

## Wine and haemostatic system Platelet aggregation, coagulation, fibrinolysis

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## Κρασί και σύστημα αιμόστασης Συσσώρευση αιμοπεταλίων, πήξη, ινωδόλυση

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**ABSTRACT:** Atherosclerosis, the underlying mechanism of cardiovascular diseases, is a multifactorial process including several cellular and molecular alterations. Haemostasis is thought to play a crucial role in the onset and perpetuation of atherosclerosis and is characterized by a delicate balance that exists between four major components: the vascular endothelium; platelets; the coagulation pathways, and fibrinolysis. Many epidemiological studies confirm an inverse relationship between light to moderate alcohol consumption and cardiovascular events, and most of them have suggested the superiority of wine among alcoholic beverages. Under this perspective, studies have been implemented in order to investigate the effect of acute or chronic wine consumption on biochemical markers associated with cardiovascular diseases. In this review, emphasis will be given in the effect of chronic wine consumption on haemostatic system.

**Key words:** Wine, atherosclerosis, haemostasis, platelet aggregation, coagulation, fibrinolysis.

**ΠΕΡΙΛΗΨΗ:** Η αθηροσκλήρωση, που αποτελεί τον βασικό μηχανισμό των καρδιαγγειακών νοσημάτων, είναι μια πολυπαραγοντική διαδικασία που περιλαμβάνει τόσο κυτταρικές όσο και μοριακές μεταβολές. Η αιμόσταση θεωρείται ότι εμπλέκεται στην έναρξη και την εξέλιξη της αθηροσκλήρωσης και χαρακτηρίζεται από μια ευαίσθητη ισορροπία μεταξύ τεσσάρων κύριων συνιστωσών: το αγγειακό ενδοθήλιο, τα αιμοπετάλια, την πήξη του αίματος και την ινωδόλυση. Πολλές επιδημιολογικές μελέτες επιβεβαιώνουν μια αντίστροφη σχέση μεταξύ της ελαφριάς έως μέτριας κατανάλωσης αλκοόλ και της εμφάνισης καρδιαγγειακών επεισοδίων, και οι περισσότερες από αυτές έχουν προτείνει την υπεροχή του κρασιού έναντι των άλλων αλκοολούχων ποτών. Σε αυτά τα πλαίσια, κλινικές δοκιμές έχουν εφαρμοστεί με σκοπό να μελετήσουν την επίδραση της άμεσης ή μακροχρόνιας κατανάλωσης κρασιού σε βιοχημικούς δείκτες που σχετίζονται με τις καρδιαγγειακές παθήσεις. Σε αυτό το άρθρο ανασκόπησης θα δοθεί έμφαση στην επίδραση της μακροχρόνιας κατανάλωσης κρασιού στο σύστημα της αιμόστασης.

**Λέξεις ευρητηρίου:** Κρασί, αθηροσκλήρωση, σύστημα αιμόστασης, συσσώρευση αιμοπεταλίων, πήξη, ινωδόλυση.

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## 1. Introduction

Cardiovascular diseases (CVDs) consist of several pathological conditions that affect the heart and the blood vessels and cause 47% of all deaths in Europe.<sup>1</sup> Death rates from coronary heart disease (CHD) are generally higher in Central and Eastern Europe than in Northern, Southern and Western Europe.<sup>1</sup> Especially in Greece, almost 43% of the deaths in 2011 were attributed to CVDs according to the Hellenic Statistical Authority.<sup>2</sup> More specifically, the first cause of deaths was stroke (31%) and the third was coronary heart disease (24%), with the 72% of them observed above the age of 75 years.<sup>2</sup>

Atherosclerosis, the underlying mechanism of CVDs, is a multifactorial process including several cellular and molecular alterations. Inflammation, oxidative stress and thrombosis underlie its onset and perpetuation.<sup>3,4</sup> The process of atherosclerotic lesion could be classified in the following essential steps: (a) endothelial dysfunction; (b) infiltration of LDL particles as well as circulating leukocytes into the subendothelium; (c) LDL oxidation; (d) monocyte-derived macrophages acquire the phenotype of foam cells; (e) smooth muscle cells (SMC) migration and proliferation in the subendothelium; (f) structural endothelial lesion followed by platelet deposition and thrombus formation.<sup>3</sup> Haemostatic system participates in the above mechanisms, which also involve platelet activation (primary haemostasis), mechanisms of coagulation and fibrinolysis (secondary haemostasis).

Atherothrombosis, in particular, is a multifocal and diffuse pathological process affecting the arterial wall, characterized by the development of atheromatous plaques and eventually their rupture and the subsequent activation of the coagulation cascade, leading to thrombosis. The thrombotic process is the main complication of atherosclerosis and results in the presentation of the acute coronary syndromes (ACS).<sup>5</sup>

Among other pro-inflammatory and thrombotic mediators Platelet-Activating Factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) and oxidized-phospholipids have been proposed to play a crucial role in the initiation and prolongation of the atherosclerotic lesion.<sup>3,6</sup>

Many epidemiological studies confirm an inverse relationship between light to moderate alcohol consumption and cardiovascular events or all-cause mor-

tality in apparently healthy individuals or patients with CVDs.<sup>7,8</sup> 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.

Renaud and De Lorgeril<sup>9</sup> in 1992, based on the findings of the MONICA (MONItoring system for CARDiovascular disease) project, a worldwide program organized by the World Health Organization, introduced the term "French Paradox" to describe the epidemiological observation that French people exhibit relatively low prevalence of CHD, despite the fact that their diet is relatively rich in saturated fats. As the consumption of alcohol, especially wine, was much higher in France than in most Western countries, Renaud and De Lorgeril concluded that wine drinking habit protect French from CHD. Following this observation, several epidemiological studies have suggested that wine may be more beneficial than other alcoholic beverages, and a J-shaped relationship between wine consumption and vascular risk as well as cardiovascular mortality has been found.<sup>10-12</sup> However, it should be mentioned that few studies do not support this superiority.<sup>13-15</sup> This scientific controversy may be attributed to the fact that many studies, especially the earlier ones, did not adequately control for potential confounders.

The superiority of wine is thought to be attributed to its micro-constituents. Apart from ethanol there are also other micronutrients, phenolic compounds and phospho- and glyco-lipids.<sup>16,17</sup> Wine compounds have been found to exert antioxidant, anti-inflammatory and anti-thrombotic properties, and therefore to inhibit or hinder several steps of atherosclerosis. Noteworthy to mention that there are already outstanding reviews about their protective actions.<sup>17-19</sup>

Moreover, it is commonly accepted that randomized controlled trials offer more concrete answers to several medical raised questions than observational studies. Under this perspective, clinical studies have been implemented, mainly in healthy volunteers and rarely in patients, in order to investigate the acute or chronic effect of wine consumption on biochemical markers associated with cardiovascular diseases. The results from these studies indicate a favorable effect on lipid biomarkers and especially on HDL particles,<sup>20</sup> while there is no evidence, at present, that wine consumption provides antioxidant benefits in healthy volun-

teers other than to counteract a possible pro-oxidative effect of the alcohol.<sup>21</sup> As far as inflammatory markers concerning, the results are not yet certified and more studies are needed in order to obtain more stable evidence.<sup>20,22</sup>

In this review, emphasis will be given in the effect of wine consumption on haemostatic system. For this reason, firstly a brief description of haemostatic system will be given followed by: (a) data concerning the *in vitro* effects of wine micro-constituents, and (b) data obtain from long-term intervention studies.

## 2. Haemostasis and its major components

Haemostasis is the physiological process which prevents the blood loss from injured vascular ensuring its wall's integrity and tightness. A delicate balance exists between four major components: the vascular endothelium; platelets; the coagulation pathways, and fibrinolysis.

Coagulation mechanism is activated through two pathways; the intrinsic pathway and the extrinsic pathway. Extrinsic proceeding is activated when tissue factor (TF) binds to blood coagulation factor VII, in order to form a VIIa-TF complex, which catalyses the X factor activation to Xa<sup>23</sup> (figure 1). Intrinsic pathway is activated by the XII factor, which is converted to XIIa when it comes in contact with specific surfaces such as the collagen from injured area. After that, a serial activation of several factors follows.<sup>24</sup> These two pathways result in the same final step, the formation of Xa factor, which catalyses prothrombin (factor II)/thrombin (factor IIa) conversion. Thrombin participates in fibrinogen remodeling and fibrin polymer formation, a fibrous mesh, which is converted into a steady three dimension clot, which traps platelets, erythrocytes and other cells, forming a thrombus.<sup>25</sup> The thrombus could be removed by fibrinolysis process. Its main enzyme, plasmin is formed by plasminogen and has high affinity interaction with fibrin.<sup>26,27</sup> Plasmin degrades the fibrin clot, leading to the production of circulating fragments (fibrin degradation products known as D-dimer)<sup>26,27</sup>(figure 1). There have been determined two main plasminogen activators, tissue-type and urokinase-type plasminogen activator (t-PA and u-PA, respectively).<sup>28</sup> On the other hand, inhibition of the fibrinolytic system may occur either at the level of the

plasminogen activators, by specific plasminogen activator inhibitors (PAIs), or at the level of plasmin, mainly by  $\alpha_2$ -antiplasmin.<sup>27</sup>

When endothelial injury occurs, the endothelial cells interact with platelets and coagulation factors such as collagen, von Willebrand factor (vWF), and fibronectin. Furthermore, the TF is expressed and PAI-1 is secreted.<sup>29</sup> Platelets<sup>30</sup> are activated and adhere, either directly or through leukocytes, on sub-endothelium of exposed tissue and aggregate to each other in order to form haemostatic plugs on the damaged area.<sup>31,32</sup> Platelets activation and aggregation is induced by several antagonists such as thrombin, collagen, ADP, PAF, serotonin, epinephrine, thromboxane A<sub>2</sub> (TXA<sub>2</sub>) etc.<sup>32</sup> Latter on endothelial cells secrete tissue t-PA, initiating fibrinolysis, causing a shift in the haemostatic balance.<sup>29</sup>

Concerning PAF, there are several substances, capable of activating PAF synthesis from endothelium including thrombin, and pro-inflammatory cytokines. PAF may control endothelial functions,<sup>33</sup> resulting in increased permeability of the endothelium<sup>30,34</sup> which is a crucial event in the initiation of atherosclerosis.

## 3. Wine compounds and their *in vitro* effects on haemostatic system

Many *in vitro* studies have estimated the biological activity of wine phenolic compounds on haemostatic system. These studies use wine extracts or standard phenolic compounds in order to exam the biological activity of wine bioactive compounds on platelet aggregation, coagulation and fibrinolysis. Among the plethora of wine phenolic compounds resveratrol and quercetin are the most studied ones. Their concentration in wines depends on the variety of the grape, the geographical location of grapes production, and the year of production. Resveratrol is the main stilbene of grapes and quercetin belongs to flavonols class. Resveratrol is found in the skin of grapes, so much more is found in red wines, because the fermentation process includes grape skin.

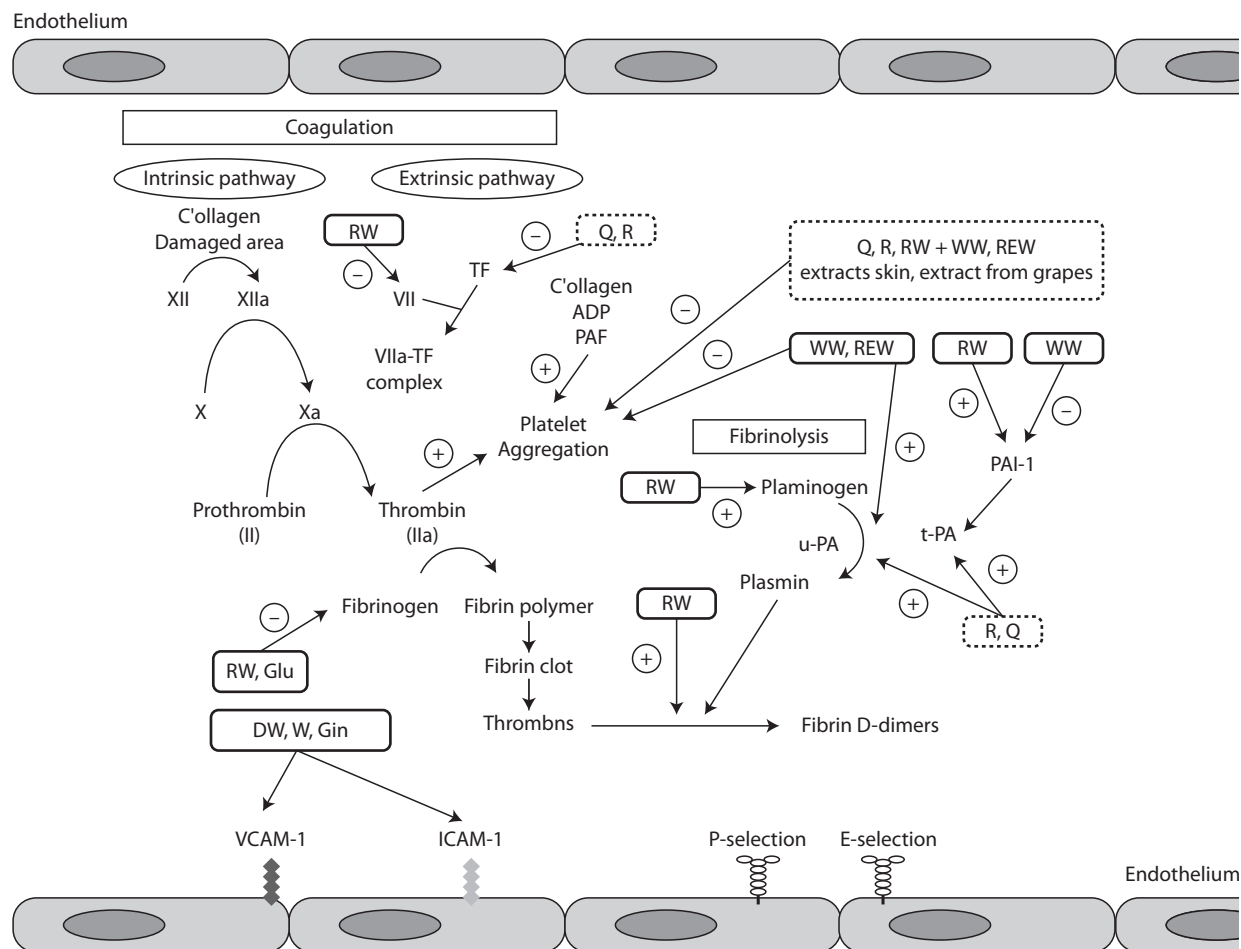
Specifically, in Cabernet Sauvignon, which is one of the most well-known varieties, resveratrol varies from 1 mg/L to 7 mg/L, and in Shiraz –another famous variety– from 1 mg/L to 5 mg/L.<sup>35</sup> Additionally, quercetin concentration in Cabernet Sauvignon is between 0,5 mg/L and 7 mg/L, and in Shiraz between 6 mg/L and 11 mg/L.<sup>35</sup> White wines contain lower resveratrol

and quercetin levels. Two of the most famous white wine varieties are Chardonnay and Sauvignon Blanc. In Chardonnay, resveratrol concentration varies from 0,05 mg/L to 0,1 mg/L, whereas in Sauvignon Blanc from 0,2 mg/L to 0,7 mg/L.<sup>36</sup> Goldberg DM et al, measured, among others polyphenols, quercetin concentration in 644 white wines from the major wine-producing regions. They reported that in most of these wines, quercetin levels were undetectable.<sup>37</sup>

### 3.1. Effects on platelet aggregation

The ability of both phenolic compounds and wine extracts to inhibit platelet aggregation has been tested usually on human or rabbit platelets.

Resveratrol inhibited thrombin, collagen, PAF or ADP-induced platelet aggregation.<sup>38-41</sup> In addition, resveratrol inhibited thromboxane B<sub>2</sub>-induced platelet aggregation through inhibition of protein kinase C actions.<sup>42</sup> Quercetin has also been reported to inhibit thrombin and ADP-induced platelet aggregation<sup>43,44</sup> and to suppress the main pathways involved in platelet aggregation through inhibition of agonist-induced platelet activation, (Ca<sup>2+</sup>)<sub>i</sub> mobilization, granule secretion, and fibrinogen binding.<sup>43</sup> Additionally, quercetin induced inhibition of platelet aggregation on vascular endothelial cells<sup>45</sup> and collagen-stimulated platelet aggregation through glycoprotein VI signal pathways.<sup>44,46-48</sup>



**Figure 1.** Effect of wine on haemostatic system.

*Explanation of symbols:* R: Resveratrol, Q: Quercetin, RW: Red Wine, WW: White Wine, DRW: De-alcoholized Red Wine, DW: De-alcoholized Wine, PAI-1: Plasminogen Activator Inhibitor -1, t-PA: tissue-type Plasminogen Activator, u-PA: urokinase-type Plasminogen Activator

*Explanation of frames:* Dashed frame: *in vitro* studies, normal frame: *in vivo* studies

*Explanation of symbols:* ⊕: increase, ⊖: decrease

As far as wine extracts are concerning it was found that red wine polyphenolic extracts<sup>49</sup> and its catechin-anthocyanidin fractions exert a significant effect on ADP-induced platelet aggregation *in vitro*.<sup>50</sup> Our research team examined wine extracts from several grape varieties, for their ability to inhibit PAF-induced platelet aggregation. The results indicated that the polar extract fraction of all wines inhibited PAF-induced platelet aggregation, and the grape variation was the main factor for the observed biological activity and not the color of the grapes. These results were based on the fact that one red wine (main variety Cabernet Sauvignon) and one white wine (main variety Rombola), appeared to be the most potent ones.<sup>51-53</sup>

### 3.2. Effects on coagulation

The studies that exam the effectiveness of wine compounds on coagulation pathways focus mainly on the TF expression and action. Kaur et al, used a cocktail containing five major phenolics from red wines and cocktails lacking one or more of the constituents, and concluded that quercetin, and not resveratrol, is the principal active ingredient among red wine phenolics to inhibit TF induction in monocytes.<sup>54</sup> However, Di Santo et al,<sup>55</sup> investigated the role of resveratrol and quercetin on TF expression by endothelial and mononuclear cells. TF activity induced by any agonist (stimulation with bacterial LPS-, IL-1b or TNFa) was significantly reduced by both resveratrol and quercetin.<sup>56</sup>

### 3.3. Effects on fibrinolysis

The cardioprotective benefit of wine may be due, in part, to a modulation of the expression of proteins involved in fibrinolysis. However, there are not many studies concerning the effect of wine phenolics on fibrinolytic activity, and there are no data concerning the effect of wine extracts. Quercetin and resveratrol increased t-PA and u-PA antigen and mRNA levels in cultured human endothelial cells.<sup>57</sup>

## 4. Long-term intervention studies

A few studies investigated the long-term effect of wine consumption on haemostatic factors (table 1). Most of them have been carried out in healthy subjects, and only three studies involved patients. The age of the study population ranged from 40 to 60 years old, while only two involved younger population (table 1).

The control group was usually abstaining from alcohol (5 studies), used gin as reference beverage (3 studies) or de-alcoholized wine (2 studies), grape juice or grape tablets (3 studies). The intervention period lasted from two to five weeks and the amount of wine, which was consumed usually with meals, ranged between 150–500 mL in men and 150–200 mL in women. In most but not all of them, in order to limit the confounder factor of diet, antioxidant substances in the diet were carefully monitored, mainly the amount of fruit, vegetables, and other foods rich in such substances, such as black and green tea as well as cocoa products and black chocolate, so that the nutrient intake of all volunteers had the same antioxidant content (table 1).

It should also be mentioned that the 12h fasting before blood collection exclude the study of the direct effects of wine, since both polyphenols and ethanol have been clearance (post-absorptive phase) from blood.

### 4.1. Effects on platelet aggregation and endothelium

The common way to test platelet aggregation is to isolate platelet rich plasma (PRP) and measure platelets ability to aggregate in the presence of several agonists, such as ADP, collagen, thrombin or PAF. Generally the effect of wine on platelet aggregation according to *in vitro* experiments could be attributed in both ethanol and wine bioactive compounds. However, the effect of ethanol on platelet aggregation *in vivo* is difficult to be distinguished since none of the studies that measured platelet aggregation used another alcoholic beverage as control group, and usually compared their results with a group that abstain from alcohol (table 1).

From the data presented on table 1 it can be concluded that wine consumption is resulting in reduced ability of platelets to aggregate.<sup>58-62</sup> It is although notable that the observed ability of platelets is different among the various agonists. Collagen-induced platelet aggregation<sup>58-60,62</sup> is the most common one to be reduced, followed by ADP and thrombin,<sup>61</sup> while no data so far exist concerning PAF-induced platelet aggregation. It should also be mentioned that one study did not observe any effect on platelet aggregation,<sup>63</sup> probably due to the fact that the volunteers were young enough. Moreover, in the same study an increase in mean platelet volume (MPV), which is identified as an expression of metabolically and enzymatically active platelets,<sup>64</sup>



Table 1. Long-term intervention studies with wine – Effect on haemostatic factors.

Subjects	Study design	Control diet	Drink	Intervention period	Measurements	Results	Reference
48 (m+w) healthy age: 43.6±10.6	crossover	No	1. RW 2. Alcohol abstinence	4 weeks: 250 mL/day, with meals	D-Dimer, factor VII, PAI Ag, t-PA Ag, fibrinogen	RW: ↑ (t-PA Ag, PAI Ag), ↓ (fibrinogen, VII), ↑ D-Dimer, no statistically significant	[76]
69 (m+w) healthy mean age: 52	4-armed	No	1. RW 2. Water+RGTs (total dose) 3. water+RGTs (1/2 dose) 4. Placebo	4 weeks 1. m: 300 mL, w: 200 mL/day (m: 38.3g, w: 25.5 g ethanol/day) 2. m: 6, w: 4 RGTs/day 3. m: 3, w: 2 RGTs/day 4. m: 6, w: 4 RGTs/day at dinner	Factor VII, factor VIIc, fibrinogen	RW: 5% ↓ fibrinogen RGTs (total dose): ↑ 10% fibrinogen All interventions: no difference VIIc	[77]
87 (m+w) healthy age: 50.2±9.6	crossover	Yes	1. RW 2. Alcohol abstinence	3 weeks: 150 mL/day (15 g ethanol/ day), whenever they wished	Fibrinogen	m+w: RW: 3% ↓ fibrinogen	[79]
40 m healthy age: 37.6 ± 7.4	crossover	Yes	1. RW 2. Gin	28 days: 30 g ethanol/ day (RW 2×160 mL or Gin 100 mL), at dinner	Fibrinogen, VCAM-1, ICAM-1	RW or Gin: ↓ (fibrinogen, VCAM-1, ICAM-1)	[70]
12 m healthy age: 21–29	crossover	Yes	RW in 4 doses: 1. No wine/day 2. 2 glasses/day 3. 4 glasses/day 4. Binge drinking	5 weeks	Collagen- and ADP - induced platelet aggregation, plasminogen, t-PA, VII	RW (all doses vs abstain): ↑ plasminogen, ↓ collagen (1 µg/mL)-induced platelet aggregation RW (4 glasses vs abstain): ↓ t-PA no effect on (VII, ADP-induced platelet aggregation, collagen (4 µg/mL)- induced platelet aggregation)	[58]
20 (m+w) healthy mean age: 43	2-armed	Yes	1. RW 2. WW	2 weeks: 300 mL/day at dinner	Collagen-induced platelet aggregation	RW, WW: ↓ collagen-induced platelet aggregation (RW>WW)	[59]

(Continued)

Table 1. (Continue).

Subjects	Study design	Control diet	Drink	Intervention period	Measurements	Results	Reference
15 m healthy age: 23.13±0.45	1-armed	Yes	RW	4 weeks: 250 mL/day	ADP- and collagen induced platelet aggregation, fibrinogen, PA, APTT, MPC, MPV, P- and E-selectin, VCAM-1, ICAM-1	RW: no difference (ADP or collagen-induced platelet aggregation, PA, APTT, MPC, P-selectin, VCAM-1), ↓ fibrinogen, ↑ (MPV, ICAM-1, E-selectin)	[63]
92 m healthy mean age: 50.3	crossover	No	1. RW 2. Alcohol abstinence	3 weeks: 250 mL/day	PLV, fibrinogen and fibrinogen subfractions	RW: ↓ (PLV, fibrinogen)	[78]
6 m healthy age: 34±6	1-armed	No	RW	2 periods of 1 week: 3 glasses/day at dinner, or during evening	PAI-1, t-PA antigen, plasmin antiplasmin complexes	No significant differences	[82]
11 m healthy	crossover	-	1. RW 2. FJ+ethanol 3. DRW	4 weeks: 320 mL/day (30 g ethanol/day) during the two main meals	ADP or collagen-induced platelet aggregation, fibrinogen, plasminogen, t-PA Ag, vWF	RW, FJ+ethanol solution: ↓ (collagen-induced platelet aggregation, fibrinogen) All interventions: no difference (ADP-induced platelet aggregation, t-PA antigen, vWF and plasminogen)	[60]
24 m healthy age: 26–45	crossover	No	1. RW 2. WW 3. CGJ 4. CGJ+trans-resveratrol	4 weeks: juices: 500 mL/day wines: 375 mL/day	Plasma TXB2, IC50 for ADP and thrombin-induced platelet aggregation	WW vs RW: ↑ IC50 for ADP-induced platelet aggregation Both wines: ↑ IC50 for thrombin-induced platelet aggregation, reduction TXB2 2 CGJ: no difference ADP-induced aggregation or TXB2 CGJ: ↓ IC50 for thrombin-induced platelet aggregation CGJ+resveratrol: dramatic ↑ IC50 for thrombin-induced platelet aggregation	[61]

(Continued)

Table 1. (Continue).

Subjects	Study design	Control diet	Drink	Intervention period	Measurements	Results	Reference
42 m healthy mean age: 22±3,4	2-armed	Yes	1. RW+MD 2. RW+HFD	30 days: 240 mL/day	Fibrinogen, Vllc and Vllc, antithrombin III (AT III), protein C, protein S, t-PA Ag, PAI-1 Ag	MD vs HFD: ↓ (fibrinogen, Vllc, Vllc), ↑ protein S, RW (both diets): ↓ (fibrinogen, Vllc), ↑ (t-PA, PAI-1)	[75]
42 m healthy mean age: 22±3,4	2-armed	Yes	1. RW+MD 2. RW+HFD	30 days: 240 mL/day	ADP-, collagen- and epinephrine-induced platelet aggregation, serotonin secretion, BT, vWF	MD vs HFD: ↑ (BT, epinephrine-induced platelet aggregation) RW (both diets): ↓ ADP-induced serotonin secretion, ↑ collagen-induced secretion and platelet aggregation, no difference (BT, vWF)	[66]
13 (m+w) healthy age: 25–56	crossover	No	1. RW 2. WW	28 days: w: 23 g ethanol/day m: 32 g ethanol/day	Fibrinogen, collagen-induced platelet aggregation, t-PA, PAI-1, TXB2, platelet membrane microviscosity	RW: ↓ collagen-induced platelet aggregation WW: ↓ (fibrinogen, PAI-1) RW, WW: ↓ TXB2, no change t-PA RW: ↓ platelet membrane microviscosity WW: ↑ platelet membrane microviscosity	[62]
20 m healthy mean age: 34±9	crossover	Yes	1. WW 2. Gin	28 days: 30 g ethanol/day	VCAM-1, ICAM-1, E-selectin, P-selectin	WW, Gin: ↓ (VCAM-1, ICAM-1, E-selectin, P-selectin)	[68]
16 (m+w) hypercholesterolemic mean age: 51.6±8.1	2-armed	Yes	1. PGJ 2. RW	2 periods of 2 weeks 1. 500 mL/day 2. 250 mL/day	ICAM-1, VCAM-1, platelet aggregation	WW vs Gin: ↓ ICAM-1 PGJ: ↓ ICAM-1 RW or PGJ: no change (platelet aggregation, VCAM-1)	[65]

(Continued)



Table 1. (Continue).

Subjects	Study design	Control diet	Drink	Intervention period	Measurements	Results	Reference
67 m, high risk age: 55-75	crossover	Yes	1. RW 2. DRW 3. Gin	4 weeks: 30 g alcohol/day	ICAM-1, E-selectin, VCAM-1	DRW vs RW, Gin: ↓ E-selectin RW, DRW, Gin: ↓ VCAM-1 RW, DRW vs Gin: ↓ ICAM-1	[69]
35 (m+w) healthy Age: 50±9,6	crossover	No	1. RW 2. Alcohol abstinence	3 weeks 150 ml/day (15 g alcohol/day)	ICAM-1, VCAM-1	RW (w Vs m): ↓ VCAM-1 no difference ICAM-1	[71]
18 with non insulin type 2 diabetes (m+w) mean age: 64	crossover	No	1. RW or WW (chosen by the volunteers) 3. Alcohol abstinence	30 days: 171 mL/day with dinner	Plasminogen	RW or WW vs abstinence: no difference plasminogen	[83]

↑: increase, ↓: reduction, m: men, w: women, Ag: antigen RW: Red wine, WW: white wine, DRW: De-alcoholised Red Wine, CGJ: commercial grape juice, FJ: fruit juice, PGJ: purple grape juice, RGTs: red grape tablets, MD: Mediterranean diet, HFD: high fat diet, PAI: Plasminogen Activator Inhibitor, t-PA: Tissue-type Plasminogen Activator, vWF: von Willebrand Factor, BT: Bleeding Time PLV: Plasma Viscosity, MPV: Mean Platelet Volume, PA: Prothrombin Activity, APTT: Activated Partial Thromboplastin Time, MPC: Mean Platelet Count, TXB2: Thromboxane B2, IC50: concentration required for 50% aggregation, ICAM-1: Intercellular Adhesion Molecule-1, VCAM-1: Vascular Cell Adhesion Molecule-1

was detected. Also, Coimbra et al, demonstrated no effect of red wine in platelet aggregation in hypercholesterolemic patients, though without reporting the specific agonist used.<sup>65</sup>

The results from the comparison between white and red wines on platelet aggregation indicate that both types are associated with favorable effect on platelet aggregation.<sup>59,61,62</sup> The effect of red wines seems to be more enhanced, probably due to their higher phenolic amount. Moreover Pace-Asciak CR et al,<sup>61</sup> found that both wines (red and white) but none of the grape juice (plain grape juice or enriched with trans-resveratrol) reduced TXB<sub>2</sub>.

Mezzano D et al, studied the effect of wine consumption in combination with the dietary habits of young healthy volunteers.<sup>66</sup> The wine intake resulted in decreased ADP-induced serotonin secretion, but increased collagen-induced secretion and platelet aggregation.<sup>66</sup> This finding is against the widespread notion that wine has a protective effect against platelet aggregation. Mezzano D et al, explain their finding, considering the rebound effect that had been observed after binge alcohol intake, since the rise of platelet aggregation happens several hours after wine consumption.<sup>66</sup> However, it should be mentioned that this phenomenon is reported to be attributed to an excess of lipid peroxidation after binge alcohol drinking known to increase platelet reactivity, especially to thrombin and not in moderate consumption of wine.<sup>67</sup> In the same point of view, Mansvelt et al, reported that in their study, volunteers who were maintaining a Mediterranean diet and consumed red instead of white wine displayed decreased platelet aggregation induced by collagen concentration that was attributed to both diet and red wine.<sup>62</sup> When lower collagen concentrations were used, both wines (white and red) showed anti-aggregation activity, which proves that white wine has also antiplatelet activity.<sup>62</sup>

Regarding the potential role of endothelium function on platelets activation, few studies have estimated the effect of long term wine consumption on the levels of adhesion molecules such as VCAM-1 and ICAM-1, and on selectins.<sup>63,65,68-71</sup> The majority of the studies revealed a decrease in VCAM-1 and ICAM-1 levels after de-alcoholised wine or wine or gin consumption, indicating that these effects may be attributed to both ethanol and phenolic compounds.<sup>68,69,71</sup> However,

Djurovic et al,<sup>71</sup> found no effect of red wine consumption on ICAM-1 in men and women, but only a decrease in VCAM-1 in women, probably through the estradiol effect on inhibition of monocyte adhesion.<sup>72</sup> The wine amount used was very small (15 g alcohol/day) and this may also explain the fact that no effect on ICAM-1 levels was observed.<sup>71</sup> In contrast, Ciancarelli MG et al, reported an increase in ICAM-1 and E-selectin after red wine consumption.<sup>63</sup> The authors conclude that increase of plasma wine phenolics is insufficient to counteract the influence of ethanol on mechanisms responsible for the endothelial activation.

#### 4.2. Effects on coagulation

In order to determine the effects on coagulation, some factors, such as fibrinogen, VII factor, vWF, plasma viscosity (PLV), TXB<sub>2</sub>, bleeding time (BT) and prothrombin activity (PA), are measured in blood plasma. Fibrinogen, factor VII and vWF are important, independent risk factors for CVDs.<sup>73,74</sup> Fibrinogen, as already mentioned, is involved in many pathophysiological mechanisms which refer to coagulation, such as platelet aggregation, endothelium function, forming the substrate for thrombin and representing the final step in the coagulation cascade.<sup>73</sup> Consequently it is the most often measured factor. Studies in which fibrinogen were estimated showed a reduction of it.<sup>60,62,63,75-79</sup> The observed decrease seems to be accompanied with alcohol consumption in any type (red/white wine or gin) and did not appear in group that abstain from alcohol or consumed de-alcoholised wine or grape juice. Although it is not yet clear how ethanol decreases fibrinogen levels, it seems that it influences fibrinogen conformation and stability.<sup>80</sup>

There are few data about the effect of long term wine consumption on FVII and vWF, and they are conflicted. Regarding FVII, Avellone G et al<sup>76</sup> showed a reduction of this factor as a result of red wine consumption, but they could not clarify whether this effect was due to ethanol or red wine polyphenols. Besides Hansen et al<sup>77</sup> did not find any changes in FVII factor after red wine intake.

Regarding vWF, Pellegrini N et al. showed no effect after red wine or fruit juice with ethanol on vWF,<sup>60</sup> even though in a meta-analysis of Rimm EB et al<sup>81</sup> was demonstrated that alcohol tended to lower vWF. In addition, Mezzano D et al, reported that independently of

the background diet wine consumption reduced FVII levels<sup>75</sup> and did not affect vWF levels or BT.<sup>67</sup>

Jensen J et al<sup>78</sup> suggested that alcohol consumption reduced PLV and mentioned that this reduction, which maintained during the 3-week alcohol abstinence period, indicated a prolonged effect of ethanol on lowering viscosity.

#### 4.3. Effects on fibrinolysis

The most commonly measured anti-coagulation indices are t-PA, PAI, fibrin degradation products (D-dimer) and plasmin/antiplasmin complexes (PAP). Sufficient function of fibrinolytic system is due to increased t-PA and/or reduced PAI.

Pikaar NA et al,<sup>58</sup> demonstrated that plasminogen increased accordingly with the wine amount that was consumed, and this could be considered as a beneficial effect. But, conversely, t-PA displayed a remarkable decrease, which compensates the beneficial effect mentioned above.<sup>58</sup>

Avellone G et al found a t-PA and PAI increase but reduced inactive t-PA/PAI complexes in blood, which lead to increase free t-PA levels.<sup>76</sup> They also referred an increase of D-dimer levels (not statistically significant), which reflects fibrin degradation, after red wine consumption.<sup>76</sup> Moreover, van Golde et al, noted that PAI-1 antigen and its activity tend to rise while t-PA activity and PAP complexes tend to drop, after red wine.<sup>82</sup> The authors conclude that since thrombocytes are a source of PAI-1 and alcohol is known to affect certain platelet functions, prolonged alcohol intake might stimulate fibrinolysis by depleting platelets from their PAI-1 content, gradually leading to lower PAI-1 concentrations in the circulation.<sup>82</sup>

In addition, Mezzano D et al, reported that independently of the background diet red wine consumption increased both t-PA and PAI-1 levels.<sup>75</sup>

In diabetic patients Bantle AE et al<sup>83</sup> did not notice any difference in plasminogen after red or white wine intake, maybe because the small amount (171 mL/day or 18 g ethanol/day) of wine consumed. This amount was exceeded the maximum intake recommended for women with diabetes, which is 15 g alcohol/day, but was less than the maximum intake recommended for men with diabetes, 30 g alcohol/day.<sup>84</sup>

## 5. Conclusions

The already existing results, mainly in vitro studies, support a potential anti-thrombotic effect of wine compounds that could participate in the protection against cardiovascular diseases. The long-term intervention studies demonstrate that wine consumption could modulate the haemostatic system. The most clearly outcome is the lower levels of fibrinogen, an effect that seems to be attributed mainly to ethanol. The effect on platelet aggregation is a more complicated process and a safe outcome is not easy to be concluded. However, wine micro-constituents seem to be mainly responsible for this favorable effect, but without easily exclude the interference of alcohol. As far as fibrinolysis is concerned, the data are not yet enough for conclusive remarks.

Overall, more controlled long-term intervention studies should be performed in order solid evidences to be obtained, concerning the effect of wine consumption on haemostatic system, and also these results should be extrapolated to patients or high CVD risk people.

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