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Alcohol beverages and biochemical changes in blood

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Abstract

Coronary atherosclerosis is the major cause of death in western civilization: one of every three in men as well as in women. It was shown that diets supplemented with moderate quantities of alcoholic beverages could lead to positive biochemical changes in blood of the consumers, which are regarded widely as indicators of improved prevention of atherosclerosis. This review summarizes the recent epidemiological, experimental and clinical investigations concerning mainly the plasma biochemical changes in lipid levels, anticoagulant and antioxidant activities.

Introduction

Atherosclerosis in general and coronary atherosclerosis in particular are still the most dangerous diseases in the industrial countries—the principal cause of death in western civilization.[1] Atherosclerosis is a multi-factorial process based on the action of various risk factors.[2] According to Ross,[2] the form and content of the advanced lesions of atherosclerosis demonstrate the results of the following fundamental biological processes: (a) accumulation of intimal smooth muscle cells, together with variable numbers of accumulated macrophages and T-lymphocytes; and (b) formation by the proliferated smooth muscle cells of large amounts of connective tissue matrix, principally in the form of cholesteryl esters and free cholesterol within the cells as well as in the surrounding connective tissues. In spite of the fact that some authors have found signs of inflammatory and immunological nature of atherosclerosis,[3–7] the oxidative theory is used widely as a basis for prevention and treatment of this disease.[8–11] The oxidative theory states that only cholesterol-rich oxidized LDL-C particles are able to penetrate arterial walls causing their occlusions.[8–11] Such pathological damage can be found practically in every artery.[12,13] The most important damage is in the coronary arteries. Coronary artery disease (CAD) is a consequence of atherosclerotic process in these arteries. Occlusions of coronary arteries lead to myocardial infarctions, which in many cases can be fatal.[14] Therefore, the international scientific community is searching for comprehensive schemes to fight this disease. Intensive multiple risk factor reduction has a beneficial effect on coronary atherosclerosis in men and women: the disease regression is twice as frequent in the risk reduction group as in the control group.[15] An integral part of these preventive measures are

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proper diets. The general principle of such diets is plenty of vegetables, fruit and cereal fibre.[16,17] Alcoholic beverages have been food throughout the ages.[18,19] In spite of the fact that even moderate consumption of alcoholic beverages leads to some structural changes in plasma circulating proteins,[20,21] these beverages are an integral part of diets in most western countries and consist about 4–6% of the average energy intake.[22] One of the reasons for widespread alcohol consumption is the claims of many investigators that alcoholic beverages have a cardioprotective effect.[23–28] These authors have observed that consumption of alcoholic beverages influence positively the main risk factors of atherosclerosis. This review is based on recent epidemiological, experimental and clinical investigations concerning the influence of moderate consumption alcohol beverages on plasma lipid levels, plasma anticoagulant and plasma antioxidant activities.

Moderate alcohol consumption and plasma lipids

Since 1913 it has been an established fact that diet containing cholesterol creates atherosclerotic changes in the arteries of laboratory animals.[29] All authors currently agree that cholesterol is also the ''building material'' for atherosclerotic plaques in humans.[30–32] Elevated levels of total cholesterol, low-density cholesterol (LDL-C), triglycerides, apolipoproteins B and C-III and reduced levels of high-density cholesterol (HDL-C) and apolipoprotein A-I are major risk factors for this disease.[30–32]

Moderate consumption of alcoholic beverages leads first to an increase in HDL-cholesterol and has little effect on total and LDL-cholesterol.[24–26] However, in experiments on laboratory animals it was demonstrated that diets supplemented with moderate quantities of alcoholic beverages led to decreased levels of plasma total cholesterol, LDL-C, triglycerides, total phospholipids and total cholesterol in liver and an increased level of HDL-cholesterol in animals fed added cholesterol.[33–35] The same tendency was observed in clinical investigations of patients suffering from hypercholesterolemia: moderate consumption of alcoholic beverages led to a decrease in the level of total cholesterol, LDL-cholesterol, triglycerides and an increase of HDL-cholesterol.[28,36]

Contrary to the above, increase of the levels of HDL-cholesterol after moderate alcoholic consumption is not disputable. An increase in the level of HDL-cholesterol was found in experiments on laboratory animals[33–35] and in investigations of patients with and without hypercholesterolaemia.[20,21,24,25,27,28,35–37] Recently, Sillanaukee *et al.*[24] have reported that HDL-cholesterol (the HDL_3 subfraction) was higher in those drinking 20–40 g alcohol daily. It was also found that the frequency of drinking was associated with a higher level of HDL-C.[25]

It was shown that paraoxonase, an HDL-associated enzyme, is related to atherosclerosis.[38–41] This organophosphate enzyme suppresses atherosclerosis in an unknown way.[41] Fuhrman & Aviram[39] have shown that paraoxonase is associated physically with HDL, and its activity has been related inversely to the risk of cardiovascular diseases. These authors found that paraoxonase can hydrolyze specific lipid peroxides in oxidized lipoproteins and in atherosclerotic lesions. Fuhrman & Aviram[39] have demonstrated that consumption of wine flavonoids preserves paraoxonase activity by reducing the oxidative stress in apolipoprotein E-deficient mice, thereby contributing to paraoxonase hydrolytic activity on lipid peroxides in oxidized lipoproteins and atherosclerotic lesions. Paraoxonase inhibits macrophage cholesterol biosynthesis and atherogenesis probably through its phospholipase-A(2)-like activity.[41,42] It was shown also in investigations of humans that paraoxonase activity is related inversely to the risk of cardiovascular diseases.[38] In this diet-controlled, randomized intervention study[38] the effects of daily moderate consumption of red wine, beer and spirits in comparison with mineral water on paraoxonase activity in serum was examined. For 3 weeks, eleven healthy middle-aged men each consumed three of the beverages (red wine, beer and spirits) with evening dinner. At the end of each 3-week period, blood samples were collected pre- and postprandially and after an overnight fast. Fasting paraoxonase activity was higher after intake of wine, beer and spirits than after water consumption ($p < 0.001$ in all three cases). The authors of this investigation suggest that increased serum paraoxonase may be one of the biological mechanisms underlying the reduced coronary heart disease risk in moderate alcohol consumers. Another study was performed to investigate the kinetics of alcohol-induced

increases in apo A-1, HDL cholesterol and paraoxonase activity.[43] This experiment was conducted with 30–40 g/day of beer. Serum apo A-I, HDL cholesterol and paraoxonase activity were increased significantly during 3 weeks of moderate alcohol consumption compared with no alcohol consumption. Moreover, the results suggest that there is a sequence in induction of these parameters. After an increase in apo A-1, HDL cholesterol is increased followed by an increase in paraoxonase activity. These authors[43] have concluded that the increased serum HDL cholesterol level and paraoxonase activity may be a mechanism of action not only in healthy middle-aged men but also in postmenopausal women, underlying the reduced coronary heart disease risk in moderate drinkers.

Moderate alcohol consumption and plasma antioxidant activity

As mentioned, some authors have found signs of the inflammatory and immunological nature of atherosclerosis.[3–7,44] One of them even claims that atherosclerosis is a true inflammatory disease.[6] However, after publication of the paper "Beyond cholesterol: modifications of low-density lipoprotein that increases its atherogenicity",[8] authors increasingly support the theory that atherosclerosis is an oxidative disease.[8–11,40] Recently, Perez *et al.*[45] have proved that oxidative stress is a central mechanism for the pathogenesis of alchaemic heart disease and atherogenesis.

Some evidence suggests that one of the important mechanisms predisposing to development of atherosclerosis is the oxidation of cholesterol-rich LDL-C particles.[9–11] Oxidation of LDL-C enhances its atherogenicity and facilitates penetration of lipids into the arterial wall.[11] Thus, according to some authors,[8–11] prevention of atherosclerosis is a fight against LDL-C oxidation.

In most western industrial countries intake of food products containing high quantities of cholesterol is correlated positively with morbidity and mortality from coronary artery disease (CAD).[26] Total consumption of saturated fats in France is equal to that of other developed countries, while French mortality from CAD is only one-third of the average.[26,45] This phenomenon is known as the French paradox for CAD.[26,46,47] The distinguishing feature of the French diet is regular consumption of red wine with meals. Therefore, it was supposed that regular consumption of red wine could explain why French mortality from CAD is only one-third of the average in other developed countries.[26,46,47]

The antioxidant properties of wine were confirmed *in vitro*: Frankel *et al.*[48,49] have shown that oxidation of human low-density lipopropein is inhibited by the phenolic substances of red wine. The same results were received a year later by using not only wine but also beer phenolic substances.[50]

In a very recent epidemiological study, a significant inverse relationship was found between oxidized LDL antibody titres and daily wine intake.[51] In experiments on laboratory animals and in investigation of humans, it was also found that red wine, white wine and beer increase plasma antioxidant activity.[28,33,34,52] However, the degree of this increase was different. The authors have explained the difference as follows. According to *in vitro* investigations, among the three widely used alcoholic beverages (red wine, white wine and beer) the highest content of total polyphenols is in red wine and the lowest in beer; this difference is significant ($p < 0.0025$).[53]

However, the influence of diet, supplemented with beer on lipid metabolism and antioxidant activity is higher than of diets supplemented with white wine.[33,34] It was found that these two beverages have different concentrations of some essential phenolics: the contents of procyanidins, epicatechin and ferulic acid are significantly higher in beer than in white wine ($p < 0.005$; $p < 0.05$ and $p < 0.0025$, respectively).[53] The high content of the above-mentioned phenolics is the most possible explanation of the higher biological activity of beer. This suggestion is supported by the findings of Paganga *et al.*[54] They have proved that the antioxidant activities of one glass of red wine (150 ml) is equivalent to 3.5 glasses of beer (500 ml) and equivalent to 12 glasses of white wine. Therefore, not only the level of total polyphenols play a role,[55] but also the content of some essential phenolics.[56]

Among the three widely used alcoholic beverages (red wine, white wine and beer), red wine has the highest content of total polyphenols and the highest content of some essential phenolics. Therefore, some authors claim that red wine is the best choice for consumers.[57]

Moderate alcohol consumption and plasma anticoagulant activity

Although not without exception, current evidence from epidemiological, experimental and clinical studies suggests a protective effect against the development of atherosclerosis with moderate consumption of alcoholic beverages. The exact nature of the protective effect remains to be established. However, mechanisms including alterations in haemostatic variables is being recognized increasingly as contributory.[58] Thrombosis of the coronary arteries is the main reason for fatal cases from myocardial infarctions due to coronary atherosclerosis.[14] As shown, moderate consumption of alcoholic beverages lowers the risk and the death rate from cardiovascular diseases.[59,60] Therefore, the decrease in the death rate could not be achieved without a beneficial influence on haemostatic risk factors.[61]

Many investigations have demonstrated that consumption of alcoholic beverages is correlated with haemostatic risk factors.[27,62–68] It was found that platelet aggregation is inhibited by moderate alcohol consumption.[26,63] According to Renaud & de Lorgeril,[26] this is the main reason for the low death rate from coronary atherosclerosis in France. It was also found in a clinical investigation that moderate beer consumption leads to a significant decrease in the prothrombin time.[27] Fibrinogen is one of the plasma circulating proteins. This protein is synthesized in liver and circulates in plasma at a concentration of 200–400 mg/dl. Fibrinogen plays an important role in blood clotting, fibrinolysis, cellular and matrix interactions. Fibrinogen is comprised of two sets of three polypeptide chains that are joined by disulfide bridging within the N-terminal E domain. These domains contain constitutive binding sites that participate in fibrinogen conversion to fibrin, fibrin assembly and platelet interactions.[69] Evidence links fibrinogen with coronary atherosclerosis and blood coagulation.[69] It was reported that fibrinogen levels predicted cardiovascular events independently of traditional risk factors.[70] Some authors have demonstrated that moderate drinking leads to a decrease in the plasma circulating fibrinogen concentration.[71–73] Wang *et al.*[72] have shown that daily consumption of moderate amounts of ethanol decreases circulating levels of fibrinogen by 18–20%. Also, some structural changes in plasma circulating fibrinogen after short-term moderate beer consumption were found using electrophoresis and spectroscopy.[36] In order to evaluate the status of the plasma anticoagulant activity Factor VIIag, Factor VIIc and PAI were studied. A decrease was found in all these tests after moderate beer consumption.[20,21,36] Another group of investigators have measured the levels of fibrinogen, plasma viscosity, von Willebrand factor, factor VII, plasminogen activator inhibitor antigen-1 and tissue plasminogen activator antigen in a cross-sectional analysis of 3223 enrolled in the Framingham Offspring Study.[67] They have found that light-to-moderate alcohol consumption is associated with lower levels of coagulatory activity, but higher intake is associated with impaired fibrinolytic potential.

All the above-mentioned epidemiological and clinical investigations have shown that moderate-only consumption of alcoholic beverages influences plasma anticoagulant activity favourably.

Moderate alcohol consumption and other biochemical changes

The positive influence of moderate alcohol consumption on plasma lipids, plasma antioxidant and plasma anticoagulant activity is well known. However, moderate consumption of alcoholic beverages leads to some other biochemical changes. It was found in a randomized, diet-controlled interventional study that moderate alcohol consumption reduces plasma C-reactive protein levels.[73] In another investigation it was demonstrated that red wine consumption influences positively preincubation of vascular smooth muscle cells, inhibits ligand binding and the subsequent tyrosine phosphorylation of the platelet-derived growth factor beta receptor, which plays a critical role in the pathogenesis of atherosclerosis.[74] There is evidence that consumption of alcoholic beverages influences positively synthase of nitric oxide, which plays a critical role in cardiovascular protection and is the responsible cardioprotective protein.[75] Also, Leikert *et al.*[76] have shown that red wine polyphenols increase endothelial nitric oxide synthase and subsequent endothelial nitric oxide release. Increased endothelial nitric oxide activity may antagonize the development of endothelial dysfunction and atherosclerosis, a hypothesis that supports the view that red wine may indeed have long-term protective cardiovascular properties mediated by its polyphenols.[75,76]

The authors of the next investigation indicated that proliferation of vascular smooth muscle cells is critical to atherosclerosis formation.[77] They found that treatment with red wine polyphenols has a potent inhibitory effect on the proliferation and DNA syntheses in cultured rats. Not all authors have found a positive influence of moderate alcohol consumption on the iron stores in the body. Therefore, one group of investigators reported that wine consumption reduces iron stores and thereby the risk of coronary heart disease.[78] However, other authors have found that light drinking increases ferritin and, by inference, body iron stores.[79] Moderate beer consumption leads to an increase in plasma magnesium and as a consequence to a decrease in the heart arrhythmias.[80]

Moderate alcohol consumption and the vascular wall functioning

In the well-known Rotterdam Study an inverse association between alcohol consumption and vascular wall functioning was found in non-smoking men and women.[81] Failure to maintain an intact endothelium, as a result of episodic and persistent injury and perturbation of the vascular endothelium, promotes formation of fatty streaks, which are considered initiation events of atherosclerosis. Cellular constituents contributing to endothelial injury include endothelial cells, monocytes, platelets and smooth muscle cells.[82] Evidence is emerging that resveratrol, a polyphenolic phytoaxelin, which is present in dietary sources including red wine, may protect against atherosclerosis and cardiovascular disease by enhancing the integrity of the endothelium. The inhibitory effect of resveratrol on protein kinase C activity and therefore on the associated signalling networks may, in part, underline the mechanisms by which this agent exerts its beneficial effects on endothelial and cardiovascular function.[82] It was shown that wine polyphenols induce vascular relaxation *in vitro* through the NO-c CMP pathway in rabbit blood vessels.[83] Zou *et al.*[83] claim that resveratrol of red wine may protect against atherosclerosis and cardiovascular disease by enhancing the integrity of the endothelium in experimental hypercholesterolemic rabbits. The authors found that administration of resveratrol, red wine or de-alcoholized red wine improved endothelial function, which in their opinion may be one of the mechanisms by which red wine polyphenols exert their alcohol-independent cardioprotective effects. The effect of red wine and wine polyphenol resveratrol on endothelial function was also investigated in humans.[84,85] It was demonstrated in a clinical study of healthy male volunteers that red wine counteracts endothelial dysfunction.[84] Cuevas *et al.*[84] have measured endothelial function as flow-mediated dilatation of the brachial artery, employing high-resolution ultrasound after an overnight fast. They have found that the deleterious effect on endothelial function by a high-fat diet was contracted by the protective role of wine. Other authors have also reported that wine in particular, but also beer, contains polyphenols, which act as antioxidants and could maintain the integrity of the endothelial function by reducing the formation of superoxide. Moreover, these antioxidants may protect against LDL oxidation and modulate the macrophage attack on the endothelium.[86,87] The above-mentioned authors have concluded that red wine polyphenolic compounds can preserve a normal vascular reactivity by acting at different stages of the cascade that leads to lipid oxidation, endothelium dysfunction and vasospasm.

Discussion

It was shown that in most western industrial countries intake of food products containing high quantities of cholesterol is correlated positively with morbidity and mortality from CAD.[2] Total consumption of saturated fats in France is equal to that of other developed countries, while French mortality from CAD is only one-third of the average.[26,46,47,74,77] The distinguishing feature of the French diet is regular consumption of red wine with meals. Therefore, it was supposed that regular consumption of red wine could explain the relatively low French mortality from CAD.[26,47,74,77] Most of the authors cited in this review have shown that moderate consumption of alcoholic beverages is cardioprotective because of the positive influence on plasma lipids, plasma antioxidant and plasma anticoagulant activity and vascular wall function.[18,88,89] The most comprehensive study to date summarizes the results of alcohol consumption and mortality among middle-aged and elderly American adults.[19] Among 490 000 men and women who used alcohol beverages, 46 000 died during 9 years of follow-up. The authors have compared cause-specific death rates and death from all causes across

categories of baseline alcohol consumption. They found that the rates of death from all cardiovascular diseases between the ages of 35 and 69 were 30–40% lower among men and women reporting one drink daily than among non-drinkers.

Currently, all investigators agree that diets supplemented with moderate quantities of alcoholic beverages lead to biochemical changes, which are regarded widely as indicators of improved prevention of atherosclerosis.[23–25] However, there is ongoing debate about the bioactive components of the alcoholic beverages which lead to this effect.

Some authors claim that ethanol itself is responsible for the cardioprotective effect of alcoholic beverages.[61,72,89] In their case–control study, Brenner *et al.*[61] have assessed and compared the effect of alcohol consumption from various sources on risk of coronary heart disease. Their sample included 312 patients with clinically stable, angiographically confirmed coronary heart disease and 479 healthy controls. The results of their investigation concluded that the protective effect of moderate alcohol consumption against coronary heart disease is mediated in part by beneficial effects of ethanol on lipids and haemostatic factors. Wang *et al.*[72] have shown that daily consumption of moderate amounts of ethanol decreases circulating levels of fibrinogen by 18–20%. However, many other authors have proved that wine polyphenols are responsible for the cardioprotective effect of alcoholic beverages.[48,50,90–98] Frankel *et al.*[48] have reported that phenolic substances of red wine inhibit oxidation of human low-density lipopropein. Mosinger[50] observed that polyphenols but not alcohol in beer and wine protect serum low-density lipoprotein against atherogenic modifications. In subsequent years a number of experiments on laboratory animals were performed.[33,34,91–93] Gorinstein *et al.* performed two experiments on rats: with dry matter of alcoholic beverages[33] and with alcohol-containing and alcohol-free different alcoholic beverages.[34] These authors reported that both original and lyophilized wine and beer increase antioxidant activity and improve lipid metabolism. There were no statistically significant differences in the results of the investigations performed on groups of rats fed basal diet supplemented with original wine and beer versus groups fed basal diet supplemented with lyophilized wine and beer. In a well-performed experiment on hypercholesterolaemic golden Syrian hamsters it was found that red wine phenolic compounds reduce plasma lipids and apolipoprotein B and prevent early aortic atherosclerosis.[93] Similar results were obtained in investigations on humans.[94–98] Fuhrman *et al.*[94] have demonstrated that consumption of red wine with meals reduces the susceptibility of human plasma and low density lipoprotein to lipid peroxidation. Serafini *et al.*[95] found that alcohol-free red wine enhances plasma antioxidant capacity. Carbonneau *et al.*[96] observed an improvement in plasma antioxidant status and in LDL-C level of subjects receiving a red wine phenolic mixture. Aviram & Fuhrman[98] proved that wine flavonoids protect against LDL oxidation and atherosclerosis. The above-mentioned experiments on laboratory animals and investigations of humans underline the role of phenolics in alcoholic beverages.

There is also a ''compromise'' opinion: both ethanol and phenolic substances of alcoholic beverages positively affect plasma lipid levels. In an already-mentioned experiment[93] the effects of a red wine phenolic extract and ethanol on plasma lipoproteins and early atherosclerosis were studied in hamsters. The aortic fatty streak area was reduced significantly in both groups receiving phenolic extract and ethanol (the later reduced the aortic fatty streak area by 60%). As far back as 1979, Leger *et al.*[88] reported a strong and specific negative association between CAD deaths and alcohol consumption of wines, beer and spirits. The report by Leger *et al.*[88] was supported by the results of the investigation by Goldberg *et al.*,[99] who found phenolic constituents even in distilled spirits. In 2000, van der Gaag *et al.*[100] claimed that the effects ''are largely independent of the type of alcoholic beverage''. This review cited many epidemiological, experimental and clinical investigations which proved that moderate consumption of alcoholic beverages is cardioprotective.

However, it must be underlined that some investigators strongly oppose the use of alcohol beverages for general population.[101,102] Llerena *et al.*[101] claim that it has not been demonstrated that alcohol intake, even in moderate amounts, is beneficial for the general population, in particular men under the age of 40 and women under 50,

because it raises mortality due to other causes, especially injury, cirrhosis of the liver and some types of cancer, thereby outweighing the benefits for coronary artery disease. Thus, alcohol consumption should not be recommended as a prophylaxis for the general population. The same opinions are shared by Corrao *et al.*[102] They have found that the estimated number of deaths attributed to alcohol use in Italy still exceeds by far the number of deaths prevented by alcohol use both for men and women.

In conclusion, (1) current evidence from the epidemiological, experimental and clinical studies shows that moderate consumption of alcoholic beverages leads to positive biochemical changes, which is believed widely to have a protective effect against the development of atherosclerosis. (2) Based on majority of the reviewed papers it can be concluded that the main biologically active compounds of alcoholic beverage are phenolics and ethanol. (3) Red wine has the highest content of total polyphenols and essential phenolics. Therefore, among alcoholic beverages red wine could be the best choice.

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