

REPORT TO
WINEGROWERS OF NEW ZEALAND
ON
EVIDENCE FOR HEALTH BENEFITS
OF MODERATE WINE CONSUMPTION

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Executive Summary

This report summarises recent literature on the evidence for health benefits of wine, and on the possible physiological and molecular mechanisms that have been proposed to explain these health benefits. Relevant background information on mechanisms of disease and adverse effects of moderate alcohol consumption is included.

Key facts are:

There is clear evidence based on population studies from a range of countries that moderate alcohol intake is associated with lower mortality rates than those seen for either zero or high alcohol intake.

It has not yet been conclusively established that wine provides greater health benefits than other alcoholic beverages, although some scientific studies support this proposal.

There are several possible mechanisms by which alcohol itself, or wine in particular, may contribute to improved health status.

Alcohol itself probably provides protection against coronary heart disease by increasing levels of high-density lipoprotein in circulation, and probably also by inhibiting platelet aggregation.

Phenolic compounds in wine may provide additional health benefits through antioxidant properties, prevention of platelet aggregation and inhibition of growth of some types of cancer cells.

The evidence as to whether red wine provides greater health benefits than white is still equivocal. Red wines often, but not always, contain higher concentrations of key phenolic compounds than white wines. Physiological studies sometimes, but not always, show greater potential health benefits for red wine. One problem is that many studies do not report the levels of key phenolic compounds in the wines that are used.

Key recommendations are:

Factual information on the potential health benefits of wine, and the possible physiological explanations for these benefits, should be made available to health professionals and others who can assess the information and promote moderate alcohol consumption with due care.

Future research areas of importance are epidemiological studies to establish evidence for health benefits of wine in New Zealand, biomedical research on the absorption and modes of action of phenolic compounds found in wine and wine research to establish concentrations of potentially beneficial compounds in New Zealand wines.

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The report was submitted to Professor Geoffrey Skurray (Centre for Advanced Food Research, University of Western Sydney) for review and comment. Following this, some further additions and updating were completed for submission back to Winegrowers of New Zealand in August 2001.

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1 Introduction

For many years, it has been generally accepted that the consumption of alcohol may have health benefits for some people. The impression that this is so arose in part from observations that people in France who have a very high intake of saturated fat have low rates of coronary heart disease (CHD) relative to people in other countries who have similar fat intakes. The observation of the anomaly in incidence rates for CHD in France became known as the 'French Paradox'. An obvious difference in diet between France and other countries is the high consumption of wine, in particular of red wine, by the French population. Since it became clear that data from France suggested a possible positive health benefit of alcohol consumption, there have been many studies carried out in other countries to see whether the effect was apparent in different populations. In general, these studies have confirmed that people who have a moderate alcohol intake have lower mortality rates from cardiovascular disease than either those who abstain or those who drink heavily. These findings are consistent even when the studies exclude those who abstain from alcohol as a result of pre-existing health problems.

There is thus little doubt now, based on statistical evidence, that the consumption of alcohol in moderate amounts can produce health benefits. The acceptance of this fact has produced an upsurge of interest in research with the aim of finding an explanation as to how alcohol consumption may produce health benefits. At this stage, the questions that remain are many: Is consumption of any type of alcohol beneficial, or are the positive effects restricted to wine consumption? Is red wine more beneficial than white wine? What are the molecular mechanisms that underlie the health benefits? What are the compounds in wine other than alcohol itself that may provide health benefits? What are the most beneficial rates of alcohol consumption?

The last of the above questions is of particular importance, since it is also well established that excess alcohol consumption has adverse health effects. The transition point from health benefit to health risk in terms of quantity of alcohol consumed per week may vary for different population groups, for males and females, and even from one individual to another. For some people, such as those who have a problem with alcohol addiction and for women who are pregnant, any alcohol consumption may produce a significant risk. It is therefore important that if alcohol in general, and wine in particular, are to be promoted as having health benefits, the level of intake that is likely to be beneficial for any individual should be defined as clearly as possible. Developing an understanding of the molecular mechanisms that account for the health benefits of alcohol and wine will help with establishing recommendations as to the amounts of these beverages that will be beneficial. Further confirmation of the statistical evidence for health benefits based on data from a wider range of population groups will also be helpful.

The current report outlines some information on disease processes that are of relevance to the potential health benefits of wine, summarises the evidence for potential health benefits, describes research on possible molecular mechanisms to explain these, and comments on possible adverse effects of moderate alcohol consumption that need to be taken into consideration.

2 Mechanisms of Disease

2.1 Antioxidants and the free radical theory

In 1956 a scientist named Denham Harman proposed the Free Radical Theory of Ageing (Beckman and Ames, 1998). The theory stated that free radicals, produced during metabolism and other biological reactions, cause cumulative cell damage that results in ageing and eventually death. (Unlike most molecules, free radicals have an odd number of electrons; it is the 'unpaired' electrons that make them very chemically reactive.) Harman also hypothesised that iron and other metals would catalyse (speed up) oxidative reactions *in vivo* thereby increasing the rate of damage. During the past forty years Harman's theory has been confirmed by many scientific studies, and free radical damage is now generally accepted as one of the mechanisms that contributes to the ageing process.

Free radicals are unstable molecules that react with cell membranes and other intracellular structures causing damage to body tissues and often generating more harmful molecules as well. Free radicals produce damage to DNA, protein, lipids and other molecules throughout the body. The damage caused by free radicals can eventually lead to diseases such as cancer and atherosclerosis (Beckman and Ames, 1998).

A natural step after the free radical theory was established was for scientists to seek compounds that could combat the harmful activities of free radicals. Two classes of compounds that can achieve this are antioxidants (as free radicals often cause damage by oxidation) and free radical scavengers. Free radical scavengers exert their effect by converting free radical species to less reactive molecules. Scavengers and antioxidants can be lipophilic (readily soluble in the lipid, or fat, components of the body), such as the tocopherols, carotenoids and ubiquinol, hydrophilic (water soluble), such as the flavonoids, ascorbate and glutathione, or enzymatic like superoxide dismutase, which hastens the conversion of the superoxide radical O_2^- to hydrogen peroxide H_2O_2 . When a small molecular antioxidant, such as glutathione, acts to reduce an oxidised biological molecule, the antioxidant itself gets oxidised. To remain effective, it then needs to be reduced again, usually by a specific enzyme.

Antioxidants are commonly found as constituents of the human diet and are found in considerable quantities in vegetables, fruit and red wine. The presence of antioxidant flavonoids in red wine has received great attention in the past few years. Red wine has been proposed as an especially palatable source of antioxidants and it is even suggested that the antioxidant activity of wine may be responsible for the good health of the Mediterranean people.

The antioxidant capacity of different animal species has been positively correlated with maximum life span (Cutler, 1991, cited from Beckman and Ames, 1998). The lifespan of houseflies has been extended by reducing oxidative stress through manipulating levels of activity or oxygen tension (Sohal and Dubey, 1994, cited from Beckman and Ames, 1998). In terms of dietary supplementation overall results from

antioxidant studies are far from consistent. There is, however, a growing excitement in the biomedical world that antioxidants may hold the key to longer and more active lives. News of theories about the life-preserving effects of antioxidants has spread down to the general public, many of whom now consume antioxidant pills or use skin creams containing antioxidants in the hope of maintaining their vitality and youthful appearance. Nutritional supplementation of antioxidants is indeed a reality, although large-scale human studies have yet to be completed and the scientific basis for some of the treatments being used is uncertain. The need for further research on the roles of dietary antioxidants has been emphasised in a recent review (Morton *et al*, 2000).

2.2 Lipoproteins and cholesterol

Over the past few years the public's awareness of cholesterol and its impact on health has increased immensely. However, there is often confusion, as some cholesterol is known to be 'good' and some 'bad'. Lipoproteins are composed of lipid and protein. The lipids are usually either triglyceride or cholesterol. The lipoproteins which carry cholesterol are intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). LDL is referred to as the 'bad' cholesterol as it is directly implicated in the pathogenesis of atherosclerosis. HDL is known as the 'good' cholesterol. It serves two functions; to transport free cholesterol from cells and tissues back to the liver for excretion (removal) and it is also thought to be capable of removing cholesterol from arterial walls. HDL levels are inversely proportional to the risk of atherosclerosis and it is thought that this protective ability results at least in part from these transporting and removing roles (Gaziano and Buring, 1998).

2.3 Platelets

Platelets are circulating cells whose main role is coagulation (blood clotting). Platelets are also involved in acute inflammation and atherosclerosis. Platelets adhere to damaged endothelial cells. (Endothelial cells are cells that line the walls of blood vessels and body organs.) This adhesion activates the platelets and initiates several chemical reactions within both the endothelial cells and the platelets. Within the platelets, arachidonic acid is metabolised to form a substance called thromboxane A₂ (TxA₂), which is secreted along with adenosine diphosphate (ADP) and several other chemicals. ADP and TxA₂ stimulate platelet aggregation, which involves more platelets adhering to those already attached to the endothelium therefore forming a clot which can further develop to become a plaque or a plug. Usually platelet aggregation is offset by another arachidonic acid metabolite, prostacyclin (PGI₂), this time produced by the endothelial cells. However, imbalances in the levels of TxA₂ and PGI₂ result in either thrombosis (formation of a solid mass of blood within the blood vessels of the heart caused by excessive platelet aggregation) or haemorrhage (uncontrolled bleeding, a failure of the blood to clot) (MacSween and Whaley, 1992; Guyton and Hall, 1996). (The prostaglandins, prostacyclin and thromboxanes, derived from arachidonic acid, are collectively known as eicosanoids. They are locally-acting hormones that alter the activities of cells in which they are synthesized and other cells in close proximity. They have roles stimulation of inflammation, and in regulation of blood flow and ion transport (Stryer, 1995).)

2.4 Atherosclerosis

The initial event leading to atherosclerosis is endothelial cell damage. Endothelial injury increases the permeability of the blood vessel thereby allowing more blood particles to flow through (Hegele, 1996). LDL is not usually oxidised in the blood plasma because of the high levels of antioxidants such as vitamin E that are present. The injury to the endothelium allows LDL to pass into the intima layer (layer of cells under the endothelial layer) where these lipoproteins can be oxidised by free radicals. The oxidation of LDL leads to enhanced uptake by macrophage cells (special cells which scavenge toxic or foreign particles), which have very few receptors for LDL and significantly more receptors for oxidised LDL. Macrophage cells which have become attached to the damaged endothelium then accumulate oxidised lipid-forming foam cells (Witzum, 1994; Puddey et al, 1998).

Changes in the smooth muscle of blood vessels can also stimulate atherosclerosis. Factors released from platelets, endothelial cells and macrophage cells cause the migration and proliferation of smooth muscle cells. These smooth muscle cells also accumulate cholesterol as the macrophages did, thus also forming foam cells within the atherosclerotic plaque.

The clumps of foam cells can eventually grow to a size where they partially or completely occlude the blood vessel, preventing blood flow. When this occurs in minor blood vessels it produces infarction, which is the death of tissue receiving inadequate blood supply. However, when this occurs in the arteries of the heart or a plug breaks off travelling towards the heart, and these vessels are occluded, sudden death by myocardial infarction ('heart attack') is likely to occur (Hendriks and van der Gaag, 1998).

2.5 Summary

Atherosclerosis arises through a combination of endothelial damage, LDL oxidation, and platelet aggregation, which may then lead to formation of clumps of foam cells which may partially or completely occlude the blood vessels. Oxidative damage due to free radicals may also lead to other types of tissue damage and to cancer.

Alcohol has been reported to increase levels of the 'good' HDL cholesterol and to inhibit platelet aggregation (Suh *et al.*, 1992; Gaziano *et al.*, 1993 cited from Goldberg *et al.*, 1995; Renaud *et al.*, 1992; Goldberg *et al.*, 1996). In addition, it has been reported that the antioxidants present in red wine can inhibit LDL oxidation and that other compounds specific to red wine have anti-aggregatory ability (Goldberg, 1995; Frankel *et al.*, 1993a; Xia *et al.*, 1998; Demrow *et al.*, 1995). Recent evidence suggests that antioxidant compounds present in wine may in some circumstances prevent initiation and growth of some types of cancer. These health benefits of wine and their possible mechanisms are discussed in more detail in sections 3, 5 and 6.

3. The French Paradox and other wine/alcohol and health associations.

3.1 The French Paradox

High intake of saturated fat is a well-known risk factor for coronary heart disease (CHD) (Renaud and de Lorgeril, 1989, cited from Renaud and de Lorgeril, 1992; Criqui and Ringel, 1994). In most countries the level of dietary saturated fat is positively associated to mortality from CHD (Renaud, 1992; Renaud and Gueguen, 1998). However, despite a high intake of saturated fat similar to other developed countries such as the USA or UK, France has a much lower incidence of CHD. In fact the incidence of CHD in France was found to be the second lowest in a study of several countries (Criqui and Ringel, 1994). The only country with a lower incidence was Japan, where people have exceptionally low saturated fat intake (Criqui and Ringel, 1994).

This phenomenon has been termed the 'French Paradox' and is thought to result from the high consumption of alcohol, particularly red wine, by French citizens (Criqui and Ringel, 1994; Finkel, 1996; Bisson, 1997; Goldberg, 1995; Renaud and de Lorgeril, 1992). St. Leger *et al.* (1979) found a strong inverse association between CHD mortality in 1970 and wine consumption for a range of countries. This observation was further supported by data from the United States that showed a negative correlation between death from acute myocardial infarction and wine consumption across all states (Werth, 1980). In 1992 Renaud and his colleagues found that France, and to a lesser extent Switzerland, showed the greatest deviation from the regression line on a plot of saturated fat intake vs CHD rate. Both these countries had a low rate of CHD despite having a greater than average level of saturated fat intake (Renaud and de Lorgeril, 1992).

In the past, scientists have been quick to suggest that wine itself must be responsible for protecting France from CHD. In a study by Criqui and Ringel it was noted that France not only had the highest level of wine consumption but the highest total alcohol intake also (Criqui and Ringel, 1994). It was also found that the ethanol in wine was slightly more inversely correlated with CHD than total wine volume. It is possible then that it is their total alcohol intake and not specifically their consumption of wine that provides the French with their cardio-protection. In a long-running study in Australia, the hazard ratio for all-causes mortality in male and female drinkers, compared with abstainers, was reported as 0.40-0.78, depending on the level of alcohol intake (Simons *et al.*, 1996) and in a further follow up on the same group (Simons *et al.*, 2000) was 0.63 for males and 0.75 for females. In the latter report, the protective effects were similar for beer and other forms of alcohol.

The proceedings of a recent conference (XXVeme Congres Mondial de la Vigne et du Vin, Sector IV, Wine and Health) cover a range of research related to health benefits of wine. In one paper in these proceedings Ellison (2000) summarises evidence of the effects of alcohol on all-cause mortality, and concludes that all the summarised evidence strongly suggests that it is the consumption of alcoholic beverage by

moderate drinkers that lowers the risk of death for these people. The author of this paper also emphasises the point that heavy drinkers had higher death rates than non-drinkers. In a second key paper Di Castelnuovo *et al.* (2000) report on a meta-analysis of studies providing specific information on wine consumption in relation to vascular risk. Analysis of the results of 13 studies indicates an overall risk of 0.66 for cardiovascular disease in wine drinkers compared to non-wine drinkers. It is also reported that the effect was dose-related, with a maximum effect at 750 ml of wine per day. This is a very misleading comment, however, as a statistically significant beneficial effect was seen only up to an intake of 300 ml/day. Beyond that level of consumption the upper limit of variation of the 95% confidence interval on the results indicates that there was an increased risk for a considerable proportion of wine drinkers. The authors conclude that overall the results of the meta-analysis support the protective role of moderate wine consumption against the risk of cardiovascular disease.

Another hypothesis is that diet and not just wine intake is responsible for the French Paradox. The diet in Toulouse, France, is characterised by low consumption of butter and high consumption of vegetables, bread, fruit, cheese, vegetable fat and wine (Renaud and de Lorgeril, 1992, Renaud *et al.*, 1994). This also describes the typical Mediterranean diet. Flavonoids, which have been hailed as the magic ingredient in red wine, are also commonly found in vegetables and fruit (Hertog *et al.*, 1993b; Muldoon and Kritchevsky, 1996; Rimm *et al.*, 1996a). It may be then that it is vegetables, which are often recommended for maintenance of good health, rather than alcohol or specifically wine, that are effective in combating CHD. However, one study that looked specifically at this possibility did not demonstrate a strong inverse relationship between intake of flavonoids from vegetables and CHD (Rimm *et al.*, 1996a).

It is also possible that the apparent association of wine or alcohol intake with decreased mortality rates is due to wine drinkers, or drinkers in general, having a higher preference on average than the general population for a healthy lifestyle. Thus a range of other lifestyle factors, rather than just wine itself, may promote a healthy body and reduce the incidence of CHD (Gordis, 1999). Evidence suggests that drinkers who prefer wine may smoke less (Klatsky *et al.*, 1990) and have a more healthy diet (Tjonneland *et al.*, 1999) than drinkers who prefer other alcoholic beverages. In addition, those who consume alcohol may tend to have more regular exercise than those who do not drink at all (Barrett *et al.*, 1995). It will be important for further research to clarify the possible significance of such general lifestyle factors in the association between alcohol consumption and decreased mortality, as there has been much debate on this point among researchers (Norrie, 1997).

It can be seen from the above comments that the French Paradox is far from completely explained. The effect may result from a combination of many aspects of Mediterranean living. However, for the moment, the French Paradox remains an intriguing contradiction that provides a strong incentive to carry out further studies that would clearly establish causal relationships.

3.2 Other wine/alcohol and health associations.

In addition to the negative association between alcohol consumption and mortality from coronary heart disease that has now been observed in many studies, some epidemiological studies have associated alcohol or wine consumption with reduced rates of incidence of other health problems. These include dementia and Alzheimer's disease (Orgogozo *et al.*, 1997), age-related macular degeneration (Obisesan and Hirsch, 1998), kidney stones (Hirvonen *et al.*, 1999; Curhan *et al.*, 1996; 1998), gallstones (Leitzman *et al.*, 1999) and rectal cancer (Tavani *et al.*, 1998). With each of these studies it must be recognised that the link between alcohol or wine intake and the reduced chance of developing a health problem is an association that requires further research to determine whether it is actually alcohol, or wine in particular, that produces the health benefit. In each case, as discussed above, there may be other lifestyle factors that contribute to, or fully explain, the effects that have been observed. The observation by Tavani *et al.* (1998) of a reduced risk of rectal cancer associated with alcohol consumption must be viewed with particular caution as numerous other studies suggest that chronic alcohol consumption is linked to increased risk of cancer of the gastrointestinal tract (Seitz *et al.*, 1998a) and research studies are beginning to address the mechanisms by which this may occur (Seitz *et al.*, 1998b). However, there may well be a threshold below which alcohol intake does not increase, and perhaps even decreases, the incidence of gastrointestinal cancer. Two other health problems to which excessive alcohol consumption may contribute are obesity and liver disease, but evidence suggests that moderate alcohol consumption (less than 40 g per day) does not lead to either weight gain (Cordain *et al.*, 1997) or increased risk of alcohol-related liver disease (Savolainen *et al.*, 1993). Overall, much evidence is accumulating through epidemiological studies that moderate alcohol consumption is not harmful, and may indeed provide significant health benefits.

4 Possible Adverse Effects of Moderate Alcohol Consumption.

The mounting evidence that moderate consumption of alcohol, and especially wine, has significant health benefits has excited the biomedical world. However, it must be remembered that alcohol in excess can have substantial adverse effects on health for all people, and that even moderate consumption is harmful for some.

The severe adverse effects of excessive alcohol consumption are well known and include cirrhosis of the liver, gastrointestinal problems, increased risk of cancers of the mouth and bowel, brain damage and, in extreme cases, death by alcohol poisoning (Guyton and Hall, 1996). Regular, heavy alcohol intake is also associated with hypertension (Klatsky, 1996a). Alcohol is, for some people, an addictive drug and alcoholism is a problem on both an economic and a social scale for many countries. In countries such as France, where the alcohol intake is high, the rate of road accidents is higher than average; driving under the influence of alcohol has long been known to be a potential road hazard (St. Leger *et al.*, 1979). This report will not deal in any more detail with the well-established negative effects of excessive

consumption. In assessing and advising on the possible beneficial effects of moderate alcohol consumption, however, any possible risks of such moderate consumption must also be carefully considered.

Many researchers examining the effect of alcohol on mortality from coronary heart disease have found what is described as either a U- or J-shaped curve resulting from the relationship between alcohol intake and incidence of heart disease (Finkel, 1996; Friedman and Kimball, 1986; Groenbaek *et al.*, 1994, cited in Groenbaek *et al.*, 1995; Kiechl *et al.*, 1998; Marmot *et al.*, 1981; Rehm and Bondy, 1998). Not only do those consuming moderate amounts of alcohol fare better than do those who abstain, there is also a substantially greater risk of coronary heart disease among those who drink heavily, or even those who just consume slightly more than the usually recommended 'moderate amount', which ranges from about 10-30 grams of alcohol daily. (An amount of 10-30 grams of alcohol is equivalent to 1-2 150 ml glasses of alcoholic beverage at an alcohol concentration of 10% weight/volume – i.e. 10 grams of alcohol per 100 ml of beverage). We do not yet know how to define exactly what amount of alcohol is likely to be maximally beneficial for any particular person.

Furthermore, alcohol even in such moderate amounts may present particular problems for some people, for part or all of their lives. Those who are at risk from alcohol addiction may never be able to drink any amount of alcohol safely. For some people, alcohol provokes migraine headaches and, in particular, some people are intolerant to red wine because of the so-called 'red wine headache' that results when they consume it. Several researchers are now investigating this phenomenon and its treatment (Jarman *et al.*, 1991; Kaufman, 1992), but for the moment this effect of red wine remains a problem for those affected.

Many Asian people have an enzyme deficiency (a lack of mitochondrial aldehyde dehydrogenase activity) that makes them unable to tolerate anything more than very small quantities of any type of alcoholic beverage. They suffer severe symptoms such as facial flushing, nausea, headaches and vomiting, which are caused by the accumulation of acetaldehyde. (Acetaldehyde is the first product of alcohol metabolism in the body, and it is normally removed by the enzyme aldehyde dehydrogenase.)

For anyone who has liver disease, for example hepatitis or liver cancer, alcohol consumption should be avoided. The additional metabolic stress placed on the liver through oxidising alcohol can make disease-related liver damage worse. Frequently those with such liver damage will have a poor tolerance for alcohol due to impaired metabolism.

For people in these categories, the possible health benefits of alcohol consumption are outweighed by the obvious, immediate, adverse effects of even moderate alcohol intake.

There are, however, two cases where possible adverse effects of moderate alcohol consumption are less obvious but perhaps, on a long-term basis, potentially much more damaging. Both of these involve women, one being the possible increase in incidence of breast cancer rates with moderate alcohol intake, the other being the risk of foetal damage with alcohol intake during pregnancy.

When consumed by expectant mothers alcohol has been proven to affect the health of the foetus and in some cases to produce a condition known as foetal alcohol syndrome. Exposure to alcohol during foetal development has been shown to result in decreased body weight (Addolaroto *et al.*, 1997) and brain development (Valles *et al.*, 1997), higher foetal and infant mortality rate and to have adverse effects on several other aspects of foetal health. Full foetal alcohol syndrome, for which the main symptoms usually include limb deformities, unusual facial features, behavioural abnormalities and mental retardation, is normally seen in children born to mothers who drink very heavily during pregnancy. Although one study suggests that there is no evidence that light alcohol consumption affects the foetus at all (Knupfer, 1991), there is a lot of other evidence that suggests that moderate alcohol consumption during pregnancy has adverse effects on brain development that result in neurodevelopmental and behavioural disorders (Sampson *et al.*, 1997; Olson *et al.*, 1997; Larroque and Kaminsky, 1998). Experiments using model animals demonstrate that prenatal alcohol exposure, even with an otherwise nutritionally well-balanced diet, can cause neurodevelopmental and behavioural deficits (Lilliquist *et al.*, 1999; Maier *et al.*, 1999). There is also evidence to suggest that prenatal exposure to alcohol, even at light to moderate levels, has long-term effects on growth of children (Day *et al.*, 1999). A recent report on a study carried out in Seattle, Washington State, U.S.A, has indicated that the combined incidence of full foetal alcohol syndrome and of alcohol-related neurodevelopmental disorders, which are milder symptoms that may result from moderate alcohol intake, may be as high as 9.1 per 1,000 live births (Sampson *et al.*, 1997). Many health professionals now believe that foetal alcohol syndrome and alcohol-related neurodevelopmental disorders are the leading cause of preventable mental disorders in many Western societies, and that development of screening for detection and of assistance programmes is vital (Roebuck *et al.*, 1999; Bearer *et al.*, 1999; Murphy-Brennan and Oei, 1999; Williams *et al.*, 1999; Bagheri *et al.*, 1998; West *et al.*, 1998; Msall *et al.*, 1998; Famy *et al.*, 1998; Kerns *et al.*, 1997). It is generally accepted that we do not currently have sufficient information to be certain about the level of alcohol intake that is completely safe during pregnancy. Therefore, the safe course of action is to recommend that pregnant women should avoid drinking alcohol. It should be noted that the metabolic situations of developing and developed brain are different; moderate alcohol consumption is probably not detrimental to the developed adult brain, and, as suggested above (Section 3.2, Orgogozo *et al.*, 1997), it may even be beneficial.

A possible link between alcohol intake and breast cancer in women has been suggested in numerous studies over the past 20 years. A recent pooled analysis of a large number of studies produced statistical evidence that the risk of breast cancer in women increased linearly with increasing alcohol intake for total intakes of up to 60 g of alcohol per day (Smith-Warner *et al.*, 1998). The results essentially supported those of another statistical analysis of a range of studies that was carried out 10 years earlier (Longnecker *et al.*, 1988), in which an alcohol intake of 2 drinks per day was indicated as giving a 1.4 fold increase in risk for breast cancer. Both these studies, however, are demonstrating an association, rather than a cause and effect.

The possible biological explanation for alcohol causing an increase in incidence of breast cancer is still the subject of debate. Alcohol may cause an increase in estrogen levels (Vishnudutt, 1998; see also discussion in Smith-Warner *et al.*, 1998), and it is

also possible that resveratrol in wine could be implicated (see discussion in Section 6.1.3.2.a of this report). It should be noted, however, that none of the studies has reported any increased risk, in terms of incidence of breast cancer, for wine consumption as compared to consumption of any other alcoholic beverage. In one study carried out in New Zealand, an increased relative risk of incidence of breast cancer of 1.8 was reported for women drinking more than 14 drinks per week, while no increased risk was found for women drinking less than 14 drinks per week (Sneyd *et al.*, 1991). Until the biological basis of the possible influence of alcohol in increasing the incidence of breast cancer has been clarified, it is advisable that recommendations on safe drinking levels for women are conservative.

A full risk/benefit analysis that might lead to provision of recommendations for safe levels of alcohol intake for women would be very complex, as the potential benefits for a decreased risk of cardiovascular disease would need to be weighed against the potential risk for breast cancer. In an attempt at such an analysis, Friedman and Klatsy (1993) indicated that for a 30-year old female drinking 1 glass of wine per day the health benefits in terms of a reduction in risk of coronary heart disease would be slightly outweighed by the health disadvantages of an increased risk of large-bowel and breast cancer. In contrast, the health benefits for a 50-year old male moderate drinker would outweigh the disadvantages. These authors indicate that their examples are over-simplified, but that they illustrate the type of analysis that is required to assess the cost/benefit ratio of moderate alcohol intake. They conclude their paper ‘Is alcohol good for your health?’ with the following statement: “Currently, indiscriminate advice to non-drinkers to take up alcohol for health reasons is inappropriate, but some people (e.g. those at high risk for coronary heart disease but low risk for problem drinking) may benefit. Risks to health must also be weighed against the non-health-related benefits of alcohol. As in other areas of health care, the patient must, with our guidance, make the final decision.” Promotion of such an approach through education of health professionals (who are probably well aware of the disadvantages) about the health advantages of alcohol consumption would probably be a safe and productive form of advertising for the wine industry.

5 Possible Mechanisms for Health Benefits of Alcohol in General and Wine in Particular.

Over the last decade it has been well established that the moderate intake of alcohol (about 10-30 g/day) is correlated with a reduced mortality rate from cardiovascular disease; those who consume a moderate level of alcohol have less incidence of mortality from cardiovascular disease than either those who are heavy drinkers or those who abstain (Marmot *et al.*, 1981; Moore and Pearson, 1986, Veenstra, 1991, Rohan 1984, cited in Hendricks *et al.*, 1994; Rimm *et al.*, 1991; Klatsky *et al.*, 1992, cited in Goldberg, 1995; Kiechel *et al.*, 1998; Yano *et al.*, 1977, Rhoads *et al.*, 1978, cited in Renaud and de Lorgeril, 1992; Kannel and Ellison, 1996 Finkel, 1996; Friedman and Kimball, 1986; Groenbaek *et al.*, 1994 cited in Groenbaek *et al.*, 1995; Constant, 1997; Doll *et al.*, 1994; Doll, 1997; Gaziano *et al.*, 1997; Wannamethee and

Shaper, 1999). This produces a pattern known as the U-shaped curve and sometimes as the J-shaped mortality curve. Some suggest that the curve results from the reduction in coronary heart disease contributed by moderate alcohol intake and the increased incidence of diseases such as cancer and cirrhosis caused by excessive and binge drinking. Heavy alcohol intake is also associated with hypertension, and it has been pointed out that the amount of alcohol taken is probably crucial in determining the balance between decreased risk of coronary heart disease and increased risk of other disorders (Klatsky, 1996b). Two recent reports have indicated that alcohol reduces the risk for peripheral arterial disease and myocardial infarction in apparently health men in the United States (Camargo *et al.*, 1997a, b). There have been fewer studies on the health benefits of alcohol in women than in men, and while there appears to be some reduction in mortality in some studies, some results have been conflicting (Fuchs *et al.*, 1995).

The benefit of alcohol has largely been attributed to its ability to raise the concentration of high density lipoprotein (HDL), which is a well-defined negative risk factor for coronary heart disease (Thornton *et al.*, 1983; Gaziano *et al.*, 1993; Suh *et al.*, 1992, cited from Goldberg, 1995; Gaziano *et al.*, 1999). However, changes in HDL concentration only account for half of the benefits alcohol affords (Gaziano *et al.*, 1993; Lieber, 1984, cited from Frolich, 1996).

Some researchers believe that the benefits of alcohol are more likely to result from its hemostatic activity. The inhibition of platelet aggregation by alcohol (Renaud *et al.*, 1992; Renaud and Ruf, 1996) and also the beneficial effects of alcohol on the fibrinolytic system (Hendriks *et al.*, 1994; Hendriks and van der Gaag, 1998) are plausible hemostatic mechanisms for reducing the incidence of coronary heart disease. Lipoprotein(a) has been proposed as an antifibrinolytic compound, and it has been suggested that alcohol may lower lipoprotein(a) levels and that this may lead to some of the cardiovascular benefits (Sharpe *et al.*, 1998). However, the overall evidence on the effect of alcohol on lipoprotein(a) levels appears to be inconclusive (Hendriks and van der Gaag, 1998). One study suggests that alcohol may exert its effects through reducing diabetes and Metabolic Syndrome X, otherwise known as insulin resistance syndrome (Bisson, 1997). This is a feasible theory as both of these conditions are plausible indicators of coronary heart disease.

A topic receiving considerable attention is the notion that wine, particularly red wine, may have superior cardioprotective ability compared with other alcoholic beverages. Wine contains phenolic compounds, some of which have significant cardioprotective roles (Anon, 1993; Frankel *et al.*, 1995; Soleas *et al.*, 1997a; Hurtado *et al.*, 1997; Viera *et al.*, 1988; Halpern *et al.*, 1998). It is suggested that these alone or in addition to the alcohol present in wine may be responsible for the French Paradox and reduction of coronary heart disease by moderate wine consumption. Both red and white wines are also an excellent source of salicylic acid (the active ingredient of aspirin) and its metabolites (Muller and Fugelsang, 1994). These compounds have known vasodilator and anti-inflammatory effects. Mortality resulting from coronary heart disease in Denmark decreased by about 30% in 15 years. Groenbak *et al.* (1995) suggested that this might be due not to an increased alcohol consumption but to a change in drinking trends which favoured wine consumption. However, attempts to demonstrate with certainty the superiority of wine over either beer or spirits have proved difficult and results of such studies have been inconsistent (White, 1996).

In a review by Rimm *et al.* (1996b), studies completed over the last decade were sorted into ecological, cohort and case-controlled to try and uncover essential flaws and to see if there was consistency in results within a single type of study. In ecological studies existing data, often collected by government agencies or international surveillance programmes, are compared and a conclusion is made based on these findings. Cohort and case-controlled studies relate the variables of interest in individual subjects and are often followed up for several years.

In the majority of ecological studies wine intake was found to have a strong inverse association with incidence of cardiovascular mortality (Criqui and Ringel, 1994; St. Leger *et al.*, 1979). Beer and spirits were found to have either a very weak correlation or none at all. In a large study carried out in California, Klatsky *et al.* (1997) reported that drinking ethanol in general appeared to protect against coronary disease, and that there were possible minor additional benefits associated with drinking both beer and wine, but not red wine in particular. However, ecological studies have important limitations and these results may be an artefact resulting from lifestyle differences, total alcohol consumption, personality traits and/or other inconsistencies.

Because something is known about the individuals being tested, cohort and case-controlled studies have the potential to be more accurate than ecological studies. Unfortunately the results from these types of studies have not been clear-cut. Some cohort studies have found an inverse relationship only between wine consumption and incidence of coronary heart disease (Groenbak *et al.*, 1995; Stampfer *et al.*, 1988, cited from Rimm *et al.*, 1996b) while in others, spirits consumption alone is strongly inversely related to coronary heart disease (Rimm *et al.*, 1991, Salonen *et al.*, 1983, cited from Rimm *et al.*, 1996b). We did not find any studies that suggested that beer consumption showed a strongly negative correlation with coronary heart disease. However some studies did show significant (but not strong) negative associations between this beverage and CHD (Yano *et al.*, 1977; Kittner *et al.*, 1983, Klatsky *et al.*, 1990, from Rimm *et al.*, 1996b; Groenbak *et al.*, 1995).

In other studies, spirits were most strongly associated with a reduction in cardiovascular disease in men (Hennekens *et al.*, 1979, cited in Rimm *et al.*, 1996b), while in women only wine was strongly associated with a reduction in risk of non-fatal myocardial infarction (Rosenberg *et al.*, 1981, cited in Rimm *et al.*, 1996b). In another case-controlled study, Kaufman *et al.* did not find any inverse association with alcoholic beverages at all (Kaufman *et al.*, 1985, cited from Rimm *et al.*, 1996b).

In summary, it appears at this stage that although alcohol itself almost certainly provides protection against cardiovascular disease, no firm conclusion can be made as to whether compounds in wine actually increase the cardioprotective ability of this beverage as compared to beer or spirits. There is, however, sufficient evidence suggesting an additional positive effect for wine to make further research on wine of particular interest. The next section of the report deals with compounds found in wine that may provide specific health benefits.

6 Compounds with Potential Health Benefits Found in Red Wine.

6.1 The chemical nature of the compounds in wine that may provide health benefits.

The compounds in wine, other than alcohol itself, that are believed to have potential health benefits belong to a general group known as ‘phenolics’, or often ‘polyphenolics’, which refers to the fact that many of the compounds have several hydroxyl groups. The general class of phenolic compounds includes two major groups, flavonoids and non-flavonoids. Flavonoids are a family of compounds that are all derivatives of 2-phenyl-1-benzopyran-4-one. The major flavonoid categories are flavonols, flavones, catechins, flavanones and anthocyanins (Howard and Kritchevsky, 1997; Soleas *et al.*, 1997). The catechins are monomeric flavan-3-ol units that may combine to form oligomers, known as the proanthocyanidins, and polymers, known as condensed tannins (Waterhouse and Teissedre, 1997). One flavonoid compound that has been of particular interest in studies on the health benefits of wine is quercetin (see Figure 1). The non-flavonoid compounds in wine include hydroxybenzoates, hydroxycinnamates and stilbenes. The most significant compounds in this group in terms of possible positive health benefits are the stilbene resveratrol (Waterhouse and Teissedre, 1997; Soleas *et al.*, 1997) and the related compounds piceid and astringin (see Figure 1).

Most of the above compounds are found in higher concentrations in red wine than in white wine since these compounds are mainly extracted from the skins and seeds of the grapes (Soleas *et al.*, 1997; Creasy and Coffee, 1988, cited in Siemann and Creasy, 1992). The skins are usually removed before the fermentation of white wine whilst they are retained in the fermentation of red wine; hence the higher concentration of phenolics in red wine (Jeandet *et al.*, 1991 cited from Ector *et al.*, 1996). While white wine may be beneficial in terms of the cardio-protective effects of alcohol itself, as discussed above, red wine may have additional protective effects due to the presence of phenolic compounds (Bradbury, 1997). Some white wines, however, do contain significant amounts of resveratrol and quercetin (McMurtrey, 1997). It is important to recognise, therefore, that studies addressing the possible health benefits of wine should ideally include measurement of the compounds of interest in the particular wine being used. The content of key phenolic compounds may vary quite widely even between different bottles of the same red wine (McMurtrey, 1997).

The following sections give details of some studies that deal specifically with the possible mechanisms of health benefits provided by compounds that are found in red wine. Some of the studies have used red wine itself, or the total fraction of phenolic compounds derived from red wine. Others have used flavonoid compounds, or specific compounds such as quercetin or resveratrol. A few researchers have sought to compare the effects of white wine with those of red wine, and results of these studies are also discussed in this section.

6.2 Antioxidant properties of phenolics.

Phenolic compounds present in red wine have been shown to inhibit the oxidation of LDL *in vitro* (Frankel *et al.* 1993a; Frankel *et al.*, 1995; Hurtado *et al.*, 1997; Henn and Stehle, 1998; Ghiselli *et al.*, 1998). Further studies have demonstrated that the phenolics present in red wine also inhibit the oxidation of LDL *in vivo* (Nigdikar *et al.*, 1998).

In 1993 a Californian group showed that polyphenolic compounds from red wine have a powerful antioxidant effect on plasma lipoproteins *in vitro* (Frankel *et al.*, 1993a). LDL peroxidation was inhibited by 60% and 98% by the addition of polyphenols from red wine at 3.8 $\mu\text{mol/L}$ and 10 $\mu\text{mol/L}$ respectively. Addition of polyphenols to platelets also resulted in decreased generation of conjugated dienes (an indicator of LDL oxidation). When compared to α -tocopherol, a compound known to have powerful antioxidant effects, wine phenolics were shown to be significantly more effective at preventing LDL oxidation. Later results supported these findings, showing a 70% reduction in LDL oxidation by mixing red wine with LDL *in vitro* (Voelker, 1995). More evidence followed in 1997 when polyphenolics were shown to delay the production of conjugated dienes (Hurtado *et al.*, 1997).

In 1995, two research groups attempted to study phenolic activity *in vivo*. An Israeli study included 17 healthy men; half of these men were given 400 mls of red wine per day for 2 weeks and the other half 400 mls of white wine per day for two weeks (Fuhrman *et al.*, 1995). Those who consumed red wine experienced a 20% reduction in plasma lipid peroxidation. In contrast, those who consumed white wine experienced a 34% increase in plasma lipid peroxidation. This finding was unexpected and was attributed to the oxidative effect of alcohol *in vivo*. Although this view was somewhat controversial (Caldu *et al.*, 1995), some evidence does exist to suggest that alcohol causes oxidation of lipids *in vivo* (Xia *et al.*, 1998). At about the same time a team of Dutch scientists had a similar study published (de Rijke *et al.*, 1995; 1996). However, their results were not in accordance with those of the Israeli study. They found that red wine did not affect the susceptibility of LDL to oxidation and that it had no benefit over white, which has insignificant amounts of phenolics. Another study by Watkins and Bierenbaum (1997) showed that white wine was more effective than red wine in providing antithrombogenic and antioxidant benefits in hyperlipemic subjects; the results reported in this study are, however, of borderline significance, and should be treated with some caution.

More recently a project was completed in the United Kingdom that supported the findings of the Israeli study. The UK study showed that red wine (specifically its phenolic compounds) reduces LDL oxidation in humans (Nigdikar *et al.*, 1998). Another study has reported an LDL-vitamin E sparing effect for red wine phenolics (Carbonneau *et al.*, 1998). In one study, red wine was claimed to be more effective than vodka in inhibiting LDL oxidation *in vivo* (Kondo *et al.*, 1994). Maxwell *et al.* (1994) reported that antioxidant activity in serum was increased in samples taken from subjects who had consumed red wine with a meal (Note: This was a brief study that attracted some negative comment about the experimental techniques – see Melville, 1994). Similar results were found by Whitehead *et al.* (1995) who reported that the serum antioxidant capacity of volunteers consuming 300 ml of red wine was

increased by 18% after 1 hour, while the same amount of white wine did not produce a significant increase.

It has also been suggested that inhibition of LDL oxidation by polyphenols could be related to binding of these compounds to LDL (Hayek *et al.*, 1997). The physiological situation may be very complex. One study has shown that alcohol in wine may increase the bioavailability of phenolic antioxidants (Serafini *et al.*, 1998) so that the end effect may be due both to the antioxidants and to the alcohol content. Phenolic compounds were also demonstrated to reduce platelet aggregation in rats (Xia *et al.*, 1998) and to produce vasorelaxation effects in rat aortic tissue (Fitzpatrick *et al.*, 1993). The effect of red wine in maintaining endothelial function as assessed by vascular relaxation in healthy male volunteers was studied by Cuevas *et al.* (2000), whose results suggested that red wine could protect against the adverse effects of a high fat diet. The studies that achieved positive results *in vivo* also suggest that at least some phenolics are absorbed *in vivo*. In a very recent study, an increased concentration of circulating levels of polyphenolic compounds and a concomitant increase in antioxidant status were observed in fasting subjects after ingestion of alcohol-free red (but not white) wine (Serafini *et al.*, 1998).

The antiatherogenic potential of polyphenolic flavonoids has been reviewed in a recent article by Aviram and Fuhrman (1998). Another recent review (Stockley, 2000) covers work that has been done in Australia on the antioxidative properties of phenolic compounds in wine. The reviewer concludes that all the Australian studies cited confirm that wine phenolic compounds have antioxidant properties, but raises the caution that in some cases the antioxidant properties of the phenolic compounds may be counteracted by pro-oxidative effects of ethanol. In Europe a project (FAIR CT 97 3261 "Wine and Cardiovascular Disease") involving seven research institutions has been established to further investigate the effects of red wine polyphenolic extracts on factors contributing to atherosclerosis and thrombosis. Results, as reviewed by Rontondo and de Gaetano (2000), indicate that wine-derived compounds can improve vascular function and may down-regulate LDL oxidation and lipid oxidation.

There is, therefore, fairly conclusive evidence that phenolic compounds present in red wine do have antioxidant properties both *in vitro* and *in vivo*. Further work however must still be completed to confirm this (Hertog *et al.*, 1997). Potentially this antioxidant ability may in part explain the anti-atherogenic quality of red wine.

6.3 Cytoprotection (cell protection) by phenolics.

A European study published in 1998 adds to the list of protective activities afforded by phenolics (Viera *et al.*, 1998). This study shows phenolic compounds are able to prevent apoptosis (programmed cell death) caused by oxidised LDL in endothelial cells. The researchers claim not only that this is mediated indirectly by the anti-oxidising effect of phenolics on LDL, but also that phenolics are able to exert a direct cytoprotective effect. Programmed cell death is an essential part of the ageing process, and this process may be enhanced by oxidative processes caused by reactive oxygen species in tissues. Phenolics may therefore play a role in slowing the ageing process.

6.4 Specific phenolic compounds

6.4.1. Flavonoids

Flavonoids occur naturally in plant foods and are a common component in the human diet, the major sources being tea, onions, soy and red wine (Howard and Kritchevsky, 1997; Hertog *et al.*, 1993b). Flavonoid intake was inversely correlated with coronary heart disease in the Zutphen Elderly Study, (Hertog *et al.*, 1993a) the Seven Countries Study, (Hertog *et al.* 1995, cited in Maxwell, 1997) and in a cohort Finnish study (Knekt *et al.*, 1996). In the Zutphen Elderly Study the flavonoid intake of 805 men aged 65-84 was assessed and the men followed up for a five year period. The results were striking even when adjusted for all major risk factors for coronary heart disease (CHD). Flavonoid intake of 0-19 mg/day was associated with a mortality rate from coronary heart disease of 18.5 per 1000 person years whilst an intake of 29.9 mg/day or more was associated with only 7.8 deaths due to coronary heart disease per 1000 person years. These findings were supported by the other two major studies (Hertog *et al.* 1995, cited in Maxwell, 1997; Knekt *et al.*, 1996). The only stronger predictor of CHD than flavonoid intake in these studies was a positive correlation seen with consumption of saturated fatty acids (Maxwell, 1997).

Several mechanisms to explain flavonoid protection have been proposed, the most likely of these being their antioxidant properties. Flavonoids protect LDL from oxidation *in vitro* (Mangipane *et al.*, 1992 and Negre-Salvayre *et al.*, 1992 both cited from Maxwell, 1997; Frankel *et al.* 1995; Jessup *et al.*, 1990). The flavonoids even out-perform α -tocopherol (vitamin E), the major endogenous radical-scavenging antioxidant that protects against oxidation of LDL (Jessup *et al.*, 1990; Frankel *et al.*, 1993a). As well as being direct radical-scavengers of the reactive oxygen species that cause LDL oxidation, flavonoids have also been shown to chelate (bind) iron, which acts as a catalyst for these oxidants (i.e. iron speeds up the rate at which they oxidise). Once chelated the iron is prevented from achieving its biological actions (Afanaslev *et al.*, 1989, cited in Maxwell, 1997; Morel *et al.* 1994). Flavonoids have further been shown to chelate other metals, such as copper, which also have roles in LDL oxidation (Degroot and Rauen, 1988). Another important antioxidant attribute of flavonoids is their ability to inhibit many enzymes that catalyse LDL oxidation. These include a group of enzymes known as lipoxygenases which have been implicated in LDL oxidation (Laughton *et al.*, 1991 cited from Maxwell, 1997; Moroney *et al.*, 1988; Takhama, 1985; Degroot and Rauen, 1998).

It should be noted that some part of the increased antioxidant activity in serum following wine consumption may be due to increased concentrations of urate, rather than to an increase in flavonoids derived from red wine (Maxwell, 1997). This possibility requires further investigation.

Other than this antioxidant protection it is claimed that flavonoids have important anti-platelet aggregation and antithrombotic activity (Corvazier and Madouf, 1985; Maalej *et al.*, 1997). Flavonoids have even been implemented in modulation of the immunological response (Fartzov *et al.*, 1994; Bratting *et al.*, 1984, Van Meer, 1984, and Hornung *et al.*, 1988, cited from Fartzov *et al.*, 1994). In one particular study

anthocyanins were shown to stimulate the activity of phagocytic immune cells (Fartzov *et al.*, 1994).

Flavonoids are commonly found in plant foods such as onions, apples and broccoli and their presence is one plausible explanation for the observed cardioprotective effects that vegetables and fruit offer (Bohm *et al.*, 1998; Hertog *et al.*, 1993a). What then, if anything, is special about the flavonoids present in red wine? Flavonoids are not always well absorbed and form precipitates through interactions with proteins. This not only removes valuable protein from the diet but may also decrease the bioavailability of the flavonoids present in food. A recent study claims that the alcohol content in wine actually reduces these protein-flavonoid interactions flavonoids (Serafini *et al.*, 1997), and this may result in increased bioavailability of both the protein and the antioxidant. If this proves to be consistently true then red wine may indeed be a superior supply of these antioxidants.

6.4.2 Quercetin.

Quercetin (Figure 1) is one of the major flavonoids found in red wine (Simmonetti *et al.*, 1997) and other sources such as tea and vegetables. Quercetin has been claimed to have antimicrobial effects (El-Gammal *et al.*, 1986, cited in Stavric., 1994), antiviral activity (Vrijssen *et al.*, 1988), antioxidant ability (Vinson *et al.*, 1995a and Negre-Salvagyre and Salvagyre *et al.*, 1992, cited in Conquer *et al.*, 1997, Jan *et al.*, 1991) and even anticarcinogenic properties (Yoshida *et al.*, 1990; Wiltout *et al.*, 1988 cited from Stavric., 1994; Soulinna *et al.*, 1975, cited from Formica and Regelson, 1995).

Quercetin was shown to inhibit copper-catalysed oxidation and cytotoxicity of LDL *in vitro* (Vinson *et al.*, 1995a; Negre-Salvagyre and Salvagyre *et al.*, 1992, cited in Conquer *et al.*, 1997) and also to be a potent free radical scavenger (Husain *et al.*, 1987; Robak and Gryglewski, 1988 cited in Conquer *et al.*, 1997; Takahama, 1995). Other cardioprotective benefits are supplied by quercetin's ability to inhibit platelet aggregation and thrombotic activity (Pace-Asciak *et al.*, 1995; 1996). In a study using rats fed a high fat diet, however, quercetin was not found to have any significant effect on plasma total cholesterol or triglyceride concentrations (Yugarami *et al.*, 1992).

6.4.3 Non-flavonoids – primarily resveratrol.

The major non-flavonoid compound on which there have been studies related to potential health benefits of wine is resveratrol (Figure 1), and details of a number of significant research findings are given below. Two other compounds, piceid (a derivative of resveratrol) and astrigin are mentioned briefly. These may also have beneficial effects, but have not been as well studied as resveratrol.

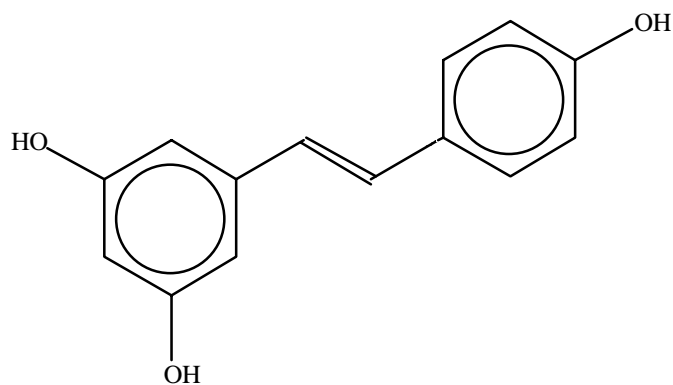
6.4.3.1 Sources of resveratrol.

In 1990 a plant physiologist named Leroy Creasy discovered that resveratrol, a trihydroxystilbene which conferred resistance in grapevines against fungal infection, was also a constituent of an Asian medicinal herb *Polygonum cuspidatum* (Waterhouse, 1993). Kojo-jon, an ancient oriental folk medicine derived from this Asian herb (Sharp, 1993), is claimed to be effective in treating arteriosclerosis and inflammatory and heart disorders (Goldberg, 1995; Soleas *et al.*, 1997b). It is not surprising then that the discovery of the presence of resveratrol in red wine generated interest in biomedical and wine industry circles. Resveratrol is found mainly in the skin of grapes and not in the flesh, and since skins are usually removed early in the processing of white wine while they are left in whilst red wine ferments, resveratrol is present in significant amounts in most red wines and only some white wines (McMurtrey, 1997). The content of resveratrol in wines varies due to factors such as temperature, fungal infection and geographic region (Skurray, 1996). It can also be affected by the use of fining agents in preparation of wine (Skurray and Dezile, 1998).

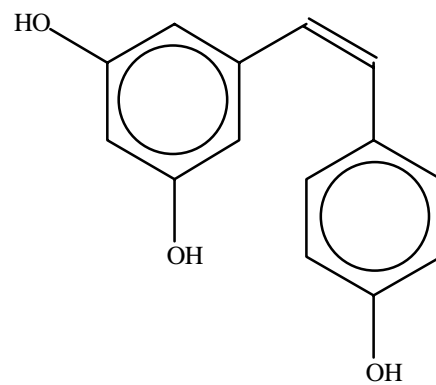
The resveratrol content of selected Australian wines has been studied, and, consistent with other observations, was found to be higher in red wines than in white wines (Skurray and Boupha, 1997). Among the red wines, resveratrol concentrations varied from 2-10 mg/litre, while white wines had concentrations of about 1-2 mg/litre.

Researchers had long been searching for a compound exclusive to wine that could prove its biomedical superiority to other alcoholic beverages and potentially explain the French Paradox. Flavonoids, as discussed above, are commonly found in fruit and vegetables as well as wine, whereas the source of resveratrol in the human diet is almost exclusively limited to red wine, with the exception of peanuts. (Arora and Strange, 1991, cited from Goldberg, 1995). Therefore resveratrol provided the missing link that researchers had been seeking and intensive and widespread study of the compound and its potential health benefits began. In recent papers using animal experiments, Bertelli *et al.* (1996;1998) claim to have shown that resveratrol can be absorbed in sufficient quantity to explain the beneficial effect of red wine on health.

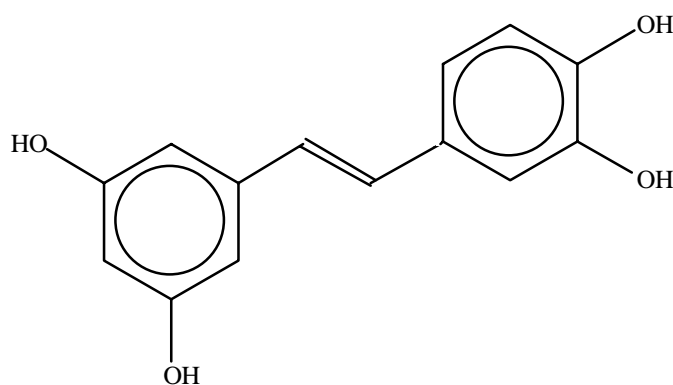
Figure 1: Structures of some compounds referred to in the text that are found in wine.



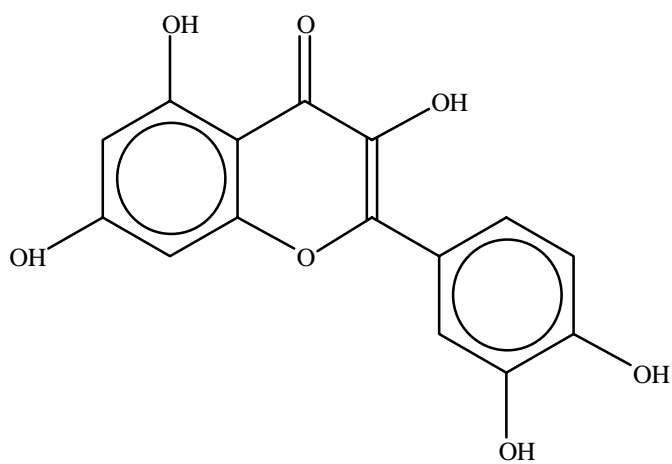
trans-resveratrol



cis-resveratrol



astringinin



quercetin

6.4.3.2 Evidence for the role of resveratrol in preventing platelet aggregation.

Platelet aggregation is known to lead to the formation of atherosclerotic plaques or plugs, which can ultimately result in thrombosis and coronary heart disease. In the laboratory, resveratrol has consistently been shown to reduce platelet aggregation significantly and therefore may have real cardio-protective benefits.

In 1985 a Japanese study, prompted by interest in the active ingredients of the herb *Polygonum cuspidatum*, showed that orally administered resveratrol lowered platelet aggregation in an *in vitro* system using rat cells (Kimura *et al.*, 1985). Several years later in another Japanese study it was shown that resveratrol is indeed a powerful inhibitor of platelet aggregation (Chung, *et al.*, 1992, cited in Goldberg, 1995). More evidence resulted from studies at the University of Toronto in Canada with resveratrol being demonstrated to reduce platelet aggregation both *in vitro* (Pace-Asciak *et al.*, 1995; Goldberg *et al.*, 1997), and *in vivo* (Pace-Asciak *et al.*, 1996).

The second study by Pace-Asciak and colleagues is interesting as it is one of very few that demonstrates resveratrol's effect in live subjects. Their study involved twenty-four healthy males each consuming the following beverages: red wine, white wine, commercial grape juice and the same grape juice enriched with *trans*-resveratrol. *Trans*-resveratrol-enriched grape juice was shown to inhibit thrombin-induced platelet aggregation and commercial grape juice alone was shown to promote it. Promotion of platelet aggregation by commercial grape juice was an anomaly that the researchers suggested may be due to sugar content and they mentioned that they were investigating this phenomenon further.

Another apparent anomaly in their study was that red wine proved no more beneficial than white wine. Here it is suggested that, *in vivo*, ethanol may be the dominant anti-aggregatory component in these beverages, which were more potent inhibitors than either grape juice. However, it was made clear that it might be due to the differences between the short-term (*in vitro*) and medium-term (*in vivo*) effects of ethanol and resveratrol. Despite these confounding issues it was concluded that, according to levels measured in the blood, sufficient resveratrol was absorbed to exercise a significant effect on eicosanoid metabolism and platelet aggregation.

A more recent study showed that *trans*-resveratrol effectively prevented the aggregation of polymorphonuclear (PMN) leukocytes *in vitro* (Rotondo *et al.*, 1998). In this work the mechanism by which resveratrol inhibits the aggregation was also elucidated. This involves resveratrol inhibiting the expression and activation of a gene called MAC-1. MAC-1 has been implicated in PMN leukocyte aggregation.

It seems then, that resveratrol is certainly able to inhibit platelet aggregation *in vitro*. It has, however, not yet been conclusively proven to have this effect *in vivo* and further studies are required to ascertain the possible significance of resveratrol in human health.

6.4.3.3 Evidence for the role of resveratrol in influencing lipid metabolism and oxidation.

High dietary fat is a leading risk factor for coronary heart disease. In a series of studies by David Goldberg and his colleagues at the University of Toronto in Canada resveratrol was demonstrated to inhibit hepatic lipid synthesis in the human liver cell line HepG2 (Goldberg, *et al.*, 1995; Goldberg, *et al.*, 1997). In the 1995 study, reduced intracellular lipid concentrations and reduced lipid secretion were shown to occur in response to resveratrol but not in a clear dose-dependent manner. However, a dose-dependent reduction of both intracellular concentration of cholesterol esters and rate of secretion of both cholesterol esters and triglycerides was obtained in the later study.

The results of the project in 1997 showed resveratrol had an overall tendency to diminish the concentration of VLDL (very low-density lipoprotein) which is converted to atherogenic LDL in circulation. This is consistent with resveratrol having an anti-atherogenic role. However, resveratrol was also found to reduce the intracellular content and secretory rate of apolipoprotein A1 (Apo A1). Apo A1 is essential for reverse cholesterol transport as well as esterification of free cholesterol. This action of resveratrol may therefore have deleterious consequences from the standpoint of protection against atherosclerosis. The claim that resveratrol has a lipid-lowering effect was also backed up by a study where resveratrol was administered to rats suffering from hyperlipemia (Arichi *et al.*, 1982, cited from Siemann and Creasy, 1992).

Around the same time scientists at the University of California were examining the effects of wine phenolics, including resveratrol, on LDL oxidation (Frankel *et al.*, 1995; Frankel *et al.*, 1993b). They discovered that resveratrol protects human LDL against copper-catalysed oxidation. Upon addition of resveratrol, peroxidation of LDL was inhibited by 81% and 70% in two healthy adult volunteers. However, resveratrol was less potent than two other flavonoid compounds, epicatechin and quercetin, which are also present in red wine. These compounds had about twice the inhibitory potency of resveratrol. It was concluded, therefore, to be unlikely that the antioxidant properties of wine are solely attributable to resveratrol. British-based scientists Nicholas Millar and Catherine Rice-Evans reached a similar conclusion to Frankel and his colleagues after work they completed in 1995 (Millar and Rice-Evans, 1995). Another study by Belguendouz *et al.* (1997) has also shown that resveratrol may protect LDL against peroxidative degradation by chelating copper.

Therefore, although resveratrol has been shown to have more powerful antioxidant status than vitamin E, it does not appear to have the dominant antioxidant activity amongst other red wine phenolics, and indeed may contribute little to red wine's overall antioxidant activity (Goldberg, 1996).

6.4.3.4 An anomaly in the evidence on metabolic effects of resveratrol.

In 1996 a study using hypercholesterolemic rabbits was undertaken at the Iowa State University. It is particularly interesting to note because of its unusual results. The results found were very different from those expected. Resveratrol was found not to inhibit aggregation of platelets but to promote it. The mechanism by which this was achieved by resveratrol also appeared to be independent of “observed differences in gross animal health, liver function, plasma cholesterol concentrations, or LDL oxidative status” (Wilson *et al.*, 1996). The researchers concluded therefore that resveratrol was “probably not the compound in red wine responsible for the French Paradox” (Wilson *et al.*, 1996). The only other hint that resveratrol has adverse effects was the previously-mentioned observation that resveratrol lowered levels of the beneficial Apo A1 (Goldberg *et al.*, 1997). Further studies in this area would be very interesting.

6.4.3.5 The possible role of resveratrol in prevention of cancer.

In 1997 reports that resveratrol might have significant anticarcinogenic properties began to surface (Brown, 1997). A group of US scientists discovered that resveratrol was a potent inhibitor of the enzyme cyclooxygenase (COX) (Jang *et al.*, 1997). COX catalyses the conversion of arachidonic acid to pro-inflammatory substances such as prostaglandins, which can stimulate tumor cell growth and suppress immune surveillance (Plescia, *et al.*, 1984, cited in Jang *et al.*, 1997). COX can also activate carcinogens to forms that damage genetic material (Zenser, *et al.*, 1983, cited in Jang *et al.*, 1997). The researchers believe that resveratrol inhibits cellular events associated with almost all stages of cancer development: tumor initiation, promotion and progression.

A later study in 1998 also demonstrates resveratrol’s anti-carcinogenic effects and suggests its use as a chemo-therapeutic drug. This work shows resveratrol induces apoptotic (programmed) cell death in the HL60 human leukemia cell line. A dose-dependent increase in tumor mortality with greater than 80% cell death by 48 hours was established (Clement *et al.*, 1998). Furthermore, Marc Fontecave and his research team demonstrated that resveratrol significantly inhibits ribonucleotide reductase, an enzyme involved in cell proliferation. This function may explain the anti-proliferative properties of resveratrol (Fontecave *et al.*, 1998).

However, a study published in 1997 reports that resveratrol belongs to a group of compounds known as phytoestrogens (Gehm *et al.*, 1997; see also comment by Kopp, 1998). These compounds mimic the action of estrogen both *in vitro* and *in vivo*. Whilst estrogenic activity is well established to confer a cardioprotective effect, it is also known to have a growth-stimulating effect on breast carcinomas. Resveratrol was indeed found to stimulate the growth of human breast cancer cells. Human breast cancers are mitogenically stimulated by estrogen (Nandi *et al.*, 1995, cited from Gehm *et al.*, 1997). In apparent contradiction a more recent study describes the dose-dependent antiproliferative activity of resveratrol in human breast epithelial cells (Mgbonyebi *et al.*, 1998), and another study has shown that resveratrol acts as an estrogen receptor antagonist in the presence of estrogen, and inhibits growth of human breast cancer cells (Lu and Serrero, 1999). Resveratrol has also, very recently, been

shown to inhibit growth of oral squamous cancer cells (ElAttar and Virji, 1999a,b) and prostate cancer cells (Hsieh and Wu, 1999).

It appears possible then that resveratrol does have anti-carcinogenic properties, but the evidence is equivocal. The estrogenic activity of resveratrol suggests a cardioprotective benefit but also raises concern for those who have a high risk of breast carcinoma. The possible link between resveratrol and breast cancer, combined with the statistical evidence for a link between levels of wine consumption in women and breast cancer (discussed in Section 4.0 of this report), means that recommendations on safe levels for wine intake in women should be conservative until the significance of these findings has been clearly evaluated.

6.4.3.6 Piceid and astringin

Piceid (polydatin) is a β -glucoside of resveratrol (resveratrol 3- β -D-glucopyranoside). It is structurally identical to resveratrol except that it has a glucose group attached to one of the free oxygen atoms (Waterhouse, 1993).

The presence of piceid in wine has important significance for two reasons. Firstly, piceid shares many of the biological activities of resveratrol; it blocks platelet aggregation (Kimura *et al.*, 1985, Shan *et al.*, 1990, cited from Sato *et al.*, 1997) and has anti-tumor properties (Jayatilake *et al.*, 1993, cited from Sato *et al.*, 1997). Secondly, piceid, like other glycosides of both *trans*- and *cis*-resveratrol isomers, can be hydrolyzed by glycosidases in the human intestinal tract (Goldberg, 1995). This means that about four times more resveratrol than previously estimated could be bioavailable in red wine.

Fauconneau and colleagues reported the presence of another stilbene, astringinin, in red wine (Fauconneau *et al.*, 1997). The structure of astringinin is very similar to that of resveratrol (see Figure 1). Astringinin, and the corresponding glucoside, astringin, were shown to have antioxidant and radical scavenging activity similar to or even superior to that of resveratrol and piceid (Fauconneau *et al.*, 1997; Merillon *et al.*, 1997). These reports further support the evidence for the antioxidant and anti-thrombogenic properties of red wine.

6.5 Summary of effects of phenolic compounds in wine.

There is now a wide range of evidence from many sources to support the theory that the health benefits of wine may, at least in part, be mediated by the actions of phenolic compounds in wine. Such compounds have been shown to act as antioxidants, prevent platelet aggregation, have anti-proliferative effects in some cancer cells and to promote vascular relaxation. The most studied compound is probably resveratrol, but many other polyphenolic compounds are now also under investigation. A note of caution is introduced by studies indicating that resveratrol may stimulate the growth of breast cancer cells in some circumstances. Such observations indicate that caution must be exercised in general promotion of the health benefits of wine until the molecular mechanisms of action of the phenolic compounds are fully understood.

7 Summary and recommendations.

7.1 Summary

7.1.1 Evidence for health benefits of alcohol consumption.

There is now substantial evidence from studies in a range of countries to indicate that moderate alcohol consumption is correlated with a reduced mortality rate from cardiovascular disease. While the statistical evidence for a correlation is now strong, the evidence to confirm the exact mechanism linking improved cardiovascular health and alcohol consumption is still lacking. There has been considerable caution expressed by some health professionals in terms of recommendations being made to encourage increased alcohol consumption on the basis that this will provide improved cardiovascular health for the general population (Whitaker and Ward, 1996; White, 1996; Wannamethee and Shaper, 1998). Before such recommendations can be made with confidence, there is a need to confirm the molecular mechanisms that could account for health benefits of alcohol in general, or wine in particular (Stockley, 1997; Waterhouse *et al.*, 1998), and to determine the safe levels of alcohol consumption for both men and women. We do not yet fully understand the significance of individual variation in parameters that are an index of cardiovascular health in response to alcohol. Nor do we yet know the full significance of the interactions between alcohol and diet. It is possible, for example, that alcohol may only provide significant benefits in association with a diet that is also rich in fresh fruit and vegetables. Alcohol, or wine, consumption has also been shown to be associated with reduced risk of a number of other health problems. In all cases, the observations remain at this stage as associations, as no causal links to alcohol or wine have been established, but such associations provide exciting prospects for further research on the possible role of alcohol consumption in maintaining health.

7.1.2 Possible mechanisms to explain the health benefits of alcohol in general and wine in particular.

The failure to establish a causal link between alcohol consumption and cardiovascular health is not a result of a lack of possible molecular mechanisms. As outlined in the above sections, there have been many proposed. Alcohol itself may increase the concentration of beneficial HDL, inhibit platelet aggregation and reduce the incidence of insulin resistance syndrome. Phenolic compounds present in wine, particularly red wine, may act as antioxidants, inhibit platelet aggregation and increase circulation through vascular relaxation. In addition to these cardioprotective roles, phenolic compounds may also stimulate the immune response, and have antimicrobial, antiviral and anticarcinogenic properties.

The problem is therefore, at this point, that there are many possible mechanisms, none of which has yet been shown conclusively to provide significant long-term health benefits in human subjects *in vivo*. The consideration of possible mechanisms to explain the protective effect is also complicated by the fact that it is still not clear whether wine provides more health benefit than other types of alcoholic beverage (White, 1996; Stockley, 1997; Wannamethee and Shaper, 1999).

The evidence for increased antioxidant status in human subjects drinking wine is reasonably strong, although there is still no overall agreement between studies as to whether this effect is greater with red wine, the same for both red and white wines, or greater with white wine. Where an effect is greater with red wine, it is easy to assume that the antioxidant activity is due to the presence of phenolic compounds in the red wine. However, there are still very few studies in which the concentration of specific phenolic compounds has been measured in circulation following ingestion of wine or of the phenolic compounds themselves by human subjects. One problem with studies comparing red and white wine is that in some cases white wine may also contain significant amounts of phenolic or other as yet unrecognised compounds that have antioxidant properties. Many studies use wines without providing measurements of their phenolic content.

7.1.3 Possible adverse effects of moderate alcohol intake and ‘safe’ drinking levels.

While there is a clear association between moderate alcohol intake and improved cardiovascular health, information on possible adverse effects of moderate alcohol intake must also be considered in weighing up the overall benefits to be gained from ‘moderate drinking’. For some groups of people, particularly pregnant mothers and abstinent alcoholics, the only completely safe recommendation is that no alcohol should be consumed. For women, the statistical evidence of a positive correlation between alcohol intake and the incidence of breast cancer means that recommended safe drinking levels should be very conservative, probably 1 drink per day or less. It is possible that some of the same phenolic compounds in wine that provide cardioprotection for men may, through their steroid-like activity, provide an increased risk of breast cancer for women. Alternatively, it may be alcohol itself that promotes breast cancer, or it may be that the statistical association does not have an underlying causal link that is directly related to drinking patterns. Further research is required on the association of alcohol intake and breast cancer, and on the mechanisms that might explain the association.

For men, ‘safe’ drinking levels can probably best be judged from intakes that are observed to give the low point on graphs of the relationship between alcohol intake and incidence of heart disease, which indicate about 1-2 drinks per day. It should be noted that the minimum point of curves for all-cause mortality vs alcohol intake may vary with the population group being studied. For example a minimum point at 1 drink per day has been reported in an American population (Criqui, 1998) while a minimum point was observed at the much higher intake of 2-3 drinks per day in a French population (Renaud and Gueguen, 1998). The minimum points for different causes of mortality (such as cancer, coronary heart disease, and accidents and

violence) plotted against alcohol intake also vary from between less than 1 to up to 3 drinks per day in these two studies. These figures illustrate the difficulties that are encountered in trying to formulate safe drinking guidelines, and the problems that would be inherent in trying to estimate the possible savings in health spending that could be generated by increasing the level of 'moderate' alcohol intake in a population. To quote from a recent review: "Thus, whilst evidence on the protective effect of drinking on CHD is conclusive at the level of association, and most likely conclusive at the level of causation, it is not on present analysis significant at the policy level. Any attempt to put about a message which encourages drinking on the basis of hoped-for gains in CHD prevention, would be likely to result in more harm to the population than benefit" (Anderson, 1998). As indicated in the recommendations that follow, this view is probably rather too pessimistic. Careful promotion of the potential health benefits of wine through the correct channels may be beneficial, and a total lack of information is denying potential benefits to those who would use wine wisely. Recommended upper limits for safe drinking suggested by the Alcohol Advisory Council of New Zealand, based on medical and scientific research from New Zealand and overseas, are 21 standard drinks per week for men, and 14 standard drinks per week for women (for more information and the definition of standard drinks see www.alcohol.org.nz/effects/upperlimits). These intakes average to three (men) or two (women) drinks per day, very similar to the minimum point on curves of mortality vs alcohol intake in a number of studies, as discussed above.

7.2 Recommendations

7.2.1 Recommendations on assessing the possible level of health benefits from wine consumption in New Zealand.

It is apparent from information already available in overseas studies that the estimation of potential health benefits due to alcohol and/or wine consumption is very complex. The shape of dose-response curves for alcohol consumption vs mortality varies for different populations, age groups, medical conditions (e.g. cancer or heart disease) and consumption levels (Rhem and Bondy, 1998). Dose response curves probably also vary with the type of alcohol being consumed and the overall diet of the population being studied. It would therefore probably be fruitless to try to apply figures from overseas studies to estimate potential health benefits in New Zealand.

One alternative approach, if this has not already been done, would be to obtain and collate as much data as possible for New Zealand for the past 30-40 years on alcohol consumption per capita for different types of beverage, all-cause mortality by age, mortality for specific relevant conditions (heart disease, cancer, accidents), diet, exercise and smoking. It would then be possible to look for a correlation that might support a protective effect of wine against coronary heart disease. From such information, it might then be possible to make an approximate estimate of savings to the health system as a result of wine consumption. New Zealand should provide an interesting situation for such an analysis, since wine consumption has increased markedly in New Zealand in the last 30 years. However, such retrospective analysis

would need to take into account changes in diet in New Zealand over the same time period. It might be difficult to separate the possible beneficial effects of wine intake from possible beneficial effects of decreases in saturated fat intake and increases in fresh fruit and vegetable intake that have probably occurred over this time.

Other approaches would be to use data from long-term studies that are already underway, or to instigate new studies, where the consumption of different types of alcoholic beverage could be correlated with health status for individuals. To provide significant results for any particular beverage, numbers of subjects involved in a study need to be high and all possible confounding variables (e.g. age, smoking, diet, cholesterol levels) need to be considered.

7.2.2 Recommendations for approaches to promoting the health benefits of wine.

As indicated in this report, the evidence to support health benefits of alcohol, and wine in particular, is now considerable. However, health professionals are still reluctant to endorse approaches that would promote alcohol or wine intake on a general basis (Gaziano and Hennekens, 1995; Anderson, 1998; Wannamethee and Shaper, 1998; Gordis, 1999). In 1995, a British Government report recommended the upward revision of 'sensible drinking' limits, based on published epidemiological data, but this recommendation has been criticised on the basis of doubts about the methodology and interpretation of some of the epidemiological studies (White, 1996).

While general advertising to increase alcohol or wine consumption based on potential health benefits may not be wise at this stage, it would be reasonable to produce factual information about the results of epidemiological and biomedical studies that could be made available to health professionals and other interested people. Such information would need to be balanced in terms of warning about the adverse effects of moderate alcohol intake for some people. However, it could provide considerable incentive for those in the age group of 40 and above, where the health benefits appear to be maximal, to increase their wine consumption. People in this age group are likely to be more interested in purchasing expensive wines, thus providing opportunities for a good profit margin on sales, and to accept advice about drinking moderately with meals. The sentiment that has been expressed that **any** attempt to encourage drinking on the basis of gains in coronary heart disease prevention would be likely to produce more harm than benefit (see quote from Anderson, 1998, at the end of Section 7.1.3 above) would seem to be too pessimistic. A total absence of promotion about the possible health benefits of wine will lead to a lack of information for those who would use it sensibly.

7.2.3 Recommendations for research.

7.2.3.1 Epidemiological research.

There is a need for both retrospective analysis of alcohol consumption and health data that is already available and for planning of new epidemiological studies in New Zealand. A well-constructed epidemiological study to investigate the effects of alcohol consumption on health would be a major undertaking. It would probably be best achieved within a wider dietary survey undertaken with the combined support of public health agencies, wine and other alcohol-related industries, and perhaps other groups such as the fruit and dairy industries.

7.2.3.2 Biomedical research.

Although there is considerable evidence that alcohol in general, and wine in particular, provide health benefits, the molecular mechanisms by which this is achieved are still far from clear. There are many possibilities for research. Those of most relevance to the wine industry would be investigation of the absorption, metabolism and modes of biological action of phenolic compounds, such as quercetin and resveratrol, which are found in wine. A related field of investigation is whether the absorption of these compounds is enhanced by alcohol; if this is the case, inclusion of alcohol in the diet may enhance the uptake of these potentially-beneficial compounds from sources such as fruit and vegetables.

7.2.3.3 Wine research.

If some of the health benefits of wine are derived from phenolic compounds found in wine, it is important to know the concentrations of these compounds in different types of wine. Wines with high concentrations of these compounds are likely to become very popular if the health benefits can be confirmed. A note of caution is that there may prove to be optimal levels of intake for some of these compounds. For example, a compound such as resveratrol may have anti-cancer activity at low concentrations but be toxic to normal cells at higher concentrations. Further biomedical research on the mechanisms of action of phenolic compounds will need to precede any promotion of high-phenolic content wines.

It should be noted that wine research to determine content of key phenolic compounds and biomedical research to determine the effects of these compounds should ideally be linked. One current problem is that some research studies on health benefits of wine consumption have failed to determine the concentrations of key compounds in the wine being used. This means that the results cannot be conclusive in attributing any health benefits to the content of any particular compound in the wine.

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