesterolaemia, smoking, and familial history of premature CHD. Also many coronary risk factors are known to reduce NO availability, making NO a popular intermediate end-point for assessing atherogenic risk (Vogel, 2003). Control of vascular relaxation is endothelium dependent and is mediated by NO (Cuevas *et al.* 2000). The mechanisms by which polyphenols could potentially influence NO production and release are: (1) an increased release of NO from intracellular stores; (2) the preservation and/or stabilisation of NO released under basal conditions; (3) the stimulation of NO

synthase activity (Cishek *et al.* 1997). The latter mechanism has support from a study performed on rabbit aortic rings, which found that acute exposure to polyphenols produces endothelium-dependent relaxation that can be abolished by inhibiting NO synthase activity (Cishek *et al.* 1997). Another suggested mechanism is the selective inhibition of NO-related oxidation and nitration reactions leading to less peroxynitrate production (Wippel *et al.* 2004).

Table 5 shows a summary of some studies performed in this area. Where endothelium-dependent relaxation of aortic rings was used as an end-point for the *in vitro* experiment, the results were generally positive. Red wine polyphenols tended to induce vessel relaxation *in vitro* (Andriambelason *et al.* 1997), but only at levels of 10 mg/l and above (Deckert *et al.* 2002). One study did not find this effect, but the dose used was only at levels equivalent in alcohol to moderate wine consumption (15 mM-alcohol in blood post-consumption) (Rendig *et al.* 2000). When specific phenolics were tested alone in the same manner, not all were seen to elicit an effect. Although quercetin alone has been shown to induce dose-related vaso-relaxation (Rendig *et al.* 2000), other more abundant phenolics gave no response, i.e. gallic, benzoic, vanillic, *p*-coumaric and caffeic acids and epicatechin (Andriambelason *et al.* 1997). A minor flavanol of red wine called leucocyanidol was seen to induce complete vessel relaxation, but the major flavanol catechin did not give a similar effect (Andriambelason *et al.* 1997). It was also shown that some of these polyphenols could cause an endothelium-dependent increase in NO content in the rat aorta. This was corroborated by a very recent study that found that red wine increased the expression of human endothelial NO production *in vitro* (Wallerath *et al.* 2003). The effects on NO production were seen with French wines, but not German wines, indicating a specific substance(s) present in one and not the other may be responsible. For instance, this could be leucocyanidol, or another minor and as yet unmeasured phenolic. However, it should also be noted that the wine concentrations used for this experiment might not reflect physiologically relevant levels (Vogel,

Table 5. Studies showing effects of wines and polyphenols on endothelium-dependent relaxation and nitric oxide

Study	Measures	Substance tested	Negative outcome	Positive outcome
Andriambeloston <i>et al.</i> (1997)	<i>In vitro</i> vaso-relaxation of thoracic rat aorta in organ bath studies	Polyphenolic compounds (powder derived from 1 litre red wine (mg/g): catechins and proanthocyanidins, 100; anthocyanins, 64; flavonols, 5; phenolic acids, 8.7) and leucocyanidol (flavan-3,4-ol, a polyphenol found in wine)	Catechin when tested alone did not induce complete vessel relaxation	Red wine polyphenolic compounds at 0.01 g/l and specifically leucocyanidol, when tested alone, induced complete vessel relaxation
Andriambeloston <i>et al.</i> (1998)	<i>In vitro</i> endothelium-dependent relaxation of the thoracic aorta of Wistar rats	Individual and fractions of red wine phenolic compounds	Gallic, benzoic, vanillic, <i>p</i> -coumaric and caffeic acids and (+)-epicatechin gave no response	Comparable concentrations of anthocyanins and oligomeric-condensed tannins fractionated from red wine showed similar effects as the full complement of red wine polyphenolic compounds
Rendig <i>et al.</i> (2000)	<i>In vitro</i> endothelium-dependent relaxation of rabbit arteries	Red wine, ethanol and quercetin	Neither red wine nor ethanol generated a significant vaso-relaxant effect at levels equivalent to moderate wine consumption	Solutions of quercetin (5.6, 8 and 30 $\mu\text{mol/l}$) gave marked dose-related vaso-relaxation
Deckert <i>et al.</i> (2002)	<i>In vitro</i> endothelium-dependent relaxation of rabbit arteries	Red wine polyphenols from Cabernet Sauvignon wine	Concentration of 1 mg/l had no effect on contraction of aortic rings	Concentrations at 10 and 100 mg/l had significant vaso-relaxation effect
Wallerath <i>et al.</i> (2003)	<i>In vitro</i> measurement of expression of human endothelial NO synthase	Various red wines from Germany and France, diluted 1, 3 and 10 % (v/v)	German red wines showed little or no effect on endothelial-type NO synthase expression	French red wines up regulated expression of endothelial-type NO synthase.

2003), as wines were simply diluted to 1, 3 and 10 % before introduction to cells and no measures of phenolic concentrations were made. In general, the studies indicated that some compounds from wine could enhance the synthesis of NO, i.e. they possessed endothelium-dependent vaso-relaxing abilities.

Endothelial function in response to wine has also been studied in man. In a 30 d, red wine supplementation trial, it was found that the presence of wine with a diet prevented fat-related changes in endothelial function. Endothelial function was measured by flow-mediated dilatation of the brachial artery with high-resolution ultrasound. In the absence of wine, high-fat diets (39.5 % total energy) in six volunteers depressed function (-2.9 (SD 2.1) %) when compared with a diet containing fat as only 27 % total energy where the change was $+3.1$ (SD 3.9) % (Cuevas *et al.* 2000). This difference was not observed when 240 ml red wine/d was supplemented with both high-fat (6.6 (SD 2.2) %) and control (5.8 (SD 4.6) %) diets. However, increased NO release from endothelium and macrophages can also cause increased LDL lipid peroxidation, therefore increasing the potential atherogenic properties of LDL (Graham *et al.* 1993; Hazen *et al.* 1999; Podrez *et al.* 1999).

A few studies have also investigated possible links between blood pressure and polyphenol consumption. One study found a decreased blood pressure with a phenolic beverage (grape juice and vinegar mix) in rats (Sugiyama *et al.* 2003). Similar effects have been seen with supplementation with single phenolics such as ferulic acid in hypertensive rats (Suzuki *et al.* 2002).

Flavonoids have been thought to modulate monocyte adhesion to endothelial cells during the inflammatory process of atherosclerosis (Harbourne & Williams, 2000). However, a new study has cast doubt on the ability of certain flavonoids to directly affect endothelial cells (Koga & Meydani, 2001). Human aortic endothelial cells were pre-treated *in vitro* with either catechin, catechin metabolites, quercetin or quercetin metabolites. Only the catechin metabolites and quercetin (but not its metabolites) inhibited monocyte adhesion to endothelial cells following treatment by H_2O_2 to create damage to the cells. It is suggested that conjugated quercetin metabolites have a reduced or abolished biological activity and that the degree of this change depends on the nature and position of the substitutions (Day *et al.* 2000). However, it is possible that *in vivo* β -glucuronidase released from neutrophils, especially at the site of inflammation, may deconjugate phenolic metabolites in serum and release the aglycone (Shimoi *et al.* 2000). Therefore extrapolation of *in vitro* findings to an *in vivo* situation may be physiologically relevant but the precise amounts of phenolic metabolites *in vivo*, their half-lives and potential reactivity require further investigation.

In vivo supplementation involves the measurement of a variety of physiological rather than cellular responses that *in vitro* experimentation would provide. Transforming growth factor β inhibits the growth of arterial smooth muscle cells, and therefore can potentially assist in preventing atherosclerosis (McCaffrey, 2000). Concentrations of plasma transforming growth factor β are raised approximately

four-fold *in vivo* after the consumption of modest amounts of red wine polyphenols in wine or in capsule form, compared with after white wine consumption (Nigdikar & Howard, 1997b). A recent study also confirmed that red wine polyphenols would inhibit the growth of rat aortic smooth muscle cells *in vitro*. When investigating the mechanism, the workers found that red wine polyphenols down regulated the expression of the cyclin A gene, a gene that is critical in DNA replication and cell cycle regulation. The authors suggest that inhibition of the cyclin A gene may be responsible for the inhibition of smooth muscle cell growth (Iijima *et al.* 2000) and this was reinforced by a later study that more fully investigated the gene (Iijima *et al.* 2002). These data appear to support the idea that polyphenols do act on aortic smooth muscle cells and that transforming growth factor β and the cyclin A gene are either directly or indirectly involved in the mechanisms.

Platelets

Platelet ability to aggregate is an important factor in CHD. *In vitro*, alcohol has a similar effect to aspirin in its ability to

decrease secondary aggregation of platelets in response to ADP. A suggestion has been made that since wine is generally consumed with food, the alcohol is absorbed slowly, therefore it can have a longer effect on the body's metabolism; for example, on blood platelet reactivity at a time when lipid levels would be high (Renaud & de Lorgeril, 1992). *In vivo* inhibition of platelet aggregation by moderate alcohol consumption was noted in the Caerphilly Prospective Heart Disease Study (Renaud *et al.* 1992) and, later, in an acute consumption study when both platelet-rich plasma and whole blood were examined (Zhang *et al.* 2000).

Both wines and grape juices can affect platelet aggregation in man, by reducing plasma thromboxane B₂ concentration and the concentration of ADP and thrombin available for platelet aggregation. Table 6 shows seven studies, five of which found lower platelet aggregation with wine phenolics both *ex vivo* and *in vitro*. One study found that red wine ingestion reduced but, surprisingly, pure alcohol increased platelet aggregation (Seigneur *et al.* 1990). It was suggested that this effect was due to the presence of aspirin in red wine but red wine has been shown to contain only negligible amounts of aspirin (Janssen *et al.* 1994).

Table 6. Studies showing effects of wines and polyphenols on platelet aggregation and related factors

Study	Measures	Substances tested	Outcome
Landolfi <i>et al.</i> (1984)	Inhibition of cyclo-oxygenase activity and human platelet aggregation and the platelet cyclic AMP response to PGI ₂ <i>in vitro</i>	Multiple flavonoids	Of flavonoids tested, most could inhibit cyclo-oxygenase activity but only flavone, chrysin, apigenin and phloretin inhibited platelet aggregation to any extent. The flavonoid glycosides and flavonone derivatives had no effect on platelet aggregation. Apigenin, chrysin and flavone reduced, while myricetin and quercetin increased, the platelet cyclic AMP response to PGI ₂
Seigneur <i>et al.</i> (1990)	ADP- or adrenaline-induced human platelet aggregation <i>ex vivo</i>	White or red wine or a synthetic alcoholic non-polyphenolic wine ingestion (0.5 litres/d) for 15 d	Red wine reduced ADP-induced platelet aggregation but pure alcohol enhanced ADP- and adrenaline-induced platelet aggregation
Demrow <i>et al.</i> (1995)	Cyclic flow reductions in canine coronary arteries	Intravenous and intragastric administration of grape juice and red wine	Eliminated reductions in cyclic flow as a measure of reduction of thrombus formation. For a full explanation, see p. 125
Pace-Asciak <i>et al.</i> (1996)	Aggregation of freshly washed human platelets <i>ex vivo</i> by ADP and thrombin plasma TxB ₂ concentration	4 weeks of moderate grape juice, grape juice enriched with resveratrol, white or red wine consumption	Aggregation strongly inhibited by red wine, unaffected by white wine and only moderately inhibited by commercial grape juice and juice enriched with <i>trans</i> -resveratrol. Reductions of 52.5 and 59.4 % in plasma TxB ₂ concentration for white and red wine consumption respectively
Janssen <i>et al.</i> (1998)	Inhibition of collagen- and ADP-induced aggregation in both human platelet-rich plasma and washed platelets <i>in vitro</i>	Six concentrations of quercetin-3-glucoside, quercetin aglycone, apigenin aglycone and catechin	Quercetin and apigenin inhibited collagen- and ADP-induced aggregation in both platelet-rich plasma and washed platelets by 80–97 %
Janssen <i>et al.</i> (1998)	Platelet aggregation, TxB ₂ production, factor VII and other haemostatic factors in human subjects <i>ex vivo</i>	Supplementation with 200 g onions/d (about 114 mg quercetin/d) or 5 g dried parsley/d (about 84 mg apigenin/d) for 7 d	No effects on platelet aggregation, TxB ₂ production, factor VII or other haemostatic factors were observed
Pignatelli <i>et al.</i> (2000)	Inhibition <i>in vitro</i> of collagen-induced human platelet aggregation and platelet adhesion to collagen	Varying concentrations of catechin and quercetin	Catechin at 50–100 μ mol/l and 10–20 μ mol quercetin/l inhibited collagen-induced platelet aggregation and platelet adhesion to collagen. When the two were combined at concentrations that did not give any reaction singly (i.e. 25 μ mol catechin/l and 5 μ mol quercetin/l), significant effects were observed

PGI, prostacyclin; TxB₂, thromboxane B₂.

Thrombus formation can cause cyclic reductions in coronary blood flow and experiments have shown an elimination of such reductions following intravenous and intragastric administration of grape juice and red wine (Demrow *et al.* 1995). Supplementation studies have shown that platelet aggregation was strongly inhibited by red wine, moderately inhibited by grape juice and unaffected by white wine. One animal study has found that platelet aggregation was reduced by ethanol, red wine and white wine in rats, but when the beverages were withdrawn, rats that had consumed red wine did not exhibit the platelet-rebound effect observed hours after drinking alcohol, which has been associated with stroke in man (Ruf *et al.* 1995). Overall, the effects of alcohol on platelet aggregation appear to be enhanced by wine or grape polyphenols.

It has also been reported that some of the individual flavonoids are effective in inhibiting platelet aggregation. Table 6 describes studies that have tested the effects of individual and combinations of different polyphenols. Quercetin and apigenin have been shown to inhibit collagen- and ADP-induced aggregation but the effect was only seen at phenolic concentrations higher than would be expected to occur *in vivo* (Janssen *et al.* 1998). The authors investigated the physiological importance of the phenolics by using a feeding study with onions (high in quercetin) and dried parsley (high in apigenin) but there were no effects on platelet aggregation or thromboxane B₂ production *in vivo*. Another interesting study using catechin and quercetin found that when the two flavonoids were combined *in vitro* at concentrations that did not give any reaction singly (i.e. 25 µmol catechin/l and 5 µmol quercetin/l), significant effects were observed on the inhibition of collagen-induced platelet aggregation and platelet adhesion to collagen (Pignatelli *et al.* 2000). The authors suggest that flavonoids may well act synergistically to inhibit platelet aggregation, and that this may be relevant after red wine consumption as the circulating concentration of each wine flavonoid is often lower than previously found to be effective to influence platelet activity.

Conclusion

Wine has been part of human culture and diet for many thousands of years and will probably continue to be for many more to come. Despite tremendous research input in this area in the past decade, we still have a great distance to cover in our understanding of the health benefits of wine. Although we know a lot more about the phenolic components of red wine, we still do not know whether the components have individual functions and which are the most important. Very little research has been done on the metabolism and biological activities of phenolics at cellular, molecular and biochemical levels. Further research is therefore required, especially human *in vivo* supplementation studies, with pure and mixtures of flavonoid compounds to identify the biological activities of their metabolites.

'It is a great error to consider a heavy tax on wine as a tax on luxury. On the contrary it is a tax on the health of our citizens.'

Thomas Jefferson (Statesman)

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Are you a Registered Nutritionist?

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The Society's registers form part of a scheme to protect the public. The registers help to set and promote high standards of education and professional development in nutrition. The register helps employers, the media and the general public to identify appropriately qualified individuals. Members on the register are expected to adhere to the Society's Code of Ethics and the Statement of Professional Conduct. Since 2000 the status and size of the scheme has risen. Increasing numbers of employers, partner professional bodies and government departments in Britain recognise registration with the Society as a sign of quality. Members, employers and the general public can find a registered nutritionist on the Society's register at www.nutrition society.org/membership/register.

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