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# French Paradox, Polyphenols and Cardiovascular Protection: The Oestrogenic Receptor-α Implication

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## 1. Introduction

Several epidemiological and clinical studies confirm an inverse correlation between a diet rich in vegetables, fruits, and red wine, in cancer development and chronic diseases such as cardiovascular diseases. This is linked to the presence in these aliments of high levels of nutrients of vegetal origin called phytonutrients. They are natural phytochemical compounds contained in plant food; they are not vitamins or minerals but they have beneficial effects on the health, sometimes acting in association with other essential nutrients. Phytonutrients are divided in three families: the terpenes, the sulfuric compounds, and the polyphenols which are the subject of this chapter.

Polyphenols are the most important group of phytonutrients. They are not only present in fruits and vegetables but also in seeds, spices, herbs and teas, at different concentrations and molecular structures in correlation with the aliment involved. The most studied polyphenolic compounds for their vascular action are resveratrol, delphinidin, quercetin and tannins contained in red wine. Indeed, the red wine is the beverage the most correlated to cardiac and vascular protection. It could reduce of 40% the risk of myocardial infarction, and of 25% the risk of vascular thrombotic events in brain.

Elevated content in polyphenols of red wine seems to play a benefic role in the mechanism of vascular and cardiac protection, not only by its anti-oxidant but also by its anti-thrombotic properties. Thus, more recently, research works were focused to study the vascular and cardiac effects of non-alcoholic fractions of red wine and have identified the oestrogenic receptor-α (ERα), as the preferential endothelial target of these molecules.

First, this chapter is focused on the "French Paradox" history. Then, we have described successively the composition and content of these compounds in food and beverages, and the epidemiological and fundamental studies showing how red wine polyphenolic compounds (RWPC) are able to improve endothelial function and cardiovascular protection. Finally, we explain the effects of oestrogens on the cardiovascular system and the implication of ERα in the beneficial cardiovascular effects of these natural molecules that could be used to prevent or treat cardiovascular diseases.

## 2. French paradox history

For a long time, it was suggested that a high fat intake is associated with an elevated risk of mortality for cardiovascular diseases in Anglo-Saxon populations. In contrast, several epidemiological studies have revealed a relatively low incidence of coronary heart diseases (CHD) in the French population, despite a high dietary intake of saturated fats. This was potentially attributable to the consumption of red wine (Renaud et al., 1999).

One of the first epidemiological studies conducted on 100,000 subjects in 1970 by Doctor Arthur Klatsky, a cardiologist of the Oakland Hospital in California, clearly evidenced that people following a diet with moderated consumption of red wine (1-3 glasses per day), showed a very little risk of death by CHD (Renaud et al., 1999). This was confirmed in 1979 by Doctor Saint-Léger which evidenced a negative correlation between wine consumption and mortality for CHD in men and women (from 55 to 64 years old), in more than 18 developed countries. Furthermore, Italy and France showed a lower level of mortality by myocardial infarction (about 3 or 5 folds less) compared to Anglo-Saxon populations such as Irish, North-American, and Scottish (Renaud et al., 1999). On the other hand, it has been demonstrated that to drink one glass of wine per day reduced death risk by CHD, but to drink more than three glasses of wine per day was associated with an increased death rate (Thun et al., 1997). In wine drinkers, the lower all-cause mortality was associated with a significant reduction in mortality from CHD, for about 45-48%, and other cardiovascular diseases (CVD), for about 39-40% (Renaud et al., 1999). Other studies have also suggested that both non-drinkers and heavy-drinkers have a higher risk of cardiovascular mortality than those who drink wine moderately (Providencia, 2006).

Then, numerous correlation studies concerning the strict relation between consumption of fat and CHD mortality have been conducted in various countries. In one of the most interesting ones, Artaud-Wild and colleagues examined the relation between CHD mortality and the intake of foodstuffs and nutrients in 40 different countries. After they have defined a cholesterol–saturated fat index (CSI), they studied this correlation in 100,000 men (aged 55 to 64 years) in all the countries studied. The findings of this epidemiological study evidenced that France had a CSI of 24 per 1000 kcal and a CHD mortality rate of 198; whereas Finland had a CSI of 26 per 1000 kcal and a CHD mortality rate of 1031 (Ferrières, 2004). The high consumption of saturated fatty acids suggests that French subjects could be exposed to a high risk of CHD (Renaud 1992), but it is in fact not the case considering the low rate of CHD mortality observed. Then, much attention has been focused on the possible superior protective effect of red wine consumption relative to those of other alcoholic beverages. So, the differential effects of wine, beer, and spirits have been examined. European research carried out in France and Denmark has shown that wine consumption was associated with a decrease of 24 up to 31% of all cause mortality; little to moderate wine drinking leads to a lower mortality rate from CVD than having an equivalent consumption of beer or spirits (Ferrières, 2004).

Nevertheless, alcohol consumption, from whatever sources, appears to have a J-shaped curve, whereby a modest intake is beneficial and either no intake or excess is harmful. This is confirmed by several studies: the Framingham study (Fuchs et al., 1995), the British Doctors study (Doll et al., 1994), the Cancer Prevention study of Thun and coworkers, conducted on about 490,000 persons (Thun et al., 1997), the Nurse Health study (Emberson

et al., 2005), and other epidemiological investigations (Gaziano et al., 2000; Suh et al., 1992). It would take too long to report on all the studies dealing with the relations between alcohol and CHD.

The mechanisms involved in the protective role of red wine include anti-platelet, anti-coagulatory, improved glucose control, and anti-inflammatory effects as shown in MONICA (multinational MONItoring of trends and determinants in CArdiovascular disease) study (Imhof et al., 2004). The World Health Organization had collected all the results of these data, evidencing the protective role of moderated red wine consumption in cardiovascular disease development. Despite the high consumption of saturated fatty acids, why the French people do not develop a high CHD risk? This is the central question behind the "French Paradox" concept. The French epidemiologist Serge Renaud evidenced for the first time this "Paradox", which is defined as the light level of incidence of CVD in people following a diet containing a high quantity of saturated fatty acids, but also having a moderated red wine consumption (Pechanova et al., 2006, Renaud et al., 1999).

The results of Criqui and colleagues (Criqui & Ringel, 1994) were found in agreement with the French Paradox. In 21 developed countries, subjects in an age range of 35 to 74, without differences linked to gender, were studied and assessed at four time periods: 1965, 1970, 1980, and 1988, respectively. The independent variables chosen were: consumption of wine, beer, spirits, animal fats, vegetables, and fruits. Ischemic heart disease and all-cause mortality were finally assessed. Wine was the beverage most strongly negatively correlated with coronary diseases. Animal fat had a tendency to positive correlation, while fruits were negatively correlated. On the light of the numerous epidemiological studies, a protective activity of wine against CVD has been widely described, suggesting that moderated consumption of wine could reduce the risk of myocardial infarction and the risk of vascular thrombosis of brain vessels.

So many questions arose next. What were the elements that differentiate the wine (especially red wine) of other spirits? What were the processes responsible for the beneficial effect of wine consumption? What, in wine, promoted this effect?

## 3. Differences in polyphenolic compositions in food and beverages

Polyphenolic compounds are the biggest group of phytochemicals characterized by one or more phenolic rings associated with one or more hydroxyl groups, free or implicated in an ester, ether or eteroside function (Richter, 1993). This family of substances includes more than 8000 phenolic structures currently known, and among them, over 4000 flavonoids have been yet identified in plants and the list is constantly growing (Bravo, 1998; Cheynier, 2005; Harborne & Williams, 2000). Flavonoids contain a structural backbone C6-C3-C6, characterized by two C6 units of phenolic nature; while the non-flavonoids are phenolic acids divided in two main types, benzoic acid and cinnamic acid derivatives, based on C1-C6 and C3-C6 backbones, respectively (Tsao, 2010). The phenolic acids are usually contained as free molecules in fruits and vegetables. Phenolic acids could be also found in the bound form in grains and seeds (Chandrasekara & Shahidi, 2010).

Polyphenols are enrolled in numerous physiological functions in vegetal organisms: cell development, latent buds, blooming, and tuber formation. These substances are involved in

the color of fruits, in particular they play a main role to confer the red color of ripe fruits, and in the savor and properties of food (Bahorun, 1997). Polyphenols include yellow, orange, red and blue pigments and various compounds implicated also in bitterness and astringency of unripe fruits, resulting from interaction of tannins with salivary proteins. Moreover, some volatile polyphenols, in particular vanillin and eugenol, are potent odorants and are responsible of the characteristic odor of cloves (Cheynier, 2005).

The content of polyphenolic compounds is particularly elevated in red wine but also in skin of red grapes, red fruits, cereals, several vegetables such as red onions, chocolate, tea, and coffee with different polyphenolic composition and percentage according to the kind of vegetal food or beverage (see Table 1) (Bravo, 1998; Tsao, 2010). Considering the diversity and wide distribution of polyphenols, they have been classified by their source of origin, biological function, and chemical structure. In plants, the majority of polyphenols exists as glycosides associated to sugar units or acylated sugars linked at different positions of the polyphenolic skeletons (Tsao, 2010).

| Food | Total polyphenols (mg/100 g of dry mutter) | Food | Total polyphenols (mg/100 g of fresh mutter) |
|---|---|---|---|
| **Cereals:** | | **Vegetables:** | |
| Barley | 1200-1500 | Onion | 100-2025 |
| Millet | 590-1060 | | |
| **Legumes:** | | **Fruits:** | |
| Black gram | 540-1200 | Apple | 27-298 |
| Green gram | 440-800 | Blackcurrant | 140-1200 |
| Pigeon peas | 380-1710 | Grapes | 50-490 |
| | | Raspberry | 37-429 |
| **Beverages** | **Total polyphenols (mg/L)** | **Beverages** | **Total polyphenols (mg/L)** |
| Orange juice | 370-7100 | Tea | 750-1050 |
| Red wine | 1000-6500 | Coffee | 1330-3670 |
| White wine | 200-300 | | |

Table 1. Plant food and beverages with high levels of total polyphenolic compounds (from Bravo, 1998).

Some flavonoids such as the isoflavones are mostly found in plants of the leguminous family. Genistein and daidzein are the two main isoflavones found in soybeans and red clovers (Tsao et al., 2006). The flavonoid subgroup of the neoflavonoids is rarely present in food plants, but the open-ring chalcones are still found in fruits, in particular in apples and hops of beers (Tsao et al., 2003; Zhao et al., 2005). In contrast, other flavonoid subgroups such as flavones, flavonols, flavanones and flavanonols are most common and ubiquitous in the plant kingdom and in particular quercetin and kaempferol (Tsao, 2010). Flavanols or flavan-3-ols, also called catechins, are found in many fruits, the skin of grapes, apple and

blueberries (Tsao et al., 2003). Catechin, epicatechin (isomer of catechin with *cis* configuration), and their derivatives, gallocatechins, are the major flavonoids contained in tea leaves and cacao beans and thus in chocolate (Si et al., 2006; Prior et al., 2001).

The red, blue and purple pigments of the majority of flower petals, fruits and vegetables and certain varieties of grains, for instance black rice, mainly contain anthocyanidins and in particular cyanidin, delphinidin, pelagonidin, and their methylated derivatives (composed up to 90% of anthocyanins). The color of these kinds of molecules can change with the pH and temperature: they are red in acidic and blue in basic conditions (Tsao, 2010). In grapes and apples, anthocyanins are found only in the red varieties (Cheynier, 2005).

Polyphenols are highly unstable species and, accordingly, their chemical structure can change during food and beverage processing and storage, leading to new compounds with different properties compared to their precursors (Xu et al., 2011). In particular, total catechin contents of fresh fruits can decrease of about 26% up to 58% after home preparation or industrial transformations (Cheynier, 2005).

Wine is a hydro-alcoholic acid solution. Indeed, its major component is water (80-90%) and ethanol (10-14%) implicated in the solubilization of polyphenols. The fraction of polyphenolic compounds contained in wine is high in red wine and its composition depends of the kind of wine. More precisely, generally red wine contains 1.2 gr/L of polyphenolic compounds while white wine contains only 0.2 gr/L of these compounds and, besides, does not contain the molecules involved in the red color such as the anthocyanidins and in particular delphinidin (see Table 2) (Pellegrini et al., 2000; Soleas et al., 1997). Interestingly, the level of these compounds in red wine is modified by the fermentation process used during wine production. Vinification variations and techniques are known to affect the phenolic composition of red wines. The fermentation of grape juice into wine is a

| Compounds (mg/L) | White young wine | White aged wine | Red young wine | Red aged wine |
|---|---|---|---|---|
| **Total phenols** | 215 | 190-290 | 1300 | 955-1215 |
| **Non flavonoides** | 175 | 160-260 | 235 | 240-500 |
| **Flavonoides** | 30 | 25 | 1060 | 705 |
| **Catechins** | 25 | 15 | 200 | 150 |
| **Anthocyanins** | 0 | 0 | 200 | 20 |
| **Soluble tannins** | 5 | 10 | 550 | 450 |

Table 2. Polyphenolic compound contents in several types of wine (from Soleas et al., 1997).

complex microbial reaction, traditionally due to the sequential development of various species of yeast and lactic acid bacteria. In the past, wine was produced by natural fermentation of grape juice by yeasts originating from grapes and winery equipment (Ribereau-Gayon et al., 2000). Nowadays, another kind of fermentation process, the carbonic maceration, is more and more used to produce wine. With this method, freshly harvested bunches of grapes are allowed to ferment in carbonic anaerobiosis, in an atmosphere saturated with carbon dioxide (Navarro et al., 2000). The absence of oxygen is important to

reduce the oxidation of polyphenolic compounds, especially the monomeric anthocyanidins such as malvidin and delphinidin. The preservation of these molecules by this new carbonic process increases their final levels in wine compared to the traditional maceration of grapes (Pellegrini et al., 2000). Furthermore, the wine ageing could modify polyphenol composition and levels in white and red wines with a time-dependent reduction of catechins and anthocyanidins contents (see Table 2) (Pellegrini et al., 2000; Soleas et al., 1997).

It is interesting to note that, after food or beverage intake, the degradation and absorption of polyphenols within the gastrointestinal tract depend on the nature of the polyphenolic compound but also of the intestinal microflora, with subsequent fermentative effect on other dietary components. Thus, these molecules are modified by intestinal bacteria but they can influence in return microflora and its fermentative capacity (Bravo, 1998). Several recent studies are focused in how processing and beverage composition might influence phenolic profiles and bioavailability of an individual polyphenol. Specifically, they showed the impact of beverage formulations and the influence of digestion on stability, bioavailability, and metabolism of bioactive polyphenolic compounds from food and beverages. For example, the co-formulation with ascorbic acid and other phytochemicals may improve absorption of these health-promoting phytochemicals (Ferruzzi, 2010). Thus, it is critical to develop beverage products designed to deliver specific health benefits.

## 4. Beneficial effects of RWPC in cardiac and vascular functions

Evidences from different experimental studies has suggested the presence of molecules with anti-oxidant properties in red wine, such as tannins and other flavonoids. These molecules could be key factors in the protective effects observed (Vidavalur et al., 2006). Red wine, might provide, through the polyphenols (non-flavonoids and flavonoids), an anti-oxidant role, leading to additional protection mechanisms in coronary arteries (Liu et al., 2007). Thus, RWPC are able to decrease oxidative stress, enhance cholesterol efflux from the vascular wall, and inhibit lipoprotein oxidation. These components may also increase nitric oxide (NO) bioavailability, thereby antagonizing the development of endothelial dysfunction. Thus, RWPC are able to modify several factors involved in the development of CDV by a direct action on vascular cells and in particular in endothelium, thus playing a preventive role in the development of atherosclerosis, hypertension and myocardial infarction. One of the most studied molecules, the resveratrol, found in grapes and wine in significant amounts, is implicated in this beneficial action because of its ability to act as an anti-oxidant and an inhibitor of platelet aggregation (Kopp, 1998; Providencia, 2006).

On the light of several recent major studies, the consumption of RWPC reduces the incidence of CVD probably by their ability to change many factors and intermediate markers implicated in these diseases. A beneficial association between consumption of food rich in polyphenols, especially flavonoids, and other chronic diseases was also investigated. People with very low consumption of flavonoids showed a higher risk to develop chronic and degenerative diseases including cardiovascular disorders, diabetes, obesity and neurodegenerative disorders compared to people with a diet rich in polyphenols (Mojzisova and Kuchta, 2001). Thus, it is important to better identify factors that may affect the bioavailability of specific phenolic components from food and beverages and to better understand how these molecules are able to act positively on organism.

### 4.1 Role on nitric oxide production

RWPC are able to improve NO production and vascular endothelium-dependent relaxation. This is possible through their action to increase endothelial nitric oxide synthase (eNOS) expression and activation *in vitro* on endothelial cells and *ex vivo* on rodent vessels.

One of the earliest works on this purpose was conducted in 1993 by Fitzpatrick and coworkers. They found that extracts from grapes and wine containing polyphenols were able to induce an endothelial-dependent vasorelaxation, probably by NO production and elevated accumulation of guanosine 3',5'-cyclic monophosphate (cGMP) (Fitzpatrick et al., 1993). The mechanisms and the identification of the molecules involved in these vascular effects were still unknown. These findings were confirmed later by another study, in which it was evidenced an endothelial and NO-dependent relaxation induced by a non-alcoholic red wine extract, RWPC, and leucocyanidol administrated directly at low concentrations (from $10^{-4}$ to $10^{-2}$ g/L) *ex vivo* on noradrenaline pre-contracted rat aortic rings (Andiambeleson et al., 1997). This was associated with an enhanced NO generation and a seven-fold increase in cGMP accumulation. A non-relevant relaxant effect was found using the structurally closely related polyphenol, catechin, at the same concentrations on the same vessels. To better determine which group(s) of polyphenols were able to cause endothelial-dependent vasorelaxation, the same team separated RWPC by chromatography in 10 fractions. These fractions were tested separately for their capacity to induce the vascular relaxation on rat aortic rings with and without endothelium. In this study, it was shown that fractions containing high polymeric condensed tannins produced a moderate vasorelaxation, at relatively high concentrations ($10^{-2}$ to $10^{-1}$ g/L) and flavan-3-ol, (+)-epicathtechin, also failed to produce endothelium-dependent vasorelaxation. In contrast, oligomeric condensed tannins and fractions containing anthocyanins, and in particular delphinidin, displayed strong vasorelaxant properties (maximal relaxation in the range of 59–77%) comparable to the original RWPC mixture (Andriambeloson et al., 1998).

The same endothelial-dependent relaxation was also found in small mesenteric arteries, but it was due to both NO and endothelium-derived hyperpolarizing factor (EDHF) and it was absent in vessels without endothelium. The NO component of the relaxation was linked to eNOS activity and absent when the NOS inhibitor, the $N^G$-nitro-L-arginine methyl ester (L-NAME), was used, while the EDHF component was abolished by partial depolarization with KCl. Thus, NO and EDHF are both required to promote endothelium-dependent relaxation produced by RWPC in mesenteric resistance arteries (Duarte et al. 2004).

Several studies conducted *in vitro* confirmed these results. In bovine aortic endothelial cells (BAECs) treated with RWPC ($10^{-2}$ g/L), it was found an increased $Ca^{2+}$-dependent eNOS activation and a subsequent increased NO production. These required the presence of extracellular $Ca^{2+}$, although polyphenolic compounds were able to mobilize $Ca^{2+}$ from intracellular stores and were also able to activate phospholipase C (PLC) and tyrosine kinase (TK) pathways. Provinols™, which contain similar types of polyphenols compared to the RWPC used by Andriambeloson and coworkers, and delphinidin displayed differences in the process leading to this increase in endothelial intracellular $Ca^{2+}$, thus illustrating multiple cellular targets of natural dietary polyphenolic compounds (Martin et al., 2002). This effect of RWPC in this cell model is associated with an increased superoxide ion ($O_2^-$) production in order to promote $Ca^{2+}$ signaling (Duarte et al., 2004). Most recently, it was found that resveratrol, a stilbenoid contained in wine, used at nanomolar concentrations,

rapidly activated extracellular-signal-regulated kinase (ERK)1/2 in BAECs and, in turn, activated eNOS (Klinge et al., 2005). The same effect of resveratrol was confirmed later in another model of endothelial cells, the human umbilical endothelial cells (HUVECs). The implication of ERα in the eNOS-pathway activation by resveratrol was also evoked (Klinge et al., 2008).

Interestingly, beneficial effects on hemodynamic parameters and on endothelial function were confirmed *in vivo* after a short-term oral administration of RWPC in normotensive rats at the dose of 20 mg/kg for 7 days. Indeed, these rats, after only 4 days of treatment, showed a significant decrease in blood pressure (129 ± 4 mmHg versus 141 ± 2 mmHg for control non-treated rats). This effect was associated, *ex vivo,* with an increased endothelium-dependent relaxation to acetylcholine in aortic rings, that was related to the enhanced endothelial NO activity. Nevertheless, RWPC induced at the same time gene expression of inducible NOS (iNOS) and inducible cyclooxigenase (COX-2), with subsequent endothelial thromboxane $A_2$ release in the arterial wall, maintaining unchanged agonist-induced contractility (Diebolt et al., 2001). The *in vivo* effects of Provinols™ (40 mg/kg per day) on hemodynamic and functional cardiovascular changes were also investigated during the inhibition of NO synthesis by L-NAME (40 mg/kg per day for 4 weeks) in rats. This model of hypertension evidenced that RWPC partially prevent L-NAME-induced hypertension, cardiovascular remodeling, and vascular dysfunction or accelerate the decrease of systolic blood pressure after L-NAME administration. These beneficial effects were mediated by the increased NO-synthase activity and the oxidative stress prevention (Bernatova et al., 2002; Pechanova et al., 2004). Nevertheless, most recently, the anti-hypertensive effects of RWPC, orally administered for 5 weeks at the dose of 40 mg/kg by gavage, was confirmed in female spontaneously hypertensive rats (SHR). The authors suggested that a chronic treatment with RWPC reduced hypertension and vascular dysfunction in this model of hypertension, rather through reduction in vascular oxidative stress (Lopez-Sepulveda et al., 2008). This findings revealed a major preventive role of these substances in cardiovascular complications linked to hypertension.

Polyphenol vascular activity in human vessels after food or beverage intake was confirmed by several studies that detected these molecules in human plasma at individual levels in the range of 0.5 to 1.6 μmol/L, comparable to the concentration required to induce 50% of the maximal relaxation, comprised between 1 and 10 μmol/L of active fractions (Paganga and Rice-Evans, 1997). Polyphenols detected in human plasma are in the range of 2.5 μg/ml after a 100 ml red wine intake (Duthie et al., 1998). Most interestingly, the vasorelaxant effect of polyphenols from red wine was confirmed also in men in which NO and normalized flow-mediated dilation were measured before and 30, 60, and 120 minutes after red wine consumption (Boban et al., 2006; Papamichael et al., 2004). Moreover, RWPC are not only able to improve NO production, for their anti-oxidant and anti-inflammatory properties but also increase the NO bioavailability in the vascular wall, by decreasing its transformation in peroxynitrite induced by $O_2^-$ during oxidative stress.

Altogether, these findings suggest a possible beneficial effect of a diet rich in these vasoactive polyphenolic compounds to prevent hypertension as the effective concentrations of these molecules can be reached in human plasma and they might act on the endothelium *in vivo*. The RWPC responsible of this effect (resveratrol, delphinidin and tannins) could be used for hypertension treatment.

### 4.2 Protective role in cardiac function and ischemic diseases

RWPC, administrated in a preventive purpose way, are able to reduce cardiac or cerebral ischemic injuries in rat models of myocardial infarct and stroke, respectively. Left ventricular hypertrophy, myocardial fibrosis and vascular remodeling were investigated in rats during chronic inhibition of NOS activity by L-NAME. The *in vivo* treatment of rats with Provinols™ (40 mg/kg per day) reduced not only the increase in blood pressure caused by L-NAME treatment, but also protein synthesis in the heart and aorta caused by the chronic inhibition of NO synthesis, finally reducing myocardial fibrosis. These effects were associated with an increase of NOS activity, a moderate enhancement of eNOS expression and a reduction of oxidative stress in the left ventricule and aorta (Pechanova et al. 2004).

The protective cardiac effect of polyphenols was confirmed by another study, conducted in rats and observing, *ex vivo*, the effects of short-term oral administration of RWPC (20 mg/kg per day for one week) on cardiac responsiveness and ischaemia-reperfusion injury. The involvement of NO in the cardiac effects of RWPC was evaluated using L-NAME (2 mg/kg per day for one week), a dose which did not affect blood pressure, in a group of rats previously treated with polyphenols. Heart reactivity was studied in perfused isolated hearts by the Langendorff method. The hearts harvested from RWPC-treated rats showed a lower basal pressure, a greater heart rate and decreased inotropic responses to either isoprenaline or carbachol, the agonists of beta-adrenoceptors or muscarinic receptors, respectively. RWPC treatment did not modify cardiac expression of eNOS or Cu/Zn superoxide dismutase, a protein involved in oxidative stress protection. However, it was found increased nitrite levels in the coronary effluent from hearts harvested from RWPC-treated rats, suggesting an increased NO production. Most interestingly, in ischaemia-reperfusion protocols, RWPC treatment reduced infarct size, oxidative stress, and the myocardial content of end products resulting from lipid peroxidation, malondialdehyde and 4-hydroxynonenal, without affecting post-ischaemic contractile dysfunction. All these observed effects were prevented by L-NAME treatment, suggesting the involvement of NO in this protective role of RWPC on heart. In conclusion, these data showed that short-term treatment with RWPC could prevent the heart injury caused by cardiac ischemia through oxidative stress decrease and NO pathway improvement (Ralay-Ranaivo et al., 2004).

The presence of melatonin in red wine was demonstrated in most recent studies. Lamont and co-workers investigated the cardio-protective role of both melatonin and resveratrol. These molecules improve heart protection via the activation of the newly discovered survivor activating factor enhancement (SAFE) pathway. This pro-survival signaling pathway involves the activation of pro-inflammatory molecules such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) and the signal transducer and activator of transcription 3 (STAT3). They realized *ex vivo* studies in isolated perfused hearts from either wild type or total TNFα receptor 2-knockout or cardiomyocyte-specific STAT3-deficient mice. The protocols of heart injury by ischemia-reperfusion showed that both resveratrol and melatonin, at concentrations found in red wine, significantly reduced infarct size in wild-type mice (25% ± 3% versus 69 ± 3% in the control non treated mice) but failed to protect hearts in both knockout mice. Perfusion with either melatonin or resveratrol increased STAT3 phosphorylation prior to ischemia by 79% and 50% versus the control, respectively. These findings suggest that both melatonin and resveratrol contained in red wine, protect heart via the SAFE pathway, in an experimental model of myocardial infarction (Lamont et al., 2011).

Concerning cerebral ischemia, Ritz and co-workers investigated the beneficial effects of chronic or acute treatment of RWPC in rats submitted to an experimental model of stroke. Rats were treated for the chronic treatment with RWPC (30 mg/kg per day) dissolved in drinking water for one week, before being subjected surgically to a transient middle cerebral artery occlusion followed by reperfusion. The volume of the ischemic lesions was assessed 24 h after reperfusion and a proteomic analysis of brain tissues was performed, to study the effects of RWPC on expression of proteins involved in cerebral stroke injury. Treatment with RWPC partially or completely prevented the increased levels of excitatory amino acids (aspartate, glutamate and taurine) that characterized the response to ischemia in control rats, significantly reduced brain infarct volumes, and enhanced residual cerebral blood flow after brain ischemia. This was associated to lower basal concentrations of energy metabolites including glucose, lactate, and free radical scavengers such as ascorbate, in the brain parenchyma, compared with untreated rats. No difference in uric acid levels was found. These effects resulted in arterial vasodilatation, as the internal diameters of several arteries were significantly enlarged after RWPC treatment. Proteomic analysis revealed that RWPC could be able to modulate *in vivo* the expression of proteins involved in maintenance of neuronal caliber and axon formation, in protection against oxidative stress, and in energy metabolism (Ritz et al., 2008a). These data were confirmed in the second work of the same team, about the protective effects of an acute treatment with RWPC (a bolus of 0.1 mg/kg), realized by an intracerebral microdialysis started at the beginning of the stroke. In this study, RWPC induced increased residual blood flow after 10 minutes of the reperfusion following ischemia and reduced size of the cerebral ischemic infarct in both cortex and striatum. The acute treatment of rats with RWPC dramatically decreased the extracellular concentrations of excitatory amino acids and, concomitantly, increased the levels of free radical scavengers such as uric and ascorbic acids (Ritz et al., 2008b). Altogether these findings provide an experimental evidence of the advantage to use RWPC for the prevention, in patients with high risk to developing ischemic events, or in the acute treatment of patients during stroke.

Angiogenesis is a main process involved in the repair of ischemic injury. The role of RWPC in angiogenesis was also investigated and several studies evidenced that these molecules are able to modulate, at the molecular and cellular levels, several actors of the pivotal pathways involved in vascular cell proliferation and migration. Previous studies had demonstrated an anti-angiogenic role of polyphenols both *in vitro* and *in vivo* (Fotsis et al., 1998; Igura et al., 2001). In contrast, most recently, Baron-Menguy and co-workers evidenced a dose-dependent effect on angiogenesis of RWPC, and in particular of delphinidin, in a model of post-ischaemic neovascularization in rats submitted to femoral artery ligature. Indeed, high doses of RWPC (i.e. 7 glasses of red wine) reduced arterial, arteriolar, and capillary densities and blood flow, inhibited the phosphoinositol 3-kinase (PI3-K)/Akt/eNOS pathway, decreased vascular endothelial growth factor (VEGF) expression, and reduced metalloproteinase-2 (MMP-2) activation. In contrast, low doses of RWPC (i.e. 1/10$^{th}$ glass of red wine) increased neovascularisation in ischemic legs compared to control level in association with an increased blood flow. The angiogenic effect was linked to the overexpression of PI3-K/Akt/eNOS pathway and to increased VEGF production, without effect on MMP-2 activation. These anti- or pro-angiogenic effects of RWPC were reproduced when they used delphinidin, administrated alone at low or high doses. This dual dose-dependent effect of polyphenols in angiogenesis is particular interesting because of its

potential applications both in the therapy of diseases requiring the block of angiogenesis such as in some cancers, and in the treatment of post-ischemic injuries to improve angiogenesis and ameliorate reperfusion of tissues, at high and low doses, respectively.

### 4.3 Role in metabolic diseases

It has been extensively evidenced the strict correlation between metabolic dysfunctions and the development of cardiovascular diseases. Endothelial dysfunction, an independent predictor of cardiovascular events, has been consistently associated with obesity and the metabolic syndrome in a complex interplay with insulin resistance. Deficiency of eNOS is considered as the primary defect that links insulin resistance and endothelial dysfunction (Cersosimo and Defronzo, 2006; Defronzo, 2006; Fornoni and Raij, 2005). Furthermore, several epidemiological studies have shown that patients affected by metabolic diseases are often also affected by hypertension and other cardio-vascular complications such as atherosclerotic plaque formation and increased levels of pro-thrombotic factors, associated to an elevated risk of mortality by vascular thrombotic events (Kopelman, 2000).

More recently, we have suggested a protective role of RWPC in metabolic syndrome (Agouni et al., 2009). In our study, Zuker fatty (ZF) rats (Fa/Fa), an experimental model of metabolic syndrome, or their "lean" littermates, received normal diet or a diet supplemented with Provinols™ for 8 weeks in food. This treatment significantly reduced the plasmatic levels of metabolic products such as glucose, fructosamine, total and LDL-cholesterol, and triglycerides, and finally improved cardiac and endothelial vascular functions. Regarding vascular function, Provinols™ corrected endothelial dysfunction in aortas and mesenteric arteries from ZF rats by improving endothelium-dependent relaxation in response to acetylcholine. This beneficial effect in endothelium was associated to an enhanced NO bioavailability due to increased NO production and eNOS activity, and reduced oxidative stress and $O_2^-$ release. The effect on eNOS activity was associated to a decreased expression of caveolin-1, a protein known to inactivate eNOS by cell membrane sequestration, while the reduction of free radical production was linked to a decrease of Nox-1 (NADPH oxidase membrane sub-unit) expression (Agouni et al., 2009). In agreement with our work, this protective effect of RWPC in plasmatic metabolic parameters and oxidative stress linked to metabolic disorders was confirmed recently in hamsters submitted to high-fat diet (Suh et al., 2011).

Because of these interesting results, polyphenols might be good candidates for prevention and treatment of metabolic syndrome and cardiovascular risk reduction. This was previously suggested by another study of Napoli and coworkers who have shown that red wine consumption improved insulin resistance in type 2 diabetic patients (Napoli et al., 2005). Thus, RWPC could represent a new class of medicinal products against obesity-associated diseases.

## 5. The oestrogenic receptors in cardiovascular protection

Several epidemiological studies suggested a protective effect of oestrogens in premenopausal women in vascular and metabolic diseases development. These numerous studies showed that the incidence of hypertension and other cardiovascular diseases is significantly lower in premenopausal females compared to males and that, after the onset of

menopause, the incidence increases dramatically, eventually approaching the level observed in age-matched males (Mendelsohn and Karas, 1999). This effect has been attributed to the fall in circulating oestrogen levels, contributing to a menopause-related increase in blood pressure, and thus to a greater predisposition to cardiovascular disease. Consistent with this, oestrogen replacement therapy has been reported to reduce the risk of cardiovascular disease, and in particular of hypertension and atherosclerosis, in postmenopausal women to that observed in premenopausal women (Barton et al., 2007; Mendelsohn and Karas, 1999). Oestrogens have been shown to have direct vasodilatory and anti-atherosclerotic effects via the oestrogen receptors expressed on human and rat arteries (Haas et al., 2007; Shaw et al., 2001). The mechanisms involved in the protective role played by these hormones is associated to vascular inflammation reduction (Nilsson, 2007), increased endothelial NO production (Chen et al., 1999) and the prevention of smooth muscle vascular cell proliferation (Pareet al., 2002). But the ability of oestrogens to elicit effects on autonomic functions involved in cardiac control appears also to constitute a major part of its beneficial effects (Spary et al., 2009). Despite wealth of evidences for its central autonomic role, the sites and mechanisms of oestrogenic action on the neural pathways of cardiovascular regulation are still poorly understood.

Oestrogens act on specific receptors which are transcription factors, the nuclear oestrogenic receptors (ERs). Two ERs have been described, ERα and ERβ, with several structurally and functionally conserved domains, and involved in genomic signaling mechanism or associated to plasma membrane, influencing cytosolic non-genomic signaling. ERα was first characterized in mid-1980 and the cloning of ERβ following in late 1995 (Kuiper et al., 1996). In addition, as a result of alternative splicing of the eight exons encoding rat ERβ, five different isoforms of this ER exist (β1, β2, β1δ3, β2δ3 and β1δ4) with a not yet completely determined role (Maruyama et al., 1998; Petersen et al., 1998; Price et al., 2000). It has been suggested that ERβ may modulate ERα gene transcription, acting in some conditions by opposite actions to ERα (Lindberg et al., 2003; Maruyama et al., 1998; Zhao et al., 2008).

In the absence of oestrogens, the receptors are conserved in an inactive state in a complex with one of the several chaperone molecules, such as heat shock protein 90 (Beato and Klug, 2000). Following binding to oestrogens, the receptor undergoes a conformational change, activating an intracellular cascade leading to the ER release from the chaperone. ER can forms homo- or hetero-dimers that interact with target gene promoters, inducing the up- or the down-regulation of several genes (Figure 1) (Hall et al, 2001). The ER subtypes have also been shown to interact differently with a range of other transcription factors, including activating protein-1 (Paech et al., 1997; Webb et al., 1999; Zhao et al., 2008). This genomic response usually occurs within hours after oestrogen exposure and is believed to be the result of a direct action, not involving the second messenger signaling pathways. In contrast, the non-genomic oestrogenic signaling is also possible but less well understood. It is associated to the cytosolic pathways with classical second messengers and occurs considerably faster than the genomic signaling (Kang et al., 2010). It is possible that these rapid non-genomic events are mediated by cytoplasmic, rather than nuclear ERα and ERβ, suggesting the involvement of another plasma membrane receptor, a particular G protein-coupled receptor (GPCR) which is not related to ERα or ERβ. To confirm this hypothesis more recently, another membrane-bound ER was emerged. This GPCR, the G protein coupled oestrogen receptor 1 (GPER1), also called GPR30, is able to bind with a high affinity

to 17β-estradiol (E2), mediating oestrogenic signals in cardiovascular and metabolic regulations (Nilsson et al., 2011). GPER1 is expressed in different vascular segments and in the heart of several species. In rats, the mRNA of this receptor was found both in endothelial and in smooth muscle cells; but in mice and humans, it seems to be expressed primarily in endothelial cells of small systemic arteries, suggesting a direct role of GPER1 in endothelial function regulation, while the effects of its activation in vascular smooth muscle cells and vascular tone are indirect, via the endothelium (Nilsson et al., 2011). GPER1 is located to the endoplasmic reticulum of vascular cells mediating the rapid oestrogen signaling (Revankar et al., 2005).

The role of GPER1 activation by its specific agonist, G-1, on vascular tone was investigated in rat vessels. Several studies showed the involvement of this receptor in vascular relaxation by reducing angiotensin II (AngII) and/or endothelin-1 (ET-1)-induced vascular contractions. This was not influenced by the endogenous oestrogenic levels and it was gender independent (Haas et al, 2009, Lindsey et al., 2009; Meyer et al., 2010). This effect was not found in serotonin-dependent vascular contraction, suggesting a direct effect of GPER1 activation on the renin-angiotensin system, probably independent of NO production (Nillson et al, 2011). In contrast, another study suggests that GPER1 causes arterial relaxation via an endothelial and a NO-dependent mechanism (Broughton et al., 2010). Thus, the involvement of endothelial NO in this vascular relaxation cannot be excluded. Moreover, an hypotensive effect of GPER1 activation was observed in ovariectomized animals, in agreement with the hypertensive phenotype of GPER1 knock-out mice (Martensson et al., 2009). Furthermore, GPER1 activation could play a protective role in atherosclerosis and/or excessive angiogenesis during cancer, reducing vascular smooth muscle or endothelial cell proliferation, respectively (Haas et al., 2009; Holm et al., 2011).

If the non-genomic effects of E2 are realized through GPER1, ERα is the receptor implicated in the anti-atherogenic effects of oestrogens. Indeed, the ERα, when stimulated by E2, induces endothelial cell proliferation, vascular re-endothelialization, endothelial NO production, vascular inflammation attenuation, and reduction of smooth muscle cell proliferation (Brouchet et al., 2001; Pare et al., 2002; Vegeto et al., 2003). Nevertheless, studies conducted on vessels harvested from ERα or ERβ knockout mice showed that both these ERs are responsible for E2-dependent vascular relaxation (Guo et al., 2005). It was previously evidenced the association of a subpopulation of ERα with the endothelial membrane and the complex structure of caveolae (Chambliss and Shaul, 2002). The binding of E2 with ERα in caveolae leads to the MAPK/Akt pathway activation, resulting in eNOS phosphorylation and activation, and subsequent increased NO production (Figure 1) (Chambliss and Shaul, 2002). This beneficial effect on vascular function played by oestrogens was confirmed by epidemiological studies, in which the presence of endogenous oestrogens and their effect on cardiovascular homeostasis appear to be closely related to the degree of atherosclerosis progression throughout a woman's life (Clarkson 2007). Experimental studies suggest that in the mouse, ERα appears to be largely responsible for the protective effects of oestrogens against atherosclerotic vascular disease (Hodgin et al., 2001). In turn, according to some studies, the abundance of both ER subtypes, ERα and ERβ, in human aorta, decreases with the progression of

atherosclerosis, aggravating the endothelial dysfunction of atherosclerotic vessels by the reduction of oestrogenic-dependent eNOS activation and NO release (Losordo et al., 1994; Nakamura et al., 2004).

On the light of the effect of E2 via ERα in eNOS pathway activation and NO production, a vascular role of oestrogens, similar to that evidenced for RWPC on endothelium, was evoked. Some researchers and our studies started to investigate if RWPC or one of the polyphenolic compounds contained in red wine, resveratrol or delphinidin, could play a role of phytoestrogens, interacting at high affinity with ERs and inducing their beneficial vascular effects via these endothelial receptors.

## 6. Oestrogenic receptor alpha and polyphenols

After the description of these encouraging findings, nobody exactly identified the pivotal compound responsible of RWPC vascular effects and, most important, how this molecule was able to interact with the vascular endothelium, thus improving endothelial function. It was previously described that resveratrol is able to enhance eNOS expression and activity, but the mechanisms by which this polyphenol induced these effects were still not well known (Wallerath et al., 2002). In a study conducted *in vitro* in BAECs, nanomolar concentrations of resveratrol induced ERK1/2 signaling activation, similar to that of E2, since this was dependent of ER activity triggering eNOS activation and NO release (Klinge et al., 2005). The same team, in another study *in vitro* (in HUVECs), better determined the mechanisms by which resveratrol was able to improve eNOS activation pathway. The authors of this work demonstrated for the first time that resveratrol increased interaction between ERα, Caveoline-1 (Cav-1) and proteins involved in eNOS activation such as Src, by a Gα-protein-coupled mechanism. A main role for ERα in the NO production induced by resveratrol in endothelial cells was suggested because they observed attenuated effects of resveratrol in cells in which ERα was depleted using a siRNA. Resveratrol and E2 did not stimulate ERβ/Cav-1 interaction (Klinge et al., 2008). Moreover ERα is 4.5 times more expressed then ERβ in HUVECs and no effect of a siRNA directed versus ERβ was found on resveratrol action in endothelium. This study implies that dietary intake of resveratrol might offer possible vascular protective effects via the activation of ERα *in vivo*.

In contrast, experiments conducted in rats did not evidence a role of oestrogen receptors in aorta endothelium-dependent relaxation to RWPC (Kane et al., 2009). The authors of this work showed that RWPC caused redox-sensitive PI3-K/Akt-dependent eNOS activation and NO-mediated relaxation in rat aortas *ex vivo*. This vascular effect was more pronounced in the aorta of female than male rats, but it was due most likely to increased expression levels of eNOS rather than activation of oestrogen receptors, because the inhibition of ER by the oestrogen antagonist, ICI 182780, did not modify the ability of RWPC to induce their vascular effects (Kane et al., 2009). Interestingly, another study conducted in female SHR rats evidenced that the chronic treatment with RWPC of ovariectomized rats induced reduction of arterial pressure and vascular dysfunction characterizing this hypertensive model in a manner independent of the ovarian function (Lopez-Sepulveda et al., 2008).

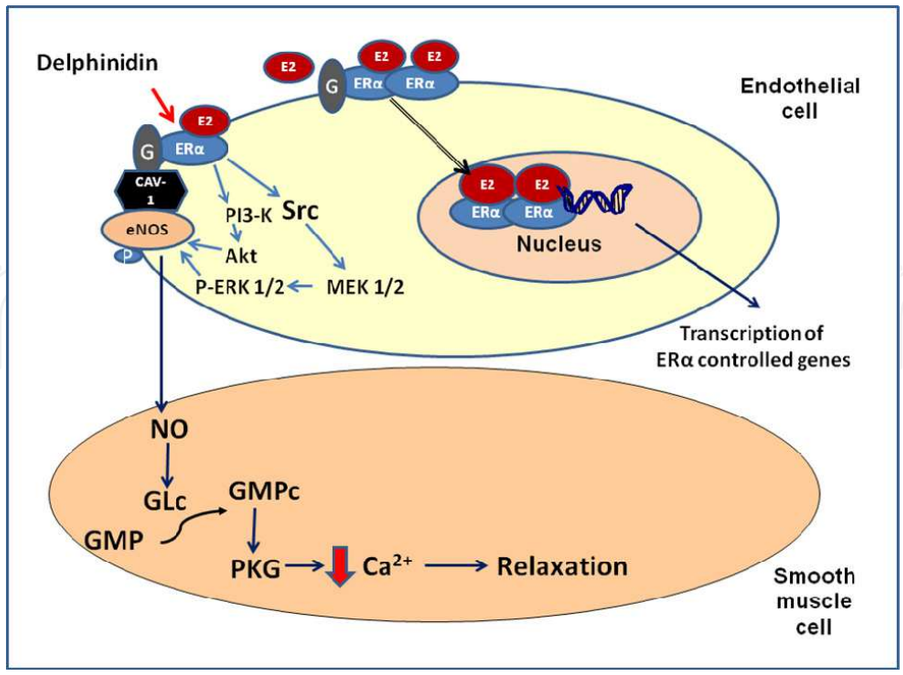


Fig. 1. ERα activation by polyphenols or oestrogens induces eNOS increased activity and NO production. Signaling pathway by which delphinidin or E2 interacting with ERα, activates rapidly eNOS and increases NO production in endothelial cells by PI3-K/Akt or via Src/ERK1/2 pathways. ERα is associated in endothelial cells caveolae with Cav-1 which links to the membrane the inactive form of eNOS. When ERα binds E2 or polyphenols, eNOS is phosphorylated in its active site, thus improving NO release. The same pathways implicated in delphinidin-ERα activation were proposed by Klinge and coworkers for endothelial cell stimulation by resveratrol at nanomolar concentrations (Klinge et al., 2008). NO is able to activate guanilyl-cyclase (GLc) in smooth muscle cells inducing increased levels of cyclic-GMP (GMPc) with subsequent protein-kinase G activation (PKG), reducing intracellular calcium and inducing vascular relaxation. On the right of the figure, is represented the homo-dimer formation and nuclear translocation of E2-activated ERα, inducing the genomic response.

Conversely, we investigated the hypothesis that ERα is one of the key targets involved *in vivo* in the vasculoprotective effects of RWPC (and in particular of delphinidin) interacting with the endothelium. Thus, the ERα implication in the French Paradox was first tested using ERα-deficient mice (Chalopin et al., 2010). We have shown the necessity of this oestrogenic receptor in the Provinols™- or delphinidin-induced endothelial-dependent relaxation, eNOS activation, and NO release. Indeed, no effect of these products on endothelium were observed in vessels harvested from ERα-deficient mice or in wild-type vessels without endothelium. The activation of ERα by RWPC or delphinidin alone induced the activation of the same pathway, evidenced by the previously described *in vitro* work of Klinge and colleagues with resveratrol. Indeed, E2 and the selective agonist of ERα, 1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole (PPT), as well as Provinols™ and delphinidin, are able to activate molecular pathways involving Src, ERK1/2, eNOS and caveolin-1 phosphorylations (see Figure 1). The mechanism involved required ERα activation because

of the absence of effect in vessels or cells from ERα-deficient mice, and after silencing, in wild-type endothelial cells, ERα activity or expression either with a pharmacological inhibitor (fulvestran) or with a siRNA, respectively. Moreover, using a binding assay and a docking study, we have shown that delphinidin fits on ERα's activation site, exerting 73% of specific inhibition against E2 on ERα, in the binding assay. Most importantly, ERα is also implicated in the *in vivo* effects observed in mice treated with Provinols™ administrated in the food, with respect to the improvement in endothelial function given by the concomitant increase in NO and decrease in $O_2^-$ release in vessels. Indeed, these vascular and anti-oxidant effects of the *in vivo* treatment with Provinols™ were not found in ERα-deficient mice (Chalopin et al., 2010). Then, we have demonstrated for the first time the physiological relevance of ERα in the *in vivo* vascular effects of RWPC.

It is important to note that ERα, ERβ, and GPER-1 are all expressed in the arterial wall of both women and men (Meyer et al., 2006; Haas et al., 2007), and that E2 has potent dilator effects on vascular tone of human coronary and internal mammary arteries harvested from patients without gender differences (Haas et al., 2007; Mugge et al., 1993). These findings suggest a potential function for oestrogen receptor also in male cardiovascular system. Thus, RWPC could have the same protective vascular properties in both women and men through ERα. In line with the fact that ERα mediates atheroprotective effects, in a man with a disruptive mutation in the ERα gene, it was noted an impaired vascular function and a premature coronary artery disease (Sudhir et al., 1997). Thus, not only the female but also the male cardiovascular system appears to be an important target for oestrogens affecting vascular disease development (Haas et al., 2007; Meyer et al., 2008). Nevertheless, studies in humans comparing oestrogen plasma concentrations and the progression of cardiovascular diseases have revealed conflicting results (Meyer et al., 2008). Actually, there is doubt about the interest to treat male patients with oestrogen receptor agonists to interfere with atherosclerosis progression.

Finally, further works are needed to confirm if ERs are implicated in all the vascular and metabolic effects of RWPC or if ERα activation by RWPC induces only the eNOS pathway improvement. For instance, the role of ERs activation by RWPC in inhibition of endothelial cell proliferation and cell cycle progression or in angiogenesis has not been investigated yet.

## 7. Conclusion

The first epidemiological studies played a main role in the demonstration of a French Paradox existence, leading to the start of about forty years of scientific findings concerning the protective properties of polyphenols and, more particularly, those contained in red wine. Currently, the numerous data obtained *in vitro, ex vivo,* and *in vivo,* on their beneficial effects in heart and vessels, validly suggest a therapeutic potential for RWPC.

The last findings have identified in delphinidin and resveratrol some of the key molecules involved in the vascular effects of RWPC via ERα activation, adding a new piece to the puzzle explaining the French Paradox (Chalopin et al., 2010; Klinge et al., 2008). Indeed, despite a previous study (Kane et al., 2008), which evidenced no implication of ERs in RWPC-dependent vascular relaxation in rats, the last studies clearly showed that the beneficial endothelial effects of RWPC require ERα activation. This is followed by a rapid response to the polyphenolic stimuli in endothelial cells, involving the pathways associated

to eNOS activation and subsequent NO release. Furthermore, the phytoestrogenic role of RWPC, and especially of delphinidin, was confirmed by binding experiences which found high affinity of delphinidin against ERα compared to its natural agonist E2 (Chalopin et al., 2010). Similar mechanisms and a phytoestrogenic role on ERα activation were suggested also for resveratrol on endothelial cells by Klinge and coworkers (Klinge et al., 2005, 2008).

In this chapter, we have focalized our attention on the red wine because it contains both, delphinidin and resveratrol, the main vasoactive compounds contained in non-alcoholic red wine extract. In particular, we wanted to explain the main mechanisms by which these compounds are able to induce cardiovascular protection against hypertension, cardiac ischemia, stroke and atherosclerotic plaque formation as one of the complications linked to metabolic syndrome. It is important to note that the effects of these substances could be different according to the concentrations employed as evidenced in experimental models of angiogenesis (Baron-Menguy et al., 2007). It is also relevant to remember of other beneficial properties of RWPC, as anti-oxidant, anti-inflammatory, anti-tumor or antithrombotic agents, that we have not extensively described here. Indeed, RWPC are also able to modulate the apoptotic, proliferative or migration processes in cells (Martin et al., 2003) by acting directly on vascular remodeling and angiogenesis (Brownson et al., 2002; Favot et al., 2003). Here, we have chosen to stress on strong properties of RWPC as vasodilators inducing endothelial NO production, because this effect implicates ERα activation as demonstrated in the last studies.

Furthermore, despite the favorable effect of some molecules contained in red wine in the prevention of several cardiovascular pathologies, alcohol is a serious problem of public health and, actually, it is important to remenber that these beneficial effects are due to the non-alcoholic fractions of red wine. Interestingly, in multinational studies it was shown an increased risk of mortality by myocardial infarction, especially in women who take no alcohol, but compared to moderate drinkers (Yusuf et al., 2004). Moreover, on the light of other epidemiological data, it seems to be developed the view that modest alcohol but neither zero nor more than modest intake reduces total mortality and cardiovascular risk by cardio and neuroprotection (Collins et al., 2009; Opie and Lecour, 2007).

According with the French Paradox, the moderate intake of wine (1 or 2 glasses per day) could be beneficial for health by reducing the risk of CVD mortality. As evidenced in Table 1, the content of these vasoactive substances is more relevant in red wine compared to other food and beverages. Finally on the light of all the epidemiological and fundamental studies analyzed in this chapter, and our works, we can suggest that RWPC, and in particular delphinidin and resveratrol, could be used for their therapeutic potential in the prevention and treatment of cardiovascular pathologies. We think that ERα activation might be the main molecular target triggering the beneficial effects of dietary supplementation of RWPC. Nevertheless, further studies are needed to verify the implication of ERα in other physiological effects of polyphenols and not only in NO release and vascular relaxation.

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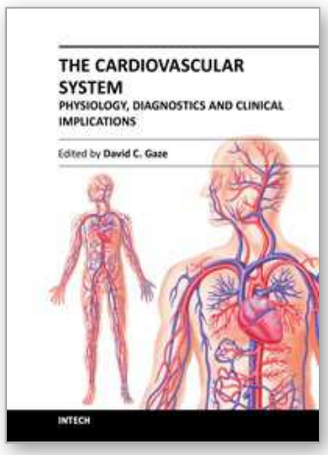


**The Cardiovascular System - Physiology, Diagnostics and Clinical Implications**

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The cardiovascular system includes the heart located centrally in the thorax and the vessels of the body which carry blood. The cardiovascular (or circulatory) system supplies oxygen from inspired air, via the lungs to the tissues around the body. It is also responsible for the removal of the waste product, carbon dioxide via air expired from the lungs. The cardiovascular system also transports nutrients such as electrolytes, amino acids, enzymes, hormones which are integral to cellular respiration, metabolism and immunity. This book is not meant to be an all encompassing text on cardiovascular physiology and pathology rather a selection of chapters from experts in the field who describe recent advances in basic and clinical sciences. As such, the text is divided into three main sections: Cardiovascular Physiology, Cardiovascular Diagnostics and lastly, Clinical Impact of Cardiovascular Physiology and Pathophysiology.



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