

Cardioprotective Effects of Dietary Polyphenols¹

Tosca L. Zern and Maria Luz Fernandez²

Department of Nutritional Sciences, University of Connecticut,
Storrs, CT 06269

ABSTRACT Dietary polyphenols have been shown to possess cardioprotective effects. For example, the most noted role of grape polyphenols is in the French Paradox, in which a diet high in saturated fat accompanied by regular consumption of red wine is associated with a low risk of coronary heart disease (CHD). Initially, the paradox was thought to be driven by the postulated major action of grape polyphenols in inhibiting LDL oxidation. Although many studies have shown inhibitory effects of polyphenols on LDL oxidation, there have been an equal number of studies that showed a null effect on this variable. Although there are contrasting viewpoints on the effects of polyphenols on LDL oxidation variables, there is increasing evidence that these compounds possess additional cardioprotective functions including altering hepatic cholesterol absorption, triglyceride assembly and secretion, the processing of lipoproteins in plasma, and inflammation. It is the purpose of this review to examine recent information on the multiple functions of dietary polyphenols, with an emphasis on grape polyphenols, in decreasing the risk of CHD by improving plasma lipid profiles and reducing inflammation. *J. Nutr.* 135: 2291–2294, 2005.

KEY WORDS: • dietary polyphenols • lipoprotein metabolism • cytokines • coronary heart disease • inflammation

Polyphenols and related compounds can be found in every plant species. In plants, polyphenols are important for pigmentation, reproduction, growth, and protection against pathogens. In industry, polyphenols are used in the production of paints, paper, and cosmetics as well as in food additives (1). However, in more recent years, polyphenols have received much attention in disease treatment and prevention due to their antioxidant capabilities.

Currently, there are >8000 known polyphenolic structures (1). Polyphenols share a common chemical structure and differ only in their additional linkages with other compounds. The majority of polyphenols have a sugar residue linked to the carbon skeleton. Glucose is a common sugar residue; however, residues can include different monosaccharides, disaccharides, or oligosaccharides. Other compounds including amines, or-

ganic acids, carboxylic acids, lipids, and other polyphenols may also be linked to the basic polyphenolic structure (1).

Because of the abundance of polyphenols in nature, it is not surprising that they can be found in fruits, vegetables, coffee, tea, chocolate, and soy (2). Once ingested, polyphenols have several possible fates, including absorption in the small intestine or colon, and/or excretion in the feces or urine. The site and rate of absorption depend on the chemical structure, degree of glycosylation/acylation, conjugation of other phenolics, molecular size, degree of polymerization, and solubility (1,2). In the small intestine, polyphenols can enter the mucosa through passive diffusion. In some instances, hydrophobic moieties must be cleaved for absorption to take place. In the colon, polyphenols are initially digested into smaller phenolic structures by gut microflora. After this initial digestion is complete, the polyphenols and their metabolites may be absorbed (1).

Once absorption has taken place, polyphenols and their metabolites are transported to the liver. Further digestion may occur there, and then polyphenol metabolites may be transported to extrahepatic tissues or to the kidneys where they are excreted in the urine. For the majority of polyphenols, the maximum concentration in the plasma is apparent 1–2 h after ingestion. Polyphenols may also be incorporated into bile, returned to the small intestine, and eventually be excreted in the feces (1).

Polyphenols have generated a great amount of scientific research due to their *in vivo* and *in vitro* antioxidant capabilities. For years, red wine was thought to have beneficial effects on cardiovascular health. This relation was clear in the French Paradox phenomenon as well as in the Mediterranean diet. The French Paradox is defined as a low incidence of coronary heart disease (CHD)³ while consuming a diet rich in saturated fat. The Mediterranean diet, rich in fruits and wine, was shown to protect against the occurrence of coronary events (3,4). In addition, the inclusion of fruits and vegetables in the diet may help reverse hyperlipidemia, alter the atherogenicity of the LDL particle (5), and protect the cholesterol in LDL from oxidation (6). For example, grapes are comprised of a wide variety of polyphenols including resveratrol (stilbene), flavonoids and its derivatives, flavons, flavonols, and anthocyanins. Polyphenols can be found in grape seeds as well as in the skin. The concentration of these compounds varies due to climate and therefore variety of grape.

In general, more than two thirds of the polyphenols consumed in the diet are flavonoids (7). The majority of flavonoid and polyphenol research has focused on the reduction of LDL oxidation *in vivo* and *in vitro*. Therefore, the following is a

³ Abbreviations used: ACAT, acyl-CoA cholesterol acyltransferase; apo, apolipoprotein; CAMs, cellular adhesion molecules; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; FC, free cholesterol; IL, interleukin; LDL-C, LDL cholesterol; LFA-1, lymphocyte-associated function antigen; LGP, lyophilized grape powder; LPL, lipoprotein lipase; MCP-1, monocyte chemoattractant protein; MTP, microsomal transfer protein; NF- κ B, nuclear factor- κ B; TC, total cholesterol; TNF, tumor necrosis factor; TG, triglyceride; VLA-4, very-late activation antigen; VLDL-C, VLDL cholesterol.

¹ Manuscript received 16 June 2005.

² To whom correspondence should be addressed.

E-mail: maria-luz.fernandez@uconn.edu.

review of the effects of polyphenols on lipid metabolism and inflammation in both in vivo and in vitro models.

Polyphenols and Hepatic Cholesterol Metabolism. Animal studies suggest that polyphenols may reduce cholesterol absorption (8) due to the interaction of these compounds with cholesterol carriers and transporters present across the brush border membrane (9,10). A study using ovariectomized rats (a model for menopause) provided evidence that green tea polyphenols lowered the lymphatic absorption of total cholesterol in a dose-dependent manner and the absorption of fatty acids in a biphasic fashion (8). Lowered cholesterol absorption would therefore result in less cholesterol being delivered to the liver by the chylomicron remnant. Furthermore, Pal et al. (11) showed that when Caco2 cells were treated with dealcoholized wine, there was a 17% reduction in apolipoprotein (apo) B48 production and a 30% reduction in secretion compared with the control cells. There were also significant decreases in both free cholesterol (FC) and total cholesterol (TC) concentrations in the dealcoholized red wine-treated cells. The authors suggested that the significant alterations in apo B48 production and secretion were due to the significant decrease in substrate availability. Apo B48 is regulated post-translationally and is degraded if lipids are not available for chylomicron production (12). The authors did not speculate on the role that dealcoholized red wine plays in cholesterol absorption (11). However, due to previous findings in green tea, there is a potential mechanism by which alteration in cholesterol absorption may disrupt the normal regulation of apo B48 production, thereby interrupting chylomicron synthesis and secretion. Consequently, significant alterations in hepatic cholesterol homeostasis including hepatic lipoprotein secretion and lipoprotein removal from the plasma compartment would occur.

In examining lipoprotein production, the majority of polyphenol research has addressed their effects on hepatic production of lipoproteins. The citrus flavonoids, naringenin and hesperitin, were shown to reduce apo B secretion in hepatocytes (13,14). This effect was associated with a reduction in cholesteryl ester (CE) mass, a selective decrease in acyl-CoA cholesterol acyltransferase (ACAT₂), and microsomal transfer protein (MTP) mRNA abundance accompanied by a decrease in MTP activity. In addition to these findings, naringenin reduced triglyceride (TG) secretion into the medium. Dealcoholized red wine treatment was also shown to reduce apo B-100 secretion from Hep G2 cells up to 50% (15). Reductions in intracellular CE, FC, and TC concentrations were also apparent after treatment. Similarly, in ovariectomized guinea pigs, a model for menopause, treatment with lyophilized grape powder (LGP) significantly lowered hepatic CE concentrations while significantly increasing FC concentrations compared with control guinea pigs (16). The significant alterations in hepatic cholesterol pools were accompanied by a 27% decrease in hepatic ACAT activity.

The mechanism by which polyphenols reduce hepatic apo B production may be through their binding with the plasma membrane transport P-glycoprotein, which inhibits cholesterol esterification (17), decreasing the incorporation of CE into nascent VLDL. A lower apo B production rate is strongly correlated with reduced hepatic ACAT activity in miniature pigs (18) and in African green monkeys (19).

Polyphenols and Plasma Lipids. Grape juice was shown to increase plasma TG in several studies due to the carbohydrate content in the juice (20,21). However, grape polyphenols were

shown to alter lipoprotein metabolism by decreasing plasma triglycerides and apo B concentrations.

In ovariectomized guinea pigs, LGP treatment reduced VLDL cholesterol (VLDL-C) and TG by 50 and 39% compared with the control (16). Furthermore, golden Syrian hamsters fed red wine phenolics had a significant decrease in plasma apo B concentrations (22). Recently in our laboratory, the same LGP used in the guinea pig study was shown to significantly decrease TG, apo B, apo E, and LDL cholesterol (LDL-C) concentrations in both pre- and postmenopausal women (23). Similar to our previous study, grape polyphenols may have altered hepatic secretion of TG-rich VLDL. This reduction is evident when observing the decreases in both plasma apo B and apo E concentrations. The significant decrease in apo E concentrations may have further reduced plasma TG concentrations. In general, apo E displaces apo C-II from the VLDL particle, thereby inhibiting lipoprotein lipase (LPL) activity and overall lipolysis. Furthermore, Huang et al. (24) showed that adding apo C-II to transgenic apo-E3-enriched VLDL increased LPL activity in a dose-dependent manner. The reductions in apo E and TG concentrations suggest less displacement by apo E, thereby promoting LPL activity and further reducing plasma TG concentrations.

Due to decreases in TG concentrations, LGP treatment was shown to affect overall lipoprotein metabolism. Decreased concentrations of plasma TG altered substrate availability in the delipidation cascade, leading to the observed decrease in LDL-C concentrations. After the 4-wk treatment period, the LGP treatment induced a significant decrease in cholesteryl ester transfer protein (CETP) activity as well. This decrease in CETP activity may be due in part to the substantial decrease in substrate availability including both plasma TG and LDL-C.

It is evident that grape polyphenols modify the packaging of VLDL through alteration in hepatic enzyme activity and apo B secretion. These modifications seem to decrease the overall secretion of the VLDL particles and therefore, decrease plasma TG and related apo concentrations. Due to alterations in TG substrate, further modifications in lipoprotein metabolism may occur.

The alteration in TG metabolism may not be the single mechanism driving the hypocholesterolemic effects of grape polyphenols. When golden Syrian hamsters were treated with dealcoholized red wine, red wine, or grape juice, similar significant reductions in both TC and LDL-C concentrations were apparent in all treatment groups compared with the control (25). Although there was a trend for a decrease in TG concentrations in all treatment groups compared with the control, the differences were not significant. That study, along with others, suggests the presence of an additional mechanism by which grape polyphenols exert a cardioprotective effect. In Hep G-2 cells, dealcoholized red wine was shown to significantly upregulate LDL receptor activity (15). This significant increase in activity was similar to the increase seen when Hep G-2 cells were treated with atorvastatin. Furthermore, when Hep G-2 cells were treated with increasing doses of red wine, LDL receptor mRNA abundance was significantly increased in a dose-responsive manner. The increase in LDL receptor activity and abundance may be a result of the homeostatic intracellular cholesterol feedback loop. In general, a decrease in intracellular cholesterol will upregulate LDL receptor expression and activity, whereas an increase in intracellular cholesterol will downregulate the receptor (26). Grape polyphenols were shown to alter hepatic cholesterol concentrations; therefore, the liver compensates for this deficit by upregulating the LDL receptor and an overall decrease in plasma

LDL concentrations occurs. A diagram showing the major sites of action of dietary polyphenols is given in Figure 1.

Polyphenols and Inflammatory Cytokines. In recent years, grape polyphenols were also shown to have a beneficial effect on inflammation. LGP treatment was shown to significantly decrease tumor necrosis factor (TNF)- α and interleukin (IL)-6 concentrations in both pre- and postmenopausal women (23). Furthermore, in an 11-wk study with a crossover design comparing red wine and gin consumption in healthy men, adhesion molecules and monocyte adhesion to endothelial cells were significantly altered due to red wine (27). After the 4-wk period of red wine consumption, concentrations of the adhesion molecules including very-late activation antigen (VLA-4), lymphocyte-associated function antigen (LFA-1), Mac-1, and monocyte chemoattractant protein (MCP-1) on monocytes and T-lymphocytes, were significantly decreased compared with baseline ($P < 0.05$). The soluble cellular adhesion molecules (CAMs), ICAM-1 and VCAM-1, were decreased by 9 and 17%, respectively (27). Similarly, monocyte adhesion to TNF- α -stimulated endothelial cells was also significantly decreased after red wine intake (28). Red wine intake was also shown to decrease MCP-1 after balloon injury to the abdominal aorta in cholesterol-fed rabbits. Furthermore, MCP-1 mRNA reduction paralleled decreases seen in aortic thickening in rabbits consuming the red wine (29). In both studies, although researchers concluded that alterations in monocyte adhesion molecules and CAMs affected the overall adhesion of monocytes to endothelial cells, they did not elaborate on the exact mechanism.

Suppression of the transcription factor, nuclear factor- κ B

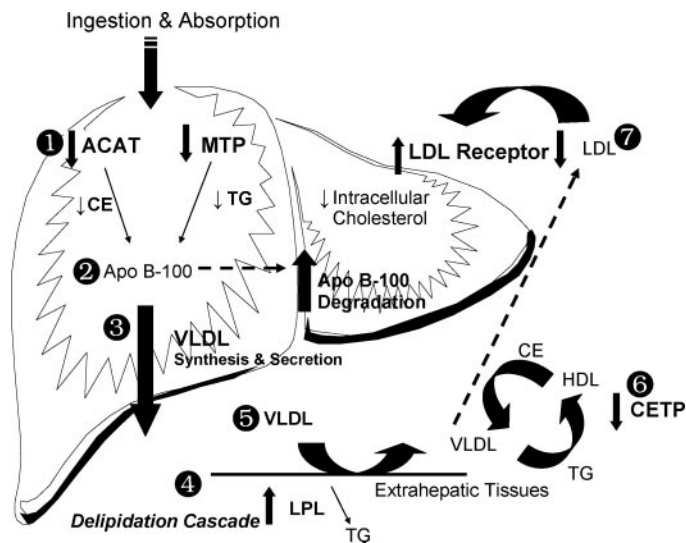


FIGURE 1 Polyphenols and their metabolites were shown to modify hepatic cholesterol and lipoprotein metabolism. 1) By decreasing both MTP and ACAT activity involved in lipid assembly for VLDL secretion. 2) Without sufficient lipid components, the degradation of apo B-100 is increased. 3) The significant decrease in substrate availability results in a decrease in both VLDL synthesis and secretion. 4) The delipidation cascade is further altered due to the decrease in VLDL particles in circulation. 5) Alterations to the VLDL particle, including significant decreases in apo E, may induce an increase in LPL activity, and therefore, decrease plasma TG and VLDL concentrations. 6) CETP activity may also be affected by the decrease in VLDL substrate. 7) The VLDL decrease yields a significant decrease in LDL concentrations. Due to the significant alteration in hepatic cholesterol concentrations, the LDL receptor may be upregulated to maintain hepatic cholesterol homeostasis.

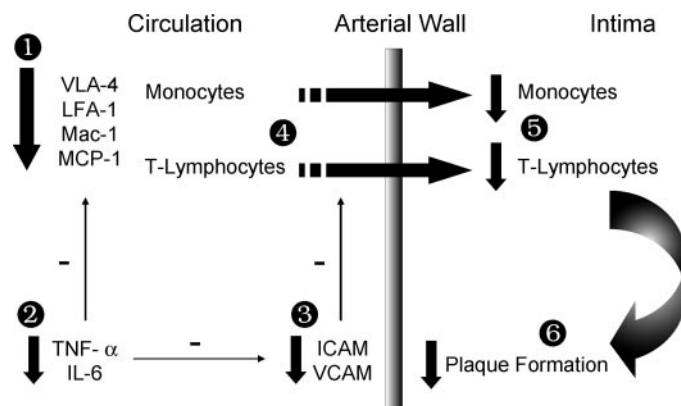


FIGURE 2 Grape polyphenols 1) decrease adhesion molecules on both monocytes and T-lymphocytes. 2) TNF- α and IL-6 concentrations are also decreased. 3) CAMs present on the arterial wall will also be decreased. 4) The overall uptake of monocytes and T-lymphocytes is significantly decreased due to the alterations in CAMs. 5) The decrease in monocytes and T-lymphocytes within the intima may alter the 6) formation of plaque and ultimately atherosclerosis.

(NF- κ B), was suggested to play a role in this mechanism. NF- κ B is responsible for activating cytokines, adhesion molecules, and procoagulant proteins (30). When activated, these inflammatory proteins can in turn increase NF- κ B levels and continue this cycle. It is important to note that oxidized LDL and other reactive oxygen species are also potent stimulators of NF- κ B (31). Red wine intake was also shown to decrease NF- κ B concentrations in monocytes. Healthy subjects were given a fat-enriched meal with or without red wine. The fat-enriched meal alone increased the expression of NF- κ B, whereas the red wine meal significantly decreased expression of NF- κ B ($P < 0.05$). The authors suggested that red wine polyphenols may have inhibited oxidation, thereby decreasing the activation of NF- κ B.

Resveratrol, a stilbene found in grapes, was found to affect a mechanism other than the inhibition of the NF- κ B pathway. The tyrosine kinase second messenger system is crucial in the regulation of the expression of CAMs and other cytokines. Resveratrol is a natural tyrosine kinase inhibitor. Therefore, researchers suggest that resveratrol affects the expression of inflammatory cytokines and adhesion molecules through its inhibition of NF- κ B as well as through inhibition of the tyrosine kinase system (32). A summary of the effects of polyphenols in inflammation is presented in Figure 2.

Summary. Polyphenols have been shown to protect against coronary heart disease, and this review has summarized the speculated mechanisms by which these compounds affect hepatic cholesterol and lipoprotein metabolism, and reduce the inflammatory response. Polyphenols may: 1) reduce cholesterol absorption. This reduction in cholesterol absorption will result in decreased delivery of cholesterol to the liver by chylomicron remnants, which in turn will 2) upregulate hepatic mRNA abundance for the LDL receptor to compensate for less substrate availability and induce reductions in plasma cholesterol. 3) Polyphenols affect apo B secretion rates, MTP, and ACAT₂ activities, resulting in a modified VLDL particle. Reductions in plasma TG were shown as a result of 4) lower MTP activity and possibly increased LPL activity. 5) Reductions in plasma TG may further alter the delipidation cascade, yielding less LDL in circulation. 6) Polyphenols also exert anti-inflammatory effects, thereby reducing the production of cytokines involved in cellular adhesion.

LITERATURE CITED

- Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev*. 1998;56:317-33.
- Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr*. 2002;130:2073S-85.
- De Lognogeril M, Salen P, Martin JL, Mamelle N, Monjaud I, Touboul P, Delaye J. Effect of a Mediterranean diet on the rate of cardiovascular complications in patients with coronary artery disease. Insights into the protective effect of certain nutrients. *J Am Coll Cardiol*. 1996;28:1103-8.
- Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S. Flavonoid intake and long term risk of cardiovascular disease in the Seven Countries Study. *Arch Intern Med*. 1995;155:381-6.
- Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies *Am J Clin Nutr*. 1999;70(Suppl):475S-90.
- Brouillard R, George F, Fougereuse A. Polyphenols produced during red wine ageing. *Biofactors*. 1997;6:403-10.
- Dell'Agli M, Busciala A, Bosisio E. Vascular effects of wine polyphenols. *Cardio Res*. 2004;63:593-602.
- Loest HB, Noh SK, Koo SI. Green tea extract inhibits the lymphatic absorption of cholesterol and α -tocopherol in ovariectomized rats. *J Nutr*. 2002;132:1282-8.
- Conseil G, Baubichon-Cortay H, Dayan G, Jault JM, Barron D, Di Pietro A. Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP- and steroid-binding sites on mouse P-glycoprotein. *Proc Natl Acad Sci USA*. 1998;95:9831-6.
- Leslie EM, Mao Q, Oleschuk CJ, Deeley RG, Cole SP. Modulation of multidrug resistance protein 1 (MRP1/ABCC1) transport and ATPase activities by interaction with dietary flavonoids. *Mol Pharmacol*. 2001;59:1171-80.
- Pal S, Ho SS, Ryusuke T. Red wine Polyphenolics suppress the secretion of apoB48 from human intestinal caco-2 cells. *J Agric Food Chem*. 2005;53:2767-72.
- Innerarity TL, Boren J, Yamanaka S, Olofsson SO. Biosynthesis of apolipoprotein B48-containing lipoproteins. Regulation by novel post-transcriptional mechanisms. *J Biol Chem*. 1996;271:2353-6.
- Borradaile NM, de Dreu LE, Barrett PHR, Huff MW. Inhibition of hepatocyte apo B secretion by naringenin: enhanced rapid intracellular degradation independent of reduced microsomal cholesteryl esters. *J Lipid Res*. 2002;43:1544-54.
- Borradaile NM, de Dreu LE, Barrett PHR, Behrsin CD, Huff MW. Hepatocyte apo-B-containing lipoprotein secretion is decreased by the grapefruit flavonoid, naringenin, via inhibition of MTP-mediated microsomal triglyceride accumulation. *Biochemistry*. 2003;42:1283-91.
- Pal S, Ho N, Santos C, Dubois P, Mamo J, Croft K, Allister E. Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of apoB 100 from human Hep G2 cells. *J Nutr*. 2003;133:700-6.
- Zern TL, West KL, Fernandez ML. Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. *J Nutr*. 2003;133:2268-72.
- Debry PE, Ash A, Neklason DW, Methelal JE. Role of multidrug resistance P-glycoprotein in cholesterol esterification. *J Biol Chem*. 1997;272:1026-31.
- Burnett JR, Wilcox LJ, Telford DE, Kleinstiver SJ, Barrett PH, Newton RS, Huff MW. Acyl inhibition of ACAT by avasimibe decreases both VLDL and LDL apolipoprotein B production in miniature pigs. *J Lipid Res*. 1999;40:1317-27.
- Carr TP, Parks SJ, Rudel LL. Hepatic ACAT activity in African green monkeys is highly correlated to plasma LDL cholesteryl ester enrichment and coronary artery atherosclerosis. *Arterioscler Thromb*. 1992;12:1274-83.
- Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary heart disease. *Circulation*. 1999;100:1050-5.
- O'Byrne DJ, Devaraj S, Grundy SM, Jialil I. Comparison of Antioxidant effects of Concord grape juice flavonoids and α -tocopherol on markers of oxidative stress in healthy adults. *Am J Clin Nutr*. 2002;76:1367-74.
- Auger C, Caporiccio B, Landraut N, Teissdre PL, Laurent C, Cros G, Rouanet J. Red wine phenolic compounds reduce plasma lipids and apolipoprotein B and prevent early atherosclerosis in hypercholesterolemic golden Syrian hamsters (*Mesocricetus auratus*). *J Nutr*. 2002;132:1207-13.
- Zern TL, Wood RJ, Greene C, West KL, Liu Y, Aggarwal D, Shachter NS & Fernandez ML. Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *J Nutr*. 2005;135:1911-7.
- Huang Y, Liu XQ, Rall SC, Taylor JM, von Eckardstein A, Assmann G, Mahley RW. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. *J Biol Chem*. 1998;273:26388-93.
- Vinson JA, Teufel K, Wu N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis*. 2001;156:67-72.
- Brown MS, Goldstein JL. Receptor-mediated pathway of cholesterol homeostasis. *Science*. 1986;232:34-47.
- Estruch R, Sacanella E, Badia E, Antunez E, Nicolas JM, Fernandez-Sola J, Rotilio D, de Gaetno G, Rubin E, Urbano-Marquez A. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: A prospective randomized crossover trial. Effects of wine on inflammatory markers. *Atherosclerosis*. 2004;175:117-23.
- Badia E, Sacanella E, Fernandez-Sola J, Nicolas JM, Antunez E, Rotilio D, de Gaetno G, Urbano-Marquez A, Estruch R. Decreased tumor-necrosis factor-induced adhesion of human monocytes to endothelial cells after moderate alcohol consumption. *Am J Clin Nutr*. 2004;80:225-30.
- Feng A, Chen Y, Chen Y, Ding Y, Lin S. Red wine inhibits monocyte chemotactic protein-1 expression and modestly reduces neointimal hyperplasia after balloon injury in cholesterol-fed rabbits. *Circulation*. 1999;100:2254-9.
- Jialal I, Devaraj S, Kaul N. The effect of α -tocopherol on monocyte proatherogenic activity. *J Nutr*. 2001;131:389S-94.
- Blanco-Colio LM, Valderrama M, Alvarez-Sala LA, Bustos C, Ortego M, Hernandez-Presa MA, Cancelas P, Gomez-Gerique J, Millan J, Egido J. Red wine intake prevents nuclear factor- κ B activation in peripheral blood mononuclear cell of healthy volunteers during postprandial lipemia. *Circulation*. 2000;102:1020-6.
- Ferrero ME, Bertelli AAE, Fulgenzi A, Pellegatta F, Corsi MM, Bonfrate M, Ferrara F, De Caterina R, Giovannini L, Bertelli A. Activity in vitro of resveratrol on granulocyte and monocyte adhesion to endothelium. *Am J Clin Nutr*. 1998;68:1208-14.