Current perspectives

The relationship between wine consumption and cardiovascular risk: from epidemiological evidence to biological plausibility

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Epidemiological studies have suggested that cardiovascular disease morbidity and mortality can be decreased by moderate alcohol consumption. Several recent studies have also separately assessed the relative risk associated with different types of alcoholic beverages. The evidence obtained strongly suggests, although does not prove, that there is a major beneficial effect from drinking a low-moderate amount of wine. A meta-analysis has recently been performed on 19 of these studies, selected on the basis of the availability of specific information on the relative risk associated with wine consumption. The results indicate a negative association of moderate (up to 300 ml per day) wine consumption with the risk of cardiovascular events.

Although some cardioprotective effects of alcoholic beverages are probably due to ethanol-induced elevation of HDL cholesterol, lowering of fibrinogen plasma levels and, perhaps, of platelet aggregation, it is reasonable to speculate that the cardiovascular protective effects of wine, observed in French and in other populations, may be attributed in part to the antioxidant, vasorelaxant, and antithrombotic properties of its polyphenolic components.

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Introduction

An inverse association between light to moderate (from few drinks per week to 1-3 drinks per day) alcohol consumption and cardiovascular disease morbidity and mortality, has been consistently demonstrated in many epidemiological studies, independent of age, sex, or smoking habits[1-4]. The beneficial effect of alcohol also applies to total mortality[5-10]. The dose-response relationship between alcohol intake and rate of cardiovascular events and of all-cause mortality has been depicted as a U- or J-shaped curve, in relation to different populations or subgroups, reference groups (abstainers vs occasional drinkers), range of the amount of alcohol consumed, and clinical endpoint[7,11,12].

The U- or J-shaped relationship between alcohol consumption and clinical events indicates that non-drinkers or occasional drinkers have higher incidence and mortality rates than light or moderate drinkers, but similar or lower rates than heavy drinkers (an example is given in figure 1)[13].

After Renaud and de Lorgeril[14] suggested wine intake as one possible explanation for the lower than expected coronary heart disease (CHD) mortality rates in France, despite the prevalence of risk factors (the "French paradox"), many ecological and observational studies have dealt with the question of whether different alcoholic beverages are equivalent in their ability to protect against cardiovascular disease, or whether a specific beverage, wine in particular, might offer greater protection[2-4], most likely related to its non-alcoholic components.

In the first part of this review we shall discuss recent major epidemiological studies reporting specific data on wine consumption in comparison to other alcoholic beverages. In the second part, we shall review the experimental results obtained with wine or wine-derived products, to highlight the possible mechanisms underlying the specific effects of wine. Other factors, different from or complementary to non-alcoholic components that may contribute to the inverse association between moderate wine consumption and cardiovascular risk will also be discussed.

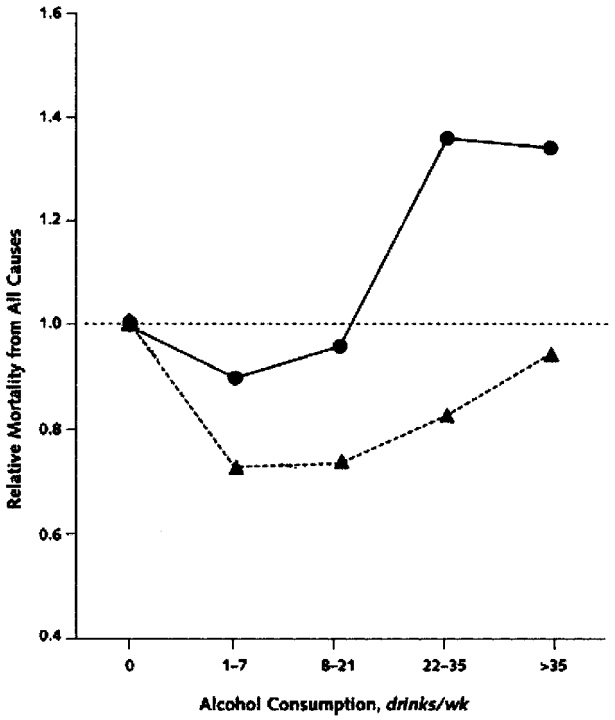

Figure 1. *Examples of U- and J-shaped curves showing the relative risk for all-cause death in relation to total alcohol intake as non-wine* (circles) *and as wine* (triangles). *Relative risk is set at 1.00 among non-drinkers (< 1 drink/week). From Gronbaek et al.[13], modified.*

Epidemiological evidence

The different ecological, case-control, and prospective studies, comparing the risk of cardiovascular disease associated with the consumption of different alcoholic beverages, do not draw homogeneous conclusions, and healthier lifestyle factors have been invoked to explain the lower rates found in wine drinkers in several studies[15-19]: among northern Californians for instance, wine drinkers usually smoke less, exercise more and suffer less from obesity[15], while in Denmark, wine drinking has been strongly associated with a healthy diet, defined as major intake of fruit, vegetables, fish, salads, and olive oil[19].

Wine consumption and cardiovascular disease. Keeping in mind these reservations, we shall mention four large prospective studies showing that the cardiovascular and all-cause mortality risk is reduced to a lower extent for consumers of wine than for consumers of beer or spirits:

• the Copenhagen City Heart Study[5] showed that the risk of dying from cardiovascular disease in a population of 13 285 subjects, followed for 10-12 years, steadily decreased with a greater intake of wine, reaching a 49% reduction for those who consumed 3-5 drinks/day (relative risk-RR 0.44; 95% confidence interval-CI 0.24-0.80) as compared to subjects who never drank wine. Intake of the same amount of beer was associated with a 28% risk reduction (RR 0.72; 95% CI 0.61-0.88), that of spirits being associated with an increased risk even though non significant (RR 1.35; 95% CI 1.00-1.83). Only wine drinking was significantly associated with a 49% decreased risk of all-cause death;

• more recently, the same group has reported data from a larger population (24 523 subjects)[13]. Compared with non-drinkers, light alcohol drinkers (from 1 to 7 drinks/week) who avoided wine had a slightly lower RR for all-cause death (RR 0.90; 95% CI 0.82-0.99), but the risk was even lower for light drinkers who drank wine (RR 0.76; 95% CI 0.55-0.77). Heavy drinkers (more than 35 drinks/week) who avoided wine were at higher risk of death than were heavy drinkers who included wine in their alcohol intake. Wine drinkers had a significantly lower mortality for both CHD and cancer than did non-wine drinkers. In particular, the RR of death from CHD was 0.76 (95% CI 0.63-0.92) for light drinkers who avoided wine and 0.58 (95% CI 0.47-0.72) for those who drank wine. The reduction in risk of CHD and of all-cause death was independent of the level of wine intake;

• Renaud et al.[20] reported that drinking up to 54 g alcohol/day (10 to 14 g being the equivalent of one standard drink in different studies) such as wine or beer was associated with a lower RR of cardiovascular disease in a population of 36 250 middle-aged healthy men in the area of Nancy, in France, followed for 12 years or more. However, the reduction in risk was higher and only significant for wine (39%, $p < 0.01$ vs 26%, $p > 0.12$). Furthermore, only wine drinking was significantly associated with a 35% ($p < 0.01$) lower RR of all-cause mortality.

In the studies mentioned above, the effect of wine was independent of common risk factors such as age, sex, smoking, and body mass index, as well as of lifestyle-related factors such as education and income;

• adjustments for common as well as other recognized risk factors such as prevalence of diabetes and cholesterol levels, or health markers such as regular medical attendance, only attenuated, but did not eliminate, the greater protection against cardiovascular mortality by wine consumption (RR 0.71; 95% CI 0.52-0.98) as compared to beer (the reference group) or spirits (RR 1.02; 95% CI 0.83-1.25) observed in the British Regional Heart Study[21] that followed up 6680 men for an average of 16.8 years. Again, only wine consumption was associated with a 20% significant decrease in risk of all-cause death.

Wine consumption and cerebrovascular disease. While the benefit of moderate alcohol consumption on overall cardiovascular disease has been well established, the specific relationship with cerebrovascular disease, mainly stroke, is more controversial. Overall evidence suggests a reduced risk of ischemic stroke, but little or no protection against hemorrhagic stroke. All cerebrovascular events substantially increase in heavy alcohol consumers[22].

Three recent studies have evaluated whether the effect on the risk of stroke depends on the type of alcohol consumed:

• the Copenhagen City Heart Study[23] prospectively examined a cohort of 13 329 subjects for 16 years and found that weekly wine consumption was significantly associated with a reduced risk of stroke (ischemic and hemorrhagic), while the association for daily wine consumption was statistically borderline (p = 0.06). Intake of either beer or spirits was not associated with reduced risk of stroke. Subjects who drank ≥ 42 units per week had a 1.5-fold significant increase in risk;
• the Northern Manhattan Stroke Study[24] found that, among 667 cases and 1139 controls, drinking up to 2 drinks per day of any type of alcoholic beverage was significantly associated with a lower first ischemic stroke risk in an elderly multiethnic population. However, those who were predominantly wine drinkers had the lowest risk (RR 0.40; 95% CI 0.23-0.70) and consumed, on average, less alcohol than those who drank beer or spirits or were combination drinkers. Intake of 7 of more drinks of alcohol per day was significantly associated with an almost 3-fold increased risk;
• finally, from an Australian case-control study among 331 matched pairs[25], wine, beer, and spirit consumption lowered intracerebral hemorrhage risk, but only wine did so significantly; in a separate analysis by sex, both wine and spirits were associated with a lower intracerebral hemorrhage risk in men, while for women wine only was protective, although not significantly. Drinking > 6 units per day of alcohol was significantly associated with a 3.4-fold increased risk.

The effects described in these studies were independent of history of diabetes, cardiac disease, hypertension, and of several common confounding factors.

Wine consumption and vascular risk: a meta-analysis. At present, a rigorous overall estimation of the relative vascular risk associated with any specific alcoholic beverage is lacking.

We have recently performed an overview of the literature[26], with the aim at evaluating the relationship between wine consumption and both cardiovascular and cerebrovascular risk, and at giving a quantitative estimate of this relationship. More than 50 studies were screened, from which only 19 could be selected that provided a quantitative estimation of the RR associated with wine consumption.

The main resultant measure was wine consumption vs the RR of morbidity and mortality from cardiovascular disease. The results obtained support the protective role of moderate wine consumption against the risk of vascular events. In fact, pooling the data from 13 prospective and case-control studies reporting only the RR of moderate (1-2 drinks per day, 150-300 ml per day) vs no wine consumption, the overall effect was a significant risk reduction of 34% (RR 0.66; 95% CI 0.57-0.75).

Furthermore, meta-analysis of six additional studies reporting trend analysis for increasing categories of consumption, allowed us to define a dose-response relation between wine intake and vascular risk, which resulted in a J-shaped curve, with a statistically significant risk reduction of about 300 ml per day (RR 0.86; 95% CI 0.74-0.99), non-drinkers being the reference group.

The choice of non-drinkers as the reference group has often been questioned since this group may include ex-drinkers who have given up because of health problems. Although attention has been drawn in the past years to this possibility, non-drinkers remain, with few exceptions, the baseline comparison group, although various statistical adjustments have been made whenever possible to eliminate the effects of differences in the prevalence of risk factors and preexisting disease in the various alcohol intake categories. In meta-analyzing the results of the various prospective and case-control studies we had to use the RR given by each single study with respect to its own reference group. However, in order to minimize any possible inaccurate evaluation due to the choice of the reference group, we performed a subanalysis, restricted to those studies which excluded ex-drinkers or had others apart from non-drinkers as reference group. Pooling the results from five such studies[21,27-30], the estimated overall risk associated with moderate wine consumption appeared to be significantly reduced by 39% (RR 0.61; 95% CI 0.57-0.75).

Biological plausibility

Studies showing that alcohol favorably influences lipid profile as well as hemostatic/thrombotic factors give experimental support to the inverse association between moderate alcohol intake and cardiovascular disease. A recent meta-analysis found strong and consistent evidence linking moderate alcohol intake (30 g ethanol/day) with higher concentrations of HDL cholesterol and apolipoprotein A-I and lower fibrinogen levels[31].

The mechanisms through which wine might exert antiatherogenic and antithrombotic effects appear to be distinct from those of alcohol and, at least in part, attributable to biological properties peculiar to its polyphenolic constituents, primarily because of their recognized antioxidant and radical scavenging properties[32]. Reactive oxygen species produced at site of vascular damage or inflammation can indeed *per se* exacerbate atherogenic and thrombotic processes through the induction of LDL oxidation, stimulation of cell growth and proliferation, and activation of both vascular and blood cells[33]. Different polyphenols have also been shown to modulate the function of cellular components involved in the process of thrombosis in several systems.

Modulation of vascular response, possibly through mechanisms linked to the nitric oxide pathway, may contribute to the maintenance of blood vessel function[34]. Interference with the arachidonic acid metabolism in both platelets and leukocytes has been reported, which re-

sulted in inhibition of platelet aggregation and reduced synthesis of prothrombotic and proinflammatory mediators *in vitro* and in experimental models. Furthermore, some polyphenols can modulate specific pathways regulating the expression and activation of genes induced by a variety of agonists; this results in down-regulation of the expression of adhesive molecules and tissue factor activity in both endothelial cells and leukocytes, and ultimately in functional modulation of cell-cell interactions and procoagulant activities[18,35].

Experimental models. In a model of mechanical stenosis and intimal damage of coronary arteries in dogs, platelet-mediated thrombus formation and consequent cyclic flow reduction in coronary blood flow were eliminated by the intravenous administration of red wine. At the same doses the effect of white wine was not significant. *Ex-vivo* platelet aggregation in response to collagen in whole blood was also decreased at the time of cyclic flow reduction elimination[36].

Bleeding time, *ex-vivo* platelet adhesion to collagen, and experimental venous thrombosis have recently been studied[37] in rats after 10 days of supplementing drinking water with either red wine or white wine or alcohol. Red wine supplementation induced significant prolongation of template bleeding time, decrease in platelet adhesion to fibrillar collagen, and reduction in thrombus weight. Alcohol-free red wine was almost as effective as the original beverage, while white wine was almost ineffective. The nitric oxide synthase inhibitor L-NAME prevented red wine from having any effect, suggesting a role for alcohol-independent factors interfering with the nitric oxide pathway. The difference in polyphenolic content between red wine and white wine and the increased radical trapping antioxidant activity found in the plasma of rats given red wine strongly favor the role of polyphenols rather than of alcohol in the observed effects.

In apolipoprotein E-deficient mice supplemented for 2 weeks with red wine (1.1% alcohol) the susceptibility to plasma LDL underwent oxidation and aggregation, and the subsequent development of atherosclerotic lesions had reduced compared to mice receiving 1.1% alcoholized water as placebo. The effect of red wine was linked to the stimulation of the activity of paraoxonase, an HDL-associated esterase, which has been postulated to reduce oxidative damage, by preventing lipid oxidation[38].

Antiatherosclerotic effects of red wine, and to a lesser extent of white wine, associated with inhibition of monocyte chemotactic protein MCP-1, have recently been shown in a model of neointimal hyperplasia after balloon injury in cholesterol-fed rabbits[39].

At the William Harvey Research Institute in London, Carrier and co-workers, our partners in the ongoing European FAIR project "Wine and Cardiovascular Disease", are studying the response to acetylcholine-induced vasodilation as a marker of endothelial dysfunction consequent to oxidative stress. In cholesterol-fed rabbits, acetylcholine-induced vasodilation was markedly impaired but, after 8 weeks administration of red wine extracts together with a cholesterol-rich diet, a significant improvement in endothelial function was apparent[40].

Studies in humans. The potential *in vivo* properties of wine and its derived products have been evaluated in terms of their ability to enhance plasma antioxidant status, considered as an index of the capacity of the organism of counteracting oxidative damage in general, as well as of reducing specific oxidative processes, in particular those related to atherogenic LDL modifications.

Several reports have documented in volunteers an increase in plasma antioxidant capacity or protection of LDL from oxidation, paralleled by an increased polyphenol concentration in plasma, after prolonged, but also after short-term consumption of red wine or alcohol-free red wine.

Despite the strong antiplatelet activity demonstrated for several polyphenols *in vitro*, the results of the studies evaluating the capacity of wine polyphenols of reducing platelet aggregation to an extent above that of alcohol are controversial[18]. This may be due to the effects of ethanol being already maximal, to the design of the study (short-term consumption, insufficient for seeing additional effects), or as will be discussed later on, to the characteristics of the population studied.

As mentioned before, endothelial dysfunction consequent to oxidative stress is observed in different pathological conditions such as hypercholesterolemia and diabetes, and is considered to play a major role in the progression of atherosclerosis and subsequent clinical manifestations, such as myocardial ischemia.

The modulatory effects of short-term ingestion of a relatively high dose of red wine on endothelial function have been shown by Shimada et al.[41], who measured changes in coronary blood flow velocity consequent to adenosine-induced vasodilation in 10 healthy volunteers before and after ingestion of red wine, white wine or vodka. Coronary flow velocity significantly increased only after red wine intake.

Interestingly, grape-derived products too may ameliorate endothelial function: in patients with coronary artery disease, impaired flow-mediated vasodilation, despite the use of lipid-lowering and antioxidant vitamin therapies, was indeed significantly increased after 2 week ingestion of purple grape juice[42].

Very recently, the ability of wine polyphenols to modulate the activation of the nuclear transcription factor-κB (NFκB), an ubiquitous factor involved in various transcriptionally controlled processes, such as inflammation and cell growth, *in vitro*, has also been demonstrated *in vivo*. Indeed, intake of red wine, but not of vodka, for 2 weeks, prevented the activation of NFκB induced by post-prandial lipemia in peripheral blood monocytes from healthy volunteers[43].

To get a more precise overview of the various effects of wine and wine-derived products related to the early stages of atherosclerosis and thrombosis, a controlled, prospective study, within the framework of the already mentioned European FAIR project, is being carried out by Dr. Urbano Marquez and co-workers at the Hospital Clinic in Barcelona[44].

The first phase of this study includes 40 healthy subjects, randomly divided into two groups, who, after a 15-day washout, receive 30 g of ethanol a day for 30 days, as red wine or as a low polyphenolic alcoholic drink, in a cross-over design. After washout, volunteers switch to an alcoholic drink or red wine as second intervention period. The preliminary results confirm an increased LDL resistance to oxidation after wine consumption: lag time of LDL oxidation is enhanced and LDL oxidation rate is significantly shorter after wine, as compared to basal assessment.

Analysis of adhesion molecules on the surface of mononuclear cells, obtained from peripheral blood of volunteers after the red wine period, showed a significant decrease in the expression of the β_2-integrins LFA-1 and Mac-1, which mediate monocyte attachment to vascular and circulating cells.

Increased expression of adhesion molecules on the surface of vascular and circulating cells plays an important role in the development of atherosclerotic and inflammatory processes and is indeed found in several conditions, like diabetes, dyslipidemia, and unstable angina. These molecules are therefore becoming a novel target for preventive and therapeutic strategies.

Wine and reduced cardiovascular risk: difficulty in establishing a causal relationship

From the experimental observations reviewed here, even though sometimes controversial, it seems plausible to hypothesize that absorption of polyphenols from wine or from wine-derived products may exert a number of effects on cellular and plasma blood components and on the vascular function that may result in a more favorable lipid and hemostatic profile, thus reducing the risk of development and progression of cardiovascular disease.

The antiatherogenic and antithrombotic effect may ultimately depend on several variables, including the dietary habits of the population studied. Thus, in men with established CHD, the platelet response to wine differed according to whether the diet was of Western or of Mediterranean type[45]. Wine ethanol was inversely correlated with platelet aggregation in the group following a Western type diet, but not a Mediterranean diet, suggesting that specific dietary patterns may influence the effect of wine ethanol on platelets. This observation is consistent with previous results from the Caerphilly study which showed that among men following a Western type diet, the strongest negative association between alcohol intake and platelet aggregation was in those with higher intake of saturated fats[46]. A major implication of these observations, if confirmed, would be that wine components might have little, if any, effect on platelet function already reduced by the regular consumption of "Mediterranean" or other healthy food.

The effects attributed to wine consumption might therefore be the result of "confounders", as was suggested by a report from a large study: in a Danish population[19] wine drinking was strongly associated with a healthy diet, defined as major intake of fruit, vegetables, fish, salads, and olive oil, a finding relevant for the interpretation of the relation between type of alcohol and total and CHD mortality in Denmark[5,13] where wine is a selective type of beverage and a potential indicator of a healthy diet.

In Italy, however, where wine is by far the most common type of alcoholic beverage, its consumption is not a correlate for a healthy diet[47] but still results in being moderately protective against non-fatal myocardial infarction, independent of recognized risk factors (serum cholesterol, diabetes, hypertension, family history of acute myocardial infarction), as shown by the case-control study designed within the framework of the GISSI-2 trial[48].

There might be however unrecognized lifestyle factors other than those already controlled for, that distinguish wine drinkers from non-wine drinkers[13].

Different drinking patterns should also be taken into account when studying the association of the different alcoholic beverages with disease and mortality risk. A recent large case-control study from Australia[49] reinforces the concept that, at the same average comsumption, subjects having a regular small intake of alcohol have a lower risk of cardiovascular death, while those who drink in excess may have no such beneficial effects. The regular, meal-associated drinking pattern of wine preferrers, as opposed to "binge" drinking, which is frequently associated with beer and spirit drinkers, may therefore help explaining the differences in the results from the various studies. In Denmark, however, wine drinkers were more likely to have "binges" than beer drinkers, suggesting that the lower cardiovascular risk among wine drinkers in this population may be negatively confounded by drinking pattern[50].

Concluding remarks

Cardiovascular disease has long since been the leading cause of death in industrialized countries, and is increasing in the developing world. The combined effect of a diet that is rich in fruit, vegetables, complex carbohydrates, and monounsaturated fat, and poor in animal fat and simple sugars, is certainly of extreme importance in lowering the rates of cardiovascular disease and possibly other chronic diseases. However, un-

til recently alcohol consumption was frequently overlooked as an important part of the diet.

Many studies have suggested that death rates for all causes and cardiovascular disease are lower for people who drink a low to moderate amount of alcohol than for people who do not drink at all and those who drink heavily.

In recent years, several studies have also been carried out to determine whether death rates differ among people who drink mostly wine or other alcoholic beverages.

Evidence obtained from the studies reviewed here suggests, but does not prove, that there is a major beneficial effect from drinking wine as compared with other types of alcoholic drinks. This suggestion is in keeping with experimental data showing that wine contains several substances that may add to the beneficial effect of a light to moderate amount of alcohol intake.

Definite proof could only be obtained by large long-term intervention trials comparing the effect of drinking wine vs non-drinking alcohol, drinking other alcoholic beverages, or assuming wine-based supplements. Such trials however appear to be unfeasible for several reasons, including the number of people to be enrolled to observe statistically meaningful clinical endpoints, ethical concern, and the huge economical investment required.

Although a causal relationship between wine consumption and cardiovascular morbidity and mortality rate cannot be unequivocally established, it is reasonable to conceive that a moderate and regular wine consumption, together with other healthy lifestyle-related factors, such as a Mediterranean-like diet (that is rich in fruit, vegetables, complex carbohydrates, and monounsaturated fat, and poor in animal fat and simple sugars) may contribute to the lower rates of cardiovascular disease observed among populations living in Southern Europe[51]. The results obtained in other countries, such as Denmark, Britain and United States, may allow to extend the beneficial effect of moderate wine consumption to different populations. In this respect, a very recent study[52] indicates that also in the United States, healthy women who adhere to lifestyle guidelines including exercise, abstinence from smoking, a diet rich in cereal fiber, marine n-3 fatty acids, folate, and at least half an alcoholic beverage per day, carry a very low risk of CHD.

Other studies indicate that light-moderate alcohol consumption may lower the risk of cardiovascular and all-cause events to the same or even to a greater extent in high risk subgroups, such as hypertensives[53], diabetics[54-56], and in patients with history of cardiovascular disease[8,57,58], although the latter finding has not been confirmed in a recent study[59]. Whether, and to what extent, the favorable results obtained in high risk populations also apply to wine preferrers has not yet been established. However it is worth mentioning that light-moderate wine consumption by diabetic patients may add to the already ethanol-related positive effect on lipid profile, thrombosis tendency and insulin levels, by reducing meal-generated oxidative stress[60].

What should the cardiologists' advice to their patients be with respect to wine consumption? First of all, patients and their relatives should be informed of what lifestyle changes might be beneficial to them. As there are presently not enough elements to encourage lifetime teetotallers to start drinking wine, it may be better to insist on the control of risk factors. Abstainers should however, in the absence of contraindications, be informed that low-moderate wine consumption, in the context of healthy eating and lifestyle, may contribute to better health, and that continued abstinence might constitute a hazard. People who are already light-moderate wine consumers should not be discouraged from continuing, even if they are at high cardiovascular risk. The hazards of excess drinking should always be highlighted, and heavy drinkers strongly urged to cut their consumption to a moderate level[61].

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