

Red Dry Wine Modulates Plasma Cholesterol Levels and Cardiac Enzymes in Humans

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Abstract: Several studies has pointed that red wine diet, rich in polyphenols, has several effects like vasodilation, possible antioxidant effect, decrease of cellular events of atherosclerosis, decrease of blood pressure, and reduction of cardiovascular risks. This paper proposes an investigation of the effects of a 21 days diet of 200 ml dialy of red dry wine for markers of muscle injury such as CK and CK-MB, besides cholesterol and its fractions, LDL and HDL, and triglycerides, in normal subjects. The results showed decreased levels of plasma total cholesterol and LDL, and a tendency to increased levels of HDL, what suggests red dry wine has effects on decrease cardiovascular risks. However, significant increase of cardiac CK-MB isoenzyme was observed what could point to increase the oxidation of muscle tissues. It can be concluded that, despite the CK-MB increase, red dry wine can be considered as a tool to decrease cardiovascular diseases risks.

Keywords: Red Wine, Polyphenols, Cholesterol, CK-MB enzyme, Cardiovascular risks.

Cardiovascular diseases are the main cause of death in several developed and non developed countries. Among the diseases, atherosclerosis has been pointed as the most important, as it can cause several cardiac events, like angina, heart failure, as well as stroke [1]. Early studies about atherosclerosis, described it as result of several vascular events in arterial bed leading to endothelial dysfunction, inflammatory cellular infiltration, lipid deposition, vascular wall cell proliferation, lumen obstruction and thrombotic complications at lesions site [2].

According to the oxidative theory, in the presence of macrophages and endothelial cells, which produces reactive oxygen species, LDL go through progressive oxidation. LDL oxidation seems to be a key event which results in increased endothelial permeability to lipoproteins, leading to atherosclerosis [3]. Atherosclerosis has been related with high plasma level of LDL lipoprotein and total cholesterol [4]. Plasmatic lipoproteins are spherical compounds who allow lipids blood transportation and include high density lipoprotein (HDL) and low density protein (LDL) [5].

Since several epidemiological studies have related plasmatic LDL levels and atherosclerosis, life styles change like weight loss, smoking cessation and

exercises are highly recommended. Diet changes are very emphasized as important factor in atherosclerosis prevention and may produce strong effects on the atherosclerosis manifestation [6]. Phenolic rich diet has been related with prevention of oxidative effects, thrombotic events and reduction of inflammatory biomarkers [7-10]. This assumption is known as French Paradox, because it's characteristic of Mediterranean Countries, such as France, where people consume foods such as olive oil, fish and red wine and are faced with much less cardiac problems [11]. A comparison between Mediterranean diet and a conventional diet of the American Heart Association, showed a significantly decrease of the incidence of new coronary events by approximately 70% by the Mediterranean diet, over a period of 4 years [12].

Some studies showed that moderate alcohol consumption *per se*, even beer or liquors, can reduce cardiovascular risks [13-15]. However it seems that the better results can be obtained with wine consumption. The antioxidant effects of wine phenolics associated with the beneficial effects of the moderate alcohol consumption seems to be related with cardiovascular risks reduction [4]. Thus, some evidences have shown that the red wine consumption can reduce the atherosclerotic plaque formation, even in cases of high fat intake [16-17] and may also reduce some of the steps of the process of atherosclerosis, such as reducing the smooth muscle cells proliferation of blood vessels [18]. It was also observed that red wine can cause vasodilation by inhibition of endothelin, a potent

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endogenous vasoconstrictor [19] and can also increase the synthesis and release of nitric oxide, which has vasodilatory effect [20-21].

There are few studies with red wine diet and plasma enzymes or biomarkers. It was observed protective effect of quercetin intake in experimental global cardiac ischemia [22]. Quercetin, a polyphenol flavonoid widely present in red wine, was able to decrease creatine phospho-kinase (CK) plasma levels after a experimental ischemia and this enzyme is considered as an efficient cardiac injury biomarker. However, it is not so specific to cardiac injury and can be altered by acute muscle injury, strenuous exercise, intramuscular injections, chronic diseases and drug use. CK has three isoenzymes composed by association with molecular groups denominated group B and group M. CK-MB is the predominant form in the heart (15% to 40% in the heart, 1% to 4% by skeletal muscle), and the enzyme dosage is currently selected for the diagnosis of myocardial injury [23].

As the red wine diet is associated with decrease of cardiovascular risks, we could expect that a red wine diet can alter plasma levels of cardiac enzymes as CK and CK-MB. Thus, the present study was designed to determine the plasma levels of muscle injury biomarkers CK and CK-MB, total and partial cholesterol as well as triglycerides before and after a 15 or 21 days of red dry wine diet.

METHODS

Subjects

Normal subjects of both sex, with 23.7 ± 4.5 years old, were supplemented 200 ml of dry red wine diet for 21 days and the measurements were performed comparing individuals with themselves at times 0 (before the diet – Group 0), 15 (15 days diet – Group 15) and 21 (21 days diet – Group 21). All the subjects were informed of the procedures used in the study and gave their written consent.

The red wine intake was performed during a meal. The subjects did not alter their eating habits and there was no change in their daily physical activity during the diet. The study was approved by Local Ethics Committee at University of Monte Serrat, Santos, SP, Brazil.

Procedure

The blood collection was performed in the laboratory of the Monte Serrat University, Santos-SP,

Brazil, by a Professional nurse, following all hygiene and asepsis. Ten ml of blood was collected in a dry tube, centrifuged at 3500rpm for 15 minutes and the samples were analysed by biochemical tests. These analyses were performed before the wine intake (Group0), after fifteen (Group15), and twenty one (Group 21) days of intake. Total cholesterol, LDL and HDL and tryglicerides were measured in Group 0 and 15 and CK and CK-MB were measured in Group 0, Group 15 and Group 21.

Clinical Tests

Clinical Tests are Carried Out Using Biochemical Kits

CK and CK-MB: kinetic system for determination of serum creatinekinase CK (CK-NAC) and CK-MB (Doles Chemical Industrie, Goiania-GO, Brazil). The samples measures were determined by the enzyme-substract reaction CK and CK-MB with di-Adenosine-5-pentaphosphate and Glucose-6-phosphate dehydrogenase (Doles Chemical Industrie, Goiania-GO, Brazil). The reaction velocity was determined by spectrophotometry and were directly proportional to CK and CK-MB measures.

Total Cholesterol, HDL and LDL cholesterol; enzyme system for determination of total cholesterol, precipitation of lipoproteins of high, low and very low density lipoproteins (LDL and VLDL) in serum samples by reaction end point methodology: Enzyme-Trinder (Labtest Chemical Industrie, Lagoa Santa-MG, Brazil). The samples measures were determined by the enzyme-substract reaction by oxidase and peroxidase of cholesterol. The reaction intensity was determined by spectrophotometry and were directly proportional to total cholesterol, HDL and LDL measures.

Triglycerides: enzyme system for determination of triglycerides in serum samples by reaction end point methodology. (Labtest Chemical Industrie, Lagoa Santa-MG, Brazil). The samples measures were determined by the enzyme-substract reaction by oxidase and peroxidase of glycerol. The reaction intensity was determined by spectrophotometry and were directly proportional to triglycerides measures.

Statistical Methods

The results were evaluated statistically with “t” Student test and values were expressed as mean \pm SD. The level of significance was set at $P < 0,05$.

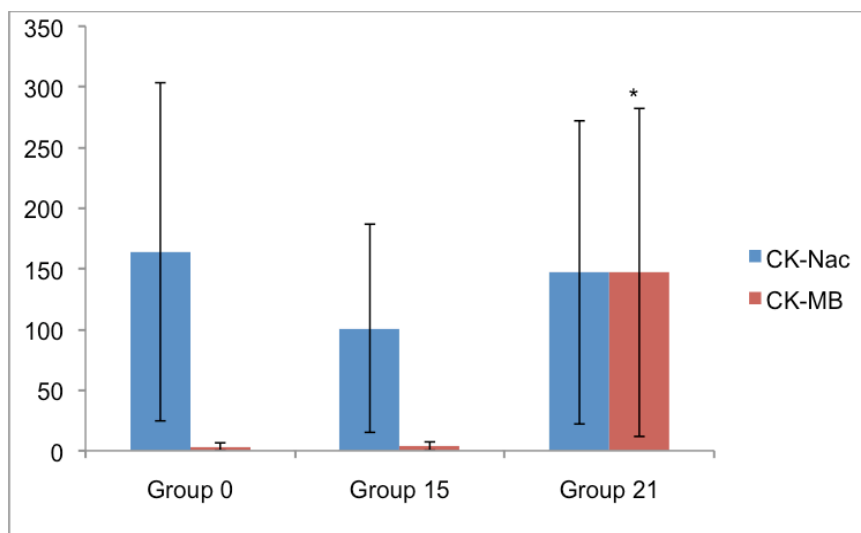


Figure 1: CK-Nac and CK-MB plasma levels for subjects before (group 0) and after 15 (group 15) and 21 (group 21) days of red wine intake. * statistical differences when compared with Group 0 and Group 15 $P < 0,05$.

RESULTS

Figure 1 shows the results of the CK-Nac and CK-MB analysis. The results of CK-Nac showed $164,1 \pm 123,2$ U/l for the Group 0, $101,1 \pm 25,7$ U/l for the Group 15 and $147 \pm 142,5$ U/l for the Group 21. We found no significant differences among the groups. However, when we observe the results for CK-MB we can see significant difference when we compared Group 21- $147 \pm 142,5$ U/l with Group 0 - $3,4 \pm 2,9$ U/l and with Group 15 - $3,8 \pm 3$ U/l.

The Figure 2 shows the measures of total cholesterol, HDL and LDL before (Group 0): $303,1 \pm 115,8$; $31,3 \pm 12,8$; $248,7 \pm 112$ mg/dl, and after 15 days of red dry wine intake (Group 15); $157,8 \pm 26,7$; $42,7 \pm 10,1$; $98,7 \pm 20,4$ mg/dl. We found significant difference between group 15 and group 0 for total cholesterol and LDL measures. However we can't observed significant differences between the groups for HDL measures.

The results for triglycerides measures before (Group 0): $115 \pm 61,5$ mg/dl and after 15 days of red dry wine intake (Group 15): $81,8 \pm 54,2$ mg/dl revealed no significant difference between the groups.

DISCUSSION

Red wine diet has been pointed as an important tool to reduce cardiovascular risks because its antioxidant effects of phenolic red wine compounds. Early case controlled studies showed that a moderate alcohol, especially wine, consumption decreased cardiovascular diseases risks [13, 15, 24, 25]. More recently several lines of evidence pointed red wine

consumption can decrease endothelin production and increase NO secretion by endothelial cells what leads to vasodilation and lower hypertension and atherosclerosis risks [26]. Some results in studies with normal or hypercholesterolemic subjects has pointed to HDL increase after a red dry wine diet but no changes were observed in total cholesterol or LDL plasma levels [13, 26, 27, 28]. Only when associated with fish and olive oil, a denominated Spanish Ketogenic Mediterranean Diet, the red wine diet showed decrease of LDL or cholesterol and increase of HDL [6].

Our results showed a LDL and total cholesterol decrease after daily consumption of 200ml of red wine for 15 days. Important to note that in other studies the subjects underwent to doses from 250 ml to 400 ml of red dry wine. We gave 200 ml, thus the lower dose, what could explain the differences among the results [13, 26, 27, 28]. The results for triglycerides showed no effects of red dry wine in triglycerides metabolism as well other studies have shown [6].

The role of red wine diet on CK and CK-MB enzymes is not found in the literature specially in humans, healthy subjects. However, in a study with quercetine, important member of dietary flavonoid family and widely present in red wine, it was observed that a 7 days of 50 mg/kg of quercetin intake could reduce in a significant way the CK plasma levels in cardiac injury rats [22]. In other study it was observed that a 10 weeks red wine diet significantly reduced CK plasma levels in rats with renal damage caused by rhabdomyolysis [29]. But, the present study is the first report on humans which is little more elaborate as compared to the rat study.

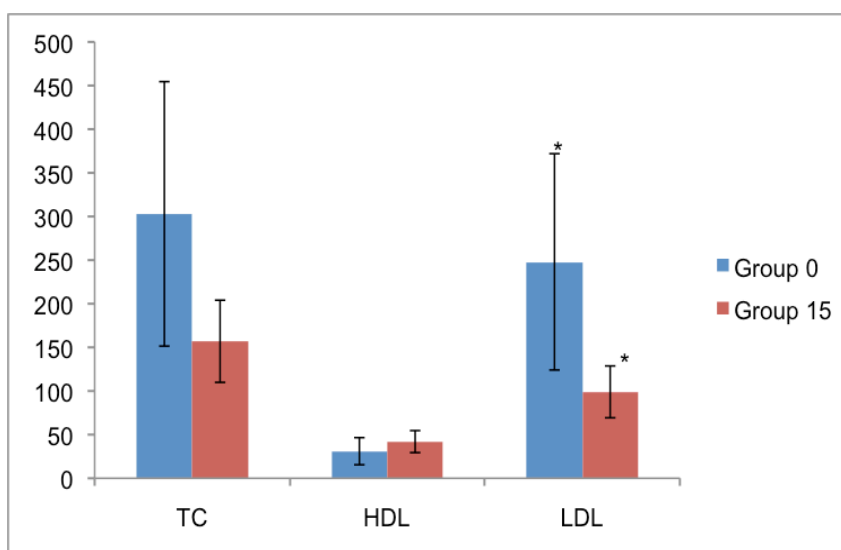


Figure 2: Total Cholesterol (TC), HDL and LDL plasma levels of subjects before (Group 0) and after 15days of red dry wine intake (Group 15). * statistical differences when compared with Group 0, $P < 0,05$.

There is no study measuring CK-MB plasma levels. CK-MB is a very specific cardiac enzyme. Our result showed a significant increase of the enzyme plasma levels after 21 days diet. These values we observed pointed to a pathological process. That point to an muscle injury or a possible increase of some oxidative muscular process, since some early studies showed that alcoholic rhabdomyolysis can alter CK-MB measures [30]. Maybe this fact could be explained by the fact that all the subjects weren't habitual consumers of alcohol. The relation of CK-MB increase with plasma total cholesterol or LDL reduction is not clear.

Our results allow us to conclude that despite de differences among the studies it is clear that red dry wine has important effects in atherosclerosis and others cardiovascular diseases manifestation and should be considered as a tool to prevent them.

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