

FLAVOURS, FRAGRANCES AND INGREDIENTS

*Essential Oils, Botanical Extracts, Cold Pressed Oils,
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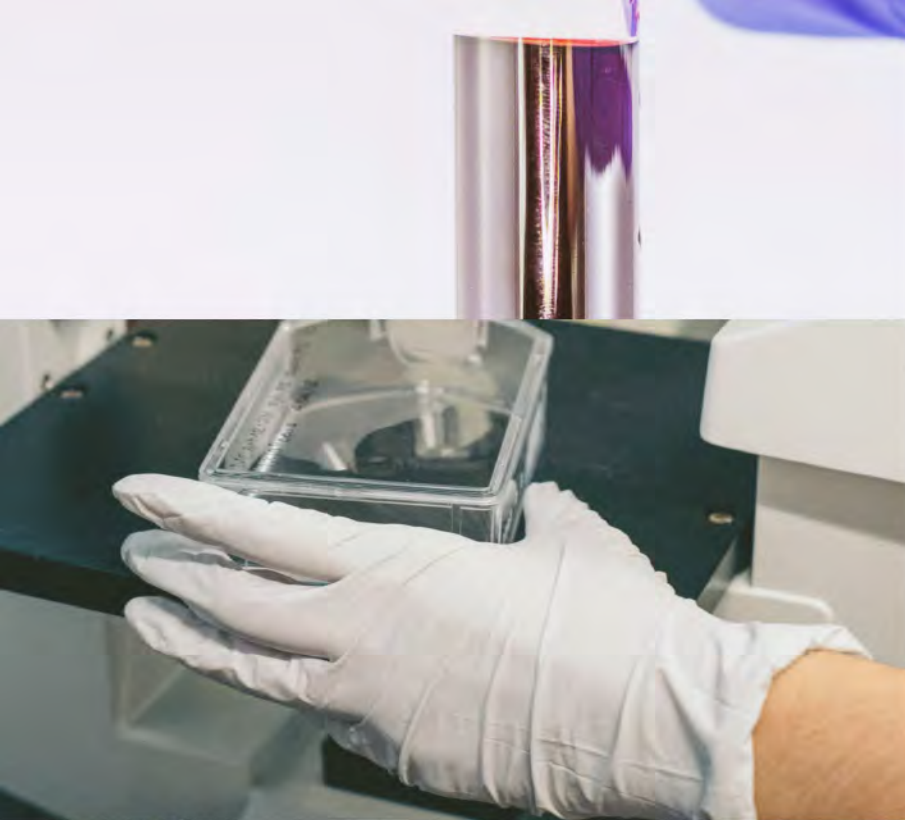
**BOTANICAL
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LITERATURE REVIEW

HEALTH BENEFITS RED WINE



ZERO ALCOHOL RED WINE
RED WINE EXTRACT POWDER
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BACKED BY SCIENCE
CONCENTRATED
ANTIOXIDANTS
POLYPHENOLS
RESVERATROL
QUERCETIN
ANTHOCYANDIN



RED WINE:
ZERO ALCOHOL
POWDER
EXTRACT
POWDER



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EXECUTIVE SUMMARY

The term **FRENCH PARADOX** is used to describe the relatively low incidence of cardiovascular disease in the French population despite the high consumption of red wine. Over the past 27 years numerous clinical studies have found a linkages with the **ANTIOXIDANTS** in particular, the **POLYPHENOLS, RESVERATROL, CATECHINS, QUERCERTIN** and **ANTHOCYANDINS** in red wine and reduced incidences of cardiovascular disease. However, the alcohol in wine limits the benefits of wine.

Studies have shown that zero alcohol red wine and red wine extract which contain the same **ANTIOXIDANTS** including **POLYPHENOLS, RESVERATROL, CATECHINS, QUERCERTIN** and **ANTHOCYANDINS** has the same is not more positive health benefits.

The following literature review details some of the most recent positive health benefits derived from the **ANTIOXIDANTS** found in red wine **POLYPHENOLS: RESVERATROL, CATECHINS, QUERCERTIN** and **ANTHOCYANDINS**.

The positive polyphenolic antioxidant effects of the polyphenols in red wine include:

- Cardio Vascular Health Benefits
- Increase antioxidants in the cardiovascular system
- Assisting blood glucose control
- Skin health
- Bone Health
- Memory
- Liking blood and brain health
- Benefits for the Central nervous system
- Neuro cognitive health
- Maintenance of a good hormonal balance
- Bone density (osteoporosis)
- Joint health (osteoarthritis)
- Muscle function (sarcopenia)
- Skin health using oral supplements and topical applications
- Blood homeostasis imbalance
- Cognitive performance
- Quality of life (vitality, mood, perception of pain)



Red Wine **Extracts and Powders**

ANTIOXIDANTS
POLYPHENOLS
RESVERATROL
QUERCETIN
ANTHOCYANDIN

RED WINE ZERO ALCOHOL POWDERS
EXTRACTS

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RED WINE EXTRACT POWDER



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BOTANICAL INNOVATIONS RED WINE EXTRACT POWDER IS A RICH SOURCE OF ANTIOXIDANTS in particular, the **POLYPHENOLS, RESVERATROL, CATECHINS, QUERCETIN** and **ANTHOCYANDINS**. These polyphenols found in red wine are linked to reduced incidences of cardiovascular disease. However, the alcohol in wine limits the benefits of wine.

Studies have shown that zero alcohol red wine and red wine extract which contain the same **ANTIOXIDANTS** including **POLYPHENOLS, RESVERATROL, CATECHINS, QUERCETIN** and **ANTHOCYANDINS** has the same is not more positive health benefits. The Botanical Innovations literature review details some of the most recent positive health benefits derived from the **ANTIOXIDANTS** found in red wine **POLYPHENOLS: RESVERATROL, CATECHINS, QUERCETIN** and **ANTHOCYANDINS**.

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- **Bone density (osteoporosis)**
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- **Muscle function (sarcopenia)**
- **Skin health using oral supplements and topical applications**
- **Blood homeostasis imbalance**
- **Cognitive performance**
- **Quality of life (vitality, mood, perception of pain)**

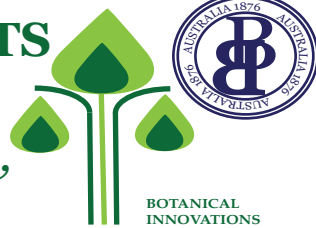
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Botanical Innovations is an Australian Bio Technology which has developed a range of unique AUSTRALIAN NUTRACEUTICAL FLAVOURS, FRAGRANCES and INGREDIENTS for functional foods and beverages, natural healthcare and cosmeceutical applications.

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ABSTRACTS

Wine as a biological fluid: history, production, and role in disease prevention.

Soleas GJ¹, Diamandis EP, Goldberg DM. J Clin Lab Anal. 1997;11(5):287-313.

Wine has been part of human culture for 6,000 years, serving dietary and socio-religious functions. Its production takes place on every continent, and its chemical composition is profoundly influenced by enological techniques, the grape cultivar from which it originates, and climatic factors. In addition to ethanol, which in moderate consumption can reduce mortality from coronary heart disease by increasing high-density lipoprotein cholesterol and inhibiting platelet aggregation, wine (especially red wine) contains a range of polyphenols that have desirable biological properties. These include the phenolic acids (p-coumaric, cinnamic, caffeic, gentisic, ferulic, and vanillic acids), trihydroxy stilbenes (resveratrol and polydatin), and flavonoids (catechin, epicatechin, and quercetin). They are synthesized by a common pathway from phenylalanine involving polyketide condensation reactions. Metabolic regulation is provided by competition between resveratrol synthase and chalcone synthase for a common precursor pool of acyl-CoA derivatives. Polymeric aggregation gives rise, in turn to the viniferins (potent antifungal agents) and procyanidins (strong antioxidants that also inhibit platelet aggregation). The antioxidant effects of red wine and of its major polyphenols have been demonstrated in many experimental systems spanning the range from in vitro studies (human low-density lipoprotein, liposomes, macrophages, cultured cells) to investigations in healthy human subjects. Several of these compounds (notably catechin, quercetin, and resveratrol) promote nitric oxide production by vascular endothelium; inhibit the synthesis of thromboxane in platelets and leukotriene in neutrophils, modulate the synthesis and secretion of lipoproteins in whole animals and human cell lines, and arrest tumour growth as well as inhibit carcinogenesis in different experimental models. Target mechanisms to account for these effects include inhibition of phospholipase A2 and cyclo-oxygenase, inhibition of phosphodiesterase with increase in cyclic nucleotide concentrations, and inhibition of several protein kinases involved in cell signalling. Although their bioavailability remains to be fully established, red wine provides a more favourable milieu than fruits and vegetables, their other dietary source in humans.

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Effects of de-alcoholated red wine and its phenolic fractions on platelet aggregation.

Russo P₁, Tedesco I, Russo M, Russo GL, Venezia A, Cicala C. Nutr Metab Cardiovasc Dis. 2001 Feb;11(1):25-9.

BACKGROUND AND AIM:

Platelet aggregation is involved in atherosclerosis and pharmacological inhibition of platelet activity may reduce the risk of coronary thrombosis and myocardial infarction. Red wine polyphenols may reduce platelet aggregability. This study evaluates the effect of de-alcoholated red wine (DRW) and its phenolic fractions on rat platelet aggregation and cyclic AMP (c-AMP) content.

METHODS AND RESULTS:

DRW was fractionated into four classes of phenolic compounds: phenolic acids (fraction 1), procyanidins, catechins and monomeric anthocyanidins (fraction 2), flavonols and resveratrol (fraction 3) and polymeric anthocyanidins (fraction 4). The effect of each fraction on ADP-induced rat platelet aggregation and c-AMP content was compared with that of DRW and pure phenolic compounds (quercetin, catechin, resveratrol, caffeic acid). DRW completely inhibited ADP-induced platelet aggregation. Fraction 2 also showed a significant anti-aggregating activity, whereas the effects of fractions 3 and 4 and the pure phenolics were not significant. A significant increase in platelet c-AMP content was observed after the addition of DRW and fraction 2.

CONCLUSIONS:

Our data indicate that DRW and its catechin-anthocyanidin fraction exert a significant effect on platelet aggregation in vitro, perhaps by enhancing platelet c-AMP levels.

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Red wine phenolic complexes and their in vitro antioxidant activity.

Sun B¹, Spranger I, Yang J, Leandro C, Guo L, Canário S, Zhao Y, Wu C. J Agric Food Chem. 2009 Sep 23;57(18):8623-7. doi: 10.1021/jf901610h.

Phenolic complexes are a major group of polyphenols in aged red wine. The objective of this work was to evaluate the in vitro antioxidant activity of the phenolic complexes. Thus, red wine polyphenols were fractionated into various fractions including monomers, oligomers, polymers, anthocyanins, and complexes. The in vitro antioxidant activities of these fractions and other phenolic standards (catechin, epicatechin, quercetin, and malvidin 3-glucoside) as well as ascorbic acid were verified by DPPH* test. On the other hand, the variation of antioxidant activities during the reaction between epicatechin and malvidin 3-glucoside mediated by acetaldehyde in a model wine solution was also monitored. The results showed that both the phenolic complex fraction and newly formed condensation products between epicatechin and malvidin 3-glucoside maintain antioxidant activities as strong as those of their compositional phenolics. This work provides, for the first time, direct evidence about the in vitro antioxidant activities of red wine phenolic complexes.

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Moderate red wine consumption and cardiovascular disease risk: beyond the "French paradox".

Lippi G¹, Franchini M, Favalaro EJ, Targher G. Semin Thromb Hemost. 2010 Feb;36(1):59-70. doi: 10.1055/s-0030-1248725. Epub 2010 Apr 13.

The term FRENCH PARADOX was coined in 1992 to describe the relatively low incidence of cardiovascular disease in the French population, despite a relatively high dietary intake of saturated fats, and potentially attributable to the consumption of red wine. After nearly 20 years, several studies have investigated the fascinating, overwhelmingly positive biological and clinical associations of red wine consumption with cardiovascular disease and mortality. Light to moderate intake of red wine produces a kaleidoscope of potentially beneficial effects that target all phases of the atherosclerotic process, from atherogenesis (early plaque development and growth) to vessel occlusion (flow-mediated dilatation, thrombosis). Such beneficial effects involve cellular signalling mechanisms, interactions at the genomic level, and biochemical modifications of cellular and plasma components. Red wine components, especially alcohol, resveratrol, and other polyphenolic compounds, may decrease oxidative stress, enhance cholesterol efflux from vessel walls (mainly by increasing levels of high-density lipoprotein cholesterol), and inhibit lipoproteins oxidation, macrophage cholesterol accumulation, and foam-cell formation. These components may also increase nitric oxide bioavailability, thereby antagonizing the development of endothelial dysfunction, decrease blood viscosity, improve insulin sensitivity, counteract platelet hyperactivity, inhibit platelet adhesion to fibrinogen-coated surfaces, and decrease plasma levels of von Willebrand factor, fibrinogen, and coagulation factor VII. Light to moderate red wine consumption is also associated with a favorable genetic modulation of fibrinolytic proteins, ultimately increasing the surface-localized endothelial cell fibrinolysis. Overall, therefore, the "French paradox" may have its basis within a milieu containing several key molecules, so that favorable cardiovascular benefits might be primarily attributable to combined, additive, or perhaps synergistic effects of alcohol and other wine components on atherogenesis, coagulation, and fibrinolysis. Conversely, chronic heavy alcohol consumption and binge drinking are associated with increased risk of cardiovascular events. In conclusion, although mounting evidence strongly supports

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beneficial cardiovascular effects of moderate red wine consumption (one to two drinks per day; 10-30 g alcohol) in most populations, clinical advice to abstainers to initiate daily alcohol consumption has not yet been substantiated in the literature and must be considered with caution on an individual basis.

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Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels.

Wang Z¹, Zou J, Cao K, Hsieh TC, Huang Y, Wu JM. Int J Mol Med. 2005 Oct;16(4):533-40.

Moderate consumption of red wine is associated with a reduced risk of coronary heart disease (CHD). This phenomenon is based on data from epidemiological observations known as the French paradox, and has been attributed to CHD-protective phytochemicals, e.g. resveratrol in red wine. Since red wine also contains alcohol, it is conceivable that alcohol interacts with resveratrol to elicit the observed cardioprotective effects. To determine whether resveratrol has alcohol-independent effects, we compared cardioprotective properties of dealcoholized Chinese red wine with alcohol-containing Chinese red wine having comparable amounts of resveratrol, using a hypercholesterolemic rabbit model and resveratrol as a reference. Animals fed a high cholesterol (1.5%) diet were simultaneously given water containing resveratrol (3 mg/kg/day) or red wine (4 ml/kg/day) containing 3.98 mg/l and 3.23 mg/l resveratrol for regular and dealcoholized red wine, respectively, for a 12-week duration. Total, HDL- and LDL-cholesterol and triglyceride levels in the plasma were measured before and after the cholesterol challenge. Atherosclerotic plaques in the thoracic aorta were evaluated using histochemical methods. Vascular and endothelial functions in the femoral artery were also assessed by ultrasonographic image analysis. High cholesterol-fed animals showed a significant increase in plasma levels of total, HDL- and LDL-cholesterol, but not triglycerides, compared to those fed a regular diet. Dietary cholesterol-elicited lipid changes were similarly observed in animals concurrently fed dealcoholized red wine, red wine or resveratrol. In contrast, whereas atherosclerotic lesions were clearly evident in specimens prepared from the thoracic aorta of high cholesterol-fed animals, the size, density, and mean area of atherosclerotic plaques, and thickness of the intima layer were significantly reduced in rabbits given dealcoholized red wine, red wine, or resveratrol. These results were in agreement with data obtained by an ultrasound analysis of endothelial function, which showed a 25% reduction in flow-mediated dilation (FMD) in rabbits fed a high cholesterol diet compared to animals on control diet. This decrease was effectively prevented by the simultaneous exposure to dealcoholized

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red wine, red wine, or resveratrol. Our study shows that animals given dealcoholized red wine exhibited cardio-active effects comparable to those of animals orally administered resveratrol, and suggests that wine polyphenolics, rather than alcohol present in red wine, suffice in exerting cardioprotective properties. The results also provide support for the notion that resveratrol and phytochemicals in red wine can suppress atherosclerosis without affecting plasma lipid levels.

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Cardioprotection of red wine: role of polyphenolic antioxidants.

Das DK¹, Sato M, Ray PS, Maulik G, Engelman RM, Bertelli AA, Bertelli A. *Drugs Exp Clin Res.* 1999;25(2-3):115-20.

Epidemiological studies suggest that the consumption of wine, particularly of red wine, reduces the incidence of mortality and morbidity from coronary heart disease. This has given rise to what is now popularly termed the "French paradox". The cardioprotective effect has been attributed to antioxidants present in the polyphenol fraction of red wine. Grapes contain a variety of antioxidants, including resveratrol, catechin, epicatechin and proanthocyanidins. Of these, resveratrol is present mainly in grape skin while proanthocyanidin is present in the seeds. In this report, we provide evidence that red wine extract as well as resveratrol and proanthocyanidins are equally effective in reducing myocardial ischemic reperfusion injury, which suggests that these red wine polyphenolic antioxidants play a crucial role in cardioprotection.

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ARTICLES

The Immune Protective Effect of the Mediterranean Diet against Chronic Low-grade Inflammatory Diseases

Rosa Casas, Emilio Sacanella, and Ramon Estruch, *Endocrine, Metabolic & Immune Disorders - Drug Targets*, 2014, 14, 245-254 245

Molecular Properties of Red Wine Compounds and Cardiometabolic Benefits

Melissa M. Markoski, Juliano Garavaglia, Aline Oliveira, Jessica Olivaes, and Aline Marcadenti, *Nutr Metab Insights*. 2016; 9: 51–57. Published online 2016 Aug 2. doi: 10.4137/NMI.S32909

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Johannes M. Breuss, Atanas G. Atanasov and Pavel Uhrin, *International Journal of Molecular Science*. Published: 27 March 2019

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Resveratrol, a natural chemopreventive agent against degenerative diseases

Ewa Ignatowicz, Wanda Baer-Dubowska *Polish Journal of Pharmacology* 2001, 53, 557-569

Wine and Cardiovascular Health A Comprehensive Review

Sohaib Haseeb ,BSc Bryce Alexande andBSc Adrian BaranchukMDFrom Division of Cardiology, Queen's University, Kingston, Ontario, Canada
<https://doi.org/10.1161/CIRCULATIONAHA.117.030387> *Circulation*. 2017;136:1434–1448

Red wine: A drink to your heart

T.S. Mohamed Saleem, S. Darbar Basha *Journal of Cardiovascular Disease Research* Vol. 1 / No 4, 171-176

The Immune Protective Effect of the Mediterranean Diet against Chronic Low-grade Inflammatory Diseases

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Abstract: Dietary patterns high in refined starches, sugar, and saturated and trans-fatty acids, poor in natural antioxidants and fiber from fruits, vegetables, and whole grains, and poor in omega-3 fatty acids may cause an activation of the innate immune system, most likely by excessive production of proinflammatory cytokines associated with a reduced production of anti-inflammatory cytokines. The Mediterranean Diet (MedDiet) is a nutritional model inspired by the traditional dietary pattern of some of the countries of the Mediterranean basin. This dietary pattern is characterized by the abundant consumption of olive oil, high consumption of plant foods (fruits, vegetables, pulses, cereals, nuts and seeds); frequent and moderate intake of wine (mainly with meals); moderate consumption of fish, seafood, yogurt, cheese, poultry and eggs; and low consumption of red meat, processed meat products and seeds. Several epidemiological studies have evaluated the effects of a Mediterranean pattern as protective against several diseases associated with chronic low-grade inflammation such as cancer, diabetes, obesity, atherosclerosis, metabolic syndrome and cognition disorders. The adoption of this dietary pattern could counter the effects of several inflammatory markers, decreasing, for example, the secretion of circulating and cellular biomarkers involved in the atherosclerotic process. Thus, the aim of this review was to consider the current evidence about the effectiveness of the MedDiet in these chronic inflammatory diseases due to its antioxidant and anti-inflammatory properties, which may not only act on classical risk factors but also on inflammatory biomarkers such as adhesion molecules, cytokines or molecules related to the stability of atheromatic plaque.

Keywords: Adhesion molecules, atheromatic plaque, atherosclerosis, cytokines, inflammation, mediterranean diet, mediterranean dietary pattern, plaque vulnerability.

INTRODUCTION

The World Health Organization (WHO) recognizes that diet plays an important role in preventing non-communicable diseases. Unhealthy nutrition, as well as other adverse lifestyle health behaviors are recognized as being part of the prime factors responsible for cardiovascular disease (CVD), diabetes, malignant cancer and chronic disease of the respiratory system [1].

Epidemiological studies such as the *Seven Countries Study* in the 60s [2] demonstrated a great interest in the Mediterranean diet (MedDiet) as a healthy eating pattern. These epidemiological studies have shown that high adherence to traditional MedDiet was associated with a lower mortality and cardiovascular disease incidence, reducing the risk of developing the metabolic syndrome, type 2 diabetes, and some neurodegenerative diseases and cancers [3].

The MedDiet is a nutritional model inspired by the traditional dietary pattern of some of the countries of the Mediterranean basin. At least 16 countries border the Mediterranean Sea. Diets vary between these countries and also between regions within the same country. Many

differences in culture, ethnic background, religion, economy and agricultural productions result in different diets, but the common Mediterranean dietary pattern (MDP) gather the following characteristics: abundant consumption of olive oil and high consumption of fruits, vegetables, cereals (preferably as whole grain), legumes, nuts and seeds. The MDP also includes moderate consumption of fish and shellfish, white meat, eggs, and fermented dairy products (cheese and yogurt), as well as relatively small amounts of red meat, processed meats, and foods rich in sugars. Frequent but moderate intake of wine, especially red wine with meals is also recommended [4, 5]. The MedDiet is characterized by a relatively high fat intake (40%–50% of total daily calories), of which saturated fatty acids (SFA) comprises $\leq 8\%$ and monounsaturated fatty acids (MUFA), mainly from olive oil, between 15%–25% of calories. It is characterized by a high omega-3 fatty acid intake from fish and plant sources and a low Omega-6:Omega-3 ratio of 2:1–1:1 compared to 14:1 in Europe [4, 6]. High consumption of dietary fiber [7], low glycemic index and glycemic load [8], anti-inflammatory effects [9], and antioxidant compounds [10, 11] may act together to produce favorable effects on health status.

Effects of the Mediterranean Diet on Inflammation

Dietary patterns high in refined starches, sugar, and saturated and trans-fatty acids, poor in natural antioxidants and fiber from fruits, vegetables, and whole grains, and poor

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evidence of a central role of inflammation in CVD, type 2 diabetes or cancer. In contrast, concentrations of anti-inflammatory adipokine adiponectin were inversely associated with CVD, type 2 diabetes, and obesity-related cancer [12, 13]. Thus, the MedDiet could be used as dietary therapy for chronic low-grade inflammation-related diseases (Fig. 1).

MEDITERRANEAN DIET AND CARDIOVASCULAR DISEASE

Fig. (1). Modulation of the Mediterranean diet on the immune factors providing protective effect against cardiovascular disease or cancer. Accumulation of oxidised low-density lipoprotein (oxLDL), starting in the fatty streaks, promotes the inflammatory response. Oxidized lipids and oxLDL trigger the expression of adhesion molecules (VCAM-1, mainly) and other mediators such as selectins and integrins, promoting the recruitment of monocytes into the subendothelial space in response to chemo-attractant cytokines. Successive accumulation of apoptotic cells, debris and cholesterol crystals form a necrotic core. Macrophages can be either classically activated (M1) or alternatively activated (M2). M1 monocytes display pro-inflammatory activity and could promote plaque vulnerability while, M2 monocytes are associated with homeostatic activity but could be pro-atherogenic in the early stages while promoting plaque stability in the later stages. Finally, in late stages atheromatic plaque may become unstable, leading to cap rupture, ensuing thrombosis and finally, cardiovascular events. The MedDiet exerts an anti-inflammatory and a modulating effect on CRP, interleukins such as IL-1, IL-6 as well as on TNF- α and its receptors, or chemoattractant molecules as MCP-1 or soluble adhesion molecules (sVCAM-1, sICAM-1, sE- and sP-Selectin). MedDiet triggers the alternative activation and exerts an immunomodulatory effect on biomarkers related to plaque stability such as IL-18, MMP-9 or TGF- β . It also can regulate the expression of leukocyte adhesion molecules including SLex, VLA-4 and LFA. IL-: interleukins; MMP-9: Metalloprotease-9; TNF- α : Tumor necrosis factor; TGF- β 1: Transforming Growth Factor- β 1; sVCAM-1: soluble vascular cell adhesion molecule; sICAM-1: Soluble intercellular adhesion molecule-1; sP-Selectin: Soluble platelet selectin; sE-Selectin: soluble endothelial selectin. Figure adapted [27, 28, 75, 76].

Western countries, although the incidence varies according to geographical origin, with Mediterranean countries and Japan showing the lowest rates worldwide [14]. Geographical differences in CHD rates are attributable, in part, to dietary and other lifestyle habits. Several epidemiological studies have reported that high adherence to the MedDiet is associated with a lower incidence of CHD and mortality rates [15-17].

Up to now, the beneficial effect of the MedDiet against CVD has been attributed to its effects in controlling classical risk factors. Thus, several clinical studies have shown that Mediterranean-style diets are protective against the development of prevalent diseases that promote CHD, such as the metabolic syndrome [18], diabetes [19], hypertension [20] or dyslipidemia [21]. Recently, some authors have suggested that an anti-inflammatory effect in the vascular wall may be another important mechanism to explain the link between the MedDiet and low cardiovascular mortality [22]. Indeed, additional evidence from clinical trials have suggested that the MedDiet reduces vascular inflammation [22], oxidative stress [23], and endothelial dysfunction [24, 25], which are factors involved in the development of atherosclerosis. Interestingly, it has also been reported that the MedDiet can favorably modulate the expression of pro-atherogenic genes [26].

The effects of Inflammation on Atherosclerotic Disease

Atherosclerosis is a chronic inflammatory disorder of the vessel wall and involves accumulation of lipids in the arterial intima which leads to formation of vascular lesions, or atheromatic plaque [27]. Monocytes and T-cells are implicated in this atherogenic process, migrating from the circulation into the intima of the arterial wall where they are differentiated into macrophages and later into foam cells after joining oxidized low-density lipoprotein (oxLDL) [28, 29]. This process triggers an activation of endothelial cells, because of the accumulation of modified LDL. Activated endothelial cells upregulate adhesion molecule expression (VCAM-1, mainly) and other mediators such as selectins, integrins, promoting the recruitment of monocytes into the subendothelial space in response to chemo-attractant cytokines [29]. The recruitment of immune cells is a crucial early step in atherogenesis. Macrophages and T-lymphocytes together release a wide array of chemokines and cytokines that can exert both pro- and antiinflammatory effects [28]. C-reactive protein (CRP), tumor necrosis factor (TNF)- α , or pro-inflammatory cytokines such as interleukin-6 (IL-6) are associated with the development of atherosclerosis. Finally, in late stages atheromatic plaque may become unstable, leading to cap rupture, ensuing thrombosis and finally, cardiovascular events [30]. In this context, some circulating molecules, such as matrix metalloprotease-9 (MMP-9) or interleukin-18 (IL-18), are considered to be early biomarkers of plaque vulnerability [31].

Several anti-inflammatory mechanisms have been proposed correlating the MedDiet and/or its components with the different steps of atherosclerotic process. Therefore, circulating markers of inflammation, such as CRP, TNF- α , and some interleukins (IL-6, IL-18) may be correlated with the development of vascular events and, in some cases,

also contribute to their pathogenesis. On the other hand, adiponectin (protein that originates from adipose tissue) also exhibits potent anti-inflammatory and antiatherosclerotic effects. Therefore, low plasma adiponectin levels are an independent risk factor for the future development of type 2 diabetes, whereas high plasma adiponectin concentrations are associated with a lower risk of myocardial infarction in men. Finally, it has become increasingly clear that inflammation strictly correlates with endothelial dysfunction and insulin resistance [13].

Immunomodulatory effects of the Mediterranean Diet

Experimental and clinical studies have shown that olive oil down-regulates the expression of VCAM-1, ICAM-1, and E-selectin in circulating lymphocytes and monocytes [32] and decreases plasma concentrations of sICAM-1, sVCAM-1, sE-selectin, IL-6, and CRP in high-risk patients [33, 34]. The observational ATTICA study evaluated 1,514 Greek men and 1,528 women. This study showed that greater adherence to a Mediterranean-style diet was associated with 20% lower CRP and 17% lower IL-6 compared with those in the lowest tertile of adherence [35]. In the Nurses' Health Study, a Mediterranean diet index score was associated with lower concentrations of biomarkers of inflammation and endothelial dysfunction (CRP, IL6, ICAM-1 and VCAM-1) [36]. A pattern similar to a MDP was inversely associated with plasma CRP and E-selectin concentrations, whereas a Western pattern, with a higher intake of red meat, sweets, fries and refined grains, was positively associated with CRP, IL-6, E-selectin, ICAM-1 and VCAM-1 concentrations [37]. A recent meta-analysis including a total 17 of trials reported that greater adherence to the MedDiet was associated with a significantly greater reduction in IL-6 and CRP compared to control intervention protocols, improving endothelial function [38]. In addition, a group of patients with metabolic syndrome following a MedDiet pattern showed reduced serum concentrations of CRP, IL-6, IL-7 and IL-18, decreased insulin resistance and improved endothelial function [22].

Other studies such as that by Llorente-Cortés *et al.* showed an increase of cyclooxygenase 2 (COX-2) and LDL-C receptor related protein, representing a decrease in the expression of monocyte chemoattractant protein 1 (MCP-1) in 49 asymptomatic individuals with high CVD risk after a 3-month intervention with the MedDiet [26]. Furthermore, the MedDiet can also exert a modulation effect on the expression of genes related to plaque stability, such as MMP-9, even in an elderly high-risk population and after a short period [39]. In addition, the MedDiet acts by improving endothelial dysfunction in healthy elderly individuals after 4-weeks of consumption, increasing the production of endothelial progenitor cells and decreasing the release of endothelial microparticles [40]. The inflammatory markers upon which the MedDiet acts are shown in Table 1.

To date, the PREDIMED study (*Prevención con Dieta Mediterránea*) is the only randomized trial that has evaluated the protective effect of a MDP supplemented with extra virgin olive oil (EVOO) or nuts versus a low-fat diet (LFD) in patients at high risk for CVD and the possible mechanisms involved in this protection. This large interventional study, including 7,447 subjects, showed that a MedDiet rich in

Table 1. Protective effects of the MedDiet on immune factors.

Mediterranean Diet						
Immune Factors						
Interleukins & CRP	Tumor Necrosis Factor & Receptors	Tumor Necrosis Factor & Receptors	Chemokines	Stability / Unstability of Atheromatic Plaque	Circulating Adhesion Molecules	Leukocyte Adhesion Molecules
IL-1	TNF- α			MMP-9	sICAM	CD11a
IL-6	TNFR60	TGF- β 1	MCP-1	TIMP-1	sVCAM-1	CD11b
IL-7	TNFR80			IL-10	sP-Selectin	CD49d
CRP				IL-18	sE-Selectin	CD40

CRP C-reactive protein; sVCAM-1: soluble vascular cell adhesion molecule; sICAM-1: Soluble intercellular adhesion molecule-1; sP-Selectin: Soluble platelet selectin; sE-Selectin: soluble endothelial selectin; TNFR, TNF receptor; ; MMP-9: Metalloprotease-9 ; TNF- α : Tumor necrosis factor; TGF- β 1: Transforming Growth Factor- β 1; IL-: interleukins; TIMP-1: metalloproteinase inhibitor 1.

olive oil or nuts reduces the risk of CVD by 30% when compared to a low-fat diet [41]. This study has also demonstrated that adherence to the MedDiet is associated with a reduced incidence of diabetes [19, 42], metabolic syndrome [43], hypertension [44], cardiovascular risk factors [9], oxidative stress [45], vascular inflammation [46-48], and endothelial dysfunction [23], all of which are factors involved in atheromatic plaque development. In the pilot study of the PREDIMED trial [9], we analyzed the effects of the MedDiet rich in olive oil or nuts and a LFD on 4 soluble adhesion molecules (ICAM-1, VCAM-1, IL-6 and CRP) on the first 772 participants recruited and after a 3-month follow up. In the short-term, plasma concentrations of IL-6, VCAM-1 and ICAM-1 decreased in the MedDiet groups supplemented with olive oil and nuts, while plasma concentrations of CRP only decreased in the MedDiet supplemented with EVOO ($P < 0.05$; all). Otherwise, plasma concentrations of VCAM-1 and ICAM-1 increased after 3 months in the LFD group ($P < 0.05$; both). In another sub-study of the PREDIMED trial, we demonstrated the anti-inflammatory effects of the MedDiet on circulating inflammatory biomarkers and immune cell activation biomarkers, all related to the atherosclerotic process, after a 3-month intervention [46]. We analyzed 106 subjects at high risk for CVD. We assessed changes in cellular and serum inflammatory biomarkers from baseline. Both MedDiets supplemented with EVOO or nuts showed down-regulated monocyte expression of CD49d and CD40 ($P < 0.05$) after 3-months with the dietary intervention. Likewise, in this sub-study we also analyzed the changes in plasma concentrations of ICAM-1, VCAM-1, IL-6, E- and P-Selectin before and after 3 months. The results showed that both MedDiets reduced ICAM-1 levels whereas VCAM-1, IL-6 and CRP decreased only for the MedDiet+VOO ($P < 0.05$). Since this had not been previously investigated, this study was a breakthrough for knowing the effects of the Med-Diet on adhesion molecule expression on circulating peripheral blood mononuclear cells linked to the development of atherosclerosis.

In a third sub-study of the PREDIMED trial [48], we analyzed the effects of 2 MedDiets (MedDiet+VOO and MedDiet+Nuts) and a LFD on 4 circulating inflammatory biomarkers related to atherogenesis (TNFR60, TNFR80, ICAM-1 and IL-6) in a total of 516 participants after a 1-year

intervention. It is known that the activation of TNFR60 can induce the expression of adhesion molecules and activate NF- κ B, and TNFR80 which play a role in T cell proliferation [49]. Thus, at baseline and after 1 year (Table 2), the MedDiet groups had lower plasma concentrations of IL-6, TNFR60, and TNFR80 ($P < 0.05$; all), whereas ICAM-1, TNFR60, and TNFR80 concentrations increased in the LFD group ($P < 0.005$; all). For the first time it was possible to link a diminution in TNFR concentrations with a MDP.

To date, data on the possible anti-inflammatory role of the MedDiet are scarce and are mainly based on observational studies [50] or short-term intervention studies [46]. In addition, serum markers related to plaque vulnerability have also been associated with cardiovascular events. Thus, high levels of MMP-9, a protease that can degrade the fibrous content of plaque and facilitate its rupture, have previously been detected in patients with acute coronary syndromes [51] or those with ulcerated plaque identified on coronary angiography [52], compared to healthy controls. Likewise, increased levels of TIMP-1 are related to a high risk of cardio- and cerebrovascular events [53], and IL-18 has been considered to be a predictor of myocardial infarction and death in patients with angina [54]. Finally, higher levels of TGF- β 1 were observed in the control diet group, but there is no consensus on whether they are related to plaque stabilization [55].

Finally, a fourth study [47] made by our group showed that adherence to the MedDiet is associated with an increase in serum markers of atheromatic plaque stability which may explain, at least in part, the protective role of MedDiet against CVD. In this case, a total of 164 participants at high risk for cardiovascular disease were randomized into one of the three diet groups described previously. Then, we assessed the 12-month effects of two enhanced MedDiets compared to a LFD on the adhesion molecules for T-Lymphocytes and monocytes (CD11a, CD11b, CD49d and CD40), as well as inflammatory biomarkers related to atherosclerosis (sVCAM-1, sICAM-1, sE- and sP-selectin) and plaque vulnerability (CRP, IL-6, IL-18, IL-10, TGF- β 1, MMP-9). As shown in Table 3, some anti-inflammatory effects were detected in the three diets studied, although they were more intense in subjects allocated to the two MedDiet

Table 2. Changes in the expression of circulating markers of plaque instability and other inflammatory biomarkers in the short- and long-term.

Inflammatory Molecules	Short-term			Long-term		
	MedDiet+ EVOO	MedDiet+ Nuts	Low-fat diet	MedDiet+ EVOO	MedDiet+ Nuts	Low-fat diet
sICAM-1, ng/mL	↓	↓	↑	↓	=	↑
sVCAM-1, ng/mL	↓	↓	↑	↓	↓	=
sE-Selectin, ng/mL	=	=	=	=	↓	=
sP-Selectin, ng/mL	=	=	=	↓	↓	=
IL-6, pg/mL	↓	↓	↑	↓	↓	↑
CRP, mg/L	↓	=	=	↓	↓	=
TNFR60, µg/L				↓	↓	↑
TNFR80, µg/L				↓	↓	↑
IL-18, pg/mL				=	↓	=
IL-10, pg/mL				=	=	=
IL-18/IL-10 ratio				↓	↓	=
MMP-9, ng/mL				=	=	↑
TIMP-1, ng/mL				=	=	=
MMP-9/TIMP-1 ratio				=	=	↑
TGF-β1, pg/mL				=	=	↑

CRP, high-sensitivity C-reactive protein; EVOO, extra virgin olive oil; LFD, low-fat diet; MedDiet+EVOO, Mediterranean diet supplemented with extra virgin olive oil; MedDiet+Nuts, Mediterranean diet supplemented with nuts; IL-: interleukins; sVCAM-1: soluble vascular cell adhesion molecule; sICAM-1: Soluble intercellular adhesion molecule-1; sP-Selectin: Soluble platelet selectin; sE-Selectin: soluble endothelial selectin.; MMP-9: Metalloproteinase-9; TNF-α: Tumor necrosis factor; TGF-β1: Transforming Growth Factor-β1; TNFR, TNF receptor; TIMP-1: metalloproteinase inhibitor 1; “↑”: increased plasma levels of biomarkers; “↓”: decreased plasma levels of biomarkers; “=”: no changes observed in the levels of the biomarkers.

interventions, supplemented with VOO and nuts, which showed a higher down-regulation of adhesion molecules in T-lymphocytes and monocytes compared to those in the control diet group. Moreover, serum levels of endothelial cell adhesion molecules were lower in subjects following both MedDiets, compared to control subjects, in whom some inflammatory molecules (e.g., sICAM-1) showed a significant increase. Likewise, parameters directly related to plaque vulnerability, such as IL-18, MMP-9, TIMP-1, TGF-β1, and IL-10 had a more favorable profile (towards stability) in participants in the MedDiet+nuts than those in the control group (Table 2). All these changes slow lymphocyte and monocyte adhesion to the endothelium and posterior transmigration to the subendothelial space to generate unstable plaque. Moreover, Casas *et al.* [47], observed a significant decrease in systolic and diastolic BP and in plasma total-cholesterol concentrations in both MedDiet groups compared to the control group. The anti-inflammatory effect of the MedDiet seems to be greater and more intense in the mid-term compared to the short-term [46], while the effect on classical cardiovascular risk factors is similar, thereby suggesting that the MedDiet exerts its effects on lipids and blood pressure relatively quickly (at 3 mo), with the maximum effect on systemic inflammatory biomarkers being achieved later (at 1 y). Thus, in the short-term the effect on blood pressure and the lipid profile is higher,

whereas in the mid-term the effect on chronic inflammatory response in the arterial wall is more pronounced. In brief, the results of this study suggest that the MedDiet, enriched with EVOO or nuts, may have a dual effect on the prevention of CVD: improving classical cardiovascular risk factors and having an intense anti-inflammatory effect.

MEDITERRANEAN DIET AND CANCER

Cancer involves a series of diseases caused primarily by exposure to environmental factors, habits and lifestyle, and is, thus, largely preventable. The MedDiet is based mainly on the consumption of plant foods, providing a high content of vitamins and antioxidants involved in cellular differentiation and proliferation in the synthesis and repair of DNA adduct formation and inhibition of the formation of carcinogenic chemicals in inflammatory response, enzyme induction and hormonal activity [56].

Since the late 50s and early 60s there is greater and growing evidence that greater adherence to a MDP is associated with a lower incidence of chronic diseases like CVD, type 2 diabetes, obesity, metabolic syndrome and certain types of cancer, as well as increased survival and longevity [5]. Several epidemiological studies have evaluated the causal relationship between the MedDiet and cancer risk. Thus, from the *Seven Countries Study* in the

Table 3. Changes in adhesion molecule expression in circulating T- lymphocytes and monocytes.

	Short-term			Long-term		
	MedDiet+ EVOO	MedDiet+ Nuts	Low-fat diet	MedDiet+ EVOO	MedDiet+ Nuts	Low-fat diet
T-LYMPHOCYTES						
CD11a	=	=	=	↓	↓	↓
CD49d	↓	↓	=	↓	=	=
CD40	=	=	=	↓	↓	=
MONOCYTES						
CD11a	=	↓	=	↓	↓	↓
CD11b	↓	↓	=	↓	↓	↓
CD49d	↓	↓	=	↓	↓	=
CD40	↓	=	=	↓	↓	=

EVOO; extra virgin olive oil; MedDiet+EVOO; Mediterranean diet supplemented with extra virgin olive oil; MedDiet +Nuts; Mediterranean diet supplemented with nuts. "↑": increased expression of adhesion molecules; "↓": decreased expression of adhesion molecules; "=": no changes observed in the expression of the adhesion molecules.

1950s to the recent European Prospective Study and to Cancer and Nutrition (EPIC) study collaboration, the evaluation of the components of diet-affecting chronic diseases such as cardiovascular disease and cancer has been crucially based on the analysis of foods and nutrients characterizing the Mediterranean dietary habits. Ongoing findings from the EPIC study (Greek cohort: n=23,349 men and women, not previously diagnosed with cancer and with a mean follow-up time of 8.5 years), showed that greater adherence to a MedDiet was related to a significant reduction in total mortality [13]. On the other hand, the whole EPIC study (142,605 men and 335,873 women) demonstrated that 4.7% of cancers in men and 2.4% in women might have been avoided if patients had had greater adherence to the MDP [57]. Other studies such as SUVIMAX (*Suppléments in Vitamines, et Minéraux Antioxydants*) have shown that to stay the risk of various cancers, the best prevention is a rich and varied diet of fruits and vegetables [58, 59]. According to the World Cancer Research Foundation, of 130,000 cancer deaths per year between 30-40% could have been prevented with the right diet [60]. Finally, a meta-analysis that included 12 studies with a total of 1,574,299 participants concluded that the 2-point increase in Mediterranean diet adherence (distributed between 0-9 points) was associated with a 6% decrease in mortality from cancer [61]. These same authors have more recently published an update of the meta-analysis cited, including the latest available works which confirm these findings [16]. When the different types of cancer are considered, a case-control study in Greece showed that a greater adherence to a MedDiet was related to a reduced risk of upper aerodigestive tract cancers [62]. Likewise, another study found a reduction in the risk of prostatic cancer for those individuals that followed a MedDiet compared with those following a Western diet [63].

Possible Biological Mechanisms Against Cancer

As we have already mentioned, the MedDiet is considered a healthy dietary pattern, a cultural model and a

lifestyle of certain countries of the Mediterranean coast. It is characterized by a high intake of olive oil, vegetables, fruits, legumes, and complex carbohydrates with a moderate consumption of fish, and a low-to-moderate amount of red wine during meals [4]. Thus, the increased quantity and quality of phytochemicals (such as vitamin C and E, folate, carotenoids and polyphenols) contained by the MedDiet could contribute to these beneficial effects due to their antioxidant and anti-inflammatory proprieties. These antioxidant compounds act in cell differentiation and proliferation, as well as in synthesis and DNA repair processes by inhibiting the endogenous formation of carcinogenic chemicals and reducing the formation of adducts in DNA [56]. Animal studies of induced breast cancer have shown that diets rich in extra virgin olive oil (EVOO) may inhibit proliferation, induce apoptosis, and minimize DNA damage [64]. A case-control study [65] including 255 newly diagnosed breast-cancer female patients (56 ± 12 yr) and 250 1-to-1 age-matched with the control patients was evaluated and showed an association between adherence to the MedDiet and its inherent constituents, with breast-cancer.

On the other hand, the MedDiet is also characterized by low consumption of red and processed meats. The latest studies have shown that a high consumption of red meat is associated with an increased risk of colorectal [66] and gastric cancer [67].

Fig. 1 shows how the MedDiet provides protective effects against CVD and cancer.

MEDITERRANEAN DIET AND NEURODEGENERATIVE DISEASES

Alzheimer's disease (AD) is the main cause of dementia among people age 65 and older and currently affects more than 25 million people in the world. AD is characterized by a progressive neurodegenerative disorder associated with cognitive impairment and neuronal cell loss, and the

pathology is characterized by the presence of several kinds of amyloid plaques and neurofibrillary tangles in the brain of AD patients. The end result is memory loss as well as personality changes [69]. It is known that AD and Parkinson's disease are two of the most common neurodegenerative diseases. In both cases, only a small percentage of cases are due to genetic mutations, although studies are now uncovering interactions with genetic and environmental factors. Animal studies have shown that dietary changes can disrupt the pattern of DNA methylation [70]. At present, APOE ϵ 4 is the only validated genetic risk factor for sporadic AD [69]. However, various non-genetic risk factors seem to be implicated in the pathophysiology of sporadic AD: brain trauma, cardiovascular diseases, stroke or transient ischemic attack, carotid atherosclerosis, and clinical history of hypertension, hypercholesterolemia and/or type-II diabetes.

Thus, higher adherence to a MedDiet has been associated with reduced cognitive decline and a protection against depression independently of the age of the patient [68]. Similarly, foods, and micro-, and macronutrients contained in the MedDiet seem to have a protective effect against dementia and pre-dementia syndromes [16, 71]. A recent meta-analysis showed that greater adherence to the MedDiet reduced cognitive impairment such as that in dementia or AD [72].

Possible Biological Mechanisms against Neurodegenerative Diseases

Several observational studies have consistently shown a protective effect of vitamin E against neurodegenerative diseases such as AD. Nevertheless, these data run counter to those shown by a recent meta-analysis in which there seems to be no evidence related to patients with AD or mild cognitive impairment [73]. Regarding fatty acids, intake of saturated fatty acids seems to increase the risk of AD. In contrast, epidemiological evidence suggests a possible association between fish consumption, monounsaturated fatty acids, and polyunsaturated fatty acids omega-3, and reduced risk of cognitive decline and dementia. However, the current evidence about light to moderate alcohol intake is only suggestive of a protective effect for vascular dementia, cognitive decline, and AD [70, 74]. However, further studies are needed to know better the mechanisms by which nutrients, foods and dietary patterns may act on cognition mechanisms.

CONCLUDING REMARKS

In summary, the results of several studies suggest that the MedDiet may have a dual effect on the prevention of CVD, improving classical cardiovascular risk factors and also having an intense anti-inflammatory effect. In fact, epidemiological studies have shown that the MedDiet may exert its effect partly through mechanisms such as improved lipid profile and reductions in blood pressure or insulin resistance. Its anti-inflammatory effect seems to be greater and more intense in the mid-term compared to the short-term while the effect on classical cardiovascular risk factors is similar, thereby suggesting that the MedDiet exerts its effects

on lipids and blood pressure relatively quickly (at 3 mo), with the maximum effect on systemic inflammatory biomarkers being achieved later (at 1 y). Thus, in the short-term the effect on blood pressure and the lipid profile is higher, whereas in the mid-term the effect on chronic inflammatory response in the arterial wall is more pronounced.

The present review also provides evidence that a MDP alone or one enriched with some of its main components (*i.e.* extra virgin olive oil and nuts), not only diminishes the classical cardiovascular risk factors, but is also associated with important reductions in circulating inflammatory biomarkers, cellular inflammatory biomarkers and improves endothelial dysfunction. Moreover, increased consumption of antioxidant-rich foods as in a MDP in general and of polyphenols in particular was associated with better cognitive performance in an elderly cohort at high cardiovascular risk.

Therefore, the choice of a healthy diet such as the MedDiet associated with regular physical activity is critical in the fight against many chronic diseases. The protection against atherosclerosis by the MedDiet can probably be extended to other chronic inflammation-related diseases, including visceral obesity, the metabolic syndrome, and type-2 diabetes, cancer or neurodegenerative diseases, among others.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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Molecular Properties of Red Wine Compounds and Cardiometabolic Benefits

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Abstract

Wine has been used since the dawn of human civilization. Despite many health benefits, there is still a lot of discussion about the real properties of its components and its actions on cells and molecular interactions. A large part of these issues permeate the fine line between the amount of alcohol that causes problems to organic systems and the amount that could be beneficial for the health. However, even after the process of fermentation, wine conserves different organic compounds from grapes, such as polysaccharides, acids, and phenolic compounds, such as flavonoids and nonflavonoids. These substances have known anti-inflammatory and antioxidant capacities, and are considered as regulatory agents in cardiometabolic process. In this study, the main chemical components present in the wine, its interaction with molecules and biological mechanisms, and their interference with intra- and extracellular signaling are reviewed. Finally, the properties of wine that may benefit cardiovascular system are also revised.

Keywords: wine, ethanol, flavonoids, cardiovascular system

Introduction

Wine is a traditional alcoholic beverage of high commercial importance, obtained by fermentation of grape must. By this definition, the quality of wine is related to the composition and variety of grape.¹ Moreover, wines can be distinguished by the geographic location of vineyards, variations in the same vineyard, different viticultural practices, and winemaking and aging techniques.²

Wine is a complex mixture of several hundred compounds, many of them found at very low concentrations; however, they play an important role in its evolution and quality.³ In general, the average concentrations of the major components of wine are water, 86%; ethanol, 12%; glycerol and polysaccharides or other trace elements, 1%; different types of acids, 0.5%; and volatile compounds, 0.5%.⁴

Wine may be classified as red, white, and rosé wines based on sweetness, alcohol content, carbon dioxide content, color, grape variety, fermentation, and maturation process or geographic origin.⁵ While red wines are obtained by the alcoholic fermentation of musts in the presence of the solid parts of the berry (skins and seeds), white wines are exclusively produced by the fermentation of grape juice.⁶

Red wine is known to contain 10-fold more phenolic compounds than white wine, resulting from the fermentation of grape juice with skins, grape pieces, and seeds ([Table 1](#)).¹ Although the antioxidant property of red wines is correlated with their phenol content, no single compound sufficiently defines the total antioxidant capacity, because of the potential synergistic antioxidant effect of other compounds.⁷

Table 1

Content of majority phenolic compounds of red and white wines, expressed in milligrams of gallic acid equivalent (mg/GAE/L).^{78,79}

PHENOLIC COMPOUNDS	RED WINE (mg/GAE/L)	WHITE WINE (mg/GAE/L)
Catequin	191	35
Epigallocatechin	82	21
Gallic acid	95	7
Cyanidin-3-glucoside	3	0
Malvidin-3-glucoside	24	1
Rutine	9	0
Quercetin	8	0
Myricetin	9	0
Caffeic acid	7.1	2.8
Resveratrol	1.5	0
Total content of phenolics	2567	239

Abbreviation: GAE, gallic acid equivalent.

Studies have shown the effect of alcohol and wine consumption on the improvement of cardiometabolic risk factors (blood pressure, serum glucose, low-density lipoprotein [LDL] and high-density lipoprotein [HDL] levels, inflammation, and endothelial function).⁸⁻¹⁰ Hyperglycemia and hypertension may contribute to the development of endothelial dysfunction, and high serum levels of

LDL oxidized by reactive oxygen species (ROS) play the main role in the initiation and progression of atherosclerosis. On the other hand, HDL exerts a protective effect in coronary heart disease by suppressing endothelial damage, LDL oxidation, vascular-LDL accumulation, inflammation, and thrombosis.¹¹ Although international guidelines suggest a light-to-moderate alcoholic beverage consumption (15–30 g/day of ethanol, about 130–250 mL of wine/day)^{12–14} for cardiovascular risk reduction, it is known that high alcohol intake (>31 g/day) may have negative effects on the cardiovascular system, including an increase in blood pressure, activation of the sympathetic system,^{15–17} and an increase in the incidence of atrial fibrillation, cardiomyopathy, and hemorrhagic stroke.¹⁸ Thus, the relationship between alcohol consumption and the risk for many cardiovascular conditions is characterized by a U- or J-shaped curve.

The aim of this mini review is to highlight the main cardioprotective molecules present in red wine and how these compounds interact with cellular systems, particularly those involving antioxidation and anti-inflammatory activities. Furthermore, we discuss how these molecules can benefit human health by pointing out results from clinical studies.

Chemical Properties of Phenolic Compounds in Red Wine

The pulp, skin, seeds, and stems of grapes of the *Vitis* genus are relatively rich in nonflavonoid compounds.¹⁹ Polyphenols are the main phenolic compounds extracted from grapes during the winemaking process, initially obtained by the crushing of the fruit, and intensified by the maceration and pumping-over processes during fermentation.

The total amount of polyphenols in red wines has been estimated to range from 2000 to 6000 mg/L,²⁰ as shown in [Table 1](#). The main bioactive polyphenols in red wines are notably flavanols, flavonols, anthocyanins, and resveratrol.²¹ Flavonoids, which account for over 85% of the phenolic components in red wine, include different molecular families such as flavonols [eg, monomeric (catechin, epicatechin), oligomeric, and polymeric compounds (proanthocyanidins, also called condensed tannins)], flavones, anthocyanins,¹ flavan-3-ols, catechins, and epicatechins.^{22,23}

Catechin and epicatechin are usually the most important flavanols in both grape skins and seeds and can represent up to 60% of total phenolic compounds present in the seed.²⁴ Both are responsible for the astringency, bitterness, and structure of wines.²⁵ Catechin and epicatechin can be extracted from grape pomace by using aqueous solutions, reaching similar level of extraction than using ethanol/water as extraction solvent.²² Red wines from Cabernet Sauvignon and Refosco grapes showed the highest polyphenol and catechin contents.²⁶

Flavonols comprise compounds such as myricetin, quercetin, kaempferol, and rutin. Quercetin is very common in different grapes; for example, it is the most abundant flavonol found in Sangiovese grapes.²⁷ Flavonols and their glycosides are important components in wine because of their impact on color, taste, and health properties.²⁸

Anthocyanins are responsible for the red color of wines and are extracted from grape skins during the winemaking process. The anthocyanins most commonly found in wines are delphinidin-3-glucoside, cyanidin-3-glucoside, and malvidin-3-glucoside,²⁹ with recognized antioxidant capacity.^{19–21}

Resveratrol is a phenolic compound of the stilbene family present in grape skin and seeds, and hence, constituent of grape juice and wines.³⁰ Although resveratrol has been considered as the major functional compound in red wine, its concentration is lower than other polyphenols.⁷

Tannins, another subgroup of phenols found in the skins and seeds of grapes, can be classified as monomeric, oligomeric, and polymeric flavan-3-ols (condensed tannins).³¹ Tannins play an important role in the quality of wine, since they contribute to sensory aspects such as color, bitterness, and astringency and structure of the wine.

The composition of wine mainly depends on grape variety, followed by the winemaking techniques. The sugar, acid, tannin, anthocyanin, phenolic, and aromatic compound contents of the grapes and their interactions play key roles in the composition of wines.³² Enological practices in winemaking can affect wine production, composition, and quality.

In summary, wine characteristics are mainly determined by the combination and interaction of phenolic compounds of grapes and its changes during the winemaking process. The main constituents of red wine, with important effects on pathophysiological mechanisms, are reported below.

Effect of Wine Constituents on Biological Functions

Wine has a varying concentration of water, alcohol, and phenolic compounds, of which tannins, resveratrol, and quercetin have been the most studied. These polyphenols have positive effects on cardiac function and prevention of cardiovascular diseases,³³ by modulating cellular and molecular mechanisms that lead to anti-inflammatory, antioxidant, and hypotensive responses.³⁴ Some of these mechanisms have been well described and explored in therapeutic and preventive approaches for cardiovascular diseases.

The effects of alcohol

High consumption of alcohol may lead to lipid peroxidation, in which ROS cause damage to the cell membranes, sometimes irreversible to the cell.³⁵ Alcohol stimulates the activity of the enzyme cytochrome P450 and alters the levels of some metals in the body, contributing to ROS production.³⁶ In tissues, exacerbated ROS generation triggers a cascading inflammatory response, which affects homeostasis and culminates in tissue injury and establishment of a disease. In this context, the negative effect of alcohol has been well described, particularly on the liver, causing severe alcohol-related liver diseases.³⁷

On the other hand, light-to-moderate consumption of alcohol can bring benefits to health. Chronic intake of light-to-moderate doses of alcohol may increase HDL levels and decrease LDL oxidation.³⁸ In addition, prior ethanol administration (ethanol preconditioning) induces a mild oxidative stress that has a protective effect against ischemia/reperfusion-induced brain damage.³⁹ In fact, the level of alcohol intake is closely related to ROS production and their deleterious effects—a low concentration is essential for the physiological degradation of polyunsaturated fatty acids, whereas high concentrations of ROS cause potential damages to cellular components, giving rise to endothelial dysfunction and other conditions.⁴⁰ Also, Agarwal pointed out the influence of moderate consumption of alcohol on preventing blood coagulation and reducing platelet aggregation.^{38,41}

Moderate alcohol consumption is also related to decreased insulin resistance in skeletal muscle, and such insulin-sensitizing activity may be related to improved production of AMP-activated protein kinase, generated by the metabolism of acetate in peripheral tissues, and involved in glucose uptake (among other functions).⁴² Finally, moderate alcohol intake also raises the paraoxonase 1 (PON1) levels,⁴³ an enzyme that, among other functions, prevents the oxidation of LDL and increases levels of homocysteine.³⁸ These beneficial effects of alcohol have been mostly associated with the phenolic compounds present in red wine.

The role of polyphenols

In vitro studies and preclinical models have demonstrated the association of wine polyphenols with activation of antioxidant and anti-inflammatory mechanisms. Flavonoids, particularly quercetins, catechins, tannins, and resveratrol,⁴⁴ also act against free radicals, allergies, inflammation, ulcers, viruses, tumors, and hepatotoxins, inhibit platelet aggregation, reduce heart disease and stroke risk, and is involved in the synthesis of estrogen. Additionally, these molecules, present in almost all varieties of red wine, have their action in cells and tissues adjacent to blood vessels, mainly in the endothelium. In addition to the already mentioned functions, they have a direct role in the reduction of cell proliferation,⁴⁵ which can be exploited for cancer therapy.

Anthoxanthins, flavans, and anthocyanidins

The main antioxidant mechanism of catechins, a flavan-3-ol quite abundant in grape and red wine, is related to the inhibition of nuclear factor kappa-B (NF- κ B), a transcription factor that activates inflammatory cytokines in tissue injury or ischemia.⁴⁶ These cytokines are released in tissue oxidative damage affecting the liver, heart, lungs, kidney, and vascular endothelium, related to chronic diseases or aging.

Epigallocatechin has been shown to mitigate the proliferation of vascular smooth muscle cells, induced by interleukin-1-beta (IL-1 β , a potent proinflammatory cytokine), and that contributes to atherosclerosis. Besides, this polyphenolic catechin also reduces the release of ROS and activates the synthesis of antioxidant enzymes.⁴⁷ In vitro experiments also showed the potential role of epigallocatechin in preventing skin aging, as it protects against oxidative stress-induced apoptosis in fibroblasts by inhibiting phosphorylation of p38 and c-Jun N-terminal kinases.⁴⁸

The hydroxylation of the catechin monomer results in proanthocyanidin polymers (the so-called condensed tannins). Proanthocyanidins have beneficial effects on human health due to antioxidant, antimicrobial, and antiallergic properties.⁴⁹ In addition, these molecules inhibit the angiotensin-converting enzyme, preventing the formation of angiotensin II, a potent vasoconstrictor.⁵⁰ Interestingly, a preclinical study carried out on dyslipidemic obese rats showed that proanthocyanidins, in association with docosahexaenoic acid, were able to modulate the expression of microRNAs, such as miR-33a and miR-122, which are the major regulators of lipid metabolism in the liver.⁵¹

Quercetin strongly induces the activity of antioxidant enzymes such as heme oxygenase, glutathione S-transferase, and thioredoxin reductase.⁵² Besides, this flavonol is able to upregulate nitric oxide synthase (NOS) expression and decrease oxidative stress. Quercetin was also reported as an anti-inflammatory compound, due to its role in mediating the reduction of the expression of Toll-like receptors (TLR2 and TLR4) by inhibiting NF- κ B translocation to the nucleus.⁵³ In addition, the reduction of excessive production of nitric oxide by phenolic compounds has also been analyzed and evidenced in aorta of rats subjected to a diet with alcohol-free red wine,⁵⁴ suggesting that both quercetin and catechin not only activate antioxidant mechanisms but are also capable to modulate them. Moreover, quercetin also seems to be associated with the inhibition of cell proliferation, attenuating the progression of some cancers,⁵⁵ and with reduction of blood pressure⁵⁶ and obesity.⁵⁷ Shimizu et al⁵⁷ showed that quercetin reduces the gene expression of apolipoproteins, including apolipoprotein B (apoB), in human enterocytes.

Stilbenoids In terms of health effects of wine constituents, resveratrol has been the most studied element, in both animal models and clinical trials. First, resveratrol is a key regulator of homeostasis, acting on gene regulation (chromatin remodeling), protein synthesis, posttranslation modifications, enzymatic function, apoptosis, signal transduction (kinase activation/inhibition), and modulation of intracellular calcium concentration.^{44,58} Considering its mechanism of action, resveratrol is able to modulate the inflammatory response in a balanced way: inflammatory cytokines, such as tumor

necrosis factor- α (TNF- α), IL-1 β , and interleukin-6 (IL-6), have already been demonstrated to be either induced or repressed by resveratrol.⁵⁹ In this scenario, resveratrol is also able to inhibit inflammatory enzymes, such as the inducible isoforms of NOS (iNOS) and cyclooxygenase-1 (COX-1), adhesion molecules, and the NF- κ B. Olas and Wachowicz showed that resveratrol is capable of inhibiting the synthesis of thromboxane and reducing platelet aggregation.⁶⁰ Further, in addition to its influence on cell signaling, inflammatory, and antioxidant profile,⁶¹ resveratrol can suppress acute and chronic pain by inhibiting the mammalian target of rapamycin (mTOR) and the extracellular signal-regulated kinase signaling in neuronal cells.⁶² Finally, Peltz et al demonstrated distinct and dynamic actions of resveratrol on human mesenchymal stem cells,⁶³ highlighting its role in tissue repair, which is very attractive to regenerative medicine.⁶⁴ Furthermore, resveratrol activates sirtuins, a class of protein deacetylases that regulate metabolism, stress responses, and aging processes.⁶⁵ In this way, resveratrol, in a dosage-dependent manner, regulates the expression of genes associated with cell cycle, cell senescence, and longevity, implicated on both cell self-renewal and differentiation capacity of mesenchymal stem cells. Together, these data corroborate the potential use of red wine as a functional food, supported by its anti-inflammatory and antioxidant functions, and its contribution to tissue repair processes.

The elucidation of cellular and molecular mechanisms modulated by phenolic constituents present in red wine contributes to the understanding of the potential beneficial effects of these compounds on the prevention and treatment of several chronic diseases such as cardiovascular and inflammatory diseases and cancer (Fig. 1).^{33,44,66} These effects become even more pronounced when a light-to-moderate wine consumption is associated with a healthy lifestyle and habits, such as the adoption of the Mediterranean diet and physical activity.^{67,68}

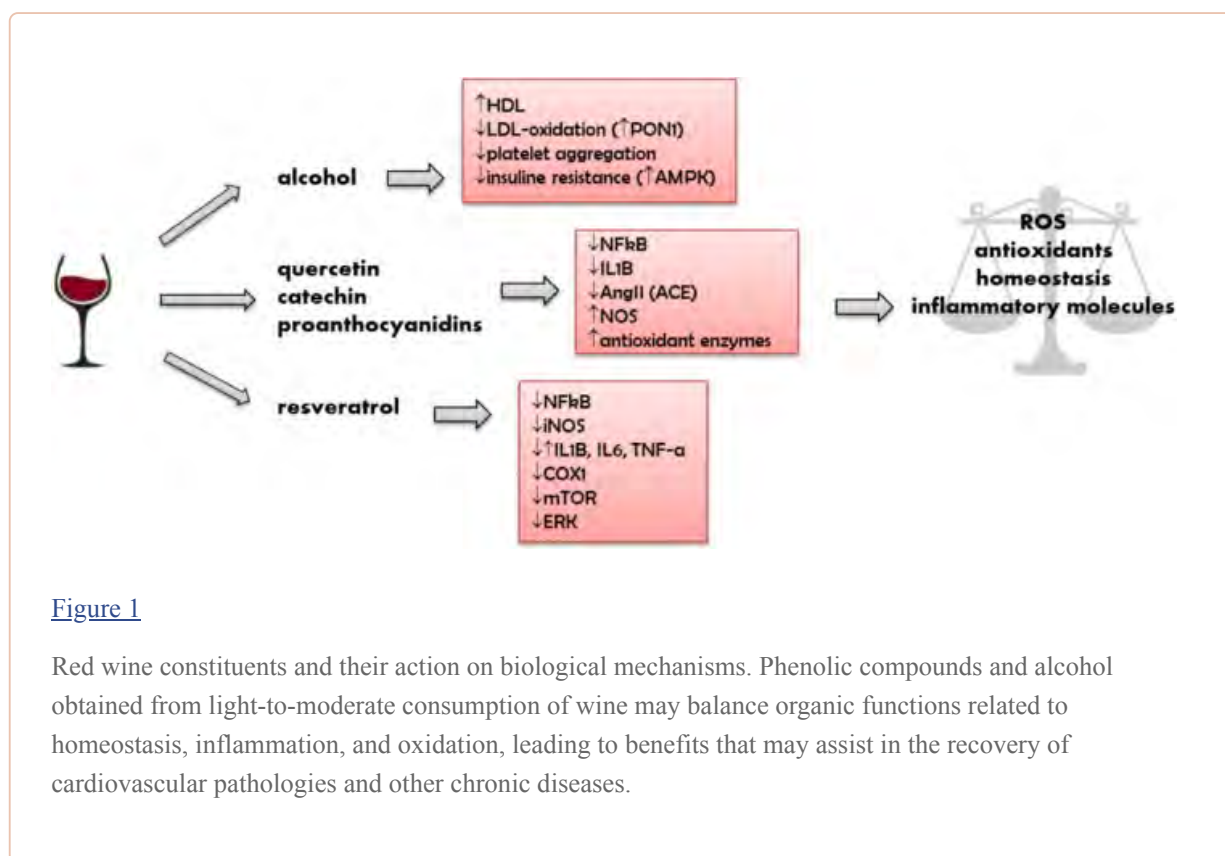


Figure 1

Red wine constituents and their action on biological mechanisms. Phenolic compounds and alcohol obtained from light-to-moderate consumption of wine may balance organic functions related to homeostasis, inflammation, and oxidation, leading to benefits that may assist in the recovery of cardiovascular pathologies and other chronic diseases.

Studies in Humans Regarding Cardiometabolic Factors and Wine

Several clinical studies have been made in both healthy volunteers and individuals with chronic diseases (dyslipidemia, hypertension, type 2 diabetes mellitus [T2DM], metabolic syndrome [MS], and coronary heart disease), regarding the effects of wine consumption on metabolic, inflammatory, and cardiovascular parameters. However, it is noteworthy that these effects are dependent on the bioavailability of the phenolic compounds, which may be affected by many factors, such as environmental, food processing (thermal treatments, cooking techniques, storage), and dietary factors (presence of positive or negative effectors of absorption, such as meals rich in fats and fibers), interactions with other compounds (polyphenols with similar mechanism of absorption), chemical structure of polyphenols and their concentrations in food, and host-related factors (intestinal factors such as enzyme activity, transit time, and microbiota; age; gender; presence of diseases; and genetic condition).⁶⁹ Some clinical trials that evaluated the beneficial effects of wine consumption (for a minimum of 15 days) on cardiometabolic factors in nonhealthy subjects are described below.

In individuals with dyslipidemia, a trend toward significance for decreased LDL/HDL ratio levels ($P = 0.05$) was detected after red wine consumption for 30 days,⁷⁰ and in hypercholesterolemic postmenopausal women, chronic consumption of red wine significantly reduced the LDL levels by 8% and increased the HDL levels by 17%.⁷¹ In patients with well-controlled T2DM, the consumption of 150 mL/day of red wine at dinner for two years significantly increased HDL and apolipoprotein A1 levels, and decreased the total cholesterol/HDL ratio.⁷² Apolipoprotein A1 and A2 and HDL levels increased in men at high cardiovascular risk who consumed 30 g alcohol/day of red wine for four weeks.⁷³

As previously mentioned, white wine is composed of a minor amount of phenolic compounds when compared to red wine, but its effects on metabolic parameters regarding lipidic, glycidic, and inflammatory profile in nonhealthy individuals have also been evaluated. Eighteen patients with MS consumed white wine for four weeks, and no changes were detected regarding total cholesterol, LDL, triglyceride, and fasting plasma glucose levels; however, homeostasis model assessment of insulin release decreased significantly ($P = 0.002$).⁷⁴ The impact of white wine in combination with extra-virgin olive oil on inflammatory profile was evaluated in patients with chronic kidney disease KDOQI stages III–IV. Subjects were allocated to two weeks of treatment with extra-virgin olive oil alone or white wine (4 mL/kg body weight, 0.48 g/kg of alcohol 12%, corresponding to 2–3 glasses/daily) plus extra-virgin olive oil. Plasma C-reactive protein (CRP) and IL-6 levels decreased after wine plus olive oil consumption, but no difference was detected after the treatment with olive oil alone.⁷⁵

Ventricular dyssynchrony and inflammatory markers were evaluated in 115 individuals with T2DM who had sustained a first nonfatal myocardial infarction and were randomized to receive red wine (during a meal) or not (control group). After one year of intervention, compared to the treatment group, all inflammatory markers (CRP, TNF- α , IL-6, IL-18, and nitrotyrosine) were increased, and echocardiographic parameters indicated ventricular dyssynchrony in the control group.⁷⁶ In another study that evaluated metabolic, autonomic, hemodynamic, and endothelial responses in subjects with hypercholesterolemia or arterial hypertension, 250 mL/day of red wine for 15 days decreased blood pressure levels and vascular resistance, enhanced muscle sympathetic fibular nerve activity in hypertensive and hypercholesterolemic individuals, and restored brachial artery flow-mediated dilation in hypercholesterolemic patients.⁷⁷

In this review, we described that alcohol and specific phenolic compounds may have different effects on different metabolic factors. Although the beneficial effects of these compounds on cardiometabolic traits have been indicated by several studies, the results of clinical studies should be interpreted with caution. Limitations of many of these studies include small sample size, short-term evaluation of wine consumption (making the extrapolation of the results to longer periods of wine consumption difficult), and lack of measurements of phenolic compounds in plasma, urine, or even in the wines used as

intervention. Besides, several issues in these studies deserve careful consideration, including the heterogeneity and genetic variability of the populations, the use of medications and their interactions with phenolic compounds, the different amounts of wine used as intervention, the lack of data regarding other dietary sources of polyphenols consumed by the subjects, and different methods used to evaluate the same outcome. Thus, further randomized, clinical trials evaluating the effects of long-term consumption of red wine are necessary, taking into account the safe limits of alcohol intake for each group. Additionally, although much has been known about the properties of wine, how different compounds of different grape varieties might help in therapeutic approaches need to be explored.

Conclusion

Studies conducted in humans have evidenced that phenolic compounds, as well as ethanol present in red wine, can have beneficial effects on health, due to its anti-inflammatory and antioxidant properties and their role in tissue repair processes. These processes are modulated due to antioxidant and anti-inflammatory capabilities of the components of the wine. Such mechanisms help the organic systems in bringing assistance to cellular and tissue functions. However, despite the protective effects of these phenolic constituents, the amount of wine consumed deserves attention, since a chronic excessive intake may lead to an exacerbated response, oxidative stress, endothelial dysfunction, and cardiovascular disease.

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Author Contributions

Contributed to the writing of the manuscript, made critical revisions, and approved the final version: MMM, JG, AO, JO, and AM.

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Review

Role of Resveratrol in Prevention and Therapy of Cancer: Preclinical and Clinical Studies

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Abstract. Resveratrol, trans-3,5,4'-trihydroxystilbene, was first isolated in 1940 as a constituent of the roots of white hellebore (*Veratrum grandiflorum* O. Loes), but has since been found in various plants, including grapes, berries and peanuts. Besides cardioprotective effects, resveratrol exhibits anticancer properties, as suggested by its ability to suppress proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers; multiple myeloma; cancers of the breast, prostate, stomach, colon, pancreas, and thyroid; melanoma; head and neck squamous cell carcinoma; ovarian carcinoma;

and cervical carcinoma. The growth-inhibitory effects of resveratrol are mediated through cell-cycle arrest; up-regulation of p21^{Cip1/WAF1}, p53 and Bax; down-regulation of survivin, cyclin D1, cyclin E, Bcl-2, Bcl-x_L and cIAPs; and activation of caspases. Resveratrol has been shown to suppress the activation of several transcription factors, including NF- κ B, AP-1 and Egr-1; to inhibit protein kinases including I κ B kinase, JNK, MAPK, Akt, PKC, PKD and casein kinase II; and to down-regulate products of genes such as COX-2, 5-LOX, VEGF, IL-1, IL-6, IL-8, AR and PSA. These

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Key Words: Resveratrol, cell signaling, chemoprevention, metastasis, transformation, invasion, tumorigenesis, apoptosis, review.

Abbreviations: TNF, tumor necrosis factor; NF- κ B, nuclear factor kappa B; PKC, protein kinase C; UV, ultraviolet; NOS, nitric oxide synthase; COX, cyclooxygenase; PMA, phorbol myristate acetate; LDL, low-density lipoprotein; PBMC, peripheral blood mononuclear cells; PMN, human polymorphonuclear leukocytes; GSH, reduced glutathione; AP-1, activator protein-1; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; TGF, transforming growth factor; PKA, protein kinase A; DMBA, 7,12-dimethylbenzoic acid; B[a]P, benzo[a]pyrene; BPDE, B[a]P diol epoxides; AhR, aryl hydrocarbon receptor; PhiP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; AOM, azoxymethane; NNK, 4-(methyl-nitrosamine)-1-(3-pyridyl)-1-butanone; ODC, ornithine decarboxylase; B-CLL, B-cell chronic lymphocytic leukemia; CTL, cytotoxic T lymphocyte; NQO, NAD(P)H quinone oxidoreductase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SBP, systolic blood pressure; EWP, extract of wine phenolics; SMC, smooth muscle cells; ROS, reactive oxygen species; EGFR, epidermal

growth factor receptor; HUVEC, human umbilical vein endothelial cells; 8-OHdG, 8-hydroxydeoxyguanosine; TBARS, thiobarbituric acid-reactive substances; AAPH, 2,2'-azobis-(2-amidinopropane) dihydrochloride; IC₅₀, concentration causing 50% inhibition; ICV, intracerebroventricular; STZ, streptozotocin; HMG, half-mustard gas; LLC, Lewis lung carcinoma; VEGF, vascular endothelial growth factor; BHA, butylated hydroxyanisole; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; MMP, matrix metalloproteinase; IL, interleukin; PARP, poly(ADP-ribose) polymerase; Egr, early growth response gene; ER, estrogen receptor; CYP, cytochrome P450; IFN, interferon; NSAID, nonsteroidal anti-inflammatory drug; H₂O₂, hydrogen peroxide; Cdk, cyclin-dependent kinases; PDGF, platelet-derived growth factor; PSA, prostate-specific antigen; ACF, aberrant crypt foci; Ach, acetylcholine; MDA, malondialdehyde; SHRSP, stroke-prone hypertensive rats; λ max, wavelength maxima; HPLC, high-pressure (performance) liquid chromatography; MS, mass spectrometric; CoA, coenzyme A; NO, nitric oxide; AIF, apoptosis-inducing factor; AML, acute myeloid leukemia; DISC, death-inducing signal complex; AR, androgen receptor; ALL, acute lymphocytic leukemia; Rb, retinoblastoma; SPT, serine palmitoyltransferase; PDE, phosphodiesterase; AZT, zidovudine; ddC, zalcitabine; ddI, didanosine; PKD, protein kinase D; LPS, lipopolysaccharide; PI3K, phosphoinositide 3-kinase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; FADD, Fas-associated death domain.

activities account for the suppression of angiogenesis by this stilbene. Resveratrol also has been shown to potentiate the apoptotic effects of cytokines (e.g., TRAIL), chemotherapeutic agents and γ -radiation. Pharmacokinetic studies revealed that the target organs of resveratrol are liver and kidney, where it is concentrated after absorption and is mainly converted to a sulfated form and a glucuronide conjugate. In vivo, resveratrol blocks the multistep process of carcinogenesis at various stages: it blocks carcinogen activation by inhibiting aryl hydrocarbon-induced CYP1A1 expression and activity, and suppresses tumor initiation, promotion and progression. Besides chemopreventive effects, resveratrol appears to exhibit therapeutic effects against cancer. Limited data in humans have revealed that resveratrol is pharmacologically quite safe. Currently, structural analogues of resveratrol with improved bioavailability are being pursued as potential therapeutic agents for cancer.

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Introduction

The history of resveratrol can be traced back thousands of years. Perhaps the first known use of grape extracts for human health can be dated over 2000 years ago, to "darakchasava", a well-known Indian herbal preparation of which the main ingredient is *Vitis vinifera* L. This "Ayurvedic" medicine is prescribed as a cardi tonic and also given for other disorders (1). The use of dried grapes (also called manakka) as a cardi tonic is well documented. High-performance liquid chromatography (HPLC) analysis of darakchasava revealed polyphenols such as resveratrol and pterostilbene. This age-old formulation became interesting in the light of recently acquired knowledge on resveratrol.

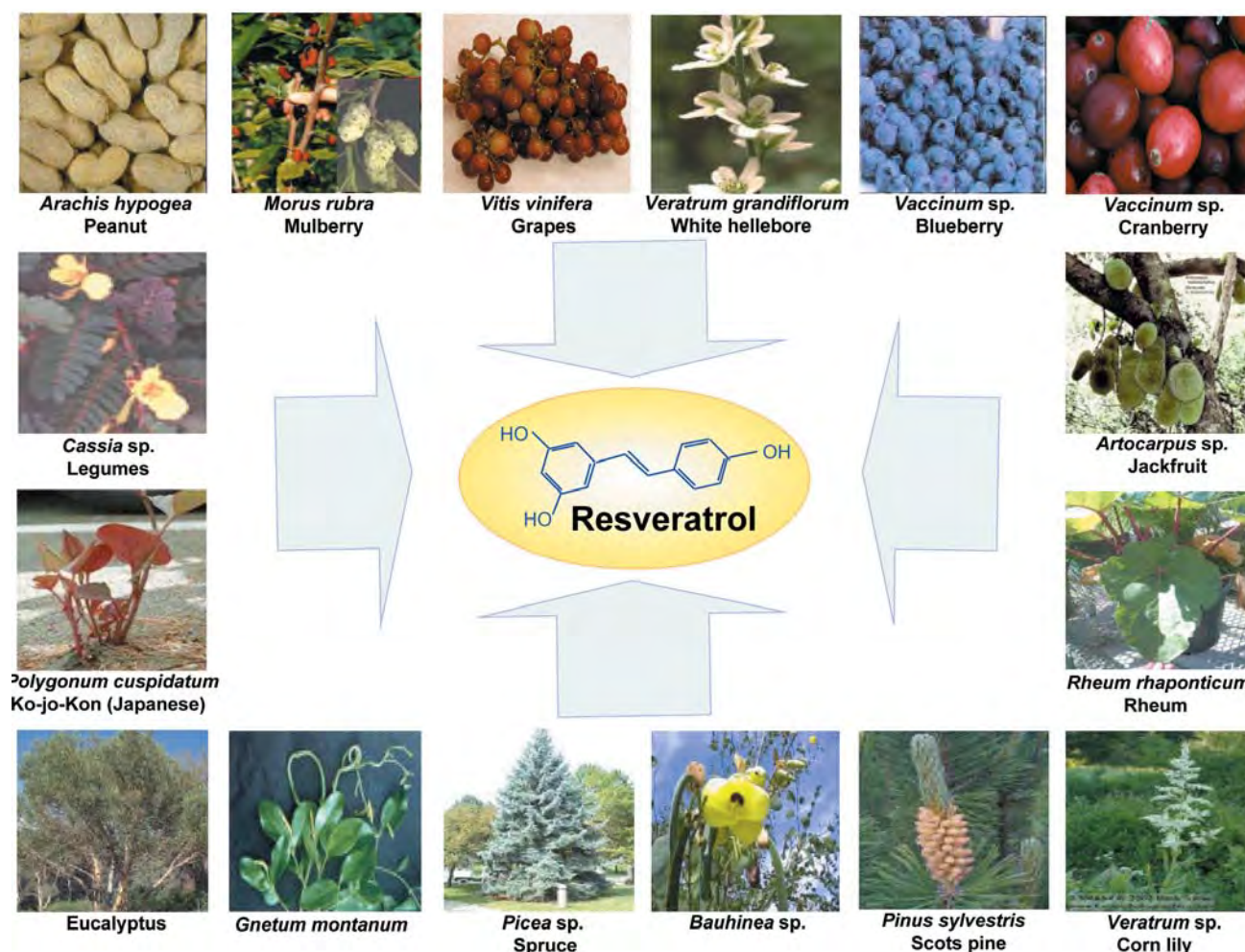


Figure 1. Sources of resveratrol from different plants.

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring phytoalexin produced by a wide variety of plants, such as grapes (*Vitis vinifera*), peanuts (*Arachis hypogea*), and mulberries in response to stress, injury, ultraviolet (UV) irradiation, and fungal (e.g., *Botrytis cinerea*) infection. Although phytoalexins have long been inferred to be important in the defense of plants against fungal infection, few reports show that they provide resistance to infection. Several plants, including grapevine, synthesize the stilbene-type phytoalexin resveratrol when attacked by pathogens. Stilbenes with fungicidal potential are formed in several unrelated plant species, such as peanut, grapevine, and pine (*Pinus sylvestris*) (Figure 1). Stilbene biosynthesis specifically requires the presence of stilbene synthase. Furthermore, the precursor molecules for the formation of hydroxy-stilbenes are malonyl-coenzyme A (CoA) and p-coumaroyl-CoA, both present in plants. Hain *et al.* isolated the stilbene

synthase gene from grapevine, transferred it into tobacco, and found that regenerated tobacco plants containing this gene are more resistant to infection by *Botrytis cinerea* (2).

Resveratrol was first identified in 1940 as a constituent of the roots of white hellebore (*Veratrum grandiflorum* O. Loes), and later in the dried roots of *Polygonum cuspidatum*, called Ko-jo-kon in Japanese, which is used in traditional Chinese and Japanese medicine to treat suppurative dermatitis, gonorrhea, favus, athlete's foot (tinea pedis), and hyperlipemia (3-6). In 1976, resveratrol was detected in the leaf epidermis and the skin of grape berries but not in the flesh (7-9). Fresh grape skins contain 50-100 mg resveratrol per gram, and the concentration in wine ranges from 0.2 mg/l to 7.7 mg/l. The epidemiological finding of an inverse relationship between consumption of red wine and incidence of cardiovascular disease has been called the "French paradox" (10, 11). For a variety of reasons, the cardioprotective effects of red wine

Table I. Sources of Resveratrol and its analogues.

Compound	Sources	References
Resveratrol (<i>trans</i> -3,5,4'-trihydroxystilbene)	Japanese knotweed (<i>Polygonum cuspidatum</i>); <i>Vitis</i> spp. (incl. grape-vines, leaves and berryskin); <i>Vaccinium</i> spp. (incl. blueberry, bilberry, cranberry); <i>Morus</i> spp. (incl. mulberry); Lily (<i>Veratrum</i> spp.); Legumes (<i>Cassia</i> spp., <i>Pterolobium hexapetallum</i>); Peanuts (<i>Arachis hypogaea</i>); <i>Rheum</i> spp.(incl. Rhubarb); Eucalyptus; Spruce (<i>Picea</i> spp.); Pine (<i>Pinus</i> spp.); Poaceae (grasses incl. <i>Festuca</i> , <i>Hordeum</i> , <i>Poa</i> , <i>Stipa</i> and <i>Lolium</i> spp.); <i>Trifolium</i> spp.; <i>Nothofagus</i> spp.; <i>Artocarpus</i> spp.; <i>Gnetum</i> spp.; <i>Pleuropterus ciliinervis</i> ; <i>Bauhinia racemosa</i> ; <i>Paeonia lactiflora</i> ; <i>Scilla nervosa</i> ; <i>Tetrastigma hypoglauca</i> ; Synthetic	(26-28, 33-38)
Dihydroresveratrol (<i>trans</i> -3,5,4'-trihydroxybibenzylstilbene)	<i>Dioscorea</i> spp.; <i>Bulbophyllum triste</i> ; Synthetic	(39, 40)
Piceatannol or astringinin (<i>trans</i> -3,4,3',5'-tetrahydroxystilbene)	White tea tree (<i>Melaleuca leucadendron</i>); Asian legume (<i>Cassia garrettiana</i>), <i>C. marginata</i> ; Rhubarb (<i>Rheum</i> spp.); <i>Euphorbia lagascae</i> ; <i>Polygonum cuspidatum</i> ; <i>Vitis vinifera</i>	(28, 40-45)
Dihydropiceatannol (<i>trans</i> -3,4,3',5'-tetrahydroxybibenzylstilbene)	<i>Cassia garrettiana</i> ; Synthetic	(42)
Gnetol (<i>trans</i> -2,6,3',5',-tetrahydroxystilbene)	<i>Gnetum</i> spp. (incl. <i>G. monatum</i> , <i>G. africanum</i> , <i>G. gnemon</i> , <i>G. ula</i>)	(36, 46, 47)
Oxyresveratrol (<i>trans</i> -2,3',4,5'-tetrahydroxystilbene)	<i>Morus</i> spp.; <i>Maclura pomifera</i> ; <i>Artocarpus gomezianus</i> ; <i>Schoenocaulon officinale</i>	(38, 48-50)
Hydroxyresveratrol (<i>trans</i> -2,3,5,4'-tetrahydroxystilbene)	<i>Polygonum cuspidatum</i>	(28)
Trans-3,4,5,4'-tetrahydroxystilbene	Synthetic	(51)
Trans-3,3',4',5,5'-pentahydroxystilbene	<i>Eucalyptus wandoo</i> ; <i>Vouacapoua americana</i> , <i>V. macropetala</i> ; Synthetic	(52, 53)
Pinosylvin (<i>trans</i> -3,) 5-dihydroxystilbene	<i>Gnetum cleistostachyum</i> ; <i>Alpinia katsumadai</i> ; <i>Polyalthia longifolia</i> ; <i>Polygonum nodosum</i> ; <i>Pinus</i> spp.(incl. Scottish pine, <i>P. sylvestris</i>); Synthetic	(51, 54-59, 361)
Dihydropinosylvin (<i>trans</i> -3,5-dihydroxybibenzylstilbene)	<i>Dioscorea batatas</i> ; Synthetic	(60-62)
Trans-2,4,4'-trihydroxystilbene	Synthetic	(61, 62)
Trans-3,5,3'-trihydroxystilbene	Synthetic	(63, 64)
Trans-3,4,5-trihydroxystilbene	Synthetic	(65)
Trans-3,4,4'-trihydroxystilbene	Synthetic	(65, 66)
Trans-3,4-dihydroxystilbene	Synthetic	(61, 62, 66)
Trans-3,4'-dihydroxystilbene	Synthetic	(63, 64)
Trans-3,3'-dihydroxystilbene	Synthetic	(63, 64)
Trans-2,4-dihydroxystilbene	Synthetic	(61, 62)
Trans- 4,4'-dihydroxystilbene	Synthetic	(61, 62, 65, 66)

continued

Table I. *continued.*

Compound	Sources	References
Trans-3-hydroxystilbene	Synthetic	(63, 64)
Trans-4-hydroxystilbene (p-hydroxystilbene)	Synthetic	(61, 62, 65)
Trans-halogenated-3,5, 4'-trihydroxystilbenes (fluoro-, chloro- and iodo-resveratrols)	Synthetic	(67, 68)
Dimethoxypinosylvin (<i>trans</i> -3,5-dimethoxystilbene)	Synthetic	(51)
Rhapontigenin or 3-methoxyresveratrol (<i>trans</i> -3,5,3',-trihydroxy- 4'-methoxystilbene)	<i>Rheum</i> spp. (incl. <i>R. rhaponticum</i> , <i>R. undulatum</i>); <i>Scilla nervosa</i> ; Synthetic	(35, 69, 70)
Isorhapontigenin (<i>trans</i> -3,5,4',- trihydroxy-3'-methoxystilbene)	<i>Gnetum</i> spp.; <i>Belamcanda chinensis</i> ; Synthetic	(36, 71, 72)
Desoxyrhapontigenin or 4-methoxyresveratrol (<i>trans</i> -3,5-dihydroxy- 4'-methoxystilbene)	<i>Gnetum cleistostachyum</i> ; <i>Rheum undulatum</i> ; <i>Knema austrosiamensis</i> ; <i>Rumex bucephalophorus</i>	(54, 73-75)
Pinostilbene or 3-methoxyresveratrol (<i>trans</i> -5,4'-dihydroxy- 3-methoxystilbene)	<i>Rumex bucephalophorus</i>	(75)
Trans-3,4'-dimethoxy- 5-hydroxystilbene	<i>Knema austrosiamensis</i> ; Synthetic	(73, 74)
Cis-3,5,3',-trihydroxy- 4'-methoxystilbene	Synthetic	(76)
Trimethylresveratrol (<i>trans</i> -3,5,4'-trimethoxystilbene)	<i>Pterolobium hexapetallum</i> ; Synthetic	(37 , 51, 77)
Gnetucleistol D or 2-methoxyoxyresveratrol (<i>trans</i> -2-methoxy-3',4, 5-trihydroxystilbene)	<i>Gnetum cleistostachyum</i>	(54)
Gnetucleistol E or 3-methoxy-isorhapontigenin (<i>trans</i> -3,3'-dimethoxy-5, 4'-dihydroxystilbene)	<i>Gnetum cleistostachyum</i>	(54)
Trans- and cis-3,5, 4'-trimethoxy-3'-hydroxystilbene	Synthetic	(76)
Trans- and cis-3,5, 3'-trimethoxy-4'-hydroxystilbene	Synthetic	(76)
Trans- and cis-3,5-dimethoxy-3', 4'-dihydroxystilbene	Synthetic	(76)

continued

Table I. *continued.*

Compound	Sources	References
Trans- and cis-3,5-dihydroxy-3'-amino-4'-methoxystilbene	Synthetic	(76)
Trans- and cis-3,5-dimethoxy-4'-aminostilbene	Synthetic	(76)
Trans-and cis-3,4',5-trimethoxy-3'-aminostilbene	Synthetic	(76)
Trans-and cis-3,5-dimethoxy-4'-nitrostilbene	Synthetic	(76)
Trans-and cis-3,4',5-trimethoxy-3'-nitrostilbene	Synthetic	(76)
Trans-5,4'-dihydroxy-3-methoxystilbene	<i>Rumex bucephalophorus</i>	(75)
Pterostilbene (<i>trans</i> -3,5-dimethoxy-4'-hydroxystilbene)	<i>Dracena cochinchinensis</i> ; <i>Pterocarpus</i> spp. (incl. <i>P. santalinus</i> , <i>P. marsupium</i>); <i>Vitis vinifera</i> ; <i>Pterolobium hexapetallum</i> ; Synthetic	(37, 76, 78)
<i>Cis</i> -3,5-dimethoxy-4'-hydroxystilbene	Synthetic	(76)
3,4,5,4'-tetramethoxystilbene	Synthetic	(51)
3,4,5,3'-tetramethoxystilbene	Synthetic	(51)
3,4,5,3',4'-pentamethoxystilbene	Synthetic	(51)
Trans-3,4,3',5'-tetra methoxystilbene	<i>Crotalaria madurensis</i>	(80)
Trans-and cis-3,3',5,5'-tetrahydroxy-4-methoxystilbene	<i>Yucca periculosa</i> , <i>Y. schidigera</i> ; <i>Cassia pudibunda</i>	(81-83)
Trans-4,4'-dihydroxystilbene	<i>Yucca periculosa</i>	(81)
Trans-3-hydroxy-5-methoxystilbene	<i>Cryptocarya idenburgensis</i>	(84)
Trans-4,3'-dihydroxy-5'-methoxystilbene	<i>Dracaena loureiri</i>	(85)
Trans-4-hydroxy-3',5'-dimethoxystilbene	<i>Dracaena loureiri</i> , <i>D. cochinchinensis</i>	(85, 86)
Piceid or polydatin or resveratrol-3-glucoside (<i>trans</i> -3,5,4'-trihydroxystilbene-3-O-β-D-glucopyranoside)	<i>Polygonum cuspidatum</i> ; <i>Rheum rhaponticum</i> ; <i>Picea</i> spp.; Lentils (<i>Lens culinaris</i>)	(27, 35, 87, 88)
Rhapontin or rhaponticin (<i>trans</i> -3,3',5-trihydroxy-4'-methoxystilbene -3-O-β-D-glucopyranoside)	<i>Rheum</i> spp.; Eucalyptus	(27, 35)
Deoxyrhapontin (<i>trans</i> -3,5-dihydroxy-4'-methoxystilbene-3-O-β-D-glucopyranoside)	<i>Rheum rhaponticum</i>	(35)
Isorhapontin (<i>trans</i> -3,4',5-trihydroxy-3'-methoxystilbene-3-O-β-D-glucopyranoside)	<i>Pinus sibirica</i> ; <i>Picea</i> spp.	(35, 87)

continued

Table I. *continued.*

Compound	Sources	References
Piceatannol glucoside (3,5,3',4'-tetrahydroxystilbene-4'- O-β-D-glucopyranoside)	<i>Rheum rhaponticum</i> ; <i>Polygonum cuspidatum</i> ; Spruce	(27, 35)
Pinostilbenoside (<i>trans</i> -3-methoxy-5-hydroxystilbene- 4'-O-β-D-glucopyranoside)	<i>Pinus koraiensis</i>	(89)
Resveratrolside or resveratrol-4'- glucopyranoside (<i>trans</i> -3,5,4'- trihydroxystilbene-4'-O-β- D-glucopyranoside)	<i>Polygonum cuspidatum</i> ; <i>Pinus</i> spp.; <i>Vitis vinifera</i>	(27, 28, 35, 90)
Astringin (<i>trans</i> -3,4,3',5'- tetrahydroxystilbene-3'-O-β- D-glucopyranoside)	<i>Picea</i> spp., <i>Vitis vinifera</i>	(28, 87, 90)
Piceid-2''-O-gallate and -2''- O-coumarate	<i>Pleuropterus ciliinervis</i>	(91)
Rhaponticin-2''-O- gallate and -6''-O-gallate	Rhubarb (<i>Rheum undulatum</i>)	(92)
Piceatannol-6''-O-gallate	Chinese rhubarb (<i>Rhei rhizoma</i>)	(93)
<i>Cis</i> -resveratrol-3,4'-O-β-diglucoside	<i>Vitis vinifera</i> (cell suspension culture)	(94)
Combretastatins and their glycosides (<i>e.g.</i> combretastatin A= <i>trans</i> -2',3'- dihydroxy-3,4,4', 5-tetramethoxystilbene)	Synthetic	(95)
5-methoxy- <i>trans</i> -resveratrol-3- O-rutinoside	<i>Elephantorrhiza goetzei</i>	(96)
Oxyresveratrol-2-O-β- glucopyranoside	<i>Schoenocaulon officinale</i>	(50)
Resveratrol-3,4'-O,O'-di-β- D-glucopyranoside	<i>Schoenocaulon officinale</i>	(50)
Mulberrosides (<i>e.g.</i> <i>cis</i> - oxyresveratrol diglucoside)	<i>Morus alba</i> (cell cultures), <i>Morus lhou</i>	(97, 98)
Gnetupendins (isorhapontigenin dimer glucosides); Gnemonosides (resveratrol oligomer glucosides)	<i>Gnetum pendulum</i> , <i>G. gnemon</i>	(98, 99)
Gaylussacin [5-(<i>b</i> -D-glucosyloxy)- 3-hydroxy- <i>trans</i> -stilbene-2- carboxylic acid]	<i>Gaylussacia baccata</i> , <i>G. frondosa</i>	(100)
Resveratrol oligomers and oligostilbenes (incl. viniferins)	Dipterocarpaceae, Gnetaceae, Vitaceae, Cyperaceae and Leguminosae plants (incl. <i>Vatica pauciflora</i> , <i>V. rassak</i> , <i>V. oblongifolia</i> ; <i>Vateria indica</i> ; <i>Shorea laevifolia</i> , <i>S. hemsleyana</i> ; <i>Paeonia lactiflora</i> ; <i>Sophora moorcroftiana</i> , <i>S. leachiana</i> ; <i>Gnetum venosum</i> ; <i>Cyperus longus</i> ; <i>Upuna borneensis</i> ; <i>Iris clarkei</i>)	(6, 101-103)
1,5,7-trimethoxy-9,10 dihydrophenanthrene-2,6-diol	<i>Nidema boothii</i>	(104)

have been attributed to resveratrol (12). These effects include suppression of lipid peroxidation and eicosanoid synthesis, inhibition of platelet aggregation, and antioxidant, anti-inflammatory and vasorelaxant activities (13). Numerous reports indicate that resveratrol has antiviral effects against HIV-1 (14) and the herpes simplex virus (15, 16). Heredia *et al.* reported that resveratrol synergistically enhances the anti-HIV-1 activity of the nucleoside analogues zidovudine (AZT), zalcitabine (ddC) and didanosine (ddI) (14).

Resveratrol also exhibits antibacterial effects (17), including inhibition of growth of different strains of *Helicobacter pylori* (18-20).

Extensive research during the last two decades has suggested that, besides cardioprotective effects, resveratrol also exhibits anticancer activities. How resveratrol manifests its anticancer properties, the cell signaling pathways affected, the transcription factors modulated, the genes induced, the enzyme activities regulated, the protein interactions, and the types of *in vitro* and *in vivo* model systems in which resveratrol has been examined are the focus of this review. Although several reviews have been written on resveratrol (21-28), none covers the aspects of this polyphenol discussed here.

A. Sources of Resveratrol

That red grapes or red wine are sources of resveratrol is well known (29). However, resveratrol has been identified in a wide variety of plants, including Japanese knotweed (*Polygonum cuspidatum*) (4); the peanut (30, 31); *Vaccinium* spp. (including blueberry, bilberry, and cranberry) (32, 33); *Reynoutria japonica*; and Scots pine (Figure 1). Other plant sources of resveratrol include *Vitis* spp. (including grapevines, leaves, and berryskins); *Morus* spp. (including mulberry); lilies (*Veratrum* spp.); legumes (*Cassia* spp., *Pterolobium hexapetallum*); *Rheum* spp. (including rhubarb); eucalyptus; spruce (*Picea* spp.); pine (*Pinus* spp.); grasses (Poaceae including *Festuca*, *Hordeum*, *Poa*, *Stipa* and *Lolium* spp.); *Trifolium* spp.; *Nothofagus* spp.; *Artocarpus* spp.; *Gnetum* spp.; *Pleuropterus ciliinervis*; *Bauhinia racemosa*; *Paeonia lactiflora*; *Scilla nervosa*; and *Tetrastigma hypoglaucom*. Isorhapontigenin, isolated from *Belamcanda chinensis*, is a derivative of stilbene. Its chemical structure is very similar to that of resveratrol and it has a potent anti-oxidative effect. Compounds that are closely related to resveratrol structurally, and thus may have similar biological effects, have been identified in a wide variety of plants (Table I).

B. Chemistry of Resveratrol

Resveratrol (Figure 2) is found widely in nature, and a number of its natural and synthetic analogues and their isomers, adducts, derivatives and conjugates are known (6,

26-28, 33-104) (Table I). It is an off-white powder (extracted by methanol) with a melting point of 253-255°C and molecular weight of 228.25. Resveratrol is insoluble in water but dissolves in ethanol and dimethylsulphoxide. The stilbene-based structure of resveratrol consists of two phenolic rings linked by a styrene double bond to generate 3,4',5', -trihydroxystilbene. Although the presence of the double bond facilitates *trans*- and *cis*-isomeric forms of resveratrol [(E)- and (Z)-diastereomers, respectively], the *trans*-isomer is sterically the more stable form (105). On spectrophotometric analysis in ethanol, *trans*-resveratrol absorbs maximally at 308 nm and *cis*-resveratrol at 288 nm, which allows for their separation by HPLC with UV detection (105, 106). Absorptivity is greater in an ethanol: water solution (1:9 v/v), but with a small shift in λ_{max} (*trans*-resveratrol λ_{max} , 306 nm; *cis*-resveratrol λ_{max} , 286 nm). Besides their differences in spectrophotometric UV absorptions, *trans*- and *cis*-resveratrol are also clearly distinguished by their chemical shifts in nuclear magnetic resonance spectroscopy (106).

Trans-resveratrol is commercially available and converts to the *cis*-form on exposure to UV irradiation (23, 24, 26-28). Trela and Waterhouse conducted trials under various conditions and showed that *trans*-resveratrol is stable for months when protected from light, except in high pH buffers (105). These workers also showed that the *cis*-isomer is extremely light-sensitive but can remain stable in the dark at ambient temperature in 50% ethanol for at least 35 days over the range of 5.3-52.8 μ M. Low pH also causes *cis*-resveratrol to isomerize to *trans*-resveratrol. Recently, Deak and Falk studied the reactions of commercially obtained *trans*-resveratrol and photochemically prepared *cis*-resveratrol (106). The free enthalpy difference between the two isomers was estimated to be of the order of that of common stilbenes, with the *trans*-isomer being more stable by about 11-14 KJ/mol. These workers also reported that the pK_a values of *trans*-resveratrol, corresponding to the mono, di- and tri-protonation of the system, were 9.3, 10.0, and 10.6, respectively. Resveratrol occurs predominantly as the *trans*-isomer, and reports of the presence of the *cis*-isomer, for example in certain wines, are attributed to photoisomeric conversion, enzyme action during fermentation, or release from resveratrol oligomers (viniferins) (23, 24, 26-28). Since reports about the *cis*-isomer are limited, when the structure of resveratrol is not specified, we refer here to *trans*-resveratrol.

Over the past decade, several HPLC and gas chromatographic methods have been developed to detect the presence and measure levels of resveratrol and its analogues (23, 24, 26-28). Much attention has been focused on method development, since studying the biological properties of resveratrol requires analyses of complex mixtures containing very low amounts of stilbenes, and

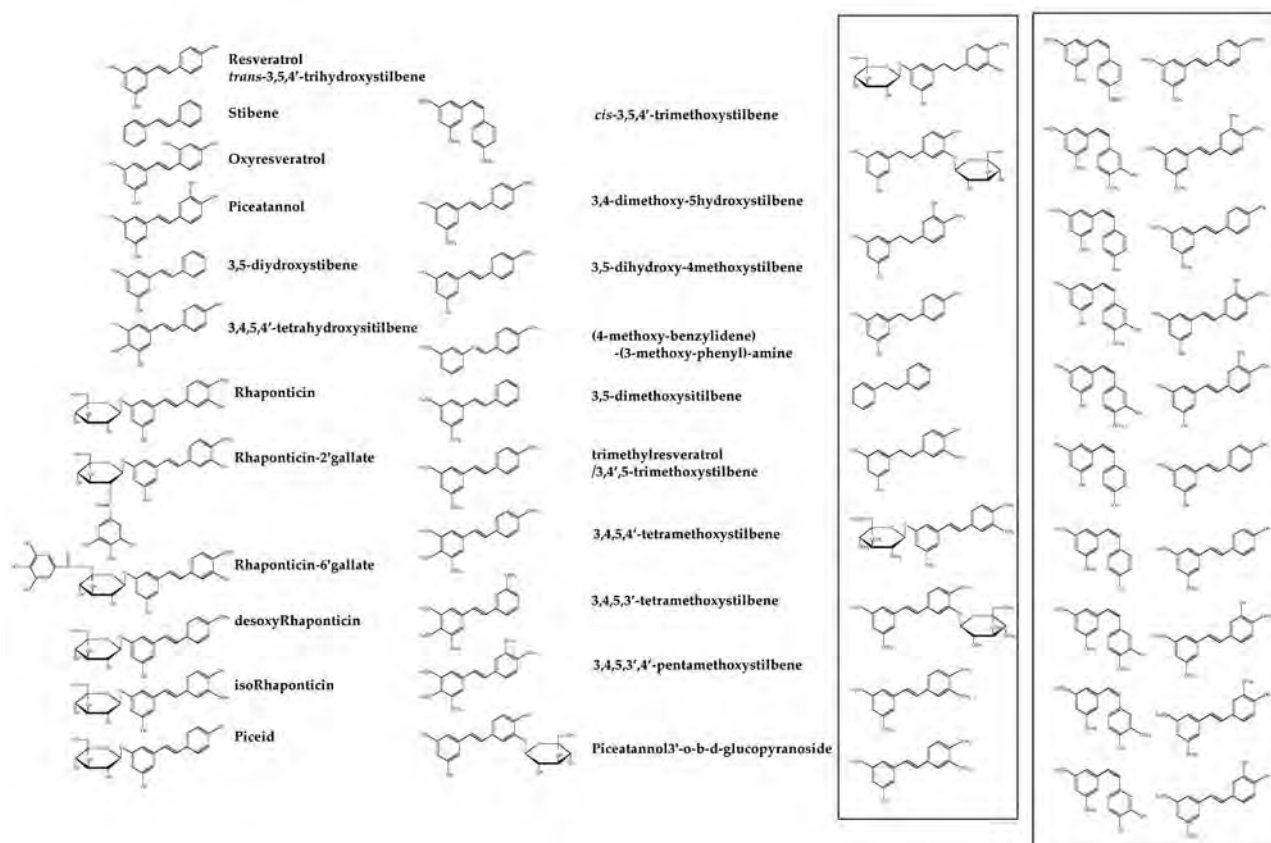


Figure 2. Resveratrol and its various analogues/derivatives.

complete and quick extractions are required to minimize losses from isomerization or denaturation. Generally, HPLC methods using reverse phase C18 columns coupled with UV detection (photodiode array or diode array detectors) can adequately distinguish resveratrol isomers and their analogues on the basis of their different absorbance maxima. However, the use of mass spectrometry (MS) fluorimetric and electrochemical detectors, which are more specific than UV detection, has considerably improved sensitivity and decreased sample size (23, 24). Gas chromatographic methods, with or without MS detection, although not as popular as HPLC, have been frequently employed but require trimethylsilyl derivatization of resveratrol and its analogues.

Since the first reported detection of *trans*-resveratrol in grapevines in 1976, and later in wine in 1992, and its implications in relation to the "French paradox" (7, 10, 107), there has been an explosion of interest in the various biological activities of this natural phytoalexin. Given the substantial number of reports on natural and synthetic analogues of resveratrol (Table I), considerable attention

has been focused on structure-activity relationship studies of these compounds. Natural and synthetic resveratrol analogues include a myriad of compounds differing in the type, number and position of substituents (hydroxyl, methoxyl, halogenated, glycosylated, esterified, *etc.*), presence or absence of stilbenic double bonds, modified stereoisomery, and oxidative dimerizations (to form oligomers). Calculations based on density functional theory studies have been used to study the structure-activity relationships of resveratrol in the chain reaction of auto-oxidation (108). The 4'-hydroxyl group of resveratrol was reported to be more reactive than the 3'- and 5'-hydroxyl groups because of resonance effects and, in conjunction with the *trans*-olefin structure of the parent stilbene skeleton, were the most important determinants of bioactivity (61-63, 108-110). Ashikawa *et al.* reported that piceatannol (a tetrahydroxyl resveratrol analogue) was considerably different in biological activity to the stilbene and rhaponticin (a methoxylated and glucosylated analogue of resveratrol) (111). Similarly, structure-activity relationship studies have shown distinct biological properties of

resveratrol oligomers and resveratrol glycosides (called polydatins and piceids) (6, 26-28). Much attention has been focused on the chemistry of resveratrol and its natural and synthetic analogues because of their biological properties and their potential in the prevention and therapy of cancer.

C. Preclinical Studies

C1. *In vitro* effects

C1a. Antiproliferative effects of resveratrol

Resveratrol has been shown to suppress proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers; breast, colon, pancreas, stomach, prostate, head and neck, ovary, liver, lung and cervical cancers; melanoma; and muscles (112-188) (Table II). Besides inhibiting proliferation, resveratrol also induces apoptosis either through the caspase-8-dependent pathway (receptor-mediated; type I) or the caspase-9-dependent pathway (mitochondrial; type II), or both. The mechanisms of suppression of cell growth and induction of apoptosis for these cell types are described here.

B-cell lymphoma: Several studies have shown the antiproliferative effects of resveratrol on B cells (112-115). Billard *et al.* investigated the effects of resveratrol on leukemic cells from patients with chronic B-cell malignancies and found that resveratrol had antiproliferative effects and induced apoptosis in leukemic B-cells that correlated with activation of caspase-3, a drop in the mitochondrial transmembrane potential, reduction in the expression of the anti-apoptotic protein Bcl-2, and reduction in expression of inducible nitric oxide synthase (iNOS) (112). In contrast, resveratrol had little effect on the survival of normal peripheral blood mononuclear cells (PBMC). Roman *et al.* reported apoptotic and growth-inhibitory effects of resveratrol in human B-cell lines derived from chronic B-cell malignancies (113). Resveratrol inhibited the expression of the antiapoptotic proteins Bcl-2 and iNOS in WSU-CLL and ESKOL cells and cells derived from patient with B-cell chronic lymphocytic leukemia (B-CLL). Dorrie *et al.* showed that resveratrol induced extensive apoptotic cell death not only in Fas/CD95-sensitive leukemia lines, but also in B-lineage leukemic cells that are resistant to Fas signaling (114). They also found that resveratrol had no cytotoxicity against normal PBMC. In each acute lymphocytic leukemia (ALL) cell line, resveratrol induced progressive loss of mitochondrial membrane potential and increase in caspase-9 activity. No evidence of caspase-8 activation or Fas signaling was observed. In BJAB Burkitt-like lymphoma cells, Wieder *et al.* demonstrated that resveratrol-induced cell death accompanied an increase in mitochondrial permeability transition and caspase-3 activation and was independent of

the Fas signaling pathway (115). Resveratrol was also found to induce apoptosis in leukemic lymphoblasts isolated from patients suffering from childhood ALL.

T-cell lymphoma: Several reports indicate that resveratrol modulates the growth of T cells (116, 117). Hayashibara *et al.* showed that resveratrol inhibited growth in five HTLV-1-infected cell lines (adult T-cell leukemia) and induced apoptosis, which correlated with a gradual decrease in the expression of survivin, an anti-apoptotic protein (116). Tinhofer *et al.* showed that resveratrol induced apoptosis in the CEM-C7H2 T-ALL cell line. They also found that resveratrol induced apoptosis *via* a novel mitochondrial pathway controlled by Bcl-2 (117) and that resveratrol-induced apoptosis was inhibited by Bcl-2. Resveratrol stimulation of C7H2 cells produced reactive oxygen species (ROS), and this production was significantly reduced by Bcl-2. As expected, pretreatment of cells with *N*-acetylcysteine protected cells from DNA fragmentation induced by resveratrol. Interestingly, resveratrol-induced apoptosis did not involve cytochrome *c* release, nor trigger activation of death receptor type II pathways, as no early processing of Bid could be detected. Resveratrol, however, caused activation of caspase-9, -2, -3 and -6 in the control cells, but not in the subclones overexpressing Bcl-2. These authors also found that DNA cleavage by resveratrol occurred downstream of mitochondrial signaling and was significantly blocked in the Bcl-2-overexpressing subclones. After various proapoptotic stimuli, the loss of mitochondrial transmembrane potential led to the release of apoptosis-inducing factor (AIF) from the mitochondrial intermembrane space, thus representing the link between mitochondria and nucleus in resveratrol-induced apoptosis. Resveratrol, however, did not induce translocation of AIF, suggesting that this pathway of caspase-independent activation of nucleases is not involved in resveratrol-induced apoptosis.

Myeloid leukemia: Resveratrol can induce apoptosis in myeloid cells (118-127). Clement *et al.* showed that resveratrol triggered Fas signaling-dependent apoptosis in HL-60 human leukemia cells (118). Resveratrol-treated cells exhibited increases in externalization of inner membrane phosphatidylserine and in cellular content of subdiploid DNA, indicating loss of membrane phospholipid asymmetry and DNA fragmentation. Resveratrol-induced cell death was mediated by intracellular caspases, as indicated by the increase in proteolytic cleavage of caspase substrate poly (ADP-ribose) polymerase (PARP) and the ability of caspase inhibitors to block resveratrol cytotoxicity. Furthermore, resveratrol treatment enhanced Fas ligand (FasLCD95L) expression on HL-60 cells, and resveratrol-mediated cell death was specifically Fas signaling-dependent. The expression of FasL was not unique to HL-60 cells but also

was induced on T47D breast carcinoma cells. Resveratrol treatment of normal human PBMC did not affect cell survival for as long as 72 h, which correlated with the absence of a significant change in either Fas or FasL expression on treated PBMC. These data showed specific involvement of the Fas-FasL system in the anticancer activity of resveratrol (Table III).

Tsan found that, in human monocytic leukemia THP-1 cells, resveratrol induced apoptosis independently of Fas signaling (119). The effect of resveratrol on THP-1 cells was reversible after its removal from the culture medium. Surh *et al.* found that resveratrol inhibited proliferation and DNA synthesis in human promyelocytic leukemia HL-60 cells (120). Resveratrol-induced cell death was characterized by internucleosomal DNA fragmentation, an increased proportion of the subdiploid cell population, and a gradual decrease in the expression of anti-apoptotic Bcl-2. In histiocytic lymphoma U-937 cells, Park *et al.* revealed that resveratrol treatment caused apoptosis and DNA fragmentation, which are associated with caspase-3 activation and phospholipase C- γ 1 degradation. Bcl-2 was found to inhibit resveratrol-induced apoptosis by a mechanism that interfered with cytochrome c release and caspase-3 activity (121).

We examined the effect of resveratrol on fresh acute myeloid leukemia (AML) cells (122). Interleukin (IL)-1 β plays a key role in proliferation of AML cells, and we found that resveratrol inhibited proliferation of AML by arresting the cells at S-phase. Resveratrol significantly reduced production of IL-1 β , suppressed IL-1 β -induced activation of NF- κ B, and suppressed colony-forming cell proliferation of fresh AML marrow cells.

Breast cancer: Several groups have investigated the effects of resveratrol on breast cancer cells (128-138). Mgbonyebi *et al.* showed that resveratrol had antiproliferative effects against the breast cancer cell lines MCF-7, MCF-10F and MDA-MB-231, and these effects were independent of the estrogen receptor (ER) status of the cells (128). Serrero *et al.* found that, in ER-positive MCF-7 breast cancer cells, resveratrol inhibited estradiol-induced cell proliferation by antagonizing the stimulation by estradiol of an ER element reporter gene construct and of progesterone receptor (PR) gene expression (129). Resveratrol also inhibited proliferation of the ER-negative human breast carcinoma cell line MDA-MB-468 by a mechanism other than ER antagonism, involving alteration in autocrine growth modulators such as transforming growth factor (TGF)- α , TGF- β , PC cell-derived growth factor and insulin-like growth factor I receptor mRNA. Nakagawa *et al.* found that resveratrol at low concentrations caused cell proliferation in ER-positive human breast cancer cell lines (KPL-1, ≤ 22 μ M; MCF-7, ≤ 4 μ M), whereas it suppressed cell growth at high concentrations (≥ 44 μ M). Growth suppression was due to apoptosis, as indicated by the

appearance of a sub-G1-phase fraction, up-regulation of Bax and Bak proteins, down-regulation of Bcl-x_L protein and activation of caspase-3. Pozo-Guisado *et al.* examined the effects of resveratrol in human breast cancer cell lines MCF-7 and MDA-MB-231 (131). They showed that, although resveratrol inhibited cell proliferation and viability in both cell lines, apoptosis was induced in a concentration- and cell-specific manner. In MDA-MB-231, resveratrol (at concentrations up to 200 μ M) lowered the expression and kinase activities of positive G1/S and G2/M cell-cycle regulators and inhibited ribonucleotide reductase activity in a concentration-dependent manner, without a significant effect on the low expression of tumor suppressors p21^{Cip1/WAF1}, p27^{Kip1} and p53. These cells died by a nonapoptotic process in the absence of a significant change in cell-cycle distribution. In MCF-7, resveratrol produced a significant (< 50 μ M) and transient increase in the expression and kinase activities of positive G1/S and G2/M regulators. Simultaneously, p21^{Cip1/WAF1} expression was markedly induced in the presence of high levels of p27^{Kip1} and p53. These opposing effects resulted in cell-cycle blockade at the S phase and induction of apoptosis in MCF-7 cells. Thus, the antiproliferative activity of resveratrol could take place through the differential regulation of the cell-cycle, leading to apoptosis or necrosis.

Colon cancer: Several reports suggest that resveratrol suppresses proliferation of colon cancer cells (143-151). In the human wild-type p53-expressing HCT116 colon carcinoma cell line and HCT116 cells with both p53 alleles inactivated by homologous recombination, Mahyar-Roemer *et al.* showed that resveratrol induced apoptosis independently of p53 and that the apoptosis was mediated primarily by mitochondria and not by a receptor pathway (143). Wolter and Stein determined that, in the colon adenocarcinoma cell line Caco-2, resveratrol enhanced the differentiation-inducing effect of butyrate, inhibited butyrate-induced TGF- β 1 secretion, and did not elevate alkaline phosphatase (ALP) activity or E-cadherin protein expression (markers of epithelial differentiation) when applied alone (144). Wolter *et al.* reported that resveratrol inhibited growth and proliferation of Caco-2 cells through apoptosis, which was accompanied by an increase in caspase-3 activity and in the expression of cyclin E and cyclin A, decrease in levels of cyclin D1 and cyclin-dependent kinase (Cdk) 4, cell-cycle arrest in S- to G2-phases at lower concentrations, and reversal of S-phase arrest at higher concentrations (145). They observed similar results for the colon carcinoma cell line HCT116 and found that cell-cycle inhibition by resveratrol was independent of COX inhibition.

Delmas *et al.* analyzed the molecular mechanisms of resveratrol-induced apoptosis in colon cancer cells, with special attention to the role of the death receptor Fas in this

Table II. Antiproliferative and pro-apoptotic effects of resveratrol against tumor cells and their mechanism.

Cell type	Mechanism	References
Leukemia		
• Inhibits proliferation of chronic B lymphocytic leukemia	• ↑ caspase 3, ↓ Bcl-2; ↓ iNOS	(112)
• Induces apoptosis in chronic B-cell leukemia	• ↓ iNOS; ↓ Bcl-2	(112)
• Inhibits growth and induces apoptosis in many lymphoid and myeloid leukemic cells	• ↑ caspases; ⊥ G2/M-phase	(113)
• Induces apoptosis in promyelocytic leukemia (HL-60) cells	• ↑ caspase-9	(114)
• Induce apoptosis in BJAB Burkitt-like lymphoma cells	• ↑ caspases	(115)
• Induces apoptosis in adult T-cell leukemia	• ↓ survivin	(116)
• Induces apoptosis in T-lymphoblastic leukemia CEM-C7H2 cells	• ↑ ROS; ↑ caspases	(117)
• Induces apoptosis in HL-60 cells	• ↑ Fas signaling-dependent apoptosis	(118)
• Induces apoptosis in monocytic leukemia (THP-1) cells	• ↑ caspases; ↑ PARP cleavage	(119)
• Induces apoptosis in HL-60 cells	• ↓ Bcl-2	(120)
• Induces apoptosis in U-937 cells	• ↑ cytochrome c; ↑ caspases	(121)
• Inhibits growth of acute myeloid leukemia (AML) OCIM2 and OCI/AML3	• ⊥ S phase; ↑ PARP cleavage; ↑ caspases	(122)
• Induces apoptosis in HL-60 cells	• ↑ Bax; ↑ cytochrome c; ↑ caspases	(123)
• Inhibits growth of HL-60 cells	• ↓ CYP1B1; ↑ DNA damage	(124)
• Inhibits growth of THP-1 cells	• ↓ tissue factor; ↓ NF-κB/Rel-dependent transcription	(125)
• Induces apoptosis in BJAB Burkitt-like lymphoma	• ↑ Mitochondrial permeability transition; ↑ caspase-3	(125)
• Inhibits cell adhesion U-937 cells to endothelial cells	• ↓ E-Selectin	(125)
• Inhibits proliferation of mitogen-, IL-2, or alloantigen-induced splenic lymphocytes	• ↓ NF-Î B, IFN-γ, IL-2, TNF and IL-12	(126)
Breast		
• Inhibits proliferation of breast epithelial (MCF-7, MCF-10F and MDA-MB-231) cells	• Mechanism is independent of ER status	(128)
• Inhibits growth of breast cancer (MCF-7, MDA-MB-468) cells	• ↓ Estradiol stimulation; ↓ TGF-α; ↑ TGF-β2	(129)
• Inhibits growth of KPL-1 and MCF-7 cells	• ↑ Bax, Bak; ↓ Bcl-x _L ; ↑ caspase-3	(130)
• Induces apoptosis in MCF-7 cells	• ↑ G1/S, G2/M-phase; ↑ p21 ^{Cip1} /WAF1; ⊥ S-phase	(131)
• Inhibits growth of MCF-7 cells	• ↓ TGF-α; ↑ TGF-β; ↓ IGF-1R	(132)
• Inhibits growth of 4T1 cells	• → Tumor take; → Tumor growth; → Metastasis	(133)
• Inhibits growth of MCF-7, T47D and MDA-MB-231 cells	• ↓ ROS	(134)
• Inhibits growth of MDA-MB-435 and MCF-7 cells	• ↑ sub G1 phase; ⊥ G2-phase; ↑ p53; ↑ cathepsin D	(135)
• Induces apoptosis in MCF-7 cells	• ↓ cyclin D; ↓ Cdk4; ↑ p53, p21 ^{Cip1} /WAF1; ↓ Bcl-2, ↑ Bax; ↑ caspase	(136)
• Induces apoptosis of MDA-MB-231	• ↑ nSMase; ↑ ceramide; ↑ serine palmitoyltransferase	(137)
• Inhibits growth of MCF-7 cells	• ↑ Adenylyl-cyclase activity	(138)
• Inhibits growth of MCF-7 cells	• ↓ TGF-α, IGR-R1 mRNA; ↑ TGF-β2 mRNA	(139)
• Inhibits growth of MCF-7 and T47D cells	• ↓ CYP1A1	(140)
Colon		
• Induces apoptosis of HCT116 cells	• ↑ p53-independent apoptosis	(143)
• Enhances the differentiation of Caco-2 with butylate	• ↓ TGF-β; ↓ p27 ^{Kip1} ; ↑ p21 ^{Cip1} /WAF1	(144)
• Induces apoptosis of Caco-2 and HCT116 cells	• ↓ cyclin D1/Cdk4 complex; ↑ cyclin E and A	(145)
• Induce apoptosis SW480	• ↑ Redistribution of Fas receptor in membrane rafts	(146)
• Induces cell-cycle arrest	• ⊥ G2-phase; ↓ Cdk 7; ↓ Cdc2	(147)
• Induces apoptosis in (col-2) cancer cells	• ⊥ sub G0-phase	(148)
• Inhibits colon carcinogenesis in F344 rats	• ↓ p21 ^{Cip1} /WAF1	(149)
• Induces apoptosis in colon cancer cells	• ↑ DNA fragmentation	(150)
• Induces apoptosis of HCT116 cells	• ↑ p53-independent apoptosis	(151)
Pancreas		
• Induces apoptosis of PANC-1 and AsPC-1 cells	• ↑ sub G0/G1-phase cells	(152)
Gastric		
• Inhibits growth of KATO-III and RF-1 cells	• ⊥ G0/G1-phase	(153)
• Inhibits proliferation of human gastric adenocarcinoma (SNU-1) cells	• ↓ DNA synthesis, ↑ iNOS	(154)

continued

Table II. *continued.*

Cell type	Mechanism	References
<ul style="list-style-type: none"> Induces apoptosis in esophageal carcinoma (EC-9706) cells 	<ul style="list-style-type: none"> ↓ Bcl-2; ↑ Bax 	(155)
Prostate		
<ul style="list-style-type: none"> Inhibits growth of LnCaP 	<ul style="list-style-type: none"> ↓ PSA 	(156)
<ul style="list-style-type: none"> Inhibits growth of LnCaP, DU145 and PC-3 cells 	<ul style="list-style-type: none"> ⊥ G1/S-phase; ↑ apoptosis; ↓ PSA 	(157)
<ul style="list-style-type: none"> Induces apoptosis in prostate cancer (DU145) cells 	<ul style="list-style-type: none"> ↑ MAPK; ↑ cellular p53; ↑ p53 binding to DNA 	(158)
<ul style="list-style-type: none"> Inhibits androgen stimulated growth of LNCaP cells 	<ul style="list-style-type: none"> ↓ PSA; ↓ kallikarin-2; ↓ ARA70 	(159)
<ul style="list-style-type: none"> Inhibits growth of LnCaP, DU145 and PC-3 cells 	<ul style="list-style-type: none"> ↓ NO secretion 	(160)
<ul style="list-style-type: none"> Inhibits growth of LnCaP 	<ul style="list-style-type: none"> ⊥ DNA synthesis; ↑ S-phase 	(161)
<ul style="list-style-type: none"> Inhibits growth of LnCaP 	<ul style="list-style-type: none"> ↓ PSA; ↓ ARA; ↓ NF-kB 	(162)
<ul style="list-style-type: none"> Inhibits growth of PC-3 	<ul style="list-style-type: none"> ↓ PKCa; ↓ ERK1/2 	(163)
Melanoma		
<ul style="list-style-type: none"> Induces apoptosis in melanoma (A375 and SK-mel28) cells 	<ul style="list-style-type: none"> ↑ Phosphorylates ERK1/2 	(164)
<ul style="list-style-type: none"> Induces apoptosis in epidermoid carcinoma (A431) cells 	<ul style="list-style-type: none"> ↑ p21^{Cip1/WAF1}; ⊥ G1-phase 	(165)
<ul style="list-style-type: none"> Inhibits proliferation of epidermoid carcinoma (A431) cells 	<ul style="list-style-type: none"> ↓ Hyperphosphorylated Rb; ⊥ G0/G1-phase 	(166)
<ul style="list-style-type: none"> Induces apoptosis in JB6 P+ mouse epidermal cell line C1 41 	<ul style="list-style-type: none"> ↑ p53-dependent apoptosis pathway 	(166)
<ul style="list-style-type: none"> Induces apoptosis of SK-Mel-28 	<ul style="list-style-type: none"> ⊥ S-phase ↑ cyclins A, E, and B1 	(167)
Lung		
<ul style="list-style-type: none"> Induce apoptosis of A549 	<ul style="list-style-type: none"> ↑ p53; ↑ p21^{Cip1/WAF1}; ↑ Bax/Bcl-2; ↑ caspase; ↓ NF-kB 	(168)
<ul style="list-style-type: none"> Induces apoptosis in Chinese hamster lung cell line 	<ul style="list-style-type: none"> ⊥ S-phase 	(169)
<ul style="list-style-type: none"> Inhibits growth of lung cancer (BEP2D) cells 	<ul style="list-style-type: none"> ↓ CYP1A1 and CYP1B1 	(170)
Liver		
<ul style="list-style-type: none"> Inhibits proliferation in rat hepatoma Fao cells 	<ul style="list-style-type: none"> ⊥ S- and G2/M-phase 	(171)
<ul style="list-style-type: none"> Suppresses hepatoma cell invasion 	<ul style="list-style-type: none"> ↓ ROS 	(172)
<ul style="list-style-type: none"> Decreases hepatocyte growth factor-induced HepG2 cell invasion 	<ul style="list-style-type: none"> Uses an unidentified post-receptor mechanism 	(173)
<ul style="list-style-type: none"> Inhibits hepatoma cell, AH 109A proliferation and invasion 	<ul style="list-style-type: none"> Antioxidant involved in anti-invasive action 	(174)
Thyroid and Head & Neck		
<ul style="list-style-type: none"> Induces apoptosis in thyroid cancer cell lines 	<ul style="list-style-type: none"> ↑ p53 and MAPK 	(175)
<ul style="list-style-type: none"> Inhibits growth and proliferation of oral squamous carcinoma (SCC-25) cells 	<ul style="list-style-type: none"> ⊥ DNA synthesis 	(176)
<ul style="list-style-type: none"> Inhibits proliferation in human gingival epithelial S-G cells 	<ul style="list-style-type: none"> ⊥ DNA synthesis 	(177)
<ul style="list-style-type: none"> Induces apoptosis in the neuroblastoma (SH-SY5Y) cell line 	<ul style="list-style-type: none"> ↑ ERK1/2 	(179)
<ul style="list-style-type: none"> Induces apoptosis in rat pheochromocytoma (PC12) cells 	<ul style="list-style-type: none"> ↓ caspase-7, ↑ PARP cleavage 	(180)
	<ul style="list-style-type: none"> ↑ DNA fragmentation; ↓ NF-kB; ↑ ROS 	(181)
Ovarian and Endometria		
<ul style="list-style-type: none"> Inhibits proliferation of endometrial adenocarcinoma cells 	<ul style="list-style-type: none"> ↑ cyclin A; ↑ cyclin E; ↓ Cdk2 	(174)
<ul style="list-style-type: none"> Inhibits cell growth and induces apoptosis in ovarian cancer (PA-1) cells 	<ul style="list-style-type: none"> ↑ NQO-1 	(182)
<ul style="list-style-type: none"> Inhibits proliferation of endometrial adenocarcinoma cells 	<ul style="list-style-type: none"> ↑ VEGF; ↓ EGF; ↓ p21^{Cip1/WAF1}; ↓ Bax 	(183)
<ul style="list-style-type: none"> Inhibited growth and induced death of five human ovarian carcinoma cell 	<ul style="list-style-type: none"> ↑ cytochrome c; ↑ caspases; ↑ autophagocytosis 	(184)
<ul style="list-style-type: none"> Inhibits proliferation of endometrial adenocarcinoma cells 	<ul style="list-style-type: none"> Exerts estrogen -dependent and -independent effects, 	(185)
<ul style="list-style-type: none"> Inhibits proliferation in cervical tumor (HeLa and SiHa) cells 	<ul style="list-style-type: none"> ⊥ S-phase, ↑ cyclins A and E 	
	<ul style="list-style-type: none"> ↓ prostaglandin biosynthesis; ⊥ S-phase 	(186)
Muscle		
<ul style="list-style-type: none"> Induces growth inhibition, apoptosis in various cell lines (MCF-7, SW480, HCE7, Seg-1, Bic-1, and HL-60) 	<ul style="list-style-type: none"> ⊥ S-phase; ↓ cyclin A1, B1, and D1; ↓ β-catenin 	(187)
<ul style="list-style-type: none"> oSuppresses mitogenesis in smooth muscle cells 	<ul style="list-style-type: none"> ⊥ G1/S-phases 	(188)

Table III. *Effects of resveratrol on different cell signaling pathways.*

Signaling pathway	References
Up-regulate Fas pathway	(118, 146, 191)
Inhibit mitochondrial pathway	(114, 117, 192)
Inhibit Rb/E2FDP pathway	(166, 168)
Activate p53 pathway	(51, 162, 175, 193-198)
Activate ceramide pathway	(137)
Inhibit tubulin polymerization pathway	(199)
Activate adenylyl-cyclase pathway	(138)
Inhibit NF- κ B signaling pathway	(120, 122, 125, 126, 168, 202-208)
Inhibit AP-1 signaling pathway	(22, 120, 201, 209-214)
Regulate Egr-1 pathway	(215, 216)
Inhibit MAPK pathway	(163, 175, 179, 195, 196, 217, 218)
Suppression of protein kinases by resveratrol	(127, 139, 153, 218-221)
Modulation of NO/NOS pathway	(92, 154, 194, 222)
Suppression of growth factor and associated protein tyrosine kinases	(129, 173, 183, 223-226)
Suppression of COX-2 and lipooxygenase	(141, 142, 212, 222, 227, 228)
Suppression of cell-cycle proteins	(122, 135, 145, 147, 151, 161, 165, 167, 187, 191, 194, 229)
Suppression of adhesion molecules	(230, 231)
Suppression of androgen receptors	(159, 285)
Suppression of PSA	(156)
Suppression of inflammatory cytokine	(211, 232-235)
Suppression of angiogenesis, invasion and metastasis	(194, 218, 237-241, 243-246, 286)
Effect on bone cells	(247, 278)
Inhibit the expression of cytochrome p450 and modulate metabolism of carcinogens:	(73, 140, 229, 248-258, 287)
Suppression of inflammation	(198, 222, 259-261)
Antioxidant effects	(71, 262-276)
Suppression of transformation	(193, 226)
Induction of cellular differentiation	(277-279)
Estrogenic/antiestrogenic effects	(132, 174, 185, 280-284, 289)
Effect on normal cells	(188, 194, 197, 237, 238, 245, 290-292)
Suppression of mutagenesis	(169, 294-298)
Radioprotective and radiosensitive	(186)
Chemosensitization	(180, 181, 304-307)
Immunomodulatory effects	(126, 236, 259, 314-316)

pathway (146). They showed that, at concentrations of 10-100 μ M, resveratrol activated various caspases and triggered apoptosis in SW480 human colon cancer cells. Caspase activation was associated with accumulation of the pro-apoptotic proteins Bax and Bak, which underwent conformational changes and relocalization to the mitochondria. Resveratrol did not modulate the expression of Fas and Fas-ligand (FasL) at the surface of cancer cells, and inhibition of the Fas/FasL interaction did not influence the apoptotic response to the molecule. Resveratrol induced the clustering of Fas and its redistribution in cholesterol and sphingolipid-rich fractions of SW480 cells, together with Fas-associated death domain protein (FADD) and procaspase-8. This redistribution was associated with the formation of a death-inducing signaling complex (DISC). Transient transfection of a dominant-negative mutant of FADD, E8, or viral protein MC159, that interfered with

DISC function, decreased the apoptotic response of SW480 cells to resveratrol and partially prevented resveratrol-induced Bax and Bak conformational changes. Altogether, these results indicated that the ability of resveratrol to induce the redistribution of Fas in membrane rafts may contribute to the molecule's ability to trigger apoptosis in colon cancer cells.

Liang *et al.* found that resveratrol inhibited proliferation of HT-29 colon cancer cells and resulted in their accumulation in the G2-phase of the cell-cycle, and that this was accompanied by inactivation of Cdc2/p34 protein kinase and an increase in the tyrosine phosphorylated (inactive) form of Cdc2 (147). Kinase assays revealed that the reduction of Cdc2 activity by resveratrol was mediated through inhibition of Cdk7 kinase activity, while Cdc25A phosphatase activity was not affected. In addition, resveratrol-treated cells were shown to have a low level of

Cdk7 kinase-Thr(161)-phosphorylated Cdc2. These results demonstrated that resveratrol induced cell-cycle arrest at the G2 phase through inhibition of Cdk7 kinase activity, suggesting that its antitumor activity might occur through disruption of cell division at the G2/M-phase.

Pancreatic cancer: Ding and Adrian demonstrated that, in human pancreatic cancer cell lines PANC-1 and AsPC-1, resveratrol inhibited proliferation through apoptosis and dramatically increased the fraction of sub-G0/G1-phase cells (152).

Gastric cancer: Resveratrol has been shown to suppress proliferation of gastric cancer cells (153-155). Atten *et al.* reported that resveratrol inhibited proliferation of nitrosamine-stimulated human gastric adenocarcinoma KATO-III and RF-1 cells (153). It arrested KATO-III cells in the G0/G1-phase of the cell-cycle and eventually induced apoptotic cell death by utilizing a proteinase kinase C (PKC)-mediated mechanism to deactivate these gastric adenocarcinoma cells. Holian *et al.* demonstrated that, in gastric adenocarcinoma cell line SNU-1, which was stimulated by hydrogen peroxide (H₂O₂), resveratrol suppressed synthesis of DNA and generation of endogenous O₂⁻ but stimulated NOS activity, which may have been responsible for inhibition of SNU-1 proliferation (154). Resveratrol also inhibited the growth of esophageal cancer cell line EC-9706 (155). Resveratrol-induced apoptosis of EC-9706 was mediated by down-regulation of *Bcl-2* and up-regulation of the expression of the apoptosis-regulated gene *Bax*.

Prostate cancer: Proliferation of both androgen-dependent and -independent prostate cancer cells is suppressed by resveratrol (156-163). Using cultured prostate cancer cells that mimic the initial (hormone-sensitive; LNCaP) and advanced (hormone-refractory; DU-145, PC-3, and JCA-1) stages of prostate carcinoma, Hsieh and Wu showed that resveratrol caused substantial decreases in growth of LNCaP, PC-3 and DU145 cells, but had only a modest inhibitory effect on proliferation of JCA-1 cells, and that it partially disrupted the G1/S transition in all three androgen-non-responsive cell lines (157). It caused a significant percentage of LNCaP cells to undergo apoptosis and significantly lowered both intracellular and secreted prostate-specific antigen (PSA) levels without affecting expression of the androgen receptor (AR). Lin *et al.* also showed, in DU145 cells, that resveratrol induced apoptosis through activation of mitogen-activated protein kinase (MAPK,) increases in cellular p53 content, serine-15 phosphorylation of p53, p53 binding to DNA and p53-stimulated increase in *p21^{Cip1/WAF1}* mRNA (158). Mitchell *et al.* found that, in a hormone-sensitive prostate cancer cell line, resveratrol repressed different classes of androgen up-regulated genes at the

protein or mRNA level, including PSA, human glandular kallikrein-2, AR-specific coactivator ARA70, and the Cdk inhibitor *p21^{Cip1/WAF1}* (159). This inhibition is probably attributable to a reduction in AR at the transcription level, inhibiting androgen-stimulated cell growth and gene expression. Kampa *et al.* reported that the antiproliferative effects of resveratrol on DU145 cells could have been mediated through a decrease in NO, although resveratrol did not affect growth of PC3 and LNCaP cells (160). Kuwajerwala *et al.* showed that, in androgen-sensitive LNCaP cells, the effect of resveratrol on DNA synthesis varied dramatically depending on the concentration and the duration of treatment (161). In cells treated for 1 h, resveratrol had only an inhibitory effect on DNA synthesis, which increased with increasing concentration (IC₅₀, 20 μM). However, when treatment duration was extended to 24 h, resveratrol had a dual effect on DNA synthesis. At 5-10 μM it caused a two- to three-fold increase in DNA synthesis, while at ≥15 μM it inhibited DNA synthesis. The increase in DNA synthesis was seen only in LNCaP cells, not in androgen-independent DU145 prostate cancer cells or in NIH/3T3 fibroblast cells. The resveratrol-induced increase in DNA synthesis was associated with enrichment of LNCaP cells in S-phase and concurrent decreases in nuclear *p21^{Cip1/WAF1}* and *p27^{Kip1}* levels. Furthermore, consistent with the entry of LNCaP cells into S-phase, there was a dramatic increase in nuclear Cdk2 activity associated with both cyclin A and cyclin E. Taken together, their observations indicate that LNCaP cells treated with resveratrol are induced to enter into S-phase, but subsequent progression through S-phase is limited by the inhibitory effect of resveratrol on DNA synthesis, particularly at concentrations greater than 15 μM. This unique ability of resveratrol to exert opposing effects on two important processes in cell-cycle progression, induction of S-phase and inhibition of DNA synthesis, may be responsible for its dual apoptotic and antiproliferative effects.

Prostate cancer prevention by key elements present in human nutrients derived from plants and fruits has been confirmed in various cell cultures and tumor models. Resveratrol has been shown to induce remarkable inhibitory effects in prostate carcinogenesis *via* diverse cellular mechanisms associated with tumor initiation, promotion and progression. Narayanan *et al.* examined whether resveratrol activates a cascade of p53-directed genes that are involved in apoptosis mechanism(s) or modifies cell growth by modifying AR and its co-activators directly or indirectly (162). They demonstrated by DNA microarray, reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot and immunofluorescence analyses that treatment of androgen-sensitive prostate cancer cells (LNCaP) with 10 μM resveratrol for 48 h down-regulated PSA, AR co-activator ARA 24, and NF- κ B p65. Altered expression of

these genes is associated with activation of p53-responsive genes such as *p53*, *PIG 7*, *p21^{Cip1/WAF1}*, *p300/CBP* and apoptosis protease activating factor-1 (*Apaf-1*). The effect of resveratrol on p300/CBP plays a central role in its cancer-preventive mechanisms in LNCaP cells. These results implicate activation of more than one set of functionally related molecular targets. At this point we have identified some of the key molecular targets associated with the AR and *p53* target genes.

Melanoma: Several studies suggest that resveratrol is effective against melanoma (164-167). Resveratrol inhibited growth and induced apoptosis in human melanoma cell lines A375 and SK-mel28 (164). It did not alter the phosphorylation of p38 MAPK or c-Jun N-terminal kinase (JNK) in either cell line. Resveratrol induced phosphorylation of extracellular receptor kinase (ERK)1/2 in A375 but not in SK-mel28 cells. Ahmad *et al.* demonstrated that resveratrol, *via* modulations in Cdk inhibitor-cyclin-Cdk machinery, resulted in a G1-phase arrest followed by apoptosis of human epidermoid carcinoma (A431) cells (165). It caused an induction of *p21^{Cip1/WAF1}* that inhibited cyclin D1/D2-Cdk6, cyclin D1/D2-Cdk4, and cyclin E-Cdk2 complexes, thereby imposing an artificial checkpoint at the G1/S-phase transition of the cell-cycle. These authors also showed, in the same cell line, the involvement of the retinoblastoma (Rb)-E2F/DP pathway in resveratrol-mediated cell-cycle arrest and apoptosis (166). They suggested that resveratrol caused a down-regulation of hyperphosphorylated Rb protein with a relative increase in hypophosphorylated Rb that, in turn, compromised the availability of free E2F, which may have resulted in stoppage of cell-cycle progression at the G1/S-phase transition, thereby leading to a G0/G1 phase arrest and subsequent apoptotic cell death. Larrosa *et al.* showed that resveratrol and the related molecule 4-hydroxystilbene induced growth inhibition, apoptosis, S-phase arrest and up-regulation of cyclins A, E and B1 in human SK-Mel-28 melanoma cells (167).

Lung cancer: Several studies suggest that resveratrol is effective against lung carcinoma (168-170). Kim *et al.* showed that resveratrol inhibited the growth of human lung carcinoma A549 cells and resulted in a concentration-dependent induction of S-phase arrest in cell-cycle progression, marked inhibition of phosphorylation of Rb and concomitant induction of Cdk inhibitor *p21^{Cip1/WAF1}*, which is transcriptionally up-regulated and is p53-dependent (168). In addition, fluorescence microscopy and flow cytometric analysis showed that treatment with resveratrol resulted in induction of apoptosis. These effects were found to correlate with activation of caspase-3 and a shift in the Bax/Bcl-x_L ratio toward apoptosis. Resveratrol treatment also inhibited

the transcriptional activity of NF- κ B. These findings suggest that resveratrol has firm potential for development as an agent for prevention of human lung cancer.

Liver cancer: Several studies suggest that resveratrol is effective against liver cancer (171-174). Delmas *et al.* examined the ability of resveratrol to inhibit cell proliferation in the rat hepatoma Fao cell line and the human hepatoblastoma HepG2 cell line (171). The results showed that resveratrol strongly inhibited cell proliferation and that Fao cells were more sensitive than HepG2 cells. Interestingly, the presence of ethanol lowered the threshold of the resveratrol effect. Resveratrol appeared to prevent or delay the entry to mitosis, since no inhibition of ³H-thymidine incorporation was observed, while the number of the cells in S- and G2/M-phases increased. Kozuki *et al.* revealed that 100 or 200 μ M of resveratrol inhibited proliferation of AH109A hepatoma cells and suppressed invasion of the hepatoma cells even at a concentration of 25 μ M (172). This anti-invasive activity of resveratrol is independent of its antiproliferative activity and may be related to its anti-oxidative action. De Ledinghen *et al.* found that resveratrol decreased hepatocyte growth factor-induced scattering of HepG2 hepatoma cells and invasion by an unidentified postreceptor mechanism (173). It decreased cell proliferation without evidence of cytotoxicity or apoptosis, with no decrease in the level of the hepatocyte growth factor receptor c-met, c-met precursor synthesis, c-met autophosphorylation, or activation of Akt-1 or ERK1/2. Moreover, resveratrol did not decrease urokinase expression and did not block the catalytic activity of urokinase.

Thyroid and head and neck cancers: Several reports suggest that resveratrol may suppress proliferation of thyroid and other head and neck cancers (174-181). Shih *et al.* showed that treatment of papillary thyroid carcinoma and follicular thyroid carcinoma cell lines with resveratrol led to apoptosis, which accompanied activation and nuclear translocation of ERK1/2 (175). Resveratrol increased the cellular abundance of p53, serine phosphorylation of p53, and abundance of *c-fos*, *c-Jun*, and *p21^{Cip1/WAF1}* mRNAs. Elattar *et al.* reported that resveratrol led to inhibition of human oral squamous carcinoma SCC-25 cell growth and DNA synthesis (176, 177). Moreover, combining 50 μ M resveratrol with 10, 25, or 50 μ M quercetin resulted in gradual and significant increases in the inhibitory effects of the two compounds. Babich *et al.* demonstrated that resveratrol irreversibly caused arrest of human gingival epithelial cell growth by inhibition of DNA synthesis (178).

Ovarian and endometrial tumors: Several studies suggest that resveratrol is effective against ovarian and endometrial tumors (174, 182-186). Yang *et al.* showed that resveratrol

inhibited cell growth and induced apoptosis in PA-1 human ovarian cancer cells and up-regulated the NAD(P)H quinone oxidoreductase 1 (NQO-1) gene, which is involved in p53 regulation (182). Bhat and Pezzuto reported that treatment of human endometrial adenocarcinoma (Ishikawa) cells with resveratrol did not significantly increase the levels of the estrogen-inducible marker enzyme ALP (174). On the contrary, it decreased 17 β -estradiol-induced ALP and PR expression and thus its effects may be mediated by both estrogen-dependent and -independent mechanisms. It inhibited Ishikawa cell proliferation by arresting cells at S-phase and increased expression of cyclins A and E but decreased Cdk2. Kaneuchi *et al.* showed that resveratrol suppressed the growth of Ishikawa cells through down-regulation of epidermal growth factor (EGF) (183).

Opipari *et al.* showed that resveratrol inhibited growth and induced death in a panel of five human ovarian carcinoma cell lines and that this response was associated with mitochondrial release of cytochrome c, formation of the apoptosome complex, and caspase activation (184). Surprisingly, even with these molecular features of apoptosis, analysis of the resveratrol-treated cells by light and electron microscopy revealed morphological and ultrastructural changes indicative of autophagocytic, rather than apoptotic, death. This suggested that resveratrol can induce cell death through two distinct pathways. Consistent with resveratrol's ability to kill cells *via* nonapoptotic processes, cells transfected to express high levels of the antiapoptotic proteins Bcl-x_L and Bcl-2 were equally as sensitive as control cells to resveratrol. Together, these findings show that resveratrol induces death in ovarian cancer cells through a mechanism distinct from apoptosis, suggesting that it may provide leverage to treat ovarian cancer that is chemoresistant on the basis of ineffective apoptosis.

C1b. Resveratrol induces apoptosis

Apoptosis is a mode of cell death that differs from necrosis. While the former is characterized by initiation of cell death from the outside of the cell, the latter is a death mechanism initiated from inside the cell, primarily from the mitochondria (189). Apoptosis is usually mediated through the activation of caspases. Mechanistically, two different type of apoptosis have been described; one that is caspase-8-dependent and receptor-mediated (type I), and the other that is caspase-9-dependent and usually mediated through the mitochondria (type II). Resveratrol has been shown to mediate apoptosis through a variety of different pathways (Figure 3) (51, 114, 117, 118, 131, 137, 138, 146, 148, 162, 166, 168, 175, 187, 190-199), as described below.

Fas pathway: Resveratrol has been shown to induce death receptors, that in turn activate apoptosis, through the type I pathway. Fas is one of the death receptors of the tumor

necrosis factor (TNF) superfamily (200). Clement *et al.* showed that resveratrol triggered FasL signaling-dependent apoptosis in human tumor cells (118). They showed that resveratrol treatment enhanced FasL expression on HL-60 cells and T47D breast carcinoma cells, and that resveratrol-mediated cell death was specifically dependent on Fas signaling. Resveratrol treatment had no effect on normal PBMC, which correlated with the absence of a significant change in either Fas or FasL expression on treated PBMC. These data showed specific involvement of the Fas-FasL system in the anticancer activity of resveratrol. In contrast to these results, those of Bernhard *et al.* found that resveratrol caused arrest in the S-phase prior to Fas-independent apoptosis in CEM-C7H2 ALL cells (191). These findings indicate that the effect of resveratrol on Fas signaling may depend on cell type. Delmas *et al.* showed that resveratrol-induced apoptosis was associated with Fas redistribution in the rafts and the formation of a DISC in colon cancer cells (146). Resveratrol did not modulate the expression of Fas and FasL at the surface of cancer cells, and inhibition of the Fas-FasL interaction did not influence the apoptotic response to the molecule. Resveratrol, however, induced the clustering of Fas and its redistribution in cholesterol- and sphingolipid-rich fractions of SW480 cells, together with FADD and procaspase-8. This redistribution was associated with formation of a DISC. Transient transfection of a dominant-negative mutant of FADD, E8, or viral protein MC159 that interferes with DISC function decreased the apoptotic response of SW480 cells to resveratrol and partially prevented resveratrol-induced Bax and Bak conformational changes. Altogether, these results indicate that the ability of resveratrol to induce redistribution of the Fas receptor in membrane rafts may contribute to the molecule's ability to trigger apoptosis in colon cancer cells.

Mitochondrial pathway: Resveratrol has also been shown to activate the type II pathway. This pathway for apoptosis is mediated through the activation of the mitochondrial pathway. Dorrie *et al.* showed that resveratrol induced extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in ALL cells and that these effects were independent of Fas signaling (114). Tinhofer *et al.* showed that resveratrol induced apoptosis *via* a novel mitochondrial pathway controlled by Bcl-2 (117).

Mitochondrial proton F₀F₁-ATPase/ATP synthase synthesizes ATP during oxidative phosphorylation. Zheng *et al.* found that resveratrol inhibited the enzymatic activity of both rat brain and liver F₀F₁-ATPase/ATP synthase (IC₅₀, 12–28 μ M) (192). The inhibition of F₀F₁-ATPase by resveratrol was non-competitive in nature. Thus the mitochondrial ATP synthase is a target for this dietary phytochemical and may contribute to its potential

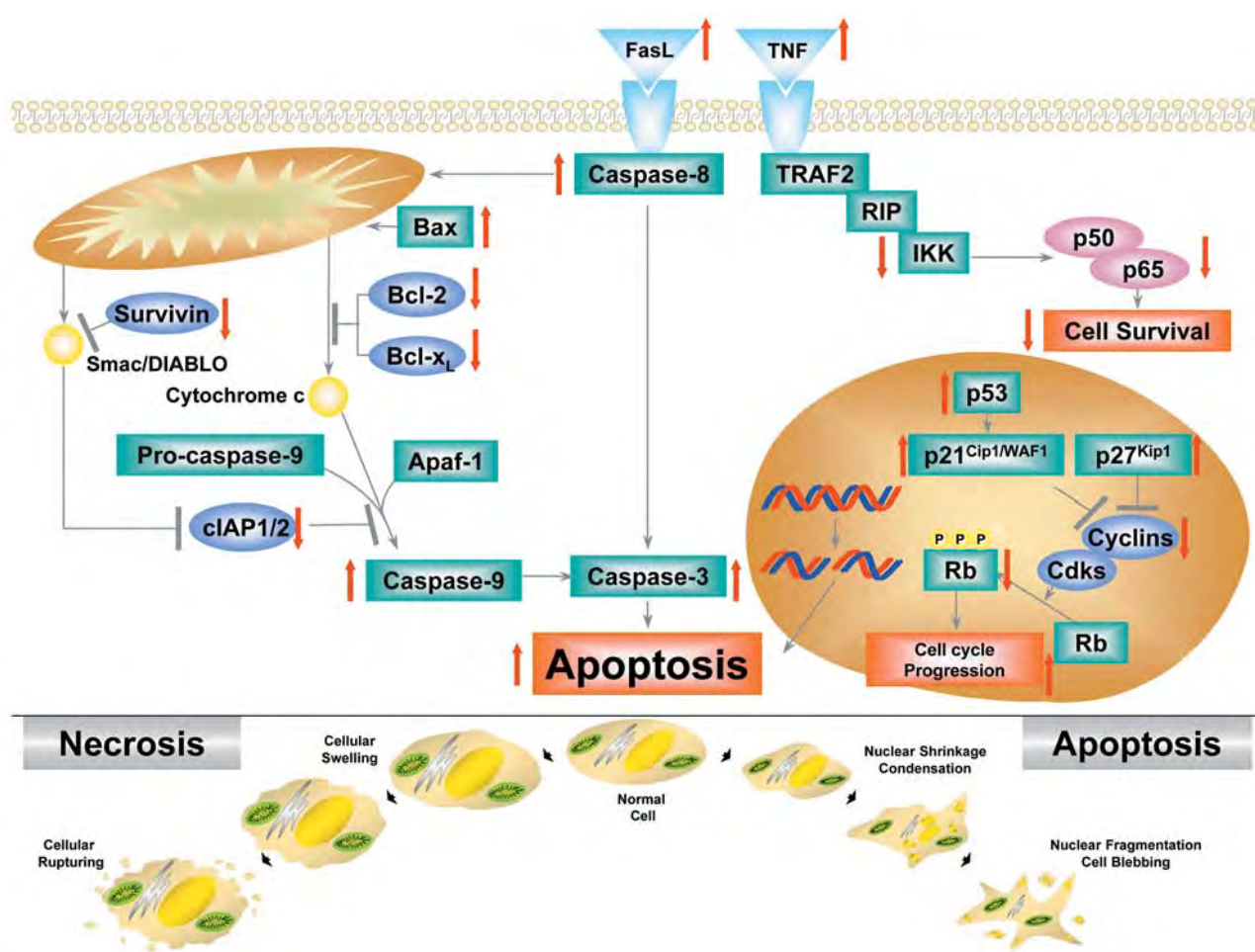


Figure 3. Various proposed mechanisms of apoptosis of tumor cells by resveratrol.

cytotoxicity. Zheng *et al.* also found that piceatannol, an analogue of resveratrol, inhibited mitochondrial F₀F₁-ATPase activity by targeting the F₁ complex (192). Piceatannol potently inhibited rat brain mitochondrial F₀F₁-ATPase activity in both solubilized and submitochondrial preparations (IC₅₀, 8-9 μ M) while having a relatively small effect on Na⁺, K⁺-ATPase activity. Piceatannol inhibited the ATPase activity of purified rat liver F₁ (IC₅₀, 4 μ M), while resveratrol was slightly less active (IC₅₀, 14 μ M). These results indicated that piceatannol and resveratrol inhibit the F-type ATPase by targeting the F₁ sector, which is located in the inner membrane of mitochondria and the plasma membrane of normal endothelial cells and several cancer cell lines.

Rb-E2F/DP pathway: Rb and the E2F family of transcription factors are important proteins that regulate the progression of the cell-cycle at and near the G₁/S-phase transition

(Figure 4). Adhami *et al.* provided evidence for the involvement of the Rb-E2F/DP pathway as an important contributor to resveratrol-mediated cell-cycle arrest and apoptosis (166). Immunoblot analysis demonstrated that resveratrol treatment of A431 melanoma cells resulted in a decrease in the hyperphosphorylated form of Rb and a relative increase in hypophosphorylated Rb. This response was accompanied by down-regulation of expression of all five E2F family transcription factors studied and their heterodimeric partners DP1 and DP2. This suggested that resveratrol causes down-regulation of hyperphosphorylated Rb protein with a relative increase in hypophosphorylated Rb that, in turn, compromises the availability of free E2F. These events may result in a stoppage of cell-cycle progression at the G₁/S-phase transition, thereby leading to a G₀/G₁-phase arrest and subsequent apoptotic cell death. Kim *et al.* showed that resveratrol treatment of A549 cells resulted in a concentration-dependent induction of S-phase

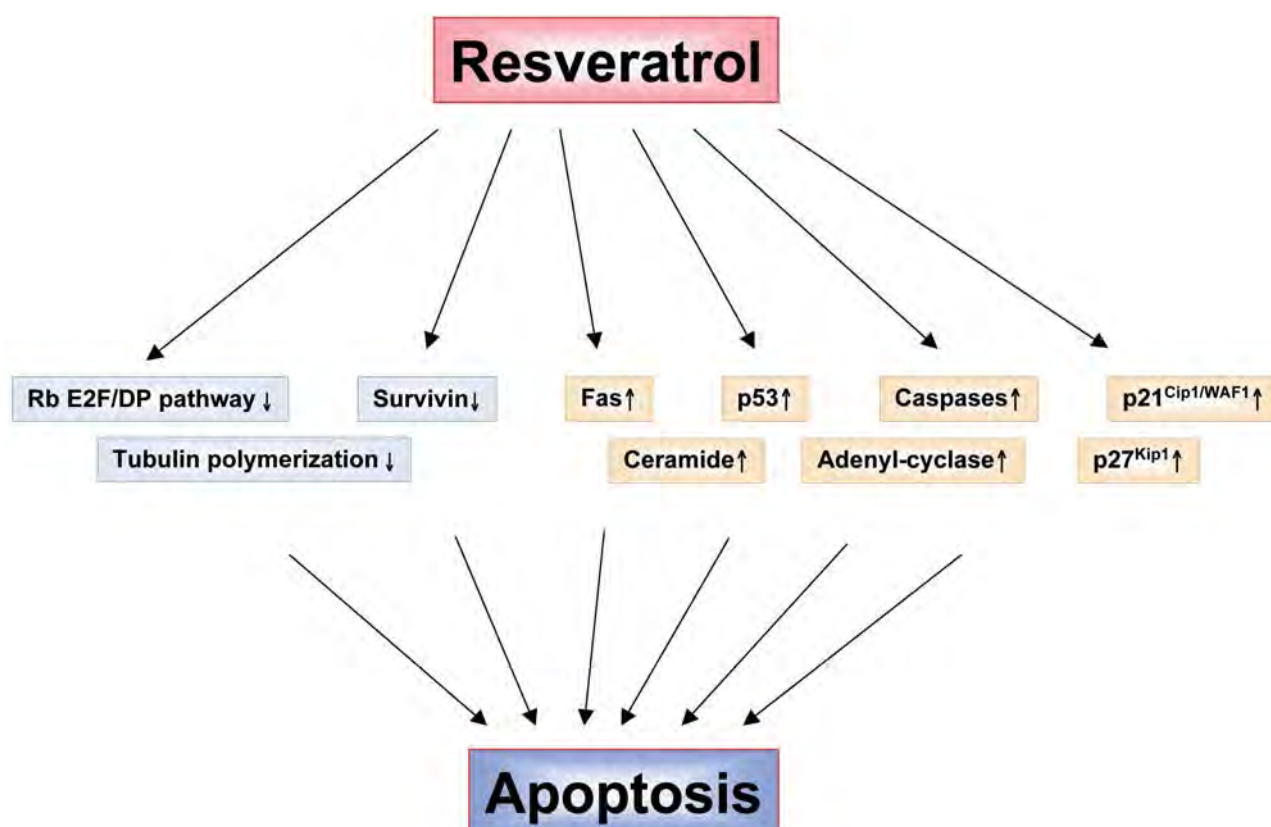


Figure 4. Effect of resveratrol on signaling proteins involved in apoptosis.

arrest in cell-cycle progression (168). This antiproliferative effect of resveratrol was associated with a marked inhibition of phosphorylation of Rb and concomitant induction of the Cdk inhibitor p21^{Cip1/WAF1}, which appears to be transcriptionally up-regulated and p53-dependent. Fluorescence microscopy and flow-cytometric analysis also revealed that treatment with resveratrol resulted in induction of apoptosis. These effects were found to correlate with activation of caspase-3 and a shift in the Bax/Bcl-x_L ratio toward apoptosis.

p53 activation pathway: p53 is a tumor suppressor gene. There are numerous reports about the role of p53 in resveratrol-induced apoptosis (51, 162, 175, 193-198). Huang *et al.* found that resveratrol-induced apoptosis occurred only in cells expressing wild-type p53 (p53^{+/+}), but not in p53-deficient (p53^{-/-}) cells, while there was no difference in apoptosis induction between normal lymphoblasts and sphingomyelinase-deficient cell lines (193). These results demonstrated for the first time that resveratrol induces apoptosis through activation of p53 activity, suggesting that resveratrol's antitumor activity may occur through induction of apoptosis. Hsieh *et al.* showed

that resveratrol inhibited proliferation of pulmonary artery endothelial cells, which correlated with suppression of cell progression through the S- and G2-phases of the cell-cycle and was accompanied by increased expression of p53 and elevation of the level of Cdk inhibitor p21^{Cip1/WAF1} (194). Lu *et al.* showed that resveratrol analogues significantly induced expression of p53, GADD45 and Bax genes and concomitantly suppressed expression of the Bcl-2 gene in human fibroblasts transformed with SV40 virus (WI38VA), but not in nontransfected WI38 cells (51). A large increase in p53 DNA-binding activity and the presence of p53 in the Bax promoter binding complex suggested that p53 was responsible for the Bax gene expression induced by resveratrol in transformed cells.

She *et al.* elucidated the potential signaling components underlying resveratrol-induced p53 activation and induction of apoptosis (195, 196). They found that, in the JB6 mouse epidermal cell line, resveratrol activated ERK1/2, JNK, and p38 MAPK and induced serine-15 phosphorylation of p53. Stable expression of a dominant-negative mutant of ERK2 or p38 MAPK or their respective inhibitors, PD98059 or SB202190, repressed phosphorylation of p53 at serine-15. In

contrast, overexpression of a dominant-negative mutant of JNK1 had no effect on the phosphorylation. Most importantly, ERK1/2 and p38 MAPK formed a complex with p53 after treatment with resveratrol. Strikingly, resveratrol-activated ERK1/2 and p38 MAPK, but not JNKs, phosphorylated p53 at serine-15 *in vitro*. Furthermore, pretreatment of the cells with PD98059 or SB202190 or stable expression of a dominant-negative mutant of ERK2 or p38 MAPK impaired resveratrol-induced p53-dependent transcriptional activity and apoptosis, whereas constitutively active MEK1 increased the transcriptional activity of p53. These data strongly suggest that both ERK1/2 and p38 MAPK mediate resveratrol-induced activation of p53 and apoptosis through phosphorylation of p53 at serine-15. Shih *et al.* also showed that resveratrol acted *via* a Ras-MAPK kinase-MAPK signal transduction pathway to increase p53 expression, serine phosphorylation of p53, and p53-dependent apoptosis in thyroid carcinoma cell lines. Haider *et al.* showed that resveratrol led to a reversible arrest in early S phase of the vascular smooth muscle cell (VSMC), accompanied by accumulation of hyperphosphorylated Rb (197). Resveratrol decreased cellular levels of the p21^{Cip1/WAF1} and p27^{Kip1} and increased the level of phosphorylated p53 protein (serine-15). The authors found that resveratrol only slightly inhibited phosphorylation of ERK1/2, protein kinase B/Akt, and p70(S6) kinase upon serum stimulation. Thus, inhibition of these kinases is not likely to contribute to the effects of the polyphenol on the cell-cycle. Importantly, the observed S-phase arrest was not linked to an increase in apoptotic cell death: there were no detectable increases in apoptotic nuclei or in levels of the proapoptotic protein Bax. This was the first study to elucidate the molecular pathways mediating the antiproliferative properties of resveratrol in VSMCs.

The expression of the nonsteroidal anti-inflammatory drug -activated gene-1 (*NAG-1*), a member of the TGF- β superfamily, has been associated with pro-apoptotic and antitumorigenic activities. Baek *et al.* demonstrated that resveratrol induced *NAG-1* expression and apoptosis through an increase in the expression of p53 (198). They showed that p53-binding sites within the promoter region of *NAG-1* played a pivotal role in controlling *NAG-1* expression by resveratrol. Derivatives of resveratrol were examined for *NAG-1* induction, and the data suggest that induction of *NAG-1* and p53 by resveratrol is not dependent on its anti-oxidant activity. The data may provide a linkage between p53, *NAG-1* and resveratrol and, in part, a new clue to the molecular mechanism of the antitumorigenic activity of natural polyphenolic compounds.

Earlier studies showed that resveratrol alters the expression of genes involved in cell-cycle regulation and apoptosis, including *cyclins*, *Cdks*, *p53*, and *Cdk* inhibitors. However, most of the p53-controlled effects related to the role of

resveratrol in transcription, either by activation or repression of a sizable number of primary and secondary target genes, have not been investigated. Narayanan *et al.* examined whether resveratrol activates a cascade of p53-directed genes that are involved in apoptosis mechanism(s) (162). They demonstrated by DNA microarray, RT-PCR, Western blot and immunofluorescence analyses that treatment of androgen-sensitive prostate cancer cells (LNCaP) with resveratrol down-regulated *PSA*, *AR* co-activator *ARA 24*, and NF- κ B *p65*. Altered expression of these genes is associated with activation of p53-responsive genes such as *p53*, *PIG 7*, *p21^{Cip1/WAF1}*, *p300/CBP* and *Apaf-1*.

Ceramide activation pathway: Apoptosis induction by various cytokines has been shown to be mediated through generation of ceramide. Whether resveratrol-induced apoptosis also involves ceramide production has been investigated. Scarlatti *et al.* showed that resveratrol can inhibit growth and induce apoptosis in MDA-MB-231, a highly invasive and metastatic breast cancer cell line, in concomitance with a dramatic endogenous increase of growth inhibitory/pro-apoptotic ceramide (137). They found that accumulation of ceramide derives from both *de novo* ceramide synthesis and sphingomyelin hydrolysis. More specifically, they demonstrated that ceramide accumulation induced by resveratrol can be traced to the activation of serine palmitoyltransferase (SPT), the key enzyme of a *de novo* ceramide biosynthetic pathway, and neutral sphingomyelinase (nSMase), a main enzyme of the sphingomyelin/ceramide pathway. By using specific inhibitors of SPT (myriocin and L-cycloserine) and nSMase (glutathione and manumycin), however, they found that only the SPT inhibitors could counteract the biological effects induced by resveratrol. Thus, resveratrol seems to exert its growth-inhibitory/apoptotic effect on the metastatic breast cancer cell line MDA-MB-231 by activating the *de novo* ceramide synthesis pathway.

Tubulin polymerization pathway: Certain chemotherapeutic agents such as taxol induce apoptosis by interfering with tubulin polymerization. Whether resveratrol could also mediate apoptosis through this pathway has been investigated. Schneider *et al.* found that a methylated derivative of resveratrol (Z-3,5,4'- trimethoxystilbene; R3) at a concentration of 0.3 μ M, exerted an 80% growth-inhibitory effect on human colon cancer Caco-2 cells and arrested growth completely at a concentration of 0.4 μ M (R3 was 100-fold more active than resveratrol) (199). The *cis* conformation of R3 was also 100-fold more potent than the *trans* isomer. R3 (0.3 μ M) caused cell-cycle arrest at the G2/M-phase transition. The drug inhibited tubulin polymerization in a dose-dependent manner (IC₅₀, 4 μ M), and it reduced by half the activities of ornithine

decarboxylase and s-adenosylmethionine decarboxylase. This caused depletion of the polyamines putrescine and spermidine, which are growth factors for cancer cells. R3 partially inhibited colchicine binding to its binding site on tubulin, indicating that R3 either partially overlaps with colchicine binding or binds to a specific site of tubulin that is not identical with the colchicine binding site, modifying colchicine binding by allosteric influences. R3 is an interesting antimitotic drug that exerts cytotoxic effects by depleting the intracellular pool of polyamines and by altering microtubule polymerization. Such a drug may be useful for the treatment of neoplastic diseases.

Adenylyl-cyclase pathway: Both cyclic GMP and cyclic AMP (cAMP) are known to regulate proliferation of cells. Whether resveratrol could modulate cell growth by modulating the levels of these nucleotides has been investigated (138). El-Mowafy *et al.* examined the effects of resveratrol on the activity of the enzymes adenylate cyclase and guanylate cyclase, two known cytostatic cascades in MCF-7 breast cancer cells (138). Resveratrol increased cAMP levels ($t_{1/2}$, 6.2 min; EC₅₀, 0.8 μ M), but had no effect on cGMP levels. The stimulatory effects of resveratrol on adenylate cyclase were not altered either by the protein synthesis inhibitor actinomycin-D (5 μ M) or the ER blockers tamoxifen and ICI182,780 (1 μ M each). Likewise, cAMP formation by resveratrol was insensitive to both the broad-spectrum phosphodiesterase (PDE) inhibitor IBMX (0.5 μ M) and the cAMP-specific PDE inhibitor rolipram (10 μ M). Instead, these PDE inhibitors significantly augmented maximal cAMP formation by resveratrol. Parallel experiments showed that the antiproliferative effects of resveratrol in these cells were appreciably reversed by the protein kinase A inhibitors Rp-cAMPS (100-300 μ M) and KT-5720 (10 μ M). Pretreatment with the cPLA2 inhibitor arachidonyl trifluoromethyl ketone (10 μ M) markedly antagonized the cytotoxic effects of resveratrol. With these findings, we demonstrated that resveratrol is an agonist for the cAMP/protein kinase A system.

C1c: Resveratrol suppresses NF- κ B activation

Because resveratrol exhibits anti-inflammatory, cell growth-modulatory and anticarcinogenic effects, that it mediates these effects by suppressing NF- κ B, a nuclear transcription factor that regulates the expression of various genes involved in inflammation, cytoprotection and carcinogenesis, has been proposed (200, 201). We investigated the effect of resveratrol on NF- κ B activation induced by various inflammatory agents. Resveratrol blocked TNF-induced activation of NF- κ B and suppressed TNF-induced phosphorylation and nuclear translocation of the p65 subunit

of NF- κ B and NF- κ B-dependent reporter gene transcription (22, 71, 73, 92, 120, 122, 125-127, 129, 132, 135, 139-142, 145, 147, 151, 153, 154, 156, 159, 161, 165, 167, 168, 173-175, 179, 182, 183, 185, 187, 191, 193-196, 198, 201-284). Suppression of TNF-induced NF- κ B activation by resveratrol was not restricted to myeloid cells (U-937); it was also observed in lymphoid (Jurkat) and epithelial (HeLa and H4) cells. Resveratrol also blocked NF- κ B activation induced by phorbol myristate acetate (PMA), LPS, H₂O₂, okadaic acid and ceramide. Holmes-McNary and Baldwin found resveratrol to be a potent inhibitor of both NF- κ B activation and NF- κ B-dependent gene expression through its ability to inhibit κ B kinase activity, the key regulator in NF- κ B activation, probably by inhibiting an upstream signaling component (202). In addition, resveratrol blocked the expression of mRNA-encoding monocyte chemoattractant protein-1, a NF- κ B-regulated gene. Heredia *et al.* found that resveratrol synergistically enhanced the anti-HIV-1 activity of the nucleoside analogues AZT, ddC, and ddI (14). Resveratrol at a concentration of 10 μ M was not toxic to cells, and by itself reduced viral replication by 20-30%. In phytohemagglutinin (PHA)-activated PBMCs infected with HTLV-III_B, 10 μ M resveratrol reduced the 90% inhibitory concentrations (IC₉₀) of AZT, ddC and ddI by 3.5-, 5.5- and 17.8-fold, respectively. Similar antiviral activity was demonstrated when ddI was combined with 5 or 10 μ M resveratrol in PBMCs infected with clinical isolates of HIV-1. The addition of resveratrol resulted in a >10-fold augmentation of ddI antiviral activity in infected monocyte-derived macrophages. In a resting cell model of T lymphocytes infected with HTLV-III_B, resveratrol plus ddI in combination, but not individually, suppressed the establishment of a productive viral infection. In addition, resveratrol plus ddI markedly inhibited the replication of four ddI-resistant viral isolates, three of which presented mutations in the reverse transcriptase gene conferring reverse transcriptase-multidrug resistance. Finally, 10 μ M resveratrol showed enhancement of ddI antiviral suppressive activity similar to that of 100 μ M of hydroxyurea. However, resveratrol had less of a cellular antiproliferative effect than hydroxyurea.

Pellegatta *et al.* reported different short- and long-term effects of resveratrol on NF- κ B phosphorylation and nuclear appearance in human endothelial cells (203). They found that the nuclear appearance of p50 and p65 acutely induced by TNF α was not modified by resveratrol, but was increased after overnight incubation with resveratrol alone or in combination with TNF α . Acute treatment with resveratrol did not modify TNF α -induced cytoplasmic κ B α serine phosphorylation but did increase κ B α tyrosine phosphorylation. Resveratrol increased tyrosine phosphorylation (but not nitrosylation) of immunoprecipitated NF- κ B, did not decrease cellular p21^{Cip1/WAF1}, and did not increase peroxisome proliferator-

activated receptor- α activity. They concluded that acute resveratrol treatment does not inhibit the nuclear appearance of NF- κ B in human umbilical vein endothelial cells (HUVEC), but overnight treatment does.

We showed that resveratrol blocks IL-1 β -induced activation of NF- κ B that leads to inhibition of proliferation, causes S-phase arrest, and induces apoptosis of AML cells (122). Adhami *et al.* showed the suppression of UV B exposure-mediated activation of NF- κ B in normal human keratinocytes by resveratrol (204). Kim *et al.* showed the involvement of NF- κ B suppression in induction of growth arrest and apoptosis by resveratrol in human lung carcinoma A549 cells (168). These results indicate that NF- κ B suppression by resveratrol may be essential for its antitumor activities.

C1d. Resveratrol suppresses AP-1 activation

Activator protein-1 (AP-1) is a transcription factor transactivated by many tumor-promoting agents, such as phorbol ester, UV radiation, asbestos and crystalline silica (209, 210). AP-1 complexes are formed by dimers of Jun proto-oncogene family members (*c-Jun*, *JunB*, and *JunD*) or heterodimers of *Jun* family members with the Fos proto-oncogene family members (*c-Fos*, *FosB*, *Fra-1*, and *Fra-2*). AP-1 binds to a specific target DNA site (also known as TRE) in the promoters of several cellular genes and mediates immediate early gene expression involved in a diverse set of transcriptional regulation processes (209, 210). Agents that activate NF- κ B also activate AP-1. Both of these factors are regulated by the redox status of the cell. AP-1 activation has been implicated in cell proliferation and chemical carcinogenesis. It has been shown to play a critical role in proliferation of cells. Whether resveratrol affects activation of AP-1 has been investigated by several groups. We showed that suppression of NF- κ B by resveratrol coincided with suppression of AP-1 (201). Resveratrol has been shown to suppress activation of AP-1 by PMA, TNF and UV. It inhibited PMA-induced IL-8 production in human monocytic U-937 cells at protein and mRNA levels which was, at least partly, due to inhibition of AP-1 activation (211). It also suppressed PMA-mediated signaling events such as induction of COX-2 and prostaglandin synthesis in human mammary and oral epithelial cells (212). Moreover, it inhibited PMA-mediated activation of PKC and induction of COX-2 promoter activity by c-Jun. PMA-mediated induction of AP-1 activity was blocked by resveratrol. Resveratrol also inhibited PMA- or UV-induced AP-1-mediated activity through inhibition of c-Src non-receptor tyrosine kinase and MAPK pathways and may also regulate gene expression of cellular defensive enzymes such as phase II detoxifying enzymes (213). It also suppressed TNF-induced AP-1 activity in various cancer cell lines (201).

Resveratrol inhibited the TNF-induced activation of MAPK and JNK, which are needed for AP-1 activation.

Yu *et al.* found that resveratrol inhibited phorbol ester and UV-induced AP-1 activation by interfering with MAPK pathways (213). They showed that pretreatment with resveratrol also inhibited the activation of ERK2, JNK1 and p38 MAPK. Selectively blocking MAPK pathways by overexpression of dominant-negative mutants of kinases attenuated the activation of AP-1 by PMA and UVC. Interestingly, resveratrol had little effect on induction of the AP-1 reporter gene by active Raf-1, MAPK/ERK kinase (MEKK)1, or MAPK kinase (MKK)6, suggesting that it inhibited MAPK pathways by targeting the signaling molecules upstream of Raf-1 or MEKK1. Indeed, incubation of resveratrol with the isolated c-Src protein tyrosine kinase and PKC diminished their kinase activities. Moreover, modulation of ER activity by 17- β -estradiol had no effect on the inhibition of AP-1 by resveratrol. In contrast to these studies, those of Wolter *et al.* showed that the AP-1 constituents c-Fos and c-Jun increased on resveratrol treatment of cells (214). While the DNA-binding activity of c-Jun remained unchanged, the DNA-binding activity of c-Fos was significantly enhanced by resveratrol and piceatannol.

C1e: Resveratrol suppresses Egr-1 activation

Early growth response-1 gene product (Egr-1) is another transcription factor that plays an important role in proliferation of cells. It is a member of a family of immediate early response genes and regulates a number of pathophysiologically relevant genes that are involved in growth, differentiation, immune response, wound healing and blood clotting. Resveratrol selectively up-regulates Egr-1 by an ERK1/2-dependent mechanism in human erythroleukemic K562 cells, induces γ -globin synthesis, and causes erythroid differentiation due to impairment of cell proliferation, increase in p21^{Cip1/WAF1} expression and inhibition of Cdk2 activity (215). Ragione *et al.* found that resveratrol increases Egr-1 and causes differentiation of HL-60 cells (216) and examined its effects on this transcription factor (215). Up-regulation of p21^{Cip1/WAF1} transcription is prevented by cycloheximide, indicating that an intermediate protein(s) is required that, in turn, regulates gene expression. Quantitative analysis of some transcription factors involved in the erythroid lineage, namely GATA-1, GATA-2 and Egr-1, indicated that resveratrol selectively up-regulates Egr-1 by an ERK1/2-dependent mechanism. The presence of an Egr-1 consensus sequence in the p21^{Cip1/WAF1} promoter suggests that this transcription factor directly regulates the expression of the Cdk inhibitor. Transfection studies with deleted gene promoter constructs, as well as electrophoretic mobility shift assay, pull-down and chromatin immunoprecipitation experiments, substantiated this view, demonstrating that Egr-1 binds *in vitro* and *in vivo* to the identified consensus sequence

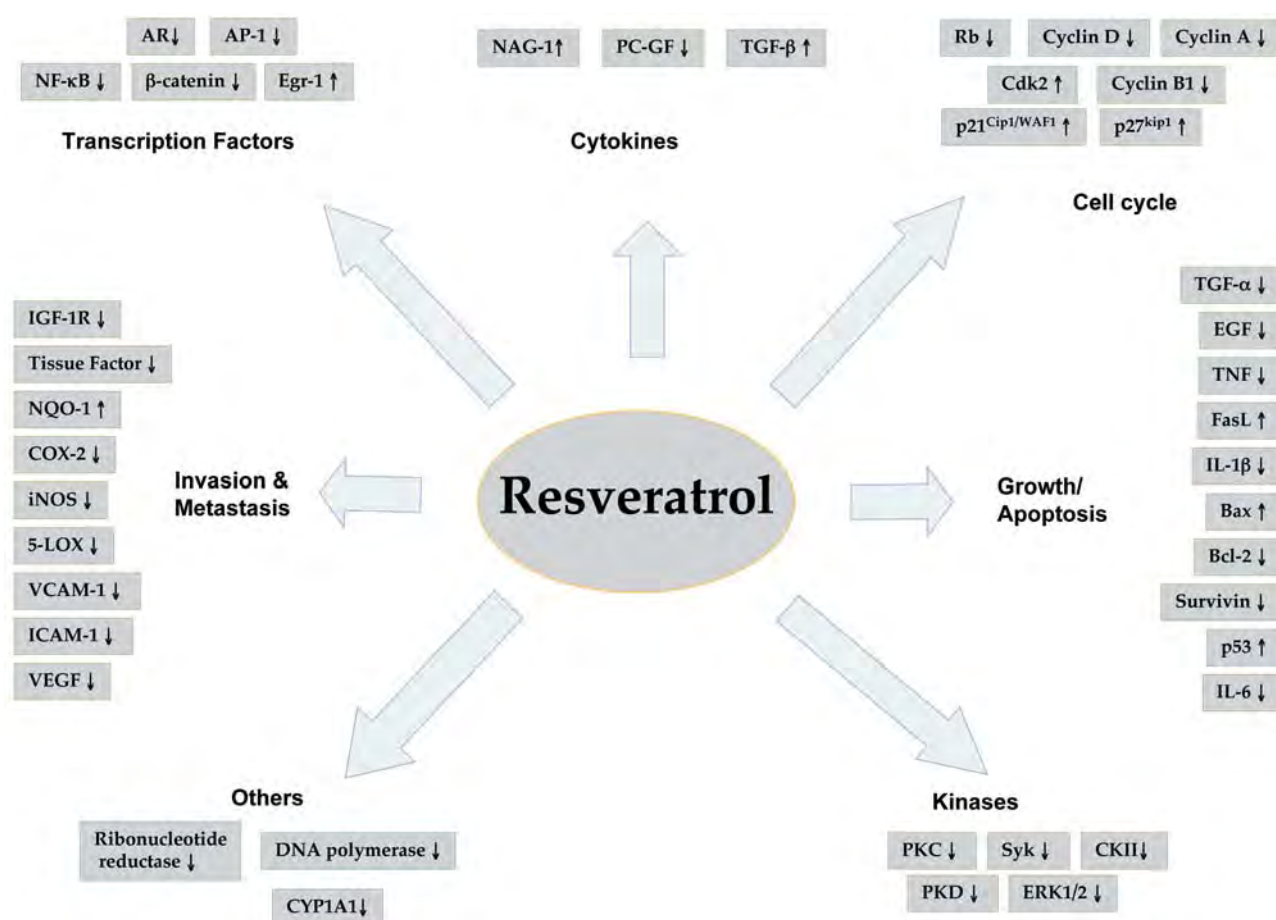


Figure 5. Identification of molecular targets of resveratrol.

of the p21^{Cip1/WAF1} promoter. Moreover, an Egr-1 phosphorothioate antisense construct hinders p21^{Cip1/WAF1} accumulation and the antiproliferative effects of resveratrol.

C1f. Suppression of MAPK by resveratrol

Three different MAPK have been identified: ERK1/2, JNK and p38 MAPK. While ERK1/2 have been implicated in the proliferation of cells, JNK and p38 MAPK are activated in response to different types of stress stimuli. JNK activation is needed for activation of AP-1; it also mediates apoptosis in some situations. Numerous studies suggest that resveratrol modulates all three of these protein kinases (163, 175, 179, 195, 196, 217, 218). Miloso *et al.* showed that resveratrol induced activation of ERK1/2 in human neuroblastoma SH-SY5Y cells (179). In undifferentiated cells, resveratrol 1 μM induced phosphorylation of ERK1/2, which was already evident at 2 min, peaked at 10 min and still persisted at 30 min. A wide range of resveratrol

concentrations (from 1 pM to 10 μM) were able to induce phosphorylation of ERK1/2, while higher concentrations (50-100 μM) inhibited phosphorylation of MAPK. In retinoic acid-differentiated cells, resveratrol (1 μM) induced an evident increase in ERK1/2 phosphorylation. El-Mowafy *et al.* found short-term treatment of porcine coronary arteries with resveratrol substantially inhibited MAPK activity (IC₅₀, 37 μM) and reduced phosphorylation of ERK1/2, JNK1 and p38 MAPK at active sites. Endothelin-1 enhanced, MAPK activity, phosphorylation and nuclear translocation in a concentration-dependent manner, but resveratrol reversed it (217). She *et al.* showed that resveratrol activated ERK1/2, JNKs and p38 MAPK in the JB6 mouse epidermal cell line and induced serine-15 phosphorylation of p53 (196). Stable expression of a dominant-negative mutant of ERK2 or p38 MAPK repressed phosphorylation of p53 at serine-15. In contrast, overexpression of a dominant-negative mutant of JNK1 had no effect on this phosphorylation. Most importantly,

ERK1/2 and p38 MAPK formed a complex with p53 after treatment with resveratrol. Strikingly, resveratrol-activated ERK1/2 and p38 MAPK, but not JNKs, phosphorylated p53 at serine-15 *in vitro*. Shih *et al.* examined the effect of resveratrol on papillary and follicular thyroid carcinoma cell lines (175). They found that treatment with resveratrol (1-10 μ M) induced activation and nuclear translocation of ERK1/2. Cellular abundance of the oncogene suppressor protein p53, serine phosphorylation of p53, and abundance of *c-fos*, *c-Jun*, and *p21^{Cip1/WAF1}* mRNAs were also increased by resveratrol. Inhibition of the MAPK pathway by either *H-Ras* antisense transfection or PD 98059, MAPK kinase inhibitor, blocked these effects. Thus, resveratrol appears to act *via* a Ras-MAPK kinase-MAPK signal-transduction pathway to increase p53 expression, serine phosphorylation of p53 and p53-dependent apoptosis in thyroid carcinoma cell lines.

She *et al.* showed the interesting involvement of JNK in resveratrol-induced activation of p53 (195). They found that resveratrol activated JNKs at the same dosage that inhibited tumor promoter-induced cell transformation. Stable expression of a dominant-negative mutant of JNK1 or disruption of the *Jnk1* or *Jnk2* gene markedly inhibited resveratrol-induced p53-dependent transcription activity and induction of apoptosis. Furthermore, resveratrol-activated JNKs were shown to phosphorylate p53 *in vitro*, but this activity was repressed in the cells expressing a dominant-negative mutant of JNK1 or in *Jnk1* or *Jnk2* knockout (*Jnk1^{-/-}* or *Jnk2^{-/-}*) cells. These data suggest that JNKs act as mediators of resveratrol-induced activation of p53 and apoptosis, which may occur partially through p53 phosphorylation. Woo *et al.* showed that resveratrol inhibited PMA-induced matrix metalloproteinase (MMP)-9 expression by inhibiting JNK (218). From these results, it is clear that resveratrol can modulate all three MAPKs, which leads to modulation of gene expression. Resveratrol appears to cause activation of MAPK in some cells and inhibition in others. This variability may depend on the cell type and the dose of resveratrol used.

Stewart and O'Brian showed that resveratrol antagonized EGFR-dependent ERK1/2 activation in human androgen-independent prostate cancer cells with associated isozyme-selective PKC- α inhibition (163). They found that resveratrol suppressed EGFR-dependent ERK1/2 activation pathways stimulated by EGF and PMA in human AI PrCa PC-3 cells *in vitro*. Resveratrol abrogation of a PKC-mediated ERK1/2 activation response in PC-3 cells correlated with isozyme-selective PKC- α inhibition.

C1g. Suppression of protein kinases by resveratrol

PKC has been shown to play a major role in tumorigenesis. The PKC isozyme subfamily consists of cPKC- α , - β and - γ , nPKC-D and - ϵ , and α PKC- ζ . Numerous reports indicate

that resveratrol can inhibit PKC (127, 139, 153, 218-221). Garcia-Garcia *et al.* showed that resveratrol was incorporated into model membranes and inhibited PKC- α activity (219). Resveratrol activated by phosphatidylcholine/phosphatidylserine vesicles inhibited PKC- α with an IC₅₀ of 30 μ M, whereas that activated by Triton X-100 micelles inhibited PKC- α with an IC₅₀ of 300 μ M. These results indicate that the inhibition of PKC- α by resveratrol can be mediated, at least partially, by membrane effects exerted near the lipid-water interface. Stewart *et al.* showed that resveratrol preferentially inhibited PKC-catalyzed phosphorylation of a cofactor-independent, arginine-rich protein substrate by a novel mechanism (139). While resveratrol has been shown to antagonize both isolated and cellular forms of PKC, the weak inhibitory potency observed against isolated PKC cannot account for the reported efficacy of the polyphenol against PKC in cells. Stewart *et al.* analyzed the mechanism of PKC inhibition by resveratrol and found that resveratrol has a broad range of inhibitory potencies against purified PKC that depend on the nature of the substrate and the cofactor dependence of the phosphotransferase reaction. Resveratrol weakly inhibited the Ca²⁺/phosphatidylserine-stimulated activity of a purified rat brain PKC isozyme mixture (IC₅₀, 90 μ M) by competition with ATP (K_i, 55 μ M). Consistent with the kinetic evidence for a catalytic domain-directed mechanism was resveratrol's inhibition of the lipid-dependent activity of PKC isozymes with divergent the regulatory domains, and it was even more effective in inhibiting a cofactor-independent catalytic domain fragment of PKC generated by limited proteolysis. This suggested that regulatory features of PKC might impede resveratrol inhibition of the enzyme. To explore this, the authors examined the effects of resveratrol on PKC-catalyzed phosphorylation of the cofactor-independent substrate protamine sulfate, which is a polybasic protein that activates PKC by a novel mechanism. Resveratrol potently inhibited protamine sulfate phosphorylation (IC₅₀, 10 μ M) by a mechanism that entailed antagonism of the activation of PKC by protamine sulfate and did not involve competition with either substrate.

Protein kinase D (PKD) is a member of the PKC superfamily with distinctive structural, enzymic and regulatory properties. Identification of the cellular function(s) of PKD has been hampered by the absence of a selective inhibitor. Stewart *et al.* compared the effects of resveratrol against the autophosphorylation reactions of PKC isozymes to those against the autophosphorylation reactions of the novel phorbol ester-responsive kinase PKD (127). They found that resveratrol inhibited PKD autophosphorylation, but had only negligible effects against the autophosphorylation reactions of representative members of each PKC isozyme subfamily (cPKC- α , - β 1 and - γ , nPKC-D and - ϵ , and α PKC- ζ). Resveratrol was

comparably effective against PKD autophosphorylation (IC_{50} , 52 μ M) and PKD phosphorylation of the exogenous substrate syntide-2 (IC_{50} , 36 μ M). The inhibitory potency of resveratrol against PKD is in line with those observed in cellular systems and against other purified enzymes and binding proteins that are implicated in the cancer chemopreventive activity of the polyphenol. Thus, PKD inhibition may contribute to the cancer chemopreventive action of resveratrol. Haworth *et al.* showed inhibition of PKD by resveratrol, not only *in vitro* but also in intact cells (220). Atten *et al.* demonstrated that resveratrol treatment significantly inhibited PKC activity of KATO-III human gastric adenocarcinoma cells and of human recombinant PKC- α (153). Woo *et al.* showed that resveratrol inhibited PMA-mediated PKC- Δ activation, which led to suppression of MMP-9 (218).

The COP9 signalosome (CSN), purified from human erythrocytes, possesses kinase activity that phosphorylates proteins such as c-Jun and p53, with consequences for their ubiquitin-dependent degradation. Uhle *et al.* showed that resveratrol could block the CSN-associated kinases protein kinase CK2 and PKD and induce degradation of c-Jun in HeLa cells (221).

C1h. Modulation of NO/NOS expression by resveratrol

Synthesis of NO is dependent on expression of an inducible enzyme, iNOS. The expression of this enzyme is regulated by the transcription factor NF- κ B. Production of NO has been shown to mediate antiproliferative effects in various cell types. NO also been linked with pro-inflammatory effects. Resveratrol has been reported to both enhance and suppress production of NO (92, 154, 194, 222). Kageura *et al.* reported that resveratrol analogues had inhibitory activity against NO production in LPS-activated macrophages (IC_{50} , 11-69 μ M) (92). Furthermore, the active stilbenes (rhapontigenin, piceatannol and resveratrol) did not inhibit iNOS activity, but they inhibited NF- κ B activation following expression of iNOS. Chung *et al.* examined the effect of α -viniferin, a trimer of resveratrol, in a mouse model of carrageenin-induced paw edema (222). They found that α -viniferin at doses >30 mg/kg (*p.o.*) or >3 mg/kg (*i.v.*) showed significant anti-inflammatory activity on this edema. α -Viniferin at doses of 3-10 μ M inhibited NO production in LPS-activated Raw 264.7 cells when α -viniferin and LPS were applied simultaneously, but not when α -viniferin was applied 12 h after LPS stimulation. α -Viniferin inhibited synthesis of the iNOS transcript with an IC_{50} value of 4.7 μ M.

Hsieh *et al.* found that resveratrol induced NOS in cultured pulmonary artery endothelial cells, which led to inhibition of their proliferation (194). Holian *et al.* found that resveratrol stimulated NOS activity in human gastric

adenocarcinoma SNU-1 cells (154). They suggested that the antioxidant action of resveratrol toward gastric adenocarcinoma cells may reside in its ability to stimulate NOS to produce low levels of NO, which, in turn, exerts antioxidant action. Thus, whether resveratrol induces or inhibits NO production depends on the cell system, inducer and other conditions.

C1i. Suppression of growth factor and associated protein tyrosine kinases by resveratrol

Because resveratrol exhibits antiproliferative effects against a wide variety of tumor cells and the effects of various growth factors are mediated through protein tyrosine kinases, it is possible that resveratrol either down-regulates the expression of growth factors and growth factor receptors or suppresses the activity of protein tyrosine kinases required for their activity. Kaneuchi *et al.* found that resveratrol treatment significantly decreased EGF expression in Ishikawa endometrial cancer cells (183). Palmieri *et al.* found that tyrosine kinase activities from particulate and cytosolic fractions of placenta were inhibited by resveratrol and piceatannol (223). Oliver *et al.* showed that piceatannol (3,4,3',5'-tetrahydroxy-*trans*-stilbene) preferentially inhibited the activity of Syk protein tyrosine kinase as compared with Lyn when added to *in vitro* assays with isolated enzymes (224). Selective inhibition of Syk in this manner blocked receptor-mediated downstream cellular responses (inositol 1,4,5-trisphosphate production, secretion, ruffling and spreading). We showed that piceatannol inhibited H₂O₂-induced NF- κ B activation through inhibition of Syk kinase (225). These reports suggest that resveratrol and its analogues can potentially suppress growth factors, growth factor receptors and their associated protein tyrosine kinases.

Resveratrol exerts an inhibitory effect in EGF-induced cell transformation (226). It also inhibits proliferation of the breast cancer cell line MDA-MB-468 through alteration in autocrine growth modulators such as TGF- α , TGF- β , PC cell-derived growth factor, and insulin-like growth factor I receptor mRNA (129). Moreover, it decreases hepatocyte growth factor-induced cell scattering and invasion by an unidentified postreceptor mechanism in HepG2 cells (173).

C1j. Suppression of COX-2 and LOX by resveratrol

The enzymes COX-2 and lipoxygenase (LOX) play important roles in inflammation. Both of these enzymes are regulated by the transcription factors NF- κ B and AP-1. The products of these enzymes also regulate proliferation of cells. Whether resveratrol modulates expression of these enzymes has been investigated by numerous groups (141, 142, 212, 222, 227, 228). Subbaramaiah *et al.* showed that resveratrol inhibits COX-2 transcription and activity in phorbol ester-

treated human mammary epithelial cells (141). Transient transfections utilizing COX-2 promoter deletion constructs and COX-2 promoter constructs, in which specific enhancer elements were mutagenized, indicated that the effects of PMA and resveratrol were mediated *via* a cAMP response element. Resveratrol inhibited the PMA-mediated activation of PKC. Overexpressing PKC- α , ERK1 and c-Jun led to 4.7-, 5.1- and 4-fold increases in COX-2 promoter activity, respectively. These effects were inhibited by resveratrol. Resveratrol blocked PMA-dependent activation of AP-1-mediated gene expression. In addition to these effects on gene expression, we found that resveratrol also directly inhibited the activity of COX-2. These data are likely to be important for understanding the anticancer and anti-inflammatory properties of resveratrol. Chung *et al.* showed that α -viniferin inhibited COX-2 activity with an IC₅₀ value of 4.9 μ M, and at doses of 3-10 μ M, inhibited synthesis of COX-2 transcript in LPS-activated murine macrophages Raw 264.7 (222). MacCarrone *et al.* demonstrated that resveratrol acted as a competitive inhibitor of purified 5-LOX and 15-LOX and prostaglandin H synthase, with inhibition constants of 4.5 μ M (5-LOX), 40 μ M (15-LOX), 35 μ M (COX activity of prostaglandin H synthase), and 30 μ M (peroxidase activity of prostaglandin H synthase) (227).

C1k. Suppression of cell-cycle proteins by resveratrol

Numerous reports indicate that resveratrol inhibits proliferation of cells by inhibiting cell-cycle progression (122, 135, 145, 147, 151, 161, 165, 167, 187, 191, 194, 229). Various reports indicate that resveratrol inhibits different cells at different stages of the cell-cycle. The arrest of cells in G1-phase (165), S-phase (122, 151, 161, 187, 191), S/G2-phase (194) and G2-phase (147) of the cell-cycle has been reported. Why the effects of resveratrol on different cell types vary so widely is not clear. Which cell-cycle proteins are modulated by resveratrol has been investigated in detail. Wolter *et al.* showed the down-regulation of the cyclin D1/Cdk4 complex by resveratrol in colon cancer cell lines (145). Yu *et al.* showed that, following treatment of H22 tumor-bearing mice with resveratrol at 10 or 15 mg/kg bodyweight for 10 days, the growth of transplantable liver cancers was inhibited by 36.3% or 49.3%, respectively (229). The levels of expression of cyclin B1 and Cdc2 protein were decreased in treated tumors, whereas the expression of cyclin D1 protein did not change. Liang *et al.* showed that resveratrol induced G2 arrest through the inhibition of Cdk7 and Cdc2 kinases in colon carcinoma HT-29 cells (147). Larrosa *et al.* showed that resveratrol and the related molecule 4-hydroxystilbene induced S-phase arrest and up-regulation of cyclins A, E and B1 in human SK-Mel-28 melanoma cells (167). Thus, it is clear that the effects of resveratrol on the cell-cycle are highly variable. Kuwajerwala *et al.* showed that resveratrol had a

dual effect on DNA synthesis (161). At concentrations of 5-10 μ M, it caused a 2- to 3-fold increase in DNA synthesis, and at doses \geq 15 μ M, it inhibited DNA synthesis. The increase in DNA synthesis was seen only in LNCaP cells, not in the androgen-independent DU145 prostate cancer cells or in NIH/3T3 fibroblast cells. The resveratrol-induced increase in DNA synthesis was associated with enrichment of LNCaP cells in S-phase and concurrent decreases in nuclear p21^{Cip1/WAF1} and p27^{Kip1} levels. Furthermore, consistent with the entry of LNCaP cells into the S-phase, there was a dramatic increase in nuclear Cdk2 activity associated with both cyclin A and cyclin E.

C1l. Suppression of adhesion molecules by resveratrol

Various cell-surface adhesion molecules, including intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and endothelial-leukocyte adhesion molecule (ELAM)-1, are regulated by NF- κ B. These molecules play an essential role in adhesion of tumor cells to endothelial cells and thus mediate tumor cell metastasis. Several groups have examined the effect of resveratrol on the adhesion of cells to the endothelial cells. Ferrero *et al.* examined the activity of resveratrol on granulocyte and monocyte adhesion to endothelium *in vitro* (230, 231). They showed that resveratrol, at concentrations as low as 1 μ M and 100 nM, significantly inhibited ICAM-1 and VCAM-1 expression by TNF α -stimulated HUVEC and LPS-stimulated human saphenous vein endothelial cells (HSVEC), respectively. They also showed that resveratrol induced significant inhibition of the adhesion of U-937 monocytoid cells to LPS-stimulated HSVEC. Such inhibition was comparable with that obtained when anti-VCAM-1 monoclonal antibody was used instead of resveratrol. Resveratrol also significantly inhibited the adhesion of neutrophils to TNF α -stimulated NIH/3T3 ICAM-1-transfected cells, whereas neutrophils activated by formyl-methionyl-leucyl-phenylalanine did not significantly modify adhesion to NIH/3T3 ICAM-1-transfected cells. Pendurthi *et al.* also showed that resveratrol suppressed agonist-induced monocyte adhesion to cultured human endothelial cells (125). Thus, it is clear that resveratrol affects the expression of adhesion molecules, most likely through down-regulation of NF- κ B.

C1m. Suppression of androgen receptors by resveratrol

Via their receptor AR, androgens play a role in prostate cancer etiology (159, 285). Mitchell *et al.* demonstrated that resveratrol had inhibitory effects on androgen action in the LNCaP prostate cancer cell line (159). They found that resveratrol repressed different classes of androgen up-regulated genes at the protein or mRNA level, including PSA, human glandular kallikrein-2, AR-specific coactivator ARA70, and the Cdk inhibitor p21^{Cip1/WAF1}. This inhibition

is probably attributable to a reduction in AR level at the transcription level, inhibiting androgen-stimulated cell growth and gene expression. These results suggest that resveratrol may be a useful chemopreventive / chemotherapeutic agent for prostate cancer.

C1n. Suppression of PSA by resveratrol

Hsieh *et al.* demonstrated that resveratrol inhibited the proliferation of LNCaP cells and expression of the prostate-specific gene PSA. A 4-day treatment with resveratrol reduced the levels of intracellular and secreted PSA by approximately 80%, as compared to controls (156). They found that this change in PSA was not due to a change in AR expression. Thus, it would appear that the prostate tumor marker PSA is down-regulated by resveratrol, by a mechanism independent of changes in AR.

C1o. Suppression of inflammatory cytokine expression by resveratrol

Because resveratrol down-regulates NF- κ B, which is known to mediate inflammation, it is possible that resveratrol also down-regulates the expression of inflammatory cytokines. Wang *et al.* showed that resveratrol inhibited IL-6 production in cortical mixed glial cells under hypoxic/hypoglycemic conditions followed by reoxygenation (232). Zhong *et al.* demonstrated the inhibitory effect of resveratrol on IL-6 release by stimulated peritoneal macrophages of mice (233). Shen *et al.* found that resveratrol suppressed IL-8 gene transcription in phorbol ester-treated human monocytic cells (211). Wadsworth *et al.* showed that resveratrol had no effect on LPS-induced TNF α mRNA in the macrophage cell line RAW 264.7, but decreased LPS-stimulated TNF α release, as measured by ELISA (234). Culpitt *et al.* determined whether resveratrol would inhibit cytokine release *in vitro* by alveolar macrophages from patients with chronic obstructive pulmonary disease (COPD) (235). They showed that resveratrol inhibited basal release of IL-8 in smokers and patients with COPD by 94% and 88%, respectively, and inhibited granulocyte-macrophage colony-stimulating factor (GM-CSF) release by 79% and 76%, respectively. Resveratrol also inhibited stimulated cytokine release. Resveratrol reduced IL-1 β -stimulated IL-8 and GM-CSF release in both smokers and COPD patients to below basal levels. Moreover, resveratrol inhibited cigarette smoke media (CSM)-stimulated IL-8 release by 61% and 51%, respectively, in smokers and COPD patients, and inhibited GM-CSF release by 49% in both subject groups.

Boscolo *et al.* elucidated the "*in vitro*" effects of resveratrol on human PBMC proliferation and cytokine release (236). Spontaneous PBMC proliferation was unaffected by resveratrol, while resveratrol at a concentration of 100 μ M

inhibited PHA-stimulated PBMC proliferation by 69%. The proliferation stimulation index (*i.e.*, the ratio of PHA-stimulated PBMC proliferation/spontaneous PBMC proliferation) of cultures containing 100 μ M resveratrol was very low in relation to the control, while the proliferation stimulation index values at resveratrol concentrations of 10 μ M and 100 nM were similar and slightly higher (without statistical significance), respectively. Resveratrol strongly inhibited PHA-stimulated interferon (IFN)- γ and TNF α release from PBMC at a concentration of 100 μ M, but not concentrations of 10 μ M or 100 nM. The concomitant immune effects of resveratrol on PBMC proliferation and release of IFN- γ and TNF α may be explained by an inhibitory effect on transcription factor NF- κ B.

C1p. Suppression of angiogenesis, invasion and metastasis by resveratrol

Angiogenesis is a process of blood vessel formation that is mediated through modulation of proliferation and gene expression by endothelial cells. This process plays an essential role in tumor growth, other diseases and wound healing. Several studies have examined the effects of resveratrol on endothelial cells and on angiogenesis (194, 218, 237-241, 243-246, 286). Szende *et al.* examined the effect of resveratrol on endothelial cells and showed that low doses (0.1-1 μ g/ml) of resveratrol enhanced HUVEC proliferation, while higher doses (10-100 μ g/ml) induced apoptosis and decreased mitotic activity, which is reflected in changes of cell number (237). Igura *et al.* found that resveratrol inhibited the growth of bovine aorta endothelial (BAE) cells in a concentration-dependent manner (6-100 μ M) (238). The migration of BAE was obviously inhibited by resveratrol. When the lengths of all tubes constructed in the 3-dimensional culture system with or without resveratrol were measured, resveratrol was found to inhibit tube formation by BAE cells. Hsieh *et al.* found that resveratrol induced NOS in cultured pulmonary artery endothelial cells, which inhibited the proliferation of cells, correlated with suppression of cell progression through S- and G2-phases of the cell-cycle, and was accompanied by an increase in the expression of protein p53 and elevation of the level of Vdk inhibitor p21^{Cip1/WAF1} (194). Using bovine pulmonary artery endothelial cells, Bruder *et al.* found an increase in NOS expression that led to morphological and structural changes (239). Lin *et al.* investigated the mechanism by which resveratrol inhibited vascular endothelial growth factor (VEGF)-induced angiogenic effects in HUVECs (240) and showed that resveratrol, at the dose of 1 or 2.5 μ M, effectively abrogated VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin and its complex partner, β -catenin. This inhibitory effect of resveratrol reflected on the retention of VE-cadherin at

cell-cell contacts as demonstrated by immunofluorescence. They showed that VEGF stimulated an evident increase of peroxide, which was strongly attenuated by resveratrol. Their data suggested that resveratrol inhibition of VEGF-induced angiogenesis was mediated by disruption of ROS-dependent Src kinase activation and the subsequent VE-cadherin tyrosine phosphorylation.

Abou-Agag *et al.* showed that resveratrol increased tissue-type plasminogen activator (*tPA*) and urokinase-type plasminogen activator (*uPA*) gene transcription in cultured human endothelial cells (241). Resveratrol yielded increases in *tPA* and *uPA* antigen levels (two- to three-fold) and mRNA levels (3- to 4-fold) and correlated increases (2- to 3-fold) in sustained (24 h), surface-localized fibrinolytic activity. Used at concentrations present in human plasma following moderate wine consumption, resveratrol inhibited adhesion molecule expression by TNF-stimulated endothelial cells (286). Resveratrol also significantly prevented cytokine-induced vascular leakage. Others have shown that resveratrol can stimulate K-Ca channels in endothelial cells, which may be the mechanism for its effect on the functional activities of endothelial cells (243). Fulgenzi *et al.* showed that TNF-induced vascular permeability changes were inhibited by resveratrol, not only *in vitro* but also *in vivo* (244).

Proteolytic degradation of the extracellular matrix and tumor metastasis correlate with expression of endopeptidases known as MMPs. The Expression of MMPs is regulated by cytokines and signal transduction pathways, including those activated by PMA. Woo *et al.* found that resveratrol significantly inhibited PMA-induced increases in *MMP-9* expression and activity (218). These effects of resveratrol were dose-dependent and correlated with suppression of *MMP-9* mRNA expression. PMA caused about a 23-fold increase in *MMP-9* promoter activity, which was suppressed by resveratrol. Transient transfection utilizing *MMP-9* constructs, in which specific transcriptional factors were mutated, indicated that the effects of PMA and resveratrol were mediated *via* an AP-1 and NF- κ B response element. Resveratrol inhibited PMA-mediated activation of JNK and PKC- Δ . Brakenhielm *et al.* found that resveratrol suppressed angiogenesis, tumor growth and wound healing (245).

C1q: Effect of resveratrol on bone cells

Bone formation is regulated by the balance between osteoclasts (bone-resorbing cells) and osteoblasts (bone-forming cells). Resveratrol has been reported to promote differentiation of murine MC3T3-E1 osteoblasts. Ulsperger *et al.* examined the effects of resveratrol on the increased proliferation of the human AHTO-7 osteoblastic cell line, induced by conditioned medium from a panel of carcinoma cell lines (247). This compound was found to modulate

AHTO-7 proliferation in a tamoxifen-sensitive mechanism at lower concentrations but, unlike vitamin D3, it failed to induce the osteoblast differentiation marker ALP. The proliferative response of AHTO-7 cells to conditioned medium from carcinoma cell lines were diminished (30-71.4% inhibition) upon pretreatment with 0.5 μ M resveratrol. The highest degree of inhibition was demonstrated for pancreas (BxPC3 and Panc-1), breast (ZR75-1) and renal (ACHN) carcinoma cell line supernatants, whereas the effects on colon carcinoma (SW620 and Colo320DM) cell-conditioned medium and prostate cancer (PC3, DU145 and LNCaP)-conditioned medium were less pronounced. Direct addition of resveratrol affected only the supernatants of cell lines (<25% inhibition) exhibiting growth-stimulatory activity for normal WI38 lung fibroblasts. Resveratrol inhibited proliferation of DU145 and LNCaP cells at concentrations exceeding 5 μ M, altered cell-cycle distribution of all prostate cancer cell lines at concentrations as low as 0.5 μ M, but did not inhibit the production of osteoblastic factors by these lines. Thus, resveratrol failed to induce ALP activity as a marker of osteoblast differentiation in human osteoblastic AHTO-7 cells, although it inhibited their response to osteoblastic carcinoma-derived growth factors at concentrations significantly lower than those needed to reduce the growth of cancer cells, thus effectively modulating tumor-osteoblast interaction.

Mizutani *et al.* found that resveratrol directly stimulated the proliferation and differentiation of osteoblastic MC3T3-E1 cells (278). It also increased the ALP activity and prolyl hydroxylase activity of MC3T3-E1 cells. Moreover, the antiestrogen tamoxifen reversed these effects. On the other hand, resveratrol inhibited prostaglandin E2 production in MC3T3-E1 cells.

C1r. Effects of resveratrol on expression of cytochrome P450 and metabolism of carcinogens

Many environmental compounds are carcinogenic only after metabolic activation. Exposure to carcinogens, such as polycyclic aromatic hydrocarbons (PAH), increases expression of the enzymes responsible for this activation. These enzymes consist of members of the cytochrome p450 (CYP) 1A and 1B subfamilies. They generate genotoxic epoxide metabolites of the parent aryl hydrocarbon, which can bind to DNA, forming adducts. These adducts, if not repaired, can cause specific mutations leading to cellular transformation. Therefore, the activity and expression of carcinogen-activating enzymes in chemically-induced carcinogenesis, and inhibition of their activity, either by direct enzyme inhibition or through modulation of their expression, is thought to be an important mechanism in the prevention of carcinogenesis.

The carcinogen activation pathway is regulated by the aryl hydrocarbon receptor (AhR), which further activates the enzymes CYP1A1 and CYP1A2 in microsomes. Different carcinogens are activated by different CYP. The carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) is a classic hydrocarbon that is activated through the CYP enzymes CYP1B1, CYP1A1 and CYP1A2.

Resveratrol inhibits the phase I drug-activating enzymes such as CYP and increases the activity/level of phase II drug-detoxifying enzymes (73, 140, 248-258, 287, 288). In human hepatic microsomes, resveratrol inhibits CYP isoenzymes, such as CYP1A1, CYP1B1 and CYP2B6, which are involved in the bioactivation of numerous carcinogens (248). Chun *et al.* found that rhapontigenin (3,3',5-trihydroxy-4'-methoxystilbene) exhibited a potent and selective inhibition of human CYP1A1 with an IC₅₀ of 0.4 μ M. The values for K_i and K_{inactivation} were 0.09 μ M and 0.06 min⁻¹, respectively, suggesting that rhapontigenin is a potent mechanism-based inactivator of human CYP1A1 (73). Others showed that resveratrol inhibits CYP1A1 through an AhR-independent posttranscriptional pathway (140). Ciolini *et al.* showed that resveratrol competitively inhibited, in a concentration-dependent manner, the activity of the carcinogen-activating enzymes CYP1A1 and CYP1A2 in microsomes (249). Resveratrol inhibits aryl hydrocarbon-induced CYP1A activity *in vitro*, by directly inhibiting CYP1A1 and CYP1A2 enzymes activity and by inhibiting the signal transduction pathway that up-regulates the expression of carcinogen-activating enzymes. Chang *et al.* found that resveratrol differentially-inhibited human CYP1 enzymes and that this occurred through two distinct mechanisms: direct inhibition (mainly CYP1B1 and CYP1A1) and mechanism-based inactivation (CYP1A2) (250).

Chan *et al.* demonstrated that resveratrol inactivated CYP3A4 in a time- and NADPH-dependent manner (251). Chang *et al.* found that resveratrol inhibited a substrate oxidation reaction catalyzed by human recombinant CYP3A4 and CYP3A5 *in vitro* (252). That resveratrol is an irreversible (probably mechanism-based) inhibitor of CYP3A4 and a non-competitive reversible inhibitor of CYP2E1 has been demonstrated (248). Yu *et al.* found that resveratrol inhibited CYP with IC₅₀ values of 11.6 μ M for CYP2C19 and 1.1 μ M for CYP3A4, but the IC₅₀ values exceeded 50 μ M for all the other CYP isozymes, indicating no inhibition (288).

CYP1B1 is expressed in a number of human tissues in which cancers occur (*e.g.*, prostate, ovary, uterus, mammary gland). CYP1B1 activates many environmental mutagens and also catalyzes the 4-hydroxylation of estrogens, considered to be an important step in hormonal carcinogenesis. The enzyme CYP1B1 is overexpressed in a wide variety of human tumors and catalyzes aromatic hydroxylation reactions. Chang *et al.* studied whether *trans*-resveratrol modulates the catalytic activity and gene expression of CYP1B1 and found that resveratrol decreased human recombinant CYP1B1-catalyzed

7-ethoxyresorufin O-dealkylation activity with an IC₅₀ value of 1.4 μ M (253). Treatment of MCF-7 cells with 10 μ M resveratrol decreased relative *CYP1B1* mRNA levels after 5 h, indicating that resveratrol both inhibited the catalytic activity and suppressed the constitutive expression of the *CYP1B1* gene. This may explain the protection against toxicity and carcinogenicity induced by compounds that undergo CYP1B1-catalyzed bioactivation. We report here that resveratrol undergoes metabolism by CYP1B1 to give a metabolite that has been identified as the known antileukemic agent piceatannol. This demonstrates that a natural dietary cancer preventive agent can be converted to a compound with known anticancer activity by an enzyme that is found in human tumors. This also provides evidence for the concept that CYP1B1 in tumors may be functioning as a growth suppressor enzyme.

Guengerich *et al.* examined the activities of several of the major allelic variants of human CYP1B1 and found that resveratrol is also an inhibitor of this enzyme (255). Further studies with rhapontigenin and synthetic stilbenes led to the discovery of 2,4,3',5'-tetramethoxystilbene, a selective inhibitor of CYP1B1 relative to other CYP enzymes. Inhibition is competitive, with a K_i value of 3 nM, and the inhibitor is resistant to metabolism. In addition to blocking 17- β -estradiol 4-hydroxylation, this stilbene also inhibited the activation of heterocyclic amines to mutagens. 2,4,3',5'-tetramethoxystilbene also suppressed expression of CYP1B1 and growth of human mammary tumor cells. 3,3',4',5,5'-pentamethoxystilbene was a selective inhibitor of CYP1A1, showing mixed inhibition, and also suppressed CYP1A1 expression in HepG2 cells.

Dubuisson *et al.* investigated the effects of resveratrol on DNA binding *via* esterification reactions with 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (N-OH-PhIP) - a metabolite of a mammary gland carcinogen present in cooked meats (256). Treatment of primary cultures of human mammary epithelial cells with 50 μ M resveratrol led to decreases in PhIP-DNA adducts ranging from 31% to 69%. Resveratrol inhibited PhIP-DNA adduct formation by O-acetyltransferase and sulfotransferase catalysis and suppressed O-acetyltransferase and sulfotransferase activities from the breast cancer cell lines MCF-7 and ZR-75-1. It also stimulated ATP-dependent cytosolic activation of N-OH-PhIP in all human samples, but not in mouse liver samples.

Moreover, resveratrol increased the activity of NQO, a detoxifying enzyme for quinone-containing substances (182).

C1s. Suppression of inflammation by resveratrol

Numerous lines of evidence suggest that resveratrol is a potent anti-inflammatory agent. As already described, resveratrol can suppress the activation of transcription factor NF- κ B, which is closely linked with inflammation. It can also suppress the

expression of proinflammatory cytokines such as TNF, IL-1, IL-6 and IL-8 (211, 232-236). Resveratrol can abrogate the expression of proteins such as iNOS, COX-2 and 5-LOX, that mediate inflammation. Kimura *et al.* showed that resveratrol inhibits the 5-LOX products 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-diHETE) and leukotriene C4 (LTC4) at IC₅₀ of 8.9 μ M, 6.7 μ M, and 1.37 μ M, respectively (259). The IC₅₀ of 5-HETE, 5,12-diHETE and LTC4 formations of synthetic 3,3',4'-trihydroxystilbene were 5.9 μ M, 6.3 μ M and 8.8 μ M, respectively. Moreover, they inhibited the release of lysosomal enzymes such as lysozyme and β -glucuronidase induced by calcium ionophore A 23187 from human polymorphonuclear leukocytes (PMN). In another study, these workers examined the effects of various stilbenes (*i.e.*, 3,4',5-trihydroxystilbene, 3,4',5-trihydroxystilbene 3-O-D-glucoside, and 2,3,4',5-tetrahydroxystilbene 2-O-D-glucoside) on COX and LOX activities in rat PMN (260). Resveratrol inhibited the 5-LOX product, 5-HETE, and the COX products, HHT and thromboxane B2, at IC₅₀ of 2.72 μ M for 5-HETE, 0.7 μ M for HHT and 0.8 mM for thromboxane B2. Piceid (3,4',5-trihydroxystilbene 3-O-D-glucoside) and 2,3,4',5-tetrahydroxystilbene 2-O-D-glucoside also inhibited the formation of 5-HETE, HHT and thromboxane B2, although less strongly. Their IC₅₀ values were, respectively, 55.3 \pm 15.3 μ M and >1000 μ M for 5-HETE, 196 μ M and 300 μ M for HHT, and 251 μ M and 366 μ M for thromboxane B2.

The expression NAG-1, a member of the TGF- β superfamily, has been shown to be associated with proapoptotic and antitumorigenic activities. Baek *et al.* demonstrated that resveratrol induced *NAG-1* expression and apoptosis in a concentration-dependent manner (198). Resveratrol increases the expression of the tumor suppressor protein p53 prior to *NAG-1* induction, indicating that induction of *NAG-1* expression by resveratrol is mediated by p53 expression. These authors also showed that the p53-binding sites within the promoter region of *NAG-1* play a pivotal role in controlling induction of *NAG-1* expression by resveratrol.

Resveratrol exerted a strong inhibitory effect on the superoxide radical (O₂[•]) and H₂O₂ produced by macrophages stimulated by LPS or PMA. Resveratrol also significantly decreased ³H-arachidonic acid release induced by LPS and PMA or by exposure to O₂[•] or H₂O₂. Resveratrol treatment caused a significant impairment of COX-2 induction stimulated by LPS and PMA or by O₂[•] or H₂O₂ exposure. These resveratrol effects were correlated with a marked reduction of prostaglandin synthesis. These results indicate that the anti-inflammatory action of resveratrol affects arachidonic acid mobilization and COX-2 induction.

Huang *et al.* examined the anti-inflammatory activity of resveratrol tetramers amurensins I-L, (+)-hopeaphenol, isohopeaphenol, vitisin A, (+)-vitisifuran A and heyneanol A

(261). Among them, (+)-hopeaphenol, isohopeaphenol, vitisin A, (+)-vitisifuran A, and heyneanol A potently inhibited biosynthesis of leukotriene B₄, and amurensins I and L strongly antagonized the histamine receptor. Chung *et al.* examined the anti-inflammatory activity of α -viniferin, a trimer of resveratrol, in an animal model of carrageenin-induced paw edema, and its inhibitory effects on COX and iNOS (222). α -viniferin, at doses >30 mg/kg (*p.o.*) or >3 mg/kg (*i.v.*), had significant anti-inflammatory activity on this edema in mice and an inhibitory effect on COX-2 activity (IC₅₀, 4.9 μ M), but a very weak inhibitory effect on COX-1 (55.2 \pm 2.1% of the control [100%] at 100 μ M). At doses of 3-10 μ M, α -viniferin inhibited synthesis of the COX-2 transcript in LPS-activated Raw 264.7 murine macrophages. α -Viniferin inhibited NO production in LPS-activated Raw 264.7 cells at in IC₅₀ of 2.7 μ M when α -viniferin and LPS were administered simultaneously, but did not inhibit NO production when α -viniferin was administered 12 h after LPS. α -viniferin inhibited synthesis of the iNOS transcript with an IC₅₀ of 4.7 μ M. The inhibitory effect of α -viniferin on the release of prostanoids and NO may provide important evidence of its anti-inflammatory action.

C1t. Anti-oxidant effects of resveratrol

Numerous lines of evidence suggest that resveratrol exerts anti-oxidant activity (71, 262-276). Jang *et al.* found that resveratrol was a potent inhibitor of ROS production in both unopsonized zymosan-stimulated RAW 264.7 cells (IC₅₀, 17 μ M) and in human monocytes (IC₅₀, 18 μ M) and neutrophils (IC₅₀, 23 μ M) (262). 3,5-Dihydroxy-4'-methoxystilbene and 3,4'-dimethoxy-5-hydroxystilbene exhibited IC₅₀ values of 63 and 73 μ M in RAW 264.7 cells, 51 and >100 μ M in human monocytes, and 10 and 37 μ M in human neutrophils. Trimethylresveratrol, piceid and 3,5-dihydroxy-4'-methoxystilbene-3-O- β -D-glucoside were weak inhibitors of ROS production. Resveratrol's potent inhibitory action on ROS production might be one biochemical mechanism related to its anti-inflammatory and anticarcinogenic activities. The number and position of hydroxy substituents in resveratrol analogues seems to play an important role in the potency of their inhibition of ROS production. Burkitt *et al.* provided evidence for hydroxyl-radical scavenging and a novel, glutathione-sparing mechanism of action for resveratrol (263). Resveratrol strongly inhibited NADPH- and ADP-Fe³⁺-dependent lipid peroxidation at the initial and propagation stages (264). Moreover, phenolic stilbenes inhibited UV-induced lipid peroxidation and efficiently scavenged 2,2'-azobis-(2-amidinopropane)-dihydrochloride peroxy radicals (264). Tadolini *et al.* found that resveratrol inhibited more efficiently than either the hydrophilic analogue of vitamin E, Trolox, or vitamin C ascorbate the Fe²⁺-catalyzed lipid

hydroperoxide-dependent peroxidation of sonicated phosphatidylcholine liposomes (265). They also showed that resveratrol inhibited lipid peroxidation mainly by scavenging lipid peroxy radicals within the membrane, like vitamin E. Although resveratrol is less effective, its capacity to spontaneously enter the lipid environment confers on it great anti-oxidant potential.

By using the Rancimat method and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging model, Wang *et al.* found that 3, 3',4,5'-tetrahydroxystilbene, 3,3',4,5,5'-pentahydroxystilbene and 3,4,4',5-tetrahydroxystilbene were more active than resveratrol (266). A dimer of resveratrol was identified as the major radical reaction product when resveratrol was reacted with DPPH radicals. Murcia *et al.* compared the anti-oxidant activities of resveratrol and several other agents and found that the abilities to scavenge hypochlorous acid (HOCl) were, in decreasing order, propyl gallate > resveratrol > vitamin E > phenol (267). Resveratrol (6.25-100 µg/ml) also has been shown to inhibit chemiluminescence and the generation of O_2^- in blood platelets (268). It has an inhibitory effect on the production of ROS and thiobarbituric acid-reactive substances (TBARS) in platelets induced by LPS or thrombin. Isorhapontigenin, isolated from *Belamcanda chinensis*, is a derivative of stilbene whose chemical structure is very similar to that of resveratrol and has a potent anti-oxidant effect. Stojanovic *et al.* examined the efficiency and mechanism of the anti-oxidant activity of *trans*-resveratrol and its analogues in radical liposome oxidation (269). They showed that the para-hydroxyl group of *trans*-resveratrol had greater radical-scavenging activity than its meta-hydroxyl groups. This was apparently confirmed by pulse radiolysis studies of the reactions of *trans*-resveratrol and its analogues with trichloromethylperoxy radicals, CCl_3OO^\bullet , which showed that the spectral and kinetic properties of the observed transients were very similar in *trans*-resveratrol and *trans*-4-hydroxystilbene reactions.

Belguendouz *et al.* found that *trans*-resveratrol, which is by far the most potent chelator of copper, does not chelate iron (270). They also found that resveratrol protected low-density lipoprotein (LDL) against peroxidative degradation, by both chelating and free radical scavenging mechanisms. Some reports, however, suggest that resveratrol can also act as a pro-oxidant (264). Martinez *et al.* showed that resveratrol exerts a strong inhibitory effect on O_2^- and H_2O_2 produced by macrophages stimulated by LPS or PMA (271). Resveratrol also significantly decreased 3H -arachidonic acid release induced by LPS and PMA or by exposure to O_2^- or H_2O_2 and significantly impaired the COX-2 induction stimulated by LPS and PMA or by O_2^- or H_2O_2 exposure. These effects were correlated with a marked reduction in prostaglandin synthesis. These results indicate that the anti-oxidant action of resveratrol affects arachidonic acid mobilization and COX-2 induction.

C1u. Suppression of transformation by resveratrol

Some reports suggest that resveratrol can suppress the transformation of cells. Huang *et al.* found that resveratrol suppressed cell transformation and induced apoptosis through a p53-dependent pathway (193). Resveratrol suppressed tumor promoter-induced cell transformation and markedly induced apoptosis, the transactivation of p53 activity, and expression of p53 protein in the same cell line and at the same dosage. Also, resveratrol-induced apoptosis occurs only in cells expressing wild-type p53 ($p53^{+/+}$), not in p53-deficient ($p53^{-/-}$) cells, while apoptosis induction is no different in normal lymphoblasts and sphingomyelinase-deficient cell lines.

She *et al.* investigated the effect of resveratrol and its structurally related derivatives on EGF-induced cell transformation (226). Their results provided evidence that one of the resveratrol derivatives exerted a more potent inhibitory effect than resveratrol on EGF-induced cell transformation but had less cytotoxic effects on normal nontransformed cells. The resveratrol derivative caused cell-cycle arrest in the G1-phase but, unlike resveratrol, did not induce p53 activation and apoptosis. Furthermore, this compound, unlike resveratrol, markedly inhibited EGF-induced phosphoinositide 3-kinase (PI3K) and Akt activation. Collectively, these data suggest that resveratrol derivative's antitumor effect may be mediated through a different mechanism, by mainly targeting PI3K/Akt signaling pathways.

C1v. Induction of cellular differentiation by resveratrol

Evidence that resveratrol is a differentiation-inducing agent has been reported in certain cell types (277-279). Using the human erythroleukemic K562 cell line as an *in vitro* model, Rodrigue *et al.* showed that 50 µM of resveratrol induced greater hemoglobin production (7-fold) than 500 µM of hydroxyurea (3.5-fold) (277). This erythroid differentiation was linked to the inhibition of cell proliferation associated with an equivalent increased expression of $p21^{Cip1/WAF1}$ mRNA, but with the level of $p21^{Cip1/WAF1}$ protein increased to a greater extent (6-fold) for cells treated with resveratrol than for those treated with hydroxyurea (1.5-fold). They also showed that 50 µM of resveratrol and 25 µM of hydroxyurea induced variable, but similar, enhancements of fetal hemoglobin synthesis in cultured erythroid progenitors for the majority of the sickle cell patients studied. These inductions were linked to, but not correlated with, variable decreases in erythroid burst-forming unit clone number. Mizutani *et al.* examined the effect of resveratrol on the proliferation and differentiation of osteoblastic MC3T3-E1 cells and found that it increased DNA synthesis (278). In addition, resveratrol increased the ALP activity and prolyl hydroxylase activity of MC3T3-E1 cells. Moreover, the

antiestrogen tamoxifen reversed resveratrol's stimulation of proliferation and ALP activity in these cells. On the other hand, resveratrol inhibited prostaglandin E2 production in MC3T3-E1 cells. These results indicate that resveratrol directly stimulates the cell proliferation and differentiation of osteoblasts.

Wang *et al.* examined the effect of resveratrol on cell growth, differentiation and death in human medulloblastoma Med-3, UW228-1, -2 and -3 cell lines (279). The results demonstrated that resveratrol could suppress growth, promote differentiation and commit its target cells to apoptosis in time- and dose-related fashions. Fas was constitutively expressed, but FasL was undetectable in the four lines in spite of resveratrol treatment. Anti-Fas antibody neither inhibited growth nor induced apoptosis of the cell lines. Up-regulated caspase-3 was found in resveratrol-treated populations and the appearance of its cleaved form was closely associated with the apoptotic event.

C1w. Estrogenic/anti-estrogenic effects of resveratrol

Resveratrol has a structural similarity to diethylstilbestrol, a synthetic estrogen. Whether it is an estrogen agonist or antagonist is highly controversial. Some reports suggest that resveratrol has estrogenic activity, while others show no such effects (132, 174, 185, 280-284, 289). Gehm *et al.* found that, at concentrations comparable to those required for its other biological effects (~ 3 -10 μM), resveratrol inhibited the binding of labelled estradiol to the ER and activated the transcription of estrogen-responsive reporter genes transfected into human breast cancer cells (280). This transcriptional activation was ER-dependent, required an estrogen response element in the reporter gene, and was inhibited by specific estrogen antagonists. In some cell types (*e.g.*, MCF-7 cells), resveratrol functioned as a superagonist (*i.e.*, produced a greater maximal transcriptional response than estradiol), whereas in others it produced an activation equal to or less than that of estradiol. Resveratrol also increased the expression of native estrogen-regulated genes, and it stimulated the proliferation of estrogen-dependent T47D breast cancer cells. The authors concluded that resveratrol is a phytoestrogen and that it exhibits variable degrees of ER agonism in different test systems.

Turner *et al.* examined the estrogenic activity of resveratrol *in vivo* and found that resveratrol treatment had no significant effect on body weight, serum cholesterol level, radial bone growth, epithelial cell height, or mRNA levels for insulin-like growth factor I (281). These results, in contrast to those of prior *in vitro* studies, suggest that resveratrol has little or no estrogen agonism on reproductive and non-reproductive estrogen target tissues and may be an estrogen antagonist. Lu *et al.* showed that resveratrol inhibited the growth of ER-positive MCF-7 cells in a dose-dependent

fashion (132). Detailed studies with MCF-7 cells demonstrated that resveratrol antagonized the growth-promoting effect of 17- β -estradiol at both the cellular (cell growth) and the molecular (gene activation) levels. At a concentration of 5 μM , resveratrol abolished the growth-stimulatory effect mediated by concentrations of 17- β -estradiol as high as 1 nM. The anti-estrogenic effect of resveratrol could be observed at concentrations of 1 μM and higher. This effect was also demonstrated at the molecular level. Resveratrol antagonized, in a dose-dependent fashion, the stimulation by 17- β -estradiol of PR gene expression in MCF-7 cells. Moreover, expression of *TGF- α* and *insulin-like growth factor-I receptor* mRNAs were inhibited, while expression of *TGF- β 2* mRNA was significantly elevated in MCF-7 cells cultivated in the presence of resveratrol (10 μM). These results show that resveratrol, a partial ER agonist itself, acts as an ER antagonist in the presence of estrogen, leading to inhibition of human breast cancer cells.

Bhat *et al.* characterized the estrogen-modulatory effects of resveratrol in a variety of *in vitro* and *in vivo* mammary models (185). The effects of resveratrol alone, and in combination with 17- β -estradiol, were assessed in MCF-7, T47D, LY2 and S30 mammary cancer cell lines. In transient transfection studies in MCF-7 cells, resveratrol showed a weak estrogenic response, but when resveratrol was combined with 17- β -estradiol (1 nM), a clear dose-dependent antagonism was observed. Similar mixed estrogenic/anti-estrogenic effects were noted in S30 cells, whereas resveratrol functioned as a pure estrogen antagonist in T47D and LY2 cells. In MCF-7 cells, furthermore, resveratrol induced PR protein expression but, when resveratrol was combined with 17- β -estradiol, expression of PR was suppressed. With T47D cells, resveratrol significantly down-regulated the steady-state and 17- β -estradiol-induced levels of PR. In LY2 and S30 cells, resveratrol down-regulated pS2 protein expression. In the mouse mammary organ culture model, resveratrol induced PR when administered alone, but suppressed the expression of PR in the presence of 17- β -estradiol (1 nM). Furthermore, resveratrol inhibited the formation of estrogen-dependent preneoplastic ductal lesions induced by DMBA in these mammary glands (IC_{50} , 3.2 μM) and reduced N-methyl-N-nitrosourea-induced mammary tumorigenesis when administered to female Sprague-Dawley rats by gavage. In the absence of 17- β -estradiol, therefore, resveratrol exerts mixed estrogen agonist/antagonist activities in some mammary cancer cell lines, but in the presence of E2, resveratrol functions as an anti-estrogen.

In rodent models, carcinogen-induced preneoplastic lesions and mammary tumors are inhibited by resveratrol. Bhat *et al.* showed that treatment of cultured human endometrial adenocarcinoma (Ishikawa) cells with resveratrol (concentrations as high as 10 μM) did not

significantly increase the levels of the estrogen-inducible marker enzyme ALP (174). On the contrary, when ALP was induced by treatment with 1 nM of 17- β -estradiol, resveratrol exhibited a decrease in activity (IC_{50} , 2.3 μ M). Furthermore, when Ishikawa cells were treated with resveratrol alone, estrogen-inducible PR was not enhanced, and PR expression induced by treatment with 17- β -estradiol was inhibited by resveratrol in a dose-dependent fashion at both the mRNA and protein levels. Moreover, resveratrol mediated the suppression of a functional activity of PR as demonstrated by down-regulation of α 1-integrin expression induced by 17- β -estradiol plus progesterone. In transient transfection experiments conducted with Ishikawa cells, anti-estrogenic effects were confirmed by dose-dependent inhibition of the 17- β -estradiol-induced estrogen response element-luciferase transcriptional activity. Resveratrol showed no discernable activity with ER- α , but with ER- β 17- β -estradiol was displaced with an IC_{50} of 125 μ M. However, ER- α but not ER- β mRNA and protein expression were suppressed in Ishikawa cells by resveratrol in the concentration range of 5-15 μ M. In the presence or absence of 17- β -estradiol, resveratrol inhibited Ishikawa cell proliferation in a time-dependent manner with cells accumulating in the S-phase of the cell-cycle in ≤ 48 h. This effect was reversible. Analysis of some critical cell-cycle proteins revealed a specific increase in expression of cyclins A and E, but a decrease in Cdk2. These data suggest that resveratrol exerts an antiproliferative effect in Ishikawa cells, and that the effect may be mediated by both estrogen-dependent and -independent mechanisms.

Basly *et al.* examined the estrogenic/anti-estrogenic and scavenging properties of (E)- and (Z)-resveratrol (282). They found that both isomers increased the *in vitro* growth of MCF-7 cell lines at concentrations of 10-25 μ M, whereas 0.1-1 μ M had no effect and 50 μ M decreased cell growth and was cytotoxic. The 25 μ M (E)-isomer alone was able to reduce the proliferation induced by the estradiol. Low concentrations of (E)- and (Z)-resveratrol (0.1-1 μ M) and moderate concentrations of (Z)-resveratrol (10 μ M) did not interfere with the ER, whereas moderate concentrations of (E)-resveratrol (10 and 25 μ M) and a somewhat higher concentration of (Z)-resveratrol (25 μ M) both functioned as superagonists of estradiol. Bowers *et al.* showed that resveratrol acts as a mixed agonist/antagonist for ER- α and ER- β (283).

Recent data have indicated that the ER- α , through interaction with p85, regulates PI3K activity, revealing a physiological, non-nuclear function potentially relevant in cell proliferation and apoptosis. Pozo-Guisado *et al.* recently showed that resveratrol modulates the PI3K pathway through an ER- α -dependent mechanism (289). They found that resveratrol increased ER- α -associated PI3K activity with a maximum stimulatory effect at concentrations close to 10 μ M;

concentrations >50 μ M decreased PI3K activity. The stimulation of PI3K activity by resveratrol was ER- α -dependent, since it could be blocked by the antiestrogen ICI 182,780. Resveratrol did not affect p85 protein expression but induced the proteasome-dependent degradation of ER- α .

C1x: Effect of resveratrol on normal cells

Resveratrol appears to affect the proliferation not only of tumor cells but also of normal cells. The proliferation of keratinocytes (290), smooth muscle cells (SMC) (188, 197, 291), and endothelial cells (194, 237, 238, 245) is suppressed by resveratrol. The proliferation of normal human PBMC, however, was unaffected by resveratrol (292). Holian *et al.* evaluated the viability and proliferation of cultured normal human keratinocytes exposed to resveratrol (290). They found that resveratrol, even at submicromolar concentrations, inhibits the proliferation of these keratinocytes *in vitro* and, at higher concentrations, is cytotoxic to these cells.

Zou *et al.* investigated the effects of resveratrol on the proliferation and cell-cycle control of cultured SMC (188). Resveratrol reduces SMC proliferation in a dose-dependent manner, with concentrations of 50-100 μ M resveratrol resulting in 70-90% reduction of SMC proliferation induced by such diverse mitogens as serum, endothelin and platelet-derived growth factor (PDGF). The antimitogenic effects of resveratrol are not mediated by the induction of apoptosis, but appear to relate to a G1/S-phase block in the cell-cycle. Mnjoyan *et al.* found that resveratrol inhibited the growth of human aortic VSMC at concentrations as low as 1 μ M, as indicated by inhibition of DNA synthesis and increased intracellular p53 and p21^{Cip1/WAF1} levels, and effectively blocked the cell-cycle progression of serum-stimulated VSMC (291). Intriguingly, however, high concentrations of resveratrol could not induce apoptosis in quiescent VSMC. These differential biological effects of resveratrol on quiescent and proliferating VSMC suggest that resveratrol may be capable of selectively eliminating abnormally proliferating VSMC of the arterial walls *in vivo*. Haider *et al.* showed that resveratrol led to reversible arrest in early S-phase of VSMC, accompanied by the accumulation of hyperphosphorylated Rb (197). In contrast to findings in other cell systems, resveratrol decreases the cellular levels of the Cdk inhibitors p21^{Cip1/WAF1} and p27^{Kip1}. This is of particular interest because phosphorylated p53 protein (serine-15) is strongly enhanced by this substance. Importantly, the observed S-phase arrest was not linked to an increase in apoptotic cell death: there were no detectable increases in apoptotic nuclei or in levels of the proapoptotic protein Bax.

Lu *et al.* synthesized a number of polyhydroxy- and polymethoxy-stilbenes and tested their antiproliferative effects in normal and transformed human cells (51). They

showed that one of the resveratrol analogues, 3,4,5,4'-tetrahydroxystilbene (R-4), specifically inhibited the growth of SV40 virally-transformed WI38 cells (WI38VA) at a concentration of 10 μ M, but had no effect on normal WI38 cells at even higher concentrations. R-4 also prominently induced apoptosis in WI38VA cells, but not in WI38 cells. An RNase protection assay showed that R-4 significantly induced the expression of *p53*, *GADD45* and *Bax* genes and concomitantly suppressed expression of the *Bcl-2* gene in WI38VA, but not in WI38 cells. A large increase in *p53* DNA-binding activity and the presence of *p53* in the *Bax* promoter binding complex suggested that *p53* was responsible for the *Bax* gene expression induced by R-4 in transformed cells. Within 4 h of treatment with R-4, the *Bax* to *Bcl-2* protein ratios in WI38 and WI38VA cells were, respectively, 0.1 and 105, a difference of three orders of magnitude. While R-4 prominently induced the *p53/Bax* pro-apoptotic genes, it also concomitantly suppressed the expression of COX-2 in WI38VA cells. Taken together, these findings suggest that induction of the *p53* gene by R-4 in transformed cells may play a key role in the differential growth inhibition and apoptosis of transformed cells.

Cavallaro *et al.* investigated the effect of resveratrol on some activities of PBMC, particularly generation of the superoxide anion O_2^- in whole blood, HOCl and NO production by isolated cells, and chemotaxis (292). Resveratrol had significant effects on all these activities. In particular, it inhibited O_2^- generation in stimulated, but not in resting, neutrophils and decreased HOCl much more than O_2^- production, indicating an effect on myeloperoxidase secretion, since HOCl production is directly and proportionally dependent on O_2^- generation and reduced cell motility. The small dose of resveratrol (4.38 nM) used is attainable by consuming a diet that includes red wine and vegetables, confirming its protective role against some pathological processes such as inflammation, coronary heart disease and cancer.

Losa *et al.* examined the effect of resveratrol on apoptosis and the oxidative metabolic status of normal human PBMC isolated *ex vivo* from healthy donors (293). Neither apoptotic nor oxidative parameters were affected by culturing PBMC in medium containing resveratrol at concentrations as high as 20 μ M for 5 days, while the frequency of cells with intermediate permeability to propidium iodide (17%) increased at a concentration of 50 μ M. Thus resveratrol was slightly toxic, but there was little apoptosis in these cells. PBMC were also grown, first in medium plus resveratrol for 24 h, and then for 96 h in medium containing resveratrol plus 10 mM of oxidant 2-deoxy-D-ribose, an oxidant sugar that is apoptogenic in human lymphocytes. The apoptotic changes triggered by 2-deoxy-D-ribose were counteracted by the phytoalexin in a dose-dependent manner, but resveratrol activity was absent

at the lowest concentration (5 μ M) and significantly reduced at the highest concentration used (50 μ M). In PBMCs co-incubated with 20 μ M of resveratrol and 10 mM of 2-deoxy-D-ribose, the anti-oxidant effect of resveratrol manifested with significant reductions of caspase-3, -8, γ -glutamyltransferase, and glutathione-S-transferase activities and intracellular lipid peroxidation content.

C1y. Suppression of mutagenesis by resveratrol

Numerous reports suggest that resveratrol exerts chemopreventive activities. The suppression of mutagenesis is one line of evidence in this direction. Sgambato *et al.* evaluated the antiproliferative activity of resveratrol on a panel of cell lines of various histogenetic origins, including normal rat fibroblasts, mouse mammary epithelial cells and human breast, colon and prostate cancer cells (294). They found that resveratrol induced significant increases in the apoptotic index, reductions in the percentage of cells in the G2/M-phase, inhibition of increases in ROS following exposure to oxidative agents (*e.g.*, tobacco-smoke condensate and H_2O_2), and reduced nuclear DNA fragmentation, as assessed by single cell gel electrophoresis (comet test), suggesting that resveratrol can act as an antimutagenic/anticarcinogenic agent by preventing oxidative DNA damage, which plays a pivotal role in the carcinogenic activity of many genotoxic agents.

Uenobe *et al.* showed that resveratrol had a suppressive effect on *umu* gene expression of the SOS response induced by 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) in *Salmonella typhimurium* (295). Revel *et al.* showed that B[a]P damaged sperm through AhR activation, phase I enzyme induction, DNA adduct formation and increased germ cell apoptosis in the testis, and that resveratrol could prevent these adverse effects. (296). B[a]P significantly increased apoptosis, and this effect was abrogated by resveratrol. Thus B[a]P caused increased sperm cell B[a]P diol epoxidite (BPDE) DNA adduct formation and apoptosis in the mouse. The natural AhR antagonist resveratrol diminished B[a]P-induced DNA adducts and apoptosis in seminiferous tubules. Matsuoka *et al.* tested the genotoxicity of resveratrol in a bacterial reverse mutation assay, an *in vitro* chromosome aberration test, an *in vitro* micronucleus test and sister chromatid exchange test (169). They found that resveratrol may preferentially induce sister chromatid exchange but not chromosome aberration, that is, it may cause S-phase arrest only when sister chromatid exchanges are induced.

Resveratrol was recently shown to induce strand breakage in DNA in the presence of copper ions. Ahmad *et al.* showed that resveratrol catalyzed the reduction of Cu(II) to Cu(I), which is accompanied by formation of "oxidized product(s)" of resveratrol, which in turn also appear to catalyze the reduction of Cu(II) (297). Strand scission by the

resveratrol-Cu(II) system was found to be biologically active, as assayed by bacteriophage inactivation. Fukuhara *et al.* demonstrated DNA cleavage by resveratrol, as indicated by relaxation of pBR322 in the presence of Cu²⁺ (298). They provided evidence that resveratrol is capable of binding to DNA, and that the Cu²⁺-dependent DNA damage is more likely to be caused by a copper-peroxide complex than by a freely diffusible oxygen species.

C1z. Radioprotective and radiosensitive effects of resveratrol

Various reports during the last few years have suggested that radioresistance is induced by the activation of NF- κ B and NF- κ B-regulated gene products such as COX-2 and 5-LOX (299, 300). Inhibitors of NF- κ B, COX-2, and 5-LOX have been shown to induce radiosensitivity (301-303). Because resveratrol has also been shown to down-regulate NF- κ B (201), COX-2 (141) and 5-LOX (227), it is possible that resveratrol will induce radiosensitization. Prostaglandins, products of COX-2, have been implicated in the cytotoxic and/or cytoprotective response of tumor cells to ionizing radiation. Using clonogenic cell survival assays, Zoberi *et al.* showed that HeLa and SiHa cell killing was enhanced by pretreatment with resveratrol prior to ionizing radiation exposure, and that this pretreatment induced an early S-phase cell-cycle checkpoint arrest (186). These results suggest that resveratrol alters both cell-cycle progression and the cytotoxic response to ionizing radiation.

C1aa. Chemosensitization by resveratrol

Several mechanisms of chemoresistance have been described. Some reports during the last few years have suggested that chemoresistance is induced by the activation of NF- κ B and NF- κ B-regulated gene products such as COX-2 and 5-LOX (299, 300). Inhibitors of NF- κ B, COX-2 and 5-LOX have been shown to induce radiosensitivity (301-303). Because resveratrol has also been shown to down-regulate NF- κ B (201), COX-2 (141) and 5-LOX (227), it is possible that resveratrol will induce chemosensitization. Kubota *et al.* studied the *in vitro* biological activity of resveratrol by examining its effect on the apoptosis induced by taxol in lung cancer cell lines A549, EBC-1 and Lu65 (304). Although simultaneous exposure to resveratrol plus taxol did not result in significant synergy, treatment with resveratrol (10 μ M, 3 days) significantly enhanced the subsequent antiproliferative effect of taxol. The same resveratrol treatment similarly enhanced the subsequent apoptotic effects of taxol: when given prior to taxol, it induced p21^{Cip1/WAF1} expression approximately 4-fold. These results suggest that lung cancer cells exposed to resveratrol have a lowered threshold for killing by taxol.

Survivin is an inhibitor of apoptotic proteins, that is expressed at high levels in most human cancers and may facilitate evasion from apoptosis and aberrant mitotic progression. Fulda *et al.* discovered that resveratrol is a potent sensitizer of tumor cells to TRAIL-induced apoptosis through p53-independent induction of p21^{Cip1/WAF1} and p21^{Cip1/WAF1}-mediated cell-cycle arrest associated with survivin depletion (305). Concomitant analysis of cell-cycle, survivin expression and apoptosis revealed that resveratrol-induced G1-phase arrest was associated with down-regulation of survivin expression and sensitization for TRAIL-induced apoptosis. Importantly, resveratrol sensitized various tumor cell lines, but not normal human fibroblasts, for apoptosis induced by death receptor ligands or anticancer drugs. This combined sensitization with resveratrol as an induction (*e.g.*, TRAIL) strategy may be a novel approach to enhancing the efficacy of TRAIL-based therapies in a variety of human cancers.

Nicolini *et al.* found that taxol induced apoptosis in the human neuroblastoma cell line SH-SY5Y (180). Addition of *trans*-resveratrol to SH-SY5Y cultures exposed to taxol significantly reduced cellular death. Resveratrol is able to inhibit the activation of caspase-7 and degradation of PARP that occur in SH-SY5Y exposed to taxol.

Jazirehi and Bonavida found that resveratrol modified the expression of apoptotic regulatory proteins and sensitized non-Hodgkin's lymphoma and multiple myeloma cell lines to taxol-induced apoptosis (306). Both resveratrol and taxol negatively-modulated tumor cell growth by arresting the cells at the G2/M-phase of the cell-cycle. Low concentrations of resveratrol exerted a sensitizing effect on drug-refractory non-Hodgkin's lymphoma and multiple myeloma cells to apoptosis induced by taxol. Resveratrol selectively down-regulated the expression of anti-apoptotic proteins Bcl-x_L and myeloid cell differentiation factor-1 and up-regulated the expression of proapoptotic proteins Bax and Apaf-1. Combination of resveratrol with taxol had minimal cytotoxicity against quiescent and mitogenically stimulated human PBMC. Inhibition of Bcl-x_L expression by resveratrol was critical for chemosensitization, and its functional impairment mimicked resveratrol-mediated sensitization to taxol-induced apoptosis. Inhibition of Bcl-x_L expression by resveratrol was due to inhibition of the ERK1/2 pathway and diminished AP-1-dependent Bcl-x_L expression.

Depending on the concentration, resveratrol may exhibit dual effects; potentiating the effect of cytokines and chemotherapeutic agents at higher concentrations and inhibiting them at lower concentrations. The protective/inhibitory effects at lower concentrations appear to be mediated through an anti-oxidant mechanism. Manna *et al.* showed that resveratrol abrogated TNF-induced cytotoxicity and caspase activation (201). Similarly, Jang

and Surh showed that resveratrol pretreatment attenuated H₂O₂-induced cytotoxicity, DNA fragmentation and intracellular accumulation of ROS, suggesting that resveratrol has the potential to prevent oxidative stress-induced cell death, owing to its anti-oxidant property (181). Recently, Ahmad *et al.* provided evidence that exposure of human leukemia cells to low concentrations of resveratrol (4-8 µM) inhibited caspase activation, DNA fragmentation and translocation of cytochrome c induced by H₂O₂ or the anticancer drug C2, which is a purified photoproduct of MC540, vincristine, and daunorubicin (307). They found that, at these concentrations, resveratrol induces an increase in intracellular superoxide and inhibits drug-induced acidification. Blocking the activation of the NADPH oxidase complex neutralized resveratrol-induced inhibition of apoptosis. Interestingly, decreasing intracellular superoxide with the NADPH oxidase inhibitor diphenyliodonium reversed the inhibitory effect of resveratrol on drug-induced H₂O₂ production.

C1ab. Direct targets of resveratrol

From the preceding description, it is clear that resveratrol exhibits numerous biological activities. How resveratrol exhibits all these activities is not fully understood. Numerous molecules with which resveratrol physically interacts have been identified. These include PKC (139), PKD (127), SYK (151), 5-LOX (227), COX-2 (141), ER (132), AR (159), AhR (249), and CYP (308). The *in vitro* efficiency of resveratrol was found to be due mainly to its capacity to chelate copper, although it also scavenges free radicals. Belguendouz *et al.* found resveratrol to associate with LDL in the order of their lipid content: high-density lipoprotein < LDL < very LDL (309). Miura *et al.* found that resveratrol associated with and inactivated creatine kinase, alcohol dehydrogenase and cholinesterase (310). Kitson *et al.* found that resveratrol inhibited alcohol dehydrogenase by binding to the aldehyde site on the enzyme (311). Zhou *et al.* found that resveratrol bound and inhibited xanthine oxidase *in vitro*, and the binding was shown to be competitive with their K_i values of 9.7 µM (312). Resveratrol competitively inhibits monoamine oxidase A with an IC₅₀ of 26.6 µM and a K_i of 47.3 µM. Fontecave *et al.* showed that resveratrol bound and inhibited ribonucleotide reductase, which might have further applications as an antiproliferative or cancer chemopreventive agent in humans (313).

C1ac. Immunomodulatory effects of resveratrol

Numerous reports suggest that resveratrol can modulate the immune system (126, 236, 259, 314-316). Falchetti *et al.* evaluated the *in vitro* effects of resveratrol in three immune response models: i) development of cytokine-producing CD4⁺ and CD8⁺ T-cells induced by stimulation of PBMC with anti-

CD3/anti-CD28; ii) specific antigen-induced generation of CTL; and iii) natural killer (NK) activity of PBMC (314). The results showed that *in vitro* exposure to resveratrol produces a biphasic effect on the anti-CD3/anti-CD28-induced development of IFN-γ-, IL2- and IL4-producing CD8⁺ and CD4⁺ T-cells, with stimulation at low resveratrol concentrations and suppression at high concentrations. Similarly, the compound was found to induce significant enhancement (at low concentrations) and suppression (at high concentrations) of both CTL and NK cell cytotoxic activities. On the whole, the results of the study indicate that resveratrol modulates several human immune cell functions and suggest that this activity may be related to its effects on cytokine production by both CD4⁺ and CD8⁺ T-cells.

Gao *et al.* investigated the effect of resveratrol on mitogen/antigen-induced proliferation of splenic lymphocytes, induction of CTL and lymphokine-activated killer (LAK) cells, and production of the cytokines IFN-γ, IL-2, TNFα and IL-12 (126). They found that mitogen-, IL-2-, or alloantigen-induced proliferation of splenic lymphocytes and development of antigen-specific CTL were suppressed significantly at resveratrol concentrations of 25-50 µM. LAK cells generated at similar concentrations were less sensitive to the suppressive effect of resveratrol. The suppression of cell proliferation and CTL generation by resveratrol was not only reversible, but in some cases the response (mitogen/IL-2-induced proliferation and CTL generation) was actually enhanced following pretreatment of cells with resveratrol. Resveratrol also inhibited the production of IFN-γ and IL-2 by splenic lymphocytes and production of TNFα and IL-12 by peritoneal macrophages. The inhibition of cytokine production by resveratrol was irreversible. Further, resveratrol blocked activation of NF-κB without affecting basal NF-κB activity. The latter result suggested that resveratrol inhibits cell proliferation, cell-mediated cytotoxicity and cytokine production, at least in part through inhibition of NF-κB activation. Gao *et al.* also compared the *in vitro* and *in vivo* effects of resveratrol on the development of various cell-mediated immune responses, including mitogen/antigen-induced T-cell proliferation, induction of CTLs, IL-2 induced LAK cells and cytokine production (315). They found significant suppression (>90%) of mitogen/antigen-induced T-cell proliferation and development of allo-antigen specific CTLs *in vitro* with resveratrol at a concentration of 25 µM. Intragastric administration of resveratrol (2 mg daily) to mice for 4 weeks showed no effect on age-related gain in body weight, peripheral blood cell counts (WBC, RBC, or platelets), or the cellularity of bone marrow or spleen. The CD4⁺ and CD8⁺ T-cells in spleen or total colony-forming units in the marrow also remained unaffected by treatment with resveratrol. Spleen cells, which were stimulated *in vitro* after being removed from mice that had been administered

resveratrol for 2 or 4 weeks, showed no significant change in IL-2- or concanavalin A-induced proliferation of T-cells or production of IL-2-induced LAK cells. Further, production of IFN- γ and IL-12 was not affected by the administration of resveratrol, but production of TNF α was reduced. Even when conducted entirely *in vivo*, treatment with resveratrol was found to only marginally reduce the allo-antigen-induced T-cell proliferation and generation of CTL in the draining lymph nodes. Thus, even though resveratrol strongly inhibits T-cell proliferation and production of cytolytic cells *in vitro*, oral administration of resveratrol for 4 weeks does not induce hematological or hematopoietic toxicity and only marginally reduces T-cell-mediated immune responses.

Rotondo *et al.* showed that resveratrol has a strong inhibitory effect on ROS produced by PMN stimulated with formyl methionyl leucyl phenylalanine (fMLP). Resveratrol prevented the release of elastase and β -glucuronidase by PMN stimulated with fMLP and C5a and also inhibited elastase and β -glucuronidase secretion and production of 5-LOX metabolites LTB₄, 6-*trans*-LTB₄ and 12-*trans*-epi-LTB₄ by PMN stimulated with the calcium ionophore A23187 (316). Resveratrol significantly reduced the expression and activation of the β_2 -integrin MAC-1 on the PBMC surface following stimulation. PMN homotypic aggregation and formation of mixed cell conjugates between PMN and thrombin-stimulated fixed platelets in a dynamic system were also prevented consistently by resveratrol. These results indicate that resveratrol interferes with the release of inflammatory mediators by activated PMN and down-regulates adhesion-dependent thrombogenic PMN functions. Kimura *et al.* found that resveratrol inhibited the 5-LOX products 5-HETE, 5,12-diHETE and LTC₄ with IC₅₀ of 8.9 μ M, 6.70 μ M and 1.37 μ M, respectively (259). The IC₅₀ of 5-HETE, 5,12-diHETE and LTC₄ formations of synthetic 3,3',4-trihydroxystilbene were 5.9 μ M, 0.63 μ M and 0.88 μ M, respectively. Moreover, these compounds inhibited the release of lysosomal enzymes such as lysozyme and β -glucuronidase induced by calcium ionophore A 23187 from human PMN-L at concentrations of 0.1-1 mM. Boscolo *et al.* elucidated the *in vitro* effects of resveratrol on human PBMC proliferation and cytokine release (236). Spontaneous PBMC proliferation was unaffected by resveratrol, while the compound inhibited PHA-stimulated PBMC proliferation by 69%. Resveratrol strongly inhibited PHA-stimulated IFN- γ and TNF α release from PBMC, which may be explained by its inhibitory effect on NF- κ B.

C1ad. Modulation of gene expression by resveratrol

The expression of numerous genes that are regulated by different transcription factors has been shown to be down-regulated by resveratrol. These include *COX-2* (141), *5-LOX* (227), *iNOS* (234), *ICAM-1* (231), *TNF* (234), *IL-1* (65),

IL-6 (233) and *IL-8* (211). Fustier *et al.* found that resveratrol is also a phytoestrogen and binds to and activates ERs that regulate the transcription of estrogen-responsive target genes such as the breast cancer susceptibility genes *BRCA1* and *BRCA2* (317). Treatment of human breast cancer cell lines (MCF-7, HBL100 and MDA-MB 231) with 30 μ M resveratrol increased expression of *BRCA1* and *BRCA2* mRNAs without any change in the expression of the proteins. Yang *et al.* examined whether resveratrol has any effect on growth and gene expression in human ovarian cancer PA-1 cells (182). They investigated the effect of resveratrol on changes of global gene expression during resveratrol-induced growth inhibition and apoptosis in PA-1 cells by using a human cDNA microarray with 7,448 sequence-verified clones. Out of the genes screened, 118 were affected in their expression levels by more than 2-fold after treatment with 50 μ M resveratrol for 24 h. Following treatment of PA-1 cells at a concentration of 50 μ M for 6, 12, 24 and 48 h, gene expression patterns was analyzed by microarray. Clustering of the genes modulated more than 2-fold at three of these points divided the genes into two groups. Within these groups, there were specific subgroups of genes whose expressions were substantially changed at the specified time points. One of the most strongly up-regulated genes was *NQO-1*, which has recently been shown to be involved in p53 regulation.

Earlier studies have shown that resveratrol alters the expression of genes involved in cell-cycle regulation and apoptosis, including cyclins, Cdks, p53 and Cdk inhibitors. Narayanan *et al.* examined whether resveratrol activates a cascade of p53-directed genes that are involved in apoptosis mechanism(s), or modifies the AR and its co-activators directly or indirectly and inhibits cell growth (162). They demonstrated by DNA microarray, RT-PCR, Western blot and immunofluorescence analyses that treatment of androgen-sensitive prostate cancer cells (LNCaP) with 100 μ M resveratrol for 48 h down-regulated PSA, AR co-activator *ARA 24*, and NF- κ B p65. Altered expression of these genes is associated with activation of p53-responsive genes such as *p53*, *PIG 7*, *p21^{Cip1/WAF1}*, *p300/CBP* and *Apaf-1*. The effect of resveratrol on *p300/CBP* plays a central role in its cancer-preventive mechanisms in LNCaP cells. These results implicated its targeting of more than one set of functionally-related molecules. Pendurthi *et al.* examined the effect of resveratrol on the induction of tissue factor expression in vascular cells that had been exposed to pathophysiological stimuli (125). The data presented herein show that resveratrol inhibited the expression of tissue factor in endothelial cells stimulated with a variety of agonists, including IL-1 β , TNF and LPS. A similar inhibition of tissue factor induction was seen in monocytes that had been pretreated with resveratrol before their stimulation

with LPS. In addition, resveratrol was shown to inhibit the LPS-induced expression of *TNF α* mRNA in endothelial cells and of *TNF α* and *IL-1 β* mRNA in monocytes.

C2. *In vivo* animal studies of resveratrol

Besides its effects *in vitro*, resveratrol has been found to be quite active *in vivo*. Its *in vivo* cancer-related effects are elaborated here.

C2a. Metabolism, pharmacokinetics, tissue distribution and clearance of resveratrol

Numerous studies have examined the metabolism, pharmacokinetics, tissue distribution and clearance of resveratrol (see Table IV). Bertilli *et al.* studied the plasma kinetics and tissue bioavailability of this compound after oral administration in rats (318). Its plasma pharmacokinetics after oral administration could be described by an open one- or two-compartment model. Tissue concentrations show a significant cardiac bioavailability and a strong affinity for the liver and kidneys. Andlauer *et al.* investigated the absorption and metabolism of resveratrol by using an isolated preparation of lumenally and vascularly perfused rat small intestine (319). A synthetic perfusate free from blood components was used as a vascular medium, with a perfluorocarbon as oxygen carrier. Vascular uptake of lumenally administered resveratrol was 20.5%. The majority of the absorbed resveratrol was conjugated to yield resveratrol glucuronide (16.8%), which was also the main luminal metabolite (11.2%). Lesser amounts of resveratrol sulfate, 3.0% and 0.3%, were found on the luminal and vascular sides, respectively, while only minute amounts of resveratrol and resveratrol conjugates (1.9%) were found in the intestinal tissue. These results demonstrate an ample uptake and metabolic conversion of resveratrol. Kuhnle *et al.* studied the absorption and metabolism of resveratrol in the jejunum in an isolated rat small intestine model (320). Only small amounts of resveratrol were absorbed unmetabolized across the enterocytes of the jejunum and ileum. The principal compound detected on the serosal side was the glucuronide conjugate of resveratrol (96.5% \pm 4.6 of the amount absorbed), indicating the susceptibility of resveratrol to glucuronidation during transfer across the rat jejunum. These findings suggest that resveratrol is most likely to be in the form of a glucuronide conjugate after crossing the small intestine and entering the blood circulation. This will have important implications for the study of the biological functions of resveratrol *in vivo*.

De Santi *et al.* examined the glucuronidation of resveratrol in human liver microsomes and whether flavonoids inhibited resveratrol glucuronidation (321). The assay employed uridine-5'-diphosphoglucuronic acid-¹⁴C

and unlabelled resveratrol. They found that resveratrol underwent glucuronidation and that the flavonoid, quercetin, inhibited resveratrol glucuronidation. These results show that resveratrol is glucuronated in the human liver, which may reduce the bioavailability of this compound; however, flavonoids inhibit resveratrol glucuronidation and this inhibition might improve the bioavailability of resveratrol. Aumont *et al.* found that glucuronidation was regioselective and stereoselective (322). It occurred at a faster rate with the *cis*-isomer and preferred the 3-position on both isomers. In addition, the glucuronidation of resveratrol was tested by using several recombinant UDP-glucuronosyltransferase (UGT) isoforms. The reaction was catalyzed by UGT of the family 1A (UGT1A1, 1A6, 1A7, 1A9, 1A10). The bilirubin-conjugating UGT1A1 was involved mainly in the 3-O-glucuronidation of *trans*-resveratrol, whereas the phenol-conjugating UGT1A6 activity was restricted to *cis*-resveratrol. The UGT1A9 and 1A10 were active toward both isomers. The activity supported by UGT2B7 and UGT2B15 was very low and restricted to *cis*-resveratrol. UGT1A3, 1A4, 2B4 and 2B11 did not form resveratrol glucuronides. Li *et al.* found that resveratrol is not a substrate for P-glycoprotein or the multidrug resistance associated proteins (243). No phase I metabolites were observed, but the phase II conjugates resveratrol-3-glucuronide and resveratrol-3-sulfate were identified by liquid chromatography mass spectrometry (LC-MS) and liquid chromatographic-tandem mass spectrometry (LC-MS-MS) analysis and comparison with synthetic standards. Although these data indicate that resveratrol diffuses rapidly across the intestinal epithelium, extensive phase II metabolism during absorption might reduce the resveratrol bioavailability.

De Santi *et al.* examined the sulfation of resveratrol in the human liver and duodenum (323). They found that resveratrol undergoes sulfation and that this sulfation is blocked by quercetin, a flavonoid present in wine, fruits and vegetables, suggesting that compounds present in the diet may inhibit the sulfation of resveratrol, thus improving its bioavailability. Bertilli *et al.* examined the kinetics of *trans*- and *cis*-resveratrol in rats after oral administration (324). Resveratrol concentrations were measured in the plasma, heart, liver and kidneys. Tissue concentrations showed a significant cardiac bioavailability and strong affinity for the liver and kidneys. They found that even modest dosages of resveratrol produced an observable pharmacological effect, and that these dosages were compatible with the resveratrol concentrations obtained after oral administration (325).

Piceatannol is a closely related stilbene, that has antileukemic activity and is also a tyrosine kinase inhibitor. Piceatannol differs from resveratrol by having an additional aromatic hydroxy group. Potter *et al.* showed that the enzyme CYP1B1 is overexpressed in a wide variety of

Table IV. Pharmacokinetics, biotransformation, tissue distribution and metabolic clearance rates of resveratrol.

Animal	Route	Dose	Remarks	References
Rats	oral	100 µg/d	• No estrogen agonism on estrogen target tissues • May be an estrogen antagonist	(281)
	oral	—	• Significant cardiac bioavailability	(324)
	<i>i.v.</i>	—	• Significant cardiac bioavailability • Strong affinity for the kidneys	(325)
	oral	single	• ¹⁴ C- <i>trans</i> -resveratrol gets preferentially fixed in the organs and biological liquids of absorption and elimination (stomach, liver, kidney, intestine, bile, urine) • Glucurono- and/or sulfoconjugates along with ¹⁴ C- <i>trans</i> -resveratrol is present in these tissues	(330)
	oral	20 mg/kg/d	• Hematological and biochemical variables were not affected. • Histopathological examination of the organs obtained at autopsy did not reveal any alterations	(353)
	oral	—	• Reduces body weight, disrupted estrous cyclicity • Induces ovarian hypertrophy	(362)
	<i>i.p.</i>	20, 40 mg/kg	• Decreases brain MDA levels	(363)
	<i>i.v.</i>	10 mg/kg	• Resveratrol increased brain glutathione	(364)
	—	2 mg/kg	• Reversed hyperalgesia induced by local tissue injury	(365)
	—	—	• Affects the locus coruleus and reproductive system	(366)
	—	—	• Abolished increase in renal genomic DNA due to 8-oxodG	(345)
Rats/Mice	<i>i.p.</i>	—	• No unconjugated resveratrol in urine or serum samples	(328)
Humans	oral	25 mg/70 kg	• Absorption is inadequate for anticancer and inflammatory effects	(356)
	oral	—	• Can be absorbed from grape juice in biologically active quantities and in amounts that will cause reduction in the risk of atherosclerosis	(357)
Mice	—	—	• Increased adenosine plasma levels	(359)
	oral	2.5 mg/kg 10 mg/kg	• Reduces tumor volume, tumor weight, and metastasis to the lung	(349)
	<i>i.g.</i>	4 mg/kg	• Promoted lymphocyte proliferation and IL-2 production	(367)
	T	—	• Significant inhibition of UVB-mediated increase in bifold skin thickness and skin edema	(368)
Gerbils	<i>i.p.</i>	30 mg/kg	• Crosses blood-brain barrier	(369)
Rabbits	<i>i.g.</i>	3 mg/kg/d	• Feeding mitigated reduction in endothelial function • Plasma ET-1 levels statistically decreased	(370)

i.v., intravenous, *i.p.*, intraperitoneal; *i.g.*, intragastric; *po.*, post-oral; d, day; *i.pl.*-intrapulmonary; T-topical

human tumors and catalyzes aromatic hydroxylation reactions (254). They found that resveratrol undergoes metabolism by CYP1B1 to produce a metabolite that has been identified as piceatannol. This observation provides a novel explanation for the cancer-preventive properties of resveratrol. It demonstrates that a natural dietary agent can be converted to a compound with known anticancer activity by an enzyme that is found in human tumors. This result gives important insight into the functional role of CYP1B1 and provides evidence for the concept that CYP1B1 in tumors may function as a growth suppressor.

Corsi *et al.* evaluated resveratrol in a human monocytic leukemia cell line at concentrations (100 nM to 1 µM) found in the bloodstream after moderate wine intake (326). As early as 4 h after intake, resveratrol exhibited antiproliferative and cytotoxic activity. At the same time, some apoptosis-like phenomena were detected, such as cell membrane perturbation (phosphatidylserine-annexin V binding), Fas expression and mitochondrial Δψ depolarization. The anticancer drug camptothecin, used as a positive control, did not significantly increase Fas levels and increased FasL only modestly. At 12 h after intake, however, resveratrol at

concentrations of 100 nM and 1 μ M did not exhibit the same antiproliferative activity, and increased cell proliferation was correlated with a significant increase in FasL expression. The authors concluded that treatment with low doses of resveratrol, such as those found after moderate wine intake, is not sufficient to stop human leukemia cell line proliferation and that cell resistance, marked by high FasL expression, could be mediated by low $\Delta\psi$ mitochondria-released anti-apoptotic factors such as Bcl-2.

Whether resveratrol could be absorbed in human and enter the systemic circulation was examined by Kaldas *et al.* (327). This was examined by measuring the transport and metabolism of resveratrol (5-40 μ M) by the human intestinal epithelial cell line Caco-2 cultured in Transwells. Transport across the Caco-2 monolayer occurred in a direction-independent manner with P_{app} values of approximately 70 nm/s, much higher than for the paracellular transport marker mannitol (approximately 4 nm/s), suggesting efficient absorption *in vivo*. At the highest resveratrol concentration, the absorption increased, possibly owing to saturation of metabolism. In sharp contrast to previous findings in the rat, the metabolism of resveratrol in Caco-2 cells involved mainly sulfation and, to a minor extent, glucuronidation. At low resveratrol concentrations, most of the sulfate conjugate was exported to the apical side, presumably by multidrug resistance protein 2, which is strongly expressed in these cells. At high concentrations, there was a shift toward the basolateral side, possibly involving multidrug resistance protein 3. These results indicate that the absorption of resveratrol *in vivo* may be high but with limited bioavailability owing to efficient sulfate conjugation.

Yu *et al.* examined *in vitro* and *in vivo* the metabolism of *trans*-resveratrol (328). The *in vitro* experiments included incubation with human liver microsomes, human hepatocytes and rat hepatocytes, and the *in vivo* studies included oral or intraperitoneal administration of resveratrol to rats and mice. No resveratrol metabolites were detected in the microsomal incubations, and no phase I metabolites, such as oxidation, reduction, or hydrolysis products, were observed in any samples. However, abundant *trans*-resveratrol-3-O-glucuronide and *trans*-resveratrol-3-sulfate were identified in rat urine, mouse serum and incubations with rat and human hepatocytes. Incubation with β -glucuronidase and sulfatase to release free resveratrol was used to confirm the structures of these conjugates. Only trace amounts of *cis*-resveratrol were detected, indicating that isomerization is not an important factor in the metabolism and elimination of resveratrol. These results indicated that *trans*-resveratrol-3-O-glucuronide and *trans*-resveratrol-3-sulfate are the most abundant metabolites of resveratrol. Virtually no unconjugated resveratrol was detected in urine or serum samples, which might have implications regarding the significance of *in vitro* studies that used only unconjugated resveratrol.

Sale *et al.* examined the pharmacokinetics in mice and growth-inhibitory properties of resveratrol and the synthetic analogue *trans*-3,4,5,4'-tetramethoxystilbene (DMU 212) (329). The latter showed greater growth-inhibitory and pro-apoptotic properties in transformed cells than in untransformed cells. The authors compared the pharmacokinetic properties of DMU 212 with those of resveratrol in the plasma, liver, kidney, lung, heart, brain and small intestinal and colonic mucosa of mice. DMU 212 or resveratrol (240 mg/kg) was administered intragastrically, and drug concentrations were measured by HPLC. Metabolites were characterised by cochromatography with authentic reference compounds and were identified by MS. The ratios of area of plasma or tissue concentration vs time curves of resveratrol over DMU 212 (AUC(res)/AUC(DMU212)) for the plasma, liver and small intestinal and colonic mucosa were 3.5, 5, 0.1 and 0.15, respectively. Thus, resveratrol afforded significantly higher levels in the plasma and liver, while DMU 212 exhibited superior availability in the small intestine and colon. Resveratrol was metabolized to its sulfate or glucuronate conjugate, while DMU 212 underwent metabolic hydroxylation or single and double O-demethylation. DMU 212 and resveratrol inhibited the growth of human-derived colon cancer cells HCA-7 and HT-29 *in vitro* with IC_{50} values of between 6 and 26 μ M.

Vitrac *et al.* investigated the distribution of ^{14}C -*trans*-resveratrol in mouse tissues after oral administration (330). Male Balb/c mice were given a single oral dose of ^{14}C -*trans*-resveratrol and were sacrificed at 1.5, 3, or 6 h later. The distribution of radioactivity in the tissues was evaluated by using whole-body autoradiography, quantitative organ-level determination and microautoradiography. Radioactive compounds in the kidney and liver were identified by HPLC. An autoradiographic survey of mice sections, as well as radioactivity quantification in various organs, revealed a preferential fixation of ^{14}C -*trans*-resveratrol in the organs and biological liquids of absorption and elimination (stomach, liver, kidney, intestine, bile, urine). Moreover, they showed that ^{14}C -*trans*-resveratrol-derived radioactivity is able to penetrate the tissues of liver and kidney, a finding supported by microautoradiography. These tissue contained intact ^{14}C -*trans*-resveratrol together with glucuronoconjugates and/or sulfoconjugates. This study demonstrated that *trans*-resveratrol is bioavailable following oral administration and remains mostly in the intact form. The results also suggest a wide range of target organs for cancer chemoprevention by wine polyphenols in humans.

Meng *et al.* examined the urinary and plasma levels of resveratrol in humans, mice and rats after ingestion of pure compounds (331). Oral administration of resveratrol in humans yielded detectable levels of resveratrol and their derivatives in the plasma and urine. After intragastric administration of resveratrol to rats (2 mg/kg), levels of resveratrol as high as 1.2 μ M were observed in the plasma.

C2b. Chemopreventive effects of resveratrol in animals

Chemoprevention can be defined as the use of substances to interfere with the process of cancer development. Chemoprevention prevents or delays the process of carcinogenesis by administration of natural or synthetic compounds. That resveratrol may have chemopreventive effects has been tested in several cancer model systems (Table V). Resveratrol has been shown to have a chemopreventive role in a wide variety of tumors including skin (332-336), liver (229, 337), colon (149), breast (185, 332, 338, 339), lung (340, 341) and esophageal (342) cancers. Resveratrol suppresses tumor initiation and tumor progression by a wide variety of inducers (Table IV). It can inhibit the tumor initiation process induced by B[a]P, DMBA, azoxymethane and nitrosamines and tumor promotion induced by PMA (149, 185, 229, 332-343).

The first report of the chemopreventive effects of resveratrol appeared in 1997, when Jang *et al.* demonstrated its cancer chemopreventive activity in assays representing three major stages of carcinogenesis (332). Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes (anti-initiation activity); it mediated anti-inflammatory effects and inhibited COX and hydroperoxidase functions (antipromotion activity); and it induced human promyelocytic leukemia cell differentiation (antipromotion activity). In addition, it inhibited the development of preneoplastic lesions in carcinogen-treated mouse mammary glands in culture and inhibited tumorigenesis in a mouse skin cancer model. In another study by the same group, resveratrol was shown to inhibit carcinogen-induced preneoplastic lesions in mouse mammary organ culture and PMA-promoted mouse skin tumors. The authors also found that resveratrol inhibited tumorigenesis in mouse skin through interference with pathways of reactive oxidants and possibly by modulating the expression of c-fos and TGF- β 1 (333). Pretreatment of mouse skin with resveratrol negated several PMA-induced effects such as elevation in the expression of COX-1, COX-2, c-myc, c-fos, c-Jun, TGF- β 1 and TNF α , which could be responsible for inhibition in mouse skin tumorigenesis. Kapadia *et al.* demonstrated that at a 50-fold molar ratio to PMA, resveratrol reduced by 60% the papillomas in DMBA-initiated and PMA-promoted mouse skin two-stage carcinogenesis protocols at 20 weeks (334). In another study in a two-stage skin cancer model, in CD-1 mice using DMBA as initiator and PMA as promoter, resveratrol moderately inhibited the rate of tumor formation in individual mice and the number of mice developing one or more tumors (335). Afaq *et al.* reported that resveratrol possesses the potential to ameliorate the damage caused by short-term UVB exposure to SKH-1 hairless mouse skin through inhibition of the UVB-mediated induction of COX, ornithine decarboxylase, and lipid peroxidation (336).

Soleas *et al.* used a two-stage CD-1 mouse skin cancer model, with DMBA as initiator and PMA as promoter, to compare the antitumorigenic activities of resveratrol (335). Animals were treated with specific polyphenols, at doses ranging from 0 to 25 μ moles (dissolved in 200 μ L acetone), twice a week for 18 weeks. The solution was applied topically to the shaved dorsal region of each animal. The relative potencies of the polyphenol were compared by evaluating the percentage inhibition of tumor formation in individual mice and the number of mice developing one or more tumors with the different dose schedules. They found that resveratrol was absorbed much more efficiently and was effective in suppressing the tumors. Ignatowicz *et al.* investigated resveratrol for its inhibitory effects on the covalent binding of DMBA to DNA *in vitro* and its suppression of the oxidative burst in PMA-stimulated human PMN (344). 32 P-postlabelling analysis of DNA incubated with DMBA in the presence of 3-methylcholanthrene-induced microsomes produced three major adducts derived from anti-, syn- and anti-dihydrodiol epoxides, respectively, through reactions with 2'-deoxyguanosine and 2'-deoxyadenosine. Phenolic compounds at the concentration of 150 μ M reduced the levels of all DMBA-DNA adducts by 55-98%. Human neutrophils showed a significant dose-related decrease of PMA-induced chemiluminescence after pretreatment with phenolic compounds. These results suggest that suppression of ROS and carcinogen-DNA adduct formation may be important for the anticarcinogenic activity of these phenolics.

Hecht *et al.* examined resveratrol and some other stilbene derivatives as a chemopreventive agent against lung tumor induction in A/J mice by the tobacco smoke carcinogens B[a]P and 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (340). Groups of 20 A/J mice were treated weekly by gavage with a mixture of B[a]P and NNK (3 mmol each) for 8 weeks, then sacrificed 26 weeks after the first carcinogen treatment. In mice treated with butylated hydroxyanisole (BHA) (20 or 40 μ mol) by gavage 2 h before each dose of B[a]P and NNK, lung tumor multiplicity was significantly reduced. Treatment with resveratrol (500 ppm) from 1 week after carcinogen treatment until termination had no effect on lung tumor multiplicity. Cadenas *et al.* found that resveratrol prevented the oxidative DNA damage induced in the kidney by the carcinogen KBrO₃ (345).

We investigated the chemopreventive potential of resveratrol by testing it against mammary carcinogenesis induced by DMBA in female Sprague-Dawley rats (338). Dietary administration of resveratrol (10 ppm) had no effect on the body weight or tumor volume, but strikingly reduced the incidence (45%; $p < 0.05$), multiplicity (55%; $p < 0.001$) and extended latency period of tumor development relative to DMBA-treated animals. Histopathological analysis of the tumors revealed that DMBA induced ductal carcinomas and focal microinvasion *in situ* (7/7), whereas treatment with

Table V. Chemopreventive and therapeutic effects of resveratrol.

Effects	Route/Dose	References
Prevention of cancer:		
Skin:		
• Inhibits DMBA-induced preneoplastic lesions in mouse skin cancer model	1-25 µM, 2/week for 18 weeks, topical	(332)
• Inhibits DMBA-induced mouse mammary cell growth and PMA-promoted mouse skin tumors	1-25 µM, topical, 30min	(333)
• Reduces papillomas in DMBA-initiated and PMA-promoted mouse skin two-stage cancer	85 nM/day, topical, daily	(334)
• Inhibits DMBA-induced tumor incidence and tumor burden in CD-1 mouse skin cancer model	1-25 µM, 2/week for 18 weeks, topical	(335)
• Protects against the damage caused by short term UVB exposed-SKH-1 hairless mouse skin		(336)
Colon:		
Inhibits AOM-induced colon cancer in F344 rats	200 mg/kg/day for 100 days, oral	(149)
Breast:		
• Inhibits estrogen-dependent preneoplastic ductal lesions induced by DMBA in mouse mammary glands, reduces MNU-induced mammary carcinoma in rats	1-10 µM, 10 days, organ culture	(185)
• Suppresses DMBA-induced mammary carcinogenesis in rats	100 µg/rat/day, diet	(338)
• Moderately accelerates MNU-induced mammary carcinoma in rats	10 or 100 mg/kg/day for 5 days subcutaneous injections	(339)
Liver:		
• Induces DNA-oxidation products in plasma, the area of GST-placental form positive foci in liver and number of ACF in F344 rats	0.001 g/kg for 10 weeks, diet	(337)
• Inhibits the growth of murine transplantable liver cancer, H22.	10-15 mg/kg for 10 days, abdominal administration	(229)
Lung:		
• Does not affect lung tumor multiplicity induced by B[a]P and NNK in A/J mice	500 ppm/week for 1 week	(340)
• Abrogates BPDE-DNA adduct induction by B[a]P in lungs of Balb/c mice and prevents against B[a]P-induced CYP1A1 expression.	50 mg/kg/week, <i>i.v.</i>	(341)
Stomach:		
• Suppresses NMBA-induced esophageal tumorigenesis in F344 rats	2mg/kg for 16 weeks, orally, <i>i.p.</i>	(342)
Therapy of cancer:		
Colon:		
• Prevents the formation of colon tumors and reduces the formation of small intestinal tumors, down-regulates cyclin D1 and D2	0.01 % in drinking water for 7 weeks, oral <i>ad libitum</i>	(350)
Liver:		
• Decreases AH-130 ascites tumor cell content in rats and increase number of cells in the G2/M-phase of cell-cycle	1 mg/kg, <i>i.p.</i>	(347)
• Possesses antitumor and immunomodulatory activity in transplanted hepatoma, H22, in mice	500-1500 mg/kg for 10 days, <i>i.v.</i>	(343)
Lung:		
• Reduces tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice	2.5 – 10 mg/kg	(349)

resveratrol suppressed DMBA-induced ductal carcinoma. Immunohistochemical and Western blot analysis revealed that resveratrol suppressed DMBA-induced COX-2 and MMP-9 expression in the breast tumors. Gel-shift analysis showed that resveratrol suppressed DMBA-induced NF- κ B activation. Treatment of human breast cancer MCF-7 cells with resveratrol suppressed NF- κ B activation and inhibited proliferation at S-, G2-, and M-phases. Overall, our results suggest that resveratrol suppresses DMBA-induced mammary carcinogenesis, and that this suppression correlates with the down-regulation of NF- κ B, COX-2 and MMP-9 expression.

Ziegler *et al.* demonstrated that resveratrol consumed *ad libitum* in the diet does not modify tumorigenesis in Apc(Min/+) mice (346). B[a]P is an agonistic ligand for the AhR and a major environmental carcinogen implicated in the etiology of lung cancer through induction of BPDE and BPDE-DNA adducts. Because B[a]P metabolism requires CYP1A1 induction through activation of AhR, Revel *et al.* hypothesized that resveratrol, a natural competitive inhibitor of AhR, could prevent B[a]P's adverse effects on the lung (341). Balb/c mice were injected for 5 weeks with corn oil, B[a]P (5 mg/kg/week), resveratrol (50 mg/kg/week) or B[a]P with resveratrol. Immunohistochemical analysis was then performed on sections of their lungs for determination of CYP1A1 protein, BPDE-DNA adducts and apoptosis. Mice exposed to B[a]P had a significantly greater induction of lung BPDE-DNA adducts than controls (H scores: control, 26, interquartile range 18-33; B[a]P, 276, interquartile range 269-288; $p < 0.01$). The induction of BPDE-DNA adduct by B[a]P was significantly abrogated by resveratrol (H score: B[a]P + resveratrol, 103, interquartile range 96-113). A similar pattern was found in the analysis for apoptosis (H scores: control, 121, interquartile range 102-137; BaP, 288, interquartile range 282-292, $p < 0.05$; B[a]P with resveratrol, 132, interquartile range 121-141, $p = \text{NS}$) and CYP1A1 (H scores: control, 170.3, interquartile range 164-175; B[a]P, 302.3, interquartile range 291-315, $p < 0.05$; B[a]P with resveratrol, 200.7, interquartile range 174-215, $p = \text{NS}$). Western blot analysis confirmed that resveratrol prevented B[a]P-induced CYP1A1 expression. This increase in CYP1A1 expression in response to B[a]P administration most probably causes B[a]P metabolism, BPDE-DNA adduct formation and subsequent apoptosis. All B[a]P-induced effects could be prevented by resveratrol, suggesting a possible chemopreventive role for this natural phytoalexin against the development of lung cancer.

Resveratrol also inhibits colon cancers in mice and rats. Resveratrol pretreatment (200 $\mu\text{g/kg/day}$ in drinking water) and treatment in the initiation phase of azoxymethane-induced colon cancer in F344 rats inhibited the number of aberrant crypt foci (ACF)/colon and their multiplicity and completely

abolished the large ACF (149). In resveratrol-treated rats, bax expression was enhanced in ACF but not in the surrounding mucosa. In both controls and resveratrol-treated rats, proliferation was higher in ACF than in normal mucosa. Resveratrol prevents colon carcinogenesis with a mechanism involving changes in Bax and p21^{Cip1}/WAF1 expression. Resveratrol also prevents colon cancer in mice. Breinholt *et al.* showed that moderate to high doses of resveratrol (1-100 mg/kg diet) induced DNA oxidation products in plasma in male F344 rats, increased the area of GST-placental form-positive foci in the liver and increased the number of ACF to a number comparable to that induced by dietary carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (337). This study suggests the possibility that long-term exposure to moderate to high doses of anti-oxidants *via* pro-oxidative mechanisms and non-oxidative mechanisms can modulate carcinogenesis.

In the transplanted hepatoma H22 murine model, the antitumor activity of resveratrol was studied by treating the tumor-bearing mice with the agent at 10 or 15 mg/kg bodyweight for 10 days. Resveratrol inhibited the growth of this murine transplantable liver cancer (229). The underlying antitumor mechanism of resveratrol might involve inhibition of cell-cycle progression by decreasing the expression of cyclinB1 and Cdc2 proteins.

In addition to several *in vitro* studies on MCF-7 human breast cancer cells showing that resveratrol has superestrogenic effects and studies in ER-transfected cell lines showing that resveratrol acts as a mixed agonist/antagonist, there are some *in vivo* studies that characterize the estrogen-modulatory effects of resveratrol. Bhat *et al.* demonstrated that resveratrol alone induced PR and, in combination with estradiol, suppressed the expression of PR in mammary glands of Balb/c mice placed in organ culture (185). Moreover, resveratrol inhibited the formation of the estrogen-dependent preneoplastic ductal lesions induced by DMBA in these mammary glands (IC_{50} , 3.2 μM). Furthermore, resveratrol reduced MNU-induced mammary tumorigenesis in female Sprague-Dawley rats. On the other hand, prepubertal treatment with resveratrol for 5 days accelerated MNU-induced mammary carcinogenesis in female Sprague-Dawley rats (339). Resveratrol (100 mg/kg) significantly increased the incidence of mammary carcinomas ≥ 1 cm and multiplicity, but did not affect latency. It did not increase body weight, but did cause slightly earlier vaginal opening. Resveratrol-treated animals exhibited significantly increased irregularity of the estrous cycle, spending more time in the estrus phase. Thus, short-term resveratrol treatment of prepubertal female rats affected the endocrine function and accelerated development of MNU-induced mammary carcinomas.

Li *et al.* investigated whether resveratrol inhibits N-nitrosomethylbenzylamine (NMBA)-induced rat esophageal tumorigenesis in F344 male rats and found that the number

of NMBA-induced esophageal tumors per rat was significantly reduced. The maximum size of tumors in each group treated with resveratrol was significantly smaller than that in the group treated with NMBA alone, which correlated with decreases in COX and prostaglandin E (342).

C2c. Antitumor effects of resveratrol in animals

Numerous reports suggest that resveratrol exerts therapeutic effects against cancer (Table V). Carbo *et al.* found that administration of resveratrol to rats inoculated with a fast-growing tumour (the Yoshida AH-130 ascites hepatoma) caused a very significant decrease (25%) in the tumor cell content (347). The effects of the diphenol were associated with an increase in the number of cells in the G2/M cell-cycle phase. Interestingly, flow cytometric analysis of the tumor cell population revealed the existence of an aneuploid peak (representing 28% of total), which suggests that resveratrol decreases tumor cell numbers by inducing apoptosis. Caltagirone *et al.* investigated the effects of resveratrol on the growth and metastatic potential of B16-BL6 melanoma cells *in vivo* (348). Intraperitoneal administration of resveratrol, at the time of intramuscular injection of B16-BL6 cells into syngeneic mice, resulted in a significant, dose-dependent delay of tumor growth without toxicity. Furthermore, the polyphenol significantly potentiated the inhibitory effect of a non-toxic dose of cisplatin. Kimura *et al.* found that resveratrol significantly reduced tumor volume (42%), tumor weight (44%) and metastasis to the lung (56%) in mice bearing highly metastatic Lewis lung carcinoma (LLC) tumors at doses of 2.5 and 10 mg/kg but not at 0.6 mg/kg (349). Resveratrol did not affect the number of CD4⁺, CD8⁺ and NK1.1⁺ T-cells in the spleen. Therefore, the inhibitory effects of resveratrol on tumor growth and lung metastasis could not be explained by NK cell or CTL activation. Resveratrol inhibited DNA synthesis most strongly in LLC cells (IC₅₀, 6.8 μM). Resveratrol at 100 μM increased apoptosis to 20.6±1.35% from 12.1±0.36% (*p*<0.05) in LLC cells, and decreased the S-phase population to 22.1±1.03% and 29.2±0.27% from 35.2±1.72% (*p*<0.05) at concentrations of 50 and 100 μM, respectively. Resveratrol inhibited tumor-induced neovascularization at doses of 2.5 and 10 mg/kg in an *in vivo* model. Moreover, it significantly inhibited the formation of capillary-like tubes from HUVEC at concentrations of 10-100 μM; the degree of inhibition of capillary-like tube formation by resveratrol was 45.5% at 10 μM, 50.2% at 50 μM and 52.6% at 100 μM. Resveratrol inhibited the binding of VEGF to HUVEC at concentrations of 10-100 μM, but not at concentrations of 1 or 5 μM. The degree of inhibition of VEGF-binding to HUVEC by resveratrol was 16.9% at 10 μM, 53.2% at 50 μM and 47.8% at 100 μM. The authors suggested that the antitumor and antimetastatic activities of resveratrol might be due to

inhibition of DNA synthesis in LLC cells and of LLC-induced neovascularization and tube formation (angiogenesis) in HUVEC.

Min mice are congenic mice genetically predisposed to develop intestinal tumors as a result of a mutation of the Apc gene. Scheider *et al.* studied the effect of oral administration of resveratrol on tumorigenesis in these mice (350). Resveratrol (0.01% in the drinking water containing 0.4% ethanol) was administered for 7 weeks to Min mice, starting at 5 weeks of age. The control group was fed the same diet and received water containing 0.4% ethanol. Resveratrol prevented the formation of colon tumors and reduced the formation of small intestinal tumors by 70%. Comparison of the expression of 588 genes in the small intestinal mucosa showed that resveratrol down-regulated genes that are directly involved in cell-cycle progression or cell proliferation (cyclins D1 and D2, DP-1 transcription factor and Y-box binding protein) and up-regulated several genes that are involved in recruitment and activation of immune cells (CTL Ag-4, leukemia inhibitory factor receptor and monocyte chemotactic protein 3) or in inhibition of the carcinogenic process and tumor expansion (tumor susceptibility protein TSG101, TGF-β, inhibin-β A subunit and desmocollin 2). Thus, the high potency and efficacy of resveratrol supported its use as a therapeutic and chemopreventive agent in the management of intestinal carcinogenesis.

Bove *et al.* found that resveratrol inhibited the *in vitro* growth of 4T1 breast cancer cells in a dose- and time-dependent manner (133). *In vivo*, however, resveratrol had no effect on the time to tumor take, tumor growth, or metastasis when administered intraperitoneally (1, 3, or 5 mg/kg) daily for 23 days starting at the time of tumor inoculation. Resveratrol had no effect on body weight, organ histology, or estrous cycling of the tumor-bearing mice. Resveratrol, therefore, is a potent inhibitor of 4T1 breast cancer cells *in vitro*, is nontoxic to mice at 1-5 mg/kg, and has no growth-inhibitory effect on 4T1 breast cancer *in vivo*.

Kimura *et al.* studied the effects of stilbene glucosides, isolated from medicinal plants and grapes, on tumor growth and lung metastasis in mice bearing highly metastatic LLC tumors (351). They also studied the inhibitory effects of stilbene glucosides on the differentiation of HUVEC to form a capillary network. Tumor growth in the right hind paw and lung metastasis were inhibited by oral administration of the stilbene glucoside piceid or 2,3,5,4'-tetrahydroxystilbene-2-O-D-glucoside for 33 consecutive days in LLC-bearing mice. As the numbers of CD8⁺ and NK1.1⁺ T-cells in the spleen were not affected, the inhibitory effects of these stilbene glucosides on tumor growth and lung metastasis could not be explained by NK or CTL activation. Piceid inhibited DNA synthesis in LLC cells at a concentration of 1000 μM, but not at lower concentrations (10-100 μM). 2,3,5,4'-tetra-hydroxystilbene-

2-O-D-glucoside also inhibited DNA synthesis in LLC cells (IC_{50} , 81 μ M). Both stilbene glucosides inhibited formation of capillary-like tube networks (angiogenesis) in HUVEC at concentrations of 100 to 1000 μ M. The authors suggested that the antitumour and antimetastatic activity of these stilbene glucosides might be due to the inhibition of DNA synthesis in LLC cells and angiogenesis of HUVEC.

Kozuki *et al.* found that resveratrol inhibited the proliferation of hepatoma cells and suppressed their invasion even at a concentration of 25 μ M (172). Sera from rats given resveratrol by mouth restrained only the invasion of AH109A cells; resveratrol and resveratrol-loaded rat serum suppressed ROS-potentiated invasive capacity. These results suggest that the anti-invasive activity of resveratrol is independent of its antiproliferative activity, and that its anti-oxidant property may be linked to its anti-invasive action.

Mishima *et al.* found that vaticanol C, a resveratrol tetramer, exhibited strong cytotoxicity against various tumor cell lines (352). They examined the antitumor activity of the ethanol extract from the stem bark of *Vateria indica*, which is used for health and healing diseases in the Indian Ayurvedic tradition. HPLC analysis showed that the extract contains bergenin, hopeaphenol, vaticanol B, vaticanol C and epsilon-viniferin. An *in vitro* assay displayed the extract's anticancer activity against mouse sarcoma 180 cells (IC_{50} , 29.5 μ M). Growth of sarcoma 180 cells subcutaneously allografted in DDY mice was significantly retarded by oral administration of the extract at the dose of 30 or 100 mg/kg body weight ($p < 0.001$). The extract did not show significant toxicity to mice even at a dosage of 1000 mg/kg body weight administered daily for 28 days. De Ledinghen *et al.* showed that liver myofibroblasts stimulated the *in vitro* invasion of hepatocellular carcinoma cell lines through a hepatocyte growth factor/urokinase-dependent mechanism (173). They further evaluated the effects of *trans*-resveratrol on invasion of the human hepatoma cell line HepG2 and demonstrated that *trans*-resveratrol decreased the hepatocyte growth factor-induced HepG2 cell invasion by an, as yet, unidentified postreceptor mechanism. Juan *et al.* evaluated whether high doses of *trans*-resveratrol have harmful effects on Sprague-Dawley rats (353). *trans*-Resveratrol was administered orally to male rats for 28 day at a daily dose of 20 mg/kg, 1000 times the amount consumed by a 70-kg person taking 1.4 g of *trans*-resveratrol/day. Neither body weight nor food and water consumption differed between rats treated with *trans*-resveratrol and the control group. Hematological and biochemical variables were not affected by the treatment. Histopathological examination of the organs obtained at autopsy revealed no alterations. These results support the view that repeated consumption of *trans*-resveratrol at 20 mg/kg/day does not adversely affect the variables tested in rats.

Mollerup *et al.* studied the effect of resveratrol on the expression of genes involved in the metabolism of PAH in the human bronchial epithelial cell line BEP2D (170). Expression of the *CYP1A1* and *CYP1B1*, microsomal epoxide hydrolase (*mEH*), and *GSTP1* genes were measured by RT-PCR. The cells were treated with either B[a]P or 2,3,7,8-tetrachlorodibenzo-p-dioxin in the presence or absence of resveratrol. Resveratrol inhibited both the constitutive and induced expression of *CYP1A1* and *CYP1B1*. In contrast, resveratrol increased the expression of the *mEH* gene and elicited no change in the expression of *GSTP1*. The altered gene expression in response to resveratrol was reflected in a reduced overall level of B[a]P metabolism. These data indicate that resveratrol may exert lung cancer chemopreventive activity through altering the expression of genes involved in the metabolism of PAH, resulting in altered formation of carcinogenic B[a]P metabolites in human bronchial epithelial cells.

Liu *et al.* examined the antitumor and immunomodulatory activity of resveratrol on experimentally-implanted H22 tumors in Balb/c mice (343). Intraperitoneal resveratrol, at a dose of 500 mg/kg, 1000 mg/kg, or 1500 mg/kg, could curb the growth of implanted H22 tumors in mice. The inhibitory rates were 31.5%, 45.6% and 48.7%, respectively ($p < 0.05$), which could raise the level of serum immunoglobulin G and plaque-forming cell response to sheep red blood cells. Intraperitoneal resveratrol at doses of 1000 mg/kg or 1500 mg/kg or bacillus Calmette-Guerin 200 mg/kg could increase the production of serum TNF α in H22 tumors in mice. The effect of resveratrol, however, was insignificant ($p > 0.05$). Thus resveratrol could inhibit the growth of H22 tumors in Balb/c mice. This antitumor effect might be related directly to the inhibition of H22 cell growth and indirectly to inhibition of the agent's potential effect on nonspecific host immunomodulatory activity.

Morales *et al.* showed that *trans*-resveratrol has a protective effect on gentamycin-induced nephrotoxicity (354). This is related to resveratrol's strong affinity for the kidneys (324, 345).

D. Clinical studies with resveratrol

Despite the fact that an enormous amount of data is available on resveratrol's anticancer effects *in vitro* and in animals, few clinical studies have been performed in humans. The data available from these studies are limited. Gautam *et al.* found that *ex vivo* purging of contaminating tumor cells may reduce the incidence of relapse in patients undergoing bone marrow transplantation (355). In this study, they demonstrated that resveratrol exhibits antileukemic activity against mouse (32Dp210 and L1210) and human (U-937 and HL-60) leukemic cell lines by inhibiting cell proliferation. Long-term exposure to resveratrol also inhibits the clonal growth of normal hematopoietic progenitor cells, but at a higher IC_{50}

than for most of the leukemia cell lines tested. The inhibitory effect of resveratrol on hematopoietic progenitors is partially reversible, whereas the effect on leukemia cells is largely irreversible. The inhibition of leukemia cells by resveratrol involves nucleosomal DNA fragmentation (apoptosis). On the other hand, resveratrol does not induce or enhance spontaneously occurring apoptotic death in normal hematopoietic progenitor cells. *In vivo* experiments, performed with untreated and resveratrol-treated bone marrow, showed comparable hematopoietic reconstitution in lethally irradiated mice (10 Gy) as determined by survival, hematological recovery and the number of hematopoietic progenitor cells present in the marrow of reconstituted animals. Taken together, these results indicate the potential for the use of resveratrol for *ex vivo* pharmacological purging of leukemia cells from bone marrow autografts without significant loss in the hematopoietic activity of progenitor cells. We showed that resveratrol suppressed the colony-forming cell proliferation of fresh AML marrow cells from five patients with newly diagnosed AML in a dose-dependent fashion, showing that resveratrol is an effective *in vitro* inhibitor of AML cells and suggesting that this compound may have a role in future therapies for AML (122).

Goldberg *et al.* reported that, after an oral dose of resveratrol (25 mg/70 kg) to healthy human subjects, the compound appears in serum and urine predominantly as glucuronide and sulfate conjugates and reaches peak concentrations (10-40 nM) in serum around 30 min after consumption (356). Free polyphenols account for 1.7-1.9% of the peak serum concentrations and more than 80% is absorbed. Pace-Asciak *et al.* reported that *trans*-resveratrol can be absorbed from grape juice in biologically active quantities and in amounts that are likely to cause reduction in the risk of atherosclerosis (357). That red wines (which have 20 times more polyphenols than white wines) show no advantages over other forms of ethanol suggests that, *in vivo*, ethanol is the dominant anti-aggregatory component in these beverages, which are more potent than grape juices in preventing platelet aggregation in humans. A study by Wang *et al.*, suggested that resveratrol (at doses of 10-1000 μ M) significantly inhibits the *in vitro* platelet aggregation induced by collagen (358). Thrombin, at a concentration of 4 mg/kg/day, inhibits ADP-induced platelet aggregation in humans and rabbits, despite not changing serum lipid levels. Resveratrol also causes an increase in plasma adenosine levels and blood nucleosides in human subjects (359).

Conclusions

From the studies described in this review, it is clear that resveratrol holds great potential in the prevention and therapy of a wide variety of tumors. Resveratrol has antiproliferative effects through the induction of apoptosis in

cell lines of various origin such as leukemias and breast, prostate, colon, pancreas, and head and neck carcinomas. It induces Fas-dependent apoptosis in some cell lines and Fas-independent apoptosis in others. Most, but not all, studies indicate that resveratrol does not induce apoptosis in normal cells. Some *in vitro* studies showing that resveratrol has antiproliferative effects at certain dose ranges but not at other doses could explain the small number of *in vivo* animal studies in which resveratrol was ineffective in inhibiting certain cancer conditions. Some studies have reported that resveratrol has a biphasic behavior with respect to its antiproliferative effects. Thus, systematic studies are required to test a range of resveratrol concentrations *in vitro* and then apply those doses *in vivo* to a wide variety of tumors. *In vivo* studies clearly demonstrate that resveratrol is pharmacologically safe and can be used for the prevention and therapy of cancer. Resveratrol's ability to radiosensitize and chemosensitize opens up additional opportunities. That the structure of resveratrol is simple, and the presence of hydroxyl groups is strongly linked with its biological activity, provides additional opportunities for structure-activity relationship studies to improve its biopotency and bioavailability. Lastly, resveratrol has potential for treating diseases other than cancer and cardiovascular ailments. Howitz *et al.* found evidence in yeast that resveratrol mimics calorie restriction and thus extends the lifespan by 70% (360).

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REVIEW

RESVERATROL, A NATURAL CHEMOPREVENTIVE AGENT AGAINST DEGENERATIVE DISEASES

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Resveratrol, a natural chemopreventive agent against degenerative diseases. E. IGNATOWICZ, W. BAER-DUBOWSKA. Pol. J. Pharmacol., 2001, 53, 557–569.

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring compound shown to modulate the risk of cardiovascular degenerative diseases (atherosclerosis) and inhibit chemical carcinogenesis in rodents. Various studies have demonstrated the effect of this phytoalexin on biological mechanisms involved in cardioprotection. These include modulation of lipid turnover, inhibition of eicosanoid production, prevention of the low-density lipoprotein oxidation and inhibition of platelet aggregation. Carcinogenesis in animal models can be divided at least into three stages: initiation, promotion and progression. Initiation occurs as result of interaction of a reactive form of carcinogen with DNA. Chemical carcinogens like polycyclic aromatic hydrocarbons are metabolized to reactive species by cytochrome P450 dependent enzymes activated through aryl hydrocarbon (Ah) receptor. The inhibition of tumor initiation by resveratrol most probably occurs through preventing the activation of Ah receptor. Resveratrol affects also several factors involved in tumor promotion and progression. Since tumor promoting agents alter the expression of genes whose products are associated with inflammation, chemoprevention of cardiovascular diseases and cancer may share the same common mechanisms. This includes principally modulation of the expression of growth factors and cytokines. Recently, chemopreventive properties of resveratrol have been associated with the inhibition of NF- κ B. This transcription factor is strongly linked to inflammatory and immune responses, regulation of cell proliferation and apoptosis, thus it is important for tumor development and many other diseases including atherosclerosis. Although the mechanisms by which resveratrol interferes with the activation of NF- κ B are not clear, it seems that inhibition of its degradation which is necessary for its cellular activation is the principal target. Based on the quantity and diversity of data available on the biological activity of resveratrol, it has to be considered a very promising chemoprotector and chemotherapeutic. Urgent investigations on its bioavailability and effects on *in vivo* systems, especially in humans, are necessary.

Key words: *resveratrol, cardioprotection, chemoprevention, reactive oxygen species, multistage carcinogenesis, transcription factor NF- κ B, atherosclerosis, low density lipoprotein oxidation*

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Abbreviations: AGE – advanced glycation end, cNOS – constitutive nitric oxide synthase, COX – cyclooxygenase, DMBA – 7,12-dimethylbenz[a]anthracene, HDL – high density lipoproteins, iNOS – inducible nitric oxide synthase, LDL – low density lipoproteins, LPS – lipopolysaccharide, MAP – mitogen activated protein, NF- κ B – nuclear transcription factor-kappa B, oxyLDL – oxidized LDL, PAH – polycyclic aromatic hydrocarbon, PKC – protein kinase C, ROS – reactive oxygen species, TF – tissue factor, TGF – transforming growth factor, VLDL – very low density lipoproteins

Resveratrol (3,5,4'-trihydroxystilbene, see Figure 1 for structure) is a phytoalexin present in a wide variety of plant species, including mulberries, peanuts and grapes, and thus is a constituent of the human diet [71]. This compound, like other members of stilbene family, is produced in response to pathogen attack, UV-irradiation and exposure to ozone [4, 38, 103]. *Vitis vinifera*, or grapes, synthesize resveratrol in response to fungal infections; thus it is found at high concentrations in wine, particularly in red wine [47]. Resveratrol found in the powdered root of *Polygonum cuspidatum* (*Polygonaceae*) is an active ingredient of Chinese and Japanese folk medicine, and since ancient times, it has been used to cure diseases which contemporary medicine described as inflammation, allergy and hyperlipemia [64]. However, with the possible exception of peanuts, grapes and related products, such as red wines, are probably the most important foodstuff containing resveratrol. A primary impetus for research on resveratrol has come from the paradoxical observation that a low incidence of cardiovascular diseases may coexist with intake of a high fat diet, a phenomenon known as the French paradox [12, 25, 26, 98]. The exact mechanism by which resveratrol acts to mitigate a high fat diet from increasing the risk for coronary heart disease has not been totally elucidated but has been attributed to its antioxidant [34, 81, 101] and anticoagula-

tive properties [9, 10, 101, 111]. Recently, resveratrol has been shown to act as a pleiotropic biological effector which regulates the multistage carcinogenesis process [8, 16, 60, 62]. These studies add a new dimension to the expanding role of resveratrol as a potential chemopreventive agent exhibiting anti-inflammatory, cell growth-modulatory and anticarcinogenic effects. The successful synthesis of resveratrol [88] along with the above observations resulted in the exponentially proliferating data on chemopreventive activity of resveratrol. The number of papers published on resveratrol in the years 1998–2000 reflects the growing interest in this molecule and will be discussed in this review.

Chemoprevention of coronary heart disease by resveratrol

Coronary heart disease and its acute form, myocardial infarction, is a complication of atherosclerosis. Abnormalities of lipid metabolism and coagulation, as well as of other pathways which contribute to this pathology, are presented briefly below as a detailed description of atherosclerosis pathogenesis is beyond the scope of this article. A normal vascular endothelium functions depend on the interaction of key endothelial mediators: nitric oxide, prostacyclin and tissue plasminogen activator. The well-balanced cooperation between these factors provides artery wall with thromboresistance and prevents atherogenesis [48]. Several mechanisms contribute to the development of atherosclerotic lesions, and the primary one is believed to depend on the prolonged retention of lipoproteins in an artery wall and resulting local inflammation [69, 102]. The site of inflammation attracts phagocytosing cells, generating reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide and hydroxyl radicals [51]. In the inflammation process the release of arachidonic acid is involved as well as its metabolism to eicosanoids, prostaglandins and hydroperoxy forms of arachidonic acid [105]. Prostaglandin synthesis is regulated by cyclooxygenase (COX) gene expression. Two separate gene products, COX-1 and -2, have similar COX and peroxidase activities, although they are differentially regulated [105, 106]. Although a variety of factors can increase the mRNA levels of both COXs, the COX-2 gene generally responds in a more dramatic fashion than COX-1 gene. The oxidative

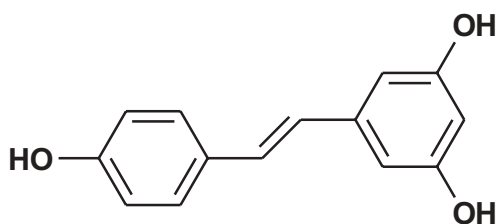


Fig. 1. Resveratrol

stress induced by the inflammation initiates a sequel of reactions within the artery wall, as well as in circulating blood cells and plasma components, which leads to oxidation of low density lipoproteins (LDL) [114]. The process is initiated by a hydroxyl radical, which exhibits high affinity for unsaturated fatty acids (PUFA) of an LDL molecule. This ROS is generated as a result of hydrogen peroxide reaction with transient metal ions. Transient metal cations of physiological importance (Fe^{2+} , Cu^{+}) are normally bound to specific proteins (ferritin, transferrin, ceruloplasmin). The local pH changes due to the inflammation release cations from the protein binding sites [57]. LDL oxidative modification is a result of the interaction between the products of PUFA oxidation and the apolipoprotein B molecules. Under physiological conditions, LDL are protected from oxidation by the concerted action of LDL-specific antioxidants (tocopherol, coenzyme Q, carotenoids). When lipoproteins are accumulated in the artery wall over a prolonged period of time, as in the case of hypercholesterolemia, the antioxidants are depleted [115]. LDL uptake in subendothelial macrophages and/or smooth muscle cells is strictly regulated by the LDL-receptor, but oxidized LDL (oxy-LDL) is abundantly incorporated into subendothelial macrophages by an unregulated "scavenging" receptor and/or phagocytosis [7].

Cells overloaded with oxy-LDL molecules are called "foam cells" and form the basis of atheromatous plaques in the artery wall [113]. Concomitantly, oxy-LDL blocks cholesterol uptake by HDL molecules and promotes platelets' adhesion to endothelium, which initiates a complex cellular reaction leading to the development of atherosclerotic lesions [33, 98, 125]. The atherogenic modifications of lipoproteins are accompanied by the enhanced blood platelets' adhesion and aggregation, and increased expression of tissue factor (TF). TF is a glycoprotein bound to the cell surface and is a primary initiator of coagulation; however it is not expressed by monocytes-macrophages and endothelial cells. Its inappropriate appearance on these cell surfaces triggers blood clotting. TF accumulates to a great extent in elements which form human atherosclerotic plaques: macrophages, smooth muscle and endothelial cells and in cell-free, cholesterol-rich layers, and it is thought to determine plaque thrombogenicity [104].

Ischemia/reperfusion injury happens during fluid resuscitation in tissues previously deprived of the proper blood supply, e.g. in trauma and/or shock. The exhaustion of cellular ATP in the ischemic tissues induces irreversible metabolic alterations: hypoxanthine accumulation and the conversion of xanthine dehydrogenase into xanthine oxidase. Paradoxically, molecular oxygen introduced into the ischemic tissues becomes a source of ROS due to the xanthine oxidase activity [66]. However, in the human myocardium after an episode of acute coronary heart disease another mechanism of ROS-mediated injury has been found. Anoxic myocardium generates hydrogen peroxide during reperfusion, which binds to myoglobin to form a highly oxidative complex [107]. The increased oxidative stress directly impairs cardiac structure and function, causing cardiomyopathy and depression of contractile functions and organ failure [65].

Resveratrol was shown to interfere with a number of mechanisms described above, leading to the diminishment of the atherogenic changes in plasma and artery wall and improving the outcome after ischemia/reperfusion injury. Resveratrol was found to prevent lipids from peroxidative degradation [18, 37, 39, 73, 118] and to stop the uptake of oxy-LDL in the vascular wall in a concentration-dependent manner [38]. Liver parenchymal cells in culture, treated with resveratrol, showed reduced secretion of esterified cholesterol and triglycerides although the intracellular triglyceride level was unchanged. It allows the supposition that resveratrol reduces the secretion of VLDL from the liver, which is transformed into LDL in blood circulation, thus blocking hepatic lipoprotein metabolism [43, 46, 50]. Treatment of hepatoma HepG2 cells with resveratrol resulted in a decreased level of the intracellular apolipoprotein B and its secretion, which may be responsible for impaired LDL and partly VLDL synthesis [46]. Resveratrol may protect LDL molecules against peroxidation through antioxidative activity and metal chelation (its ability regarding copper chelation was described elsewhere) [6]. The common recognition of resveratrol as natural antioxidant was elucidated by Zini et al. [129] who proposed three different mechanisms through which this phytoalexin exerts its antioxidative action. Resveratrol is supposed to compete with coenzyme Q and to decrease the oxidative chain complex III, the site of ROS generation. It also scavenges superoxide radicals formed in the

mitochondria and inhibits lipid peroxidation induced by Fenton reaction products [129]. Other suggestions on antioxidative abilities of resveratrol came from Jang and Pezzuto [64]. They described the normalization of myeloperoxidase and oxidized-glutathione reductase activities upon resveratrol treatment. The inhibition of the inducible nitric oxide synthase (iNOS) by resveratrol, which may prevent cytotoxic effects of nitric oxide, was also noticed [64, 82, 122]. Resveratrol has been proved to scavenge peroxy and hydroxyl radicals in reperfused postischemic isolated rat hearts, to limit infarct size and to reduce the formation of malondialdehyde, a non-specific marker of lipid peroxidation occurring under oxidative stress [97, 101]. Resveratrol was shown to modulate platelet coagulation through multiple mechanisms. It inhibited platelet adhesion to type I collagen which is the first step of platelet activation. This compound also reduced platelet aggregation induced by thrombin and ADP treatment and altered eicosanoid metabolism in favor of increased prostacycline and decreased thromboxane B_2 synthesis in the activated cells. The antioxidative properties of resveratrol were postulated as the mechanism underlying its diverse effects and as possible explanation of the abovementioned findings [9, 10, 38, 89, 90, 131, 132]. However, these effects were observed mainly *in vitro* in isolated platelets. In the whole blood the antiplatelet activity of resveratrol was less evident [68]. It has also been suggested that resveratrol blocks the *in vitro* aggregation due to the inhibition of mitogen activated protein (MAP) kinases in platelets [68]. The reduction of TF expression in vascular cells may also contribute to the anti-aggregatory effect of resveratrol [91].

Another explanation of anti-platelet action of this phytoalexin has been proposed by Dobrydyneva et al. [29]. Resveratrol was found to inhibit Ca^{2+} influx into thrombin-stimulated platelets through interference with store-operated Ca^{2+} channels. A similar effect of resveratrol on calcium influx into cultured murine macrophages has been noticed, and this action led further to the suppression of proinflammatory interleukin-6 synthesis [128]. The studies of calcium channels in endothelial cells after exposure to resveratrol have shown the ability of this phytoalexin to control vasorelaxation mediated by nitric oxide. This effect was reversed by the constitutive nitric oxide synthase (cNOS) inhibitor, N ω -nitro-L-arginine. The non-nitric oxide-

-modulated pathway was also postulated in endothelium-denuded aortic tissue: the vasodilation upon resveratrol treatment was not reversed by the NOS inhibitor [20]. Moreover, resveratrol has been found to diminish the proliferation of smooth muscle cells from intima of the vessel wall and this antimitogenic activity appears to be related to a G1 \rightarrow S block in the cell cycle [54, 130]. The data on the involvement of the steroid receptor on the cell membrane has been contradictory [32, 59]. The vasorelaxative activity of resveratrol depends also on direct stimulation of K^+/Ca^{2+} channels in endothelial cells [74].

In human polymorphonuclear neutrophils, resveratrol decreased the amount of 5-lipoxygenase proinflammatory products (5-hydroxyeicosatetraenoic acid, 5,12-dihydroxyeicosatetraenoic and leukotriene C4) [67], inhibited the lysosomal enzymes (lysozyme and β -glucuronidase) release upon calcium ionophore exposure, and decreased ROS generation [61, 99]. Another mechanism to account for the anti-inflammatory and cardioprotective effects of resveratrol is suppression of phospholipase A_2 and COX activities, along with inhibition of phosphodiesterase leading to an increase in the amount of cyclic nucleotide and inhibition of protein kinases involved in cell signaling [112].

Diabetes is considered a promoting factor of atherosclerosis in humans. Hyperinsulinemia following type 2 diabetes and insulin resistance are thought to be independent cardiovascular risk factors. In the tissues and blood of diabetic patients, reduced antioxidant content was found. Moreover, the formation of advanced glycation end-products (AGE) as a result of nonenzymatic glycation and oxidation of proteins has been observed [5]. AGE increase coagulation through various mechanisms involving the vascular endothelium and platelet activation [27]. The interaction of AGE with their receptor results in increased ROS production and activation of protein kinases [70]. AGE-stimulated proliferation of smooth muscle cells in the artery wall and increased DNA synthesis were inhibited by resveratrol in an animal experimental model [85].

The structural similarity of resveratrol to diethylstilbestrol, a synthetic estrogen, has led to the hypothesis that it might express a phytoestrogenic function. Endogenous estrogens have known cardioprotective properties and it seems very likely that phytoestrogens present in red wine could exert similar action [15, 44]. However, the estrogenic activity

of resveratrol may also result in the stimulation of human breast cancer cell proliferation [60, 77].

Chemoprevention of multistage carcinogenesis by resveratrol

The major stages of carcinogenesis were deduced over the past 50 years, primarily from animal model studies (particularly in mouse skin). These stages are termed: initiation, promotion and progression [1, 109]. Tumor initiation begins when DNA in a cell or population of cells is damaged by exposure to exogenous or endogenous carcinogens. If this damage is not repaired, it can lead to genetic mutations. The responsiveness of the mutated cells to their microenvironment can be altered and may give them a growth advantage over normal cells. In the classic two-stage carcinogenesis system in mouse skin, a low dose of polycyclic aromatic hydrocarbon (PAH), 7,12-dimethylbenz[a]anthracene (DMBA) causes permanent DNA damage (the initiating event) but does not give rise to tumors over the lifespan of the mouse unless a tumor promoter, such as 12-O-tetradecanoylphorbol-13-acetate (TPA), is repeatedly applied [1, 108, 109]. The tumor promotion stage is characterized by selective clonal expansion of the initiated cells, a result of TPA-induced oxidative stress, and the altered expression of genes whose products are associated with hyperproliferation, tissue remodelling and inflammation [58, 109]. During tumor progression, preneoplastic cells develop into tumors through a process of clonal expansion that is facilitated by progressive genomic instability and altered gene expression [95]. Most animal models used in carcinogenesis research were developed before the identification of the major cancer-related genes, the recognition of the importance of host susceptibility to a carcinogenic insult, or the realization that mitogenesis and apoptosis together regulate cell number. Nonetheless, these animal models have contributed significantly to our current understanding of carcinogenesis and the ways to interfere with that process. Current strategies for the prevention of cancer use mechanism-based approaches to block the carcinogenesis process at all stages along the pathway. Thus, the main targets for anti-initiation strategies are: i) modulation of carcinogen activation, ii) scavenging electrophiles and oxygen species, iii) carcinogen detoxification, iv) DNA repair processes. Targets for antipromotion and antiprogession strategies

include epigenetic changes in cell signaling, inflammation, proliferation and apoptosis [56].

Resveratrol has been suggested as a potential chemopreventive agent based on its striking inhibitory effects on diverse cellular events associated with tumor initiation, promotion and progression. The cancer chemopreventive potential of resveratrol was established in 1997 when it was found that this compound inhibited DMBA-induced preneoplastic lesion formation in mouse mammary organ culture and reduced the incidence and multiplicity of DMBA/TPA-induced papillomas in the two-stage mouse skin model [60]. These studies showed also that resveratrol administration decreased the number of preneoplastic lesions induced by DMBA in the mouse mammary gland organ culture model [72]. The antimutagenic activity of resveratrol was also demonstrated against foodborne heterocyclic amine, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) and 2-aminofluorene in *Salmonella* bacterial tester strains [64, 123]. Most chemical carcinogens are genotoxic, causing DNA damage by reacting with DNA bases. The carcinogens form covalent adducts with DNA. DMBA like other polycyclic aromatic hydrocarbons requires metabolic conversion to DNA-reactive intermediates. The metabolic activation is catalyzed by cytochrome P450 through oxidation of the carcinogen molecule. DMBA is metabolized by multiple forms of cytochrome(s) P450 which are characterized by different regio-selectivity. CYP1A1 is the major isozyme that catalyzes the two step-oxidation of most PAH to their bay region diol-epoxide intermediates in both rodent and human tissues. Recent studies have indicated that CYP1B1 and 2C6 are also involved in DMBA metabolism [14]. The transcriptional induction of the CYP1A1 is regulated by the Ah receptor, a cytosolic protein present in a number of rodent and human tissues [52]. The binding of ligands like PAH to the Ah receptor usually results in coordinated expression of genes encoding not only CYP1A1, but also CYP1A2 and the I and II phase enzymes responsible for conjugation (detoxification) of the reactive metabolites. Studies *in vitro* showed that resveratrol affects the expression of cytochrome P450 and is one of the most selective inhibitors of human P450 1A1 [22]. Inhibition of CYP1A1 transcription may occur by preventing activation of the Ah receptor. Resveratrol promotes Ah receptor translocation to the nucleus and binding to DNA at dioxin-respon-

sive elements, but transactivation does not take place [17, 23]. Resveratrol also induces the phase II enzyme NAD(P)H, quinone oxidoreductase, which detoxifies many quinones. This enzyme is part of the gene battery activated through the Ah receptor [87]. The effect of resveratrol on the other isozymes of P450 have not been studied in detail, but the inhibition of alkoxyresorufine dealkylases, markers of CYP1A and 2B1/2 *in vitro*, was demonstrated [120]. Resveratrol also decreased the levels of DMBA-DNA adduct formation in an *in vitro* system [2]. Beside P450, there are other enzyme systems involved in carcinogen activation such as peroxidases, including cyclooxygenases and certain transferases such as N-acetyltransferase and sulfotransferase [31, 49]. Each of these enzymes provides a potential target for modulating carcinogen activation. It has been reported that resveratrol decreased the activity of COXs [64, 94].

Tumor promoting agents are not mutagenic as carcinogens are, but rather alter the expression of genes whose products are associated with hyperproliferation, apoptosis, tissue remodelling and inflammation. Over the past few years it has become clear that apoptosis and mitogenesis are equally important in the homeostasis of cell number, and that the growth advantage manifested by initiated cells during promotion is usually the net effect of increased proliferation and decreased apoptosis. Thus, in addition to cell proliferation, apoptosis has emerged as a critical target for prevention [78]. Changes in gene expression as a consequence of external tumor promoter stimuli usually activate (but sometimes inactivate) specific signal transduction pathways. The major target for phorbol esters and other promoters is protein kinase C (PKC). Its activation seems to be a critical event in carcinogenesis. By activating PKC, phorbol esters and related tumor promoters appear to bypass the normal cellular mechanisms for regulating cell proliferation [56]. Resveratrol has been shown to inhibit isolated and cellular PKC in model systems. Phosphorylation of substrates that resemble protamine sulphate was particularly affected [116]. Resveratrol has been incorporated into model phospholipid membranes, altering the phospholipid phase polymorphism, and has inhibited PKC α enzymatic activity *in vitro* [42]. Several lines of evidence suggest that tumor promoters generally increase the expression of a number of growth factors and cytokines. TPA induces the transforming growth factors

TGF- α , TGF- β , tumor necrosis factor- α , the granulocyte-macrophage stimulating factor, and interleukins 1 and 6. The profile of growth factor induction differs for promoters with various initial mechanisms of action, although most seem to induce TGF- α messenger RNA (mRNA) expression. TPA treatment also increases expression of the epidermal growth factor receptor, possibly as a consequence of activating c-Ha-ras [28].

In addition to inducing changes in gene expression by activating specific signaling pathways, tumor promoters can elicit the production of proinflammatory cytokines, such as tumor necrosis factor, and several interleukin and nonprotein factors, such as nitric oxide, involved in inflammation and carcinogenesis [35]. Of critical importance to the promotion process is the release of arachidonic acid and its metabolism to eicosanoids [41]. Eicosanoids are involved not only in the inflammation process, but also in the immune response, tissue repair, and cell proliferation. Suppression of prostaglandin biosynthesis through selective inhibition of COX is, hence, now regarded as an important cancer chemopreventive strategy. TPA increases significantly the COX-2 mRNA level and has been referred to as a phorbol ester-inducible immediate early gene product [106]. Resveratrol was shown to inhibit COX-1 activity in microsomes derived from sheep seminal vesicles [60]. More recently, Subbaramaiah et al. [117] have reported that resveratrol inhibits the catalytic activity of the COX-2 in cultured human mammary epithelial cells with and without TPA treatment. Likewise, human recombinant COX-2 expressed in baculovirus was inhibited by resveratrol. Moreover, resveratrol effectively suppressed the COX-2 promoter-dependent transcriptional activity in human colon cancer cells [86]. Besides inhibiting the catalytic activity of COX-2, this compound also blocked TPA-mediated induction of *cox-2* mRNA in cultured human mammary epithelial cells through repression of transcription factor-AP-1-dependent transactivation [75, 117].

The tumor promoting activity mediated by TPA has also been associated with oxidative stress, as exemplified by increased production of superoxide anion radicals, H_2O_2 , reduction of superoxide dismutase activity, which is able to detoxify superoxide anion radicals, and interference with glutathione metabolism, a key intracellular component capable of protecting cellular constituents from attack of peroxide and free radicals [40, 92, 110]. Be-

side scavenging ROS, resveratrol was also found to block the production of carbon or nitrogen-centered free radicals, such as the phenylbutazone peroxyl radical and the benzidine-derived radical. Resveratrol, along with other antioxidants like vitamin E and melatonin, prevented the oxidative DNA damage induced in rat kidneys by KBrO_3 [13]. Topical application of resveratrol onto the dorsal skin of CD-1 mice led to profound attenuation of oxidative stress and expression of epidermal TGF- β 1 and *c-fos* induced by TPA; but the induction of *cox-1*, *cox-2*, *c-myc*, *c-jun* and TNF- α mRNAs was not affected [63].

Some anti-inflammatory chemopreventive agents have been found to suppress the growth and proliferation of transformed cells through induction of apoptosis [93, 100]. Little information is available with regard to the apoptosis-inducing capability of resveratrol in tumor cells. However, the inhibition by resveratrol of the growth of several types of human breast epithelial cells, which was independent on the estrogen receptor status of the cells by resveratrol was reported [84]. In this regard it was shown that resveratrol inhibited the growth of estrogen receptor-positive MCF-7 cells and human oral squamous carcinoma cells (SCC-25) [30]. The data on growth modulation of estrogen-dependent T47D breast carcinoma cells are contradictory. In some experiments, resveratrol suppressed their growth, in others, stimulation was observed [24, 44]. The proliferation of K-562 human erythroleukemia cells and P-815 was also suppressed by resveratrol treatment, which might be associated with the inhibition of ribonucleotide reductase [36]. The suppression of human promyelocytic leukemia (HL-60) cells by resveratrol was shown to be mediated *via* induction of apoptosis, as determined by nuclear fragmentation, chromatin condensation, time-related increase in the frequency of subdiploid (apoptotic) cells, and internucleosomal DNA fragmentation [119]. Besides suppression of proliferation, the compound induced differentiation of HL-60 cells, which appears to be associated with reversible cell cycle arrest at the S-phase check point [96, 119]. Moreover, resveratrol was found to induce apoptosis in the same cells by triggering the CD95 signaling system [24]. In the mouse JB6 epidermal cell line, resveratrol induced apoptosis through activation of p53 activity, however apoptosis occurred only in cells expressing a wild type of p53 [55].

In summary, the presented data indicate that resveratrol promotes homeostasis and affects the earliest and the late stages of carcinogenesis. Thus, resveratrol may be considered not only a potential chemopreventive, but also a chemotherapeutic agent to control tumor development.

Association of the chemopreventive properties of resveratrol with inhibition of activation of the nuclear factor-kappaB

Recently, much data has shown up indicating the interference of resveratrol with the nuclear factor-kappaB (NF- κ B). This transcription factor is strongly linked to inflammatory and immune responses [52]. NF- κ B is also important for the regulation of cell proliferation and apoptosis, cell transformation and tumor development [3, 45, 79, 124] and many other diseases including atherosclerotic lesions [11]. NF- κ B controls the gene expression of cytokines, chemokines, growth factors, and cell adhesion molecules as well as some acute phase proteins, including the inflammatory mediators iNOS and COX-2 [126, 127]. NF- κ B was first identified as a B-cell nuclear factor and given its name on the basis of its ability to bind to an intronic enhancer of the immunoglobulin κ -light chain gene. Since then NF- κ B has been identified in numerous cell types and is found to be activated by a wide range of inducers, including ultraviolet irradiation, cytokines, inhaled occupational particles, and bacterial or viral products. In resting cells, NF- κ B resides in cytoplasm in an inactive form bound to an inhibitory protein known as I κ B. Upon cellular activation by extracellular stimuli, I κ B is phosphorylated and proteolytically degraded or processed by proteasomes and other proteases. This proteolytic process activates NF- κ B, which then translocates into the nucleus. In nuclei, NF- κ B, can initiate or regulate early-response gene transcription by binding to decameric motif – κ B, found in the promoter or enhancer regions of specific genes [19]. Presently, five mammalian NF- κ B family members have been identified and cloned. All these family members share a highly homologous domain (Rel) composed of ~300 amino acid residues that are responsible for DNA binding, dimerization, and interactions with I κ B. Evidence for a potential role of NF- κ B in carcinogenesis was provided by the observation that activation of NF- κ B is required in oncogenic Ras-induced transformation [83]. Upon inhibition of

NF- κ B activation with the superrepressor form of I κ B α , oncogenic Ras transformed cells exhibited a loss of cell viability, indicating that oncogenic Ras requires the cell survival-promoting function of NF- κ B to overcome the role of the death signal initiated in transformed cells. The mechanisms by which resveratrol can interfere with the activation of NF- κ B are not clear. One possibility is that resveratrol can interact with ankyrin domains present in I κ B because the phosphorylation of I κ B is inhibited by resveratrol. Such interaction could conceivably hinder I κ B phosphorylation and subsequent dissociation of NF- κ B. In addition resveratrol blocked the expression of mRNA-encoding monocyte chemoattractant protein-1, a NF- κ B regulated gene. A cell treated with lipopolysaccharide (endotoxin, LPS) can generate ROS which activate protein tyrosine kinase. Furthermore, resveratrol has been found to possess potent protein kinase inhibitory activity and antioxidant activity. Protein tyrosine kinase has been implicated in NF- κ B activation [76]. Therefore, resveratrol might inhibit the activation of NF- κ B, the LPS-induced phosphorylation and degradation of NF- κ B [80]. Since activation of NF- κ B is necessary for LPS-triggered induction of iNOS, inhibition of this transcription factor may also result in a decrease in exogenous nitric oxide synthesis which is responsible for cytotoxic effects of this signal molecule [122, 126]. On the other hand, endogenous induction of nitric oxide inhibits NF- κ B and interfere with several signaling pathways that lead to activation of this transcription factor [53].

Resveratrol also induced apoptosis in fibroblasts after induced expression of oncogenic *H-Ras* [53]. Thus, resveratrol is likely to function by inhibiting inflammatory and oncogenic diseases, at least in part, through the inhibition of NF- κ B activation by blocking I κ B kinase activity. These data may also explain some aspects of the "French paradox" and provide a molecular rationale for the role of a potent chemopreventive compound in blocking the initiation of inflammation and carcinogenesis.

Final remarks

The presented chemopreventive abilities of resveratrol do not limit its activities in other fields. Some relatively new and unexplored directions of research on this molecule involve allergy and brain function. A preliminary study reported by Cheong

et al. [21] showed anti-allergic properties of resveratrol, as assessed by the β -hexosaminidase release from cells. Resveratrol induces phosphorylation of mitogen activated protein (MAP) kinases in the human neuroblastoma cells. MAP kinases are involved in signal transduction in cells. In particular, enzyme type ERK2 has been related to synaptic changes linked to learning processes [121]. As authors postulate the reduction of dementia upon moderate wine intake, this finding may enlarge our understanding of the protective role of resveratrol. Nevertheless, till now most of the data comes from *in vitro* studies. Indeed, up to the present, the evidence for resveratrol absorption and metabolism in humans is scant and the question arises if the strong biological activity of resveratrol *in vitro* can be fully reproduced *in vivo* in a comparable dose range.

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Wine and Cardiovascular Health

A Comprehensive Review

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Abstract

Alcoholic beverages have been consumed for thousands of years, attracting great human interest for social, personal, and religious occasions. In addition, they have long been debated to confer cardioprotective benefits. The French Paradox is an observation of a low prevalence of ischemic heart disease, with high intakes of saturated fat, a phenomenon accredited to the consumption of red wine. Although many epidemiological investigations have supported this view, others have attributed it to beer or spirits, with many suggesting that the drink type is not important. Although excessive consumption of alcoholic beverages is commonly regarded to be detrimental to cardiovascular health, there is a debate as to whether light-to-moderate intake is cardioprotective. Although there is extensive epidemiological support for this drinking pattern, a consensus has not been reached. On the basis of published work, we describe the composition of wine and the effects of constituent polyphenols on chronic cardiovascular diseases.

Alcoholic beverages have been consumed for thousands of years, predating biblical times and spanning as far back as early human emergence. Before there was wine, beer, or spirits, primates lived on a diet predominantly consisting of fruits and vegetables, with water serving as the main fluid for survival. Until proper methods of water delivery and decontamination were conceived, our ancestors relied on fresh water from streams, rivers, and precipitation, but fermented fruits including berries and even mead could have been regularly consumed in the form of drinks. Following the Neolithic era ≈ 10000 BC, cultivation and maintenance of crops allowed for the production of the earliest forms of what we now consider wine and beer; however, alcohol was almost certainly consumed much earlier. Fruits, grains, and even honey were fermented to produce alcoholic beverages, and alcohol became a staple of consumption to our hunter-gatherer predecessors.¹

There is no doubt that wine and alcohol have attracted great human interest for recreational and personal use.¹ The culture of drinking has only grown since its initiation, and fermented products including fruits and grains have been used to produce alcoholic beverages. In addition, scientific intrigue has also grown extensively for alcohol since the 20th century, as epidemiological evidence amassing large prospective, cross-cultural studies emerged in support for the hypothesis of a negative correlation with moderate consumption of alcohol and ischemic heart disease (IHD).² Such

correlation has also been reported individually for red wine.³ Although evidence of these cardiovascular benefits is inconsistent and heavily debated by physicians and scientists alike,⁴ epidemiological studies have strongly supported this view being specific to wine,⁵ especially red wine. More specifically, some postulate that red wine's bioactive constituents, polyphenols, impart cardioprotective effects.⁶ Others argue that there may be an equilibrium between alcohol and wine polyphenols, which in concert would be accountable for the cardioprotective benefits in the human body.⁷

The French Paradox is a term derived from the observation of a decreased incidence of IHD despite a high intake of saturated fat.⁸ This is linked to France and led scientists to attribute this phenomenon to the high consumption of wine.⁸ The French Paradox started extensive research into wine and led to the identification of many compounds, namely polyphenols, that are thought to be the basis of wine's apparent cardioprotective potential. Red wine, among other constituents, is also included in the Mediterranean diet, and this diet has been labeled as beneficial by scientific advisory committees.⁹

Although excessive or binge drinking of alcoholic beverages is regarded to be detrimental to cardiovascular and general health, light-to-moderate intake of regular amounts is recommended in the literature.¹⁰ Adverse effects of acute and chronic alcohol consumption are dependent on the doses of intake; however, differing opinions exist regarding red wine's potential as a therapeutic agent, regardless of the pattern of drinking.¹¹ From a public health perspective, alcohol consumption is regarded as a risk factor for chronic diseases, and globally it contributes to an increase in disease burden.¹² Although these detrimental risks are present and may outweigh the benefits of alcohol consumption, wine and alcohol will continue to be ever-present in our society.

With a popular drink like wine surrounded by such scientific intrigue, it is desirable to provide a comprehensive account, from a cardiovascular point of view, of its anatomy, mechanisms of actions, and risks and benefits of consumption. Most reviews and meta-analyses to date have focused on the individual characteristics of wine, but a much more inclusive review of the literature on wine and its comparisons with other alcoholic beverages is needed. This review aims to investigate wine and its cardioprotective potential, highlight the importance of individual components of wine and their interactions with the cardiovascular system at large, and present up-to-date epidemiological and experimental evidence of wine's impact on chronic cardiovascular diseases. We also address the debate around the light-to-moderate intake of consumption, variable definitions of drinking, and current recommendations for consumption.

Definitions of Consumption

What Constitutes a Standard Drink?

Alcoholic intake, if not monitored, can contribute to various adverse conditions that affect day-to-day life.¹³ Hence, governments and international institutions have defined a unit called a "standard drink."¹⁴ Epidemiological and experimental studies take advantage of this metric to quantify consumption and assess population risk. Therefore, a standard drink size has become an important, but often publicly misunderstood metric, for population-based studies assessing

phenomena related to the intake of alcohol. Quite apparent from the literature is the definition of a standard drink, which varies across country borders and across the scientific literature.¹⁵

A standard drink, or a unit of alcohol in the United Kingdom, is a national concept that is expressed in amounts of pure ethanol.¹⁶ To the general public, it is presented in amounts of beer, wine, or spirits, because they are the most commonly consumed beverages.¹⁶ The units of alcohol in a beverage can vary depending on the source, but most common ones include standard drink, grams, milliliters, ounces, and alcoholic concentration by volume.¹⁷

The guidelines from the World Health Organization (WHO) on standard drink assume 1 standard drink to be 10 g of pure ethanol, with recommendations of not exceeding 2 standard drinks per day, with at least 2 nondrinking days during the week.¹⁸ This definition is not widely adopted across country borders and carries great country-to-country variation. Kalinowski and Humphreys¹⁴ systematically gathered international guidelines on standard drink definitions. Their methods of analysis and most important findings are summarized in Table 1. They identified 75 governments that did not adopt such a definition. Their analysis included 37 countries that did adopt the WHO standard drink measure but cited large variability between countries, ranging from 8 to 20 g. The authors further reported the 10-g WHO guideline to be the modal definition between countries, with variations ranging from 10 g/d to 56 g/d for low-risk alcohol consumption. The standard drink is defined in terms of pure ethanol. Red wine, beer, and spirits are all composed of some percentage of pure ethanol. The WHO guidelines present a guide on calculating the alcoholic content of a beverage.¹⁸ The method of calculation, variables, and values are presented in Table 2. The alcoholic content within a drink depends on 2 variables: size of beverage and percent strength of pure ethanol.¹⁸ These variables vary across countries, cultures, and even vendors, but this guideline presents the most common scenario as a tool for drinkers to calculate the amount of pure ethanol in a drink.

Table 1. Most Important Methods and Findings of Governmental Standard Drink Definitions and Low-Risk Consumption		
Outcomes		Comments
Data collection period	May to August 2015	–
Mode of data collection	Government health nutrition websitesHealth ministriesFAOISPORPersonal communications with country-specific expertsPersonal contacts	Most data accumulation through Internet searches.Personal communication with country-specific experts was through email.

FAO indicates Food and Agriculture Organization of the United Nations; ISPOR, International Society for Pharmacoeconomics and Outcomes Research; and WHO, World Health Organization.

Adapted from Kalinowski and Hymphreys¹⁴ with permission of the publisher. Copyright © 2016, John Wiley & Sons.

Type of Beverage	Approximate Size of Beverage, mL*	Strength, % Pure Ethanol†	Conversion Factor‡	Formula for Standard Drink
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Standard drink=beverage size × strength × conversion factor

Type of Beverage	Approximate Size of Beverage, mL [*]	Strength, % Pure Ethanol [†]	Conversion Factor [‡]	Formula for Standard Drink
Spirits	40	24.3–90	0.79	

^{*}Beverage containers vary in size but are approximately in this range.

[†]The common strengths of beverage as described from the WHO report.

[‡]Conversion factor allows for a conversion of volume (of ethanol) to grams (of ethanol).

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A quantitative measure of a standard drink is essential; however, given the mathematical nature, the general public has difficulty comprehending it and would rather prefer a more qualitative, day-to-day metric to regulate their consumption. The WHO guidelines define a standard drink in terms of glasses of wine, beer, liquor, and shots of spirits (Table 3). They claim that these amounts roughly equal 1 standard drink.

Table 3. The World Health Organization’s Estimates of a Standard Drink for Conventional Alcoholic Beverages		
Type of Beverage	Standard Drink Equivalent	Quantitative Metric
Wine	1 glass of wine; 1 small glass of sherry	140 mL (12% strength); 90 mL (18% strength)
Beer	1 can of beer	330 mL (5% strength)
Spirits	1 shot of whisky, gin, vodka; 1 small glass of liquor	40 mL (40% strength); 70 mL (25% strength)

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Light, Moderate, and Excessive Consumption

Voskoboinik and coauthors¹⁹ define light alcohol consumption as <7 standard drinks (std) per week, moderate as 7–21 std/wk, and excessive as >21 std/wk. This translates to <1 std/d for light consumption, 1–3 std/d for moderate, and >3 std/d for excessive consumption, where 1 standard drink is defined as 12 g of pure ethanol.

Importance of Wine As a Biological Beverage

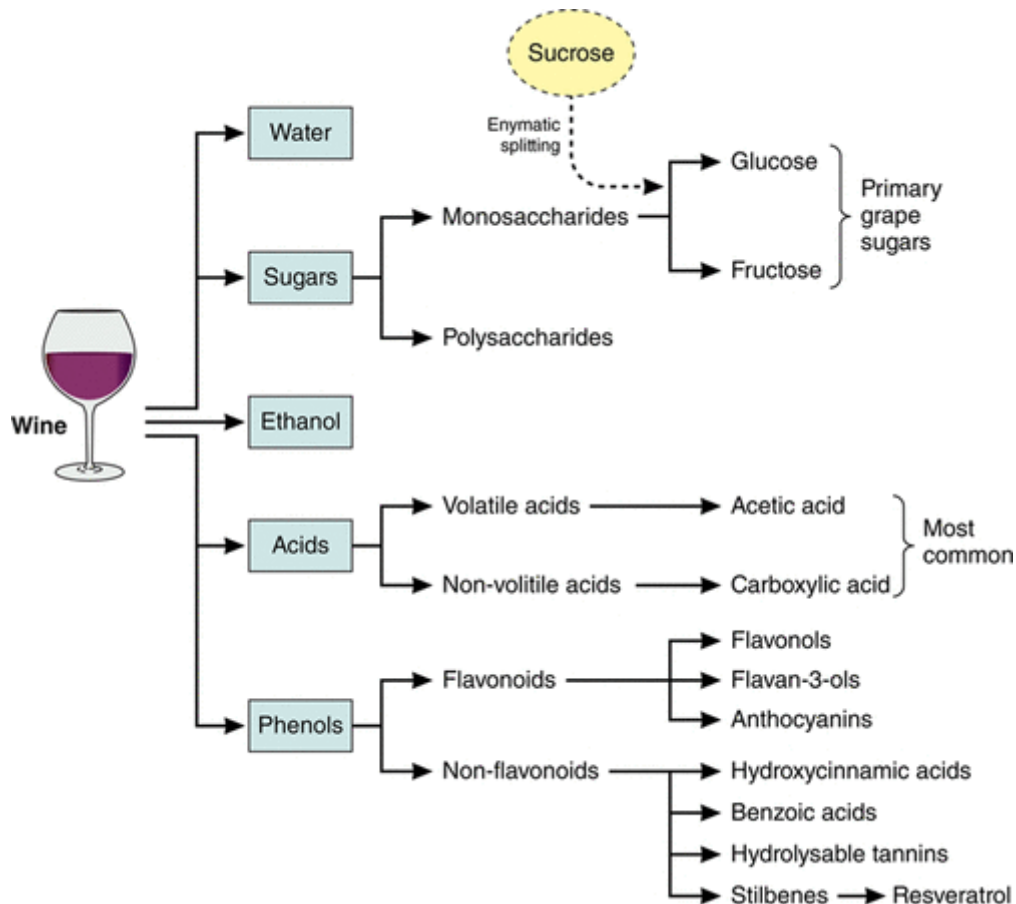
The molecular properties of wine and their interactions with the human body were extensively researched after a negative correlation for IHD was reported with alcohol consumption. Since then, researchers have focused on pinpointing how this beverage imparted cardioprotection against chronic cardiovascular diseases. After fermentation, wine still possesses a mixture of compounds known as polyphenols that, besides ethanol, have emerged as key players in explaining red wine's antioxidant, anti-inflammatory, and cytoprotective properties.²⁰

Why Red Wine? The Hypothesis

St Leger and colleagues³ reported a negative correlation between alcohol consumption and IHD deaths, and attributed this observation predominantly to wine. Renaud and de Lorgeril⁸ subsequently described what they called the French Paradox, referring to the indirect observation that the French population consumed red wine with their diet, which was mostly high in saturated fat, so this correlation between wine and cardiovascular mortality was attributed to the consumption of red wine.²¹ Since then, numerous studies have come out in favor of wine and alcohol conferring cardiovascular benefits, but with one important caveat: most of these investigations, although involving large sample sizes with cross-cultural and geographical comparisons, were epidemiological. Scientists have thus questioned these results, but intrigue still surrounds the community. Several explanations for the French Paradox have been postulated,²¹ with epidemiologists presenting strong correlations in favor of wine (both white and red), with other scientific literature criticizing these observations.

Red Wine Composition

Wine is an alcoholic beverage of complex composition that is obtained through the fermentation of grape must, and thus the quality and variety of grapes used in the vinification process have an impact on the composition of wine.²² Red wine is composed of >500 compounds, with the most important constituents being water, alcohol (ethanol), and polyphenols.²³ They can be divided into 2 primary groups: the flavonoids and nonflavonoids. The general composition of wine can be viewed in **Figure 1**. Flavonoids have been known to provide taste and color to wine while being important for health, while resveratrol (nonflavonoid) is debated to contribute to the potential bioactive properties of wine. Polyphenols amount to only a fraction of wine's total content, but are of particular interest in cardiology for their potential biological and cardioprotective properties.



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Figure 1. Overview of the chemical components of wine.

Red Wine Versus White Wine

Red wine is known to be 10-fold higher in polyphenolic content than white wine, and this variability arises because of red wine's grape must fermentation.²² This is why white wine is given much less importance than red wine in the literature. In addition, apart from the varying sugar content, polyphenols are the only major component different between red and white wines. There are few studies directly comparing the effects of the 2 drinks; therefore, conclusive evidence regarding the comparison of the 2 wines is poor. Because the polyphenolic ratio in red and white wines differs significantly, white wines' protective mechanisms may be different from red wines'. Bertelli²⁴ postulates that relatively unknown active compounds identified in white wines, namely tyrosols, caffeic acid, and shikimic acids, might explain the biological basis of white wine's cardioprotective effects.

Bioactive Components of Red Wine

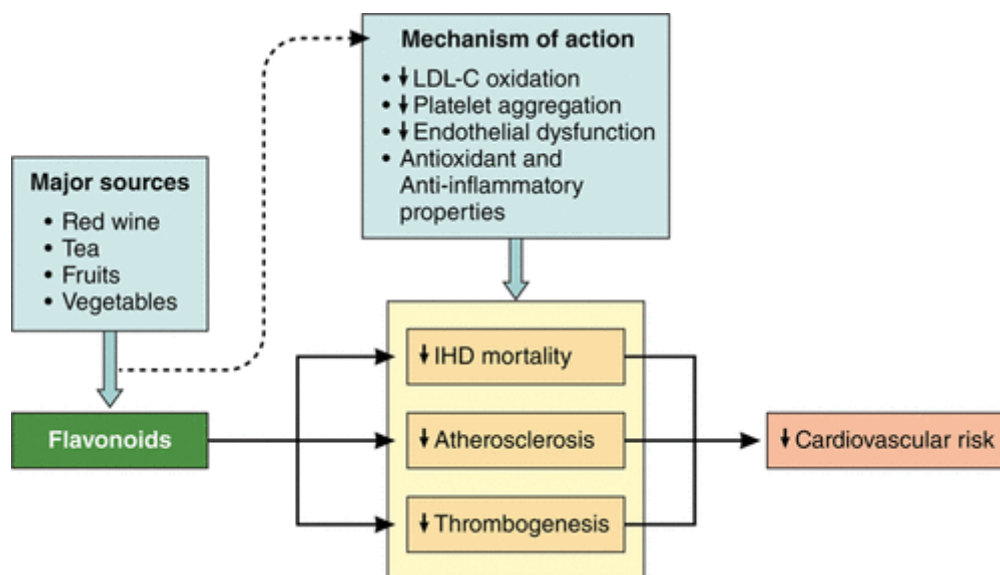
Bioavailability of Polyphenols

The biological properties of polyphenols are only utilizable if they are bioavailable, which is dictated primarily by their chemical structure. Most polyphenols cannot be absorbed in their native form and

are chemically modified after ingestion. Post-wine consumption, polyphenols are structurally modified and metabolized fairly quickly; thus, a component of the biological activity from wine is derived from metabolized polyphenols.²⁵ The bioavailability of wine polyphenols is low; however, there is consistent evidence that bioavailable concentrations after acute and chronic consumption of wine are able to exert beneficial biological effects in vivo.²⁴

Flavonoids

Flavonoids are plant-based antioxidants that are included in the family of polyphenols. They are found primarily in vegetables, fruits, and beverages such as tea and wine.²⁶ Flavonoids can be synthesized only by plants and have been investigated for their protective abilities against chronic cardiac diseases (Figure 2). In the previous few decades, flavonoids have received much attention after epidemiological investigations specifically discovered that dietary flavonoids were inversely associated with IHD mortality.²⁷ Flavonoids from red wine have been credited to inhibit low-density lipoprotein (LDL) oxidation²⁸ and prevent endothelial dysfunction,²⁹ which is postulated to increase atherosclerosis development.³⁰ Hence, flavonoids have antiatherosclerotic properties that are particularly apparent when wine is studied devoid of ethanol, as dealcoholized wine (see Dealcoholized Red Wine: Cardioprotection of Wine Polyphenols Beyond Ethanol). Of the flavonoids, quercetin is known to be a potent antioxidant because it has been shown to have a negative correlation with cardiovascular mortality.²⁹ In a dietary investigation of >1000 participants, high quercetin intakes were associated with lower IHD mortality (relative risk [RR], 0.79; 95% confidence interval [CI], 0.63–0.99; $P=0.02$) and lung cancer incidence (RR, 0.42; 95% CI, 0.25–0.72; $P=0.001$).³¹



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Figure 2. The cardioprotective effects and implicated mechanisms of flavonoids in cardiovascular risk reduction. IHD indicates ischemic heart disease; and LDL-C, low-density lipoprotein cholesterol.

Quercetin

Quercetin, a plant polyphenol from the flavonoid group, has amassed much scientific intrigue. It is an important dietary flavonoid that is prominent in red wine and the Mediterranean diet.³² Of the flavonoid group, it is the most abundant dietary flavonoid that has been investigated for its antihypertensive, anti-inflammatory, acute platelet thrombogenesis, and protective capabilities against IHDs.³³

Quercetin is found as a conjugated derivative in plasma, and those metabolites subsequently exert physiological effects.³⁴ Quercetin is known to be an effective free radical scavenger that prevents LDL oxidation in humans.³⁵ It causes endothelium-dependent vasodilation of vascular smooth muscles and inhibits platelet aggregation, which can subsequently contribute to atherosclerosis.²⁹ In apolipoprotein E gene-deficient mice, Loke and colleagues³⁶ studied quercetin and other dietary flavonoids' capabilities to reduce atherosclerotic lesions. This group demonstrated that quercetin attenuated lesions by inhibiting inflammation, improving nitric oxide (NO) bioavailability, and inducing heme oxygenase-1, which all seemed to have been protective. In human subjects, however, contradictory results have come to light, showing that dietary quercetin did not change biomarkers of inflammation.³⁷ To elucidate causation, further dose-response, randomized, and placebo-controlled studies are needed.

In dogs with experimental myocardial infarction, quercetin intervention led to an improvement in left ventricular contractile function.³⁸ Similarly, quercetin has further been documented to protect the myocardial tissue against reperfusion injury and global ischemia.³⁹

Resveratrol

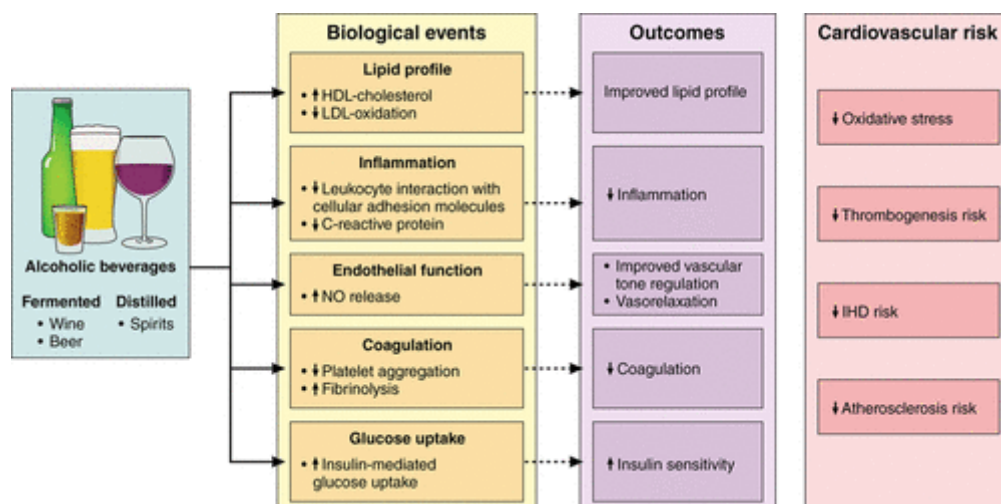
Resveratrol is a nonflavonoid stilbene derivative produced by plants that is prominently present in red wine and grapes.⁴⁰ Resveratrol from red wine is postulated to be an important contributor in explaining the French Paradox. The increased publicity of resveratrol's bioactive potential and treatment of chronic disorders such as cardiovascular diseases and cancer has engaged public interest in resveratrol supplements.⁴¹

A number of preclinical and clinical studies have demonstrated a very low oral bioavailability for resveratrol⁴²; however, Goldberg and coworkers⁴³ demonstrated that resveratrol and other polyphenols reached peak concentrations 30 minutes postprandial, with the absorption of transresveratrol being 20-fold more efficient than the flavonoid catechin.⁴⁴ However, broadly speaking for all polyphenols, the bioavailability is enough to exert antioxidant effects. A wealth of data has been collected on resveratrol and its effects on hypertension, atherosclerosis, stroke, myocardial infarction, and heart failure.⁴⁵ In general, beneficial effects of the administration of resveratrol as a supplement have been reported for the aforementioned diseases and may be one of the reasons why this compound is being heavily advertised as a cardioprotective supplement.

Putative Mechanisms of Action

According to reports, the exact mechanisms of action that underlie cardioprotection for red wine have not yet been causally interpreted; however, in vitro, in vivo, and human epidemiological studies have shown cardiovascular benefits of drinking red wine, and have postulated interesting

explanations for cardioprotection. A summary of the biological events and triggering chemicals is presented in [Figure 3](#).



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Figure 3. Schematic representation of the biological mechanisms of alcohol intake. HDL indicates high-density lipoprotein; IHD, ischemic heart disease; LDL, low-density lipoprotein; and NO, nitric oxide.

Protective Mechanisms: Red Wine Polyphenols and Ethanol

Red wine polyphenols reduce platelet aggregation⁴⁶ and improve fibrinolysis.⁴⁷ The endothelium controls NO release, which subsequently regulates vascular tone, relaxes vascular smooth muscle cells, and inhibits platelet aggregation.⁴⁸ Moderate wine consumption increases NO production,⁴⁹ which induces vasodilation.²⁹ Endothelial dysfunction reduces NO bioavailability that is associated with cardiovascular diseases including atherosclerosis, thrombosis, and hypertension.⁴⁸ In regard to hypertension, alcohol consumption in moderation is linked to a reduction in systolic and diastolic blood pressure.⁵⁰

Polyphenols are strong antioxidants that improve the lipid profile by reducing the susceptibility of LDL to oxidation.²⁸ Conversely, the intake of red wine increases blood high-density lipoprotein (HDL) levels and triglycerides.⁵¹ A meta-analysis of 42 human studies⁵² found that an experimental dose of 30 g ethanol/d increases concentrations of HDL cholesterol (3.99 mg/dL; 95% CI, 3.25–4.73), triglycerides (5.69 mg/dL; 95% CI, 2.49–8.89), and apolipoprotein AI (8.82 mg/dL; 95% CI, 7.79–9.86), a main protein in HDL cholesterol. Case presentations have supported the hypothesis of HDL cholesterol and apolipoprotein AI deficiencies playing a significant role in an increased risk of atherosclerosis.⁵³

Alcohol intake at regular intervals is observed to be beneficial for diabetes mellitus. Light-to-moderate consumption enhances insulin sensitivity by increasing insulin-mediated glucose uptake.⁵⁴ Increased insulin sensitivity is proposed to be associated with greater HDL cholesterol and apolipoprotein AI levels⁵⁵ and possibly contributes to a decreased incidence of IHD.⁵⁶

Pathophysiological Mechanisms: Alcohol and Atrial Fibrillation

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice.⁵⁷ Consumption of alcohol may trigger AF, and sustained consumption may cause atrial electric remodeling.¹⁹ Voskoboinik and coworkers¹⁹ reviewed alcohol's effects on AF and concluded that its consumption was a risk factor for AF, causing increased recurrence and higher rates of paroxysmal and persistent AF. They further commented that the cardioprotective benefits of alcohol on IHD do not apply to AF. In a UK cohort (N=703777), moderate-to-high consumption of alcohol was identified as an independent risk factor for progression of paroxysmal AF to persistent AF (odds ratio, 2.7; 95% CI, 1.2–6.0).⁵⁸ In 115 patients with a recorded idiopathic AF episode, patients who experienced recurrence (n=32) had a significantly higher incidence of consuming alcohol regularly ($P=0.014$).⁵⁹ Alcohol's effects on cardiac conduction have also been investigated. In a pilot study of 14 patients with a reported heart disease, acute excessive alcohol intake slowed intra-atrial conduction and shortened ventricular myocardial refractory periods.⁶⁰ As for interatrial conduction, which is usually measured by the P-wave duration and has been found to be a predictor of AF;⁶¹ a study showed that average P-wave duration was significantly affected after alcohol intake in normal healthy subjects (107 ± 9 versus 125 ± 11 ; $P<0.05$).⁶² However, the duration was more altered after alcohol consumption for patients with a documented history of paroxysmal AF than in control subjects (125 ± 11 versus 158 ± 29 ; $P<0.05$).

The French Paradox and Beyond

Renaud and de Lorgeril⁸ first coined the term French Paradox in 1992, an observation of low IHD and associated mortality despite the high intake of saturated fat in southern France. The authors attributed the cardioprotective effects of this phenomenon to the moderate consumption of alcoholic beverages, especially red wine, which was highly consumed in the area. Although Renaud and de Lorgeril strengthened their association between wine consumption and IHD by controlling for dairy fat intake,⁶³ other authors have argued that there were characteristics and confounding variables not controlled for in their analysis (drinking patterns, lifestyle characteristics, dietary intake, human behavior) which may have led to such a correlation.⁶⁴

Role of Wine Versus Other Alcoholic Beverages on IHD and Total Mortality

A J- or U-shaped relationship between alcohol consumption and total mortality has been well documented,⁶⁵ with the first incidence being reported as a U-shaped curve between IHD mortality for heavy male smokers and nonsmokers in the Framingham Heart Study.⁶⁶ Since then, several investigators have focused on exploring the effects of specific alcoholic beverages on IHD. An L-shaped relation between cardiovascular mortality and alcohol intake has been shown,⁶⁷ implying that a low dose of alcohol intake can have protective effects that do not decrease with elevated intakes. Many studies have reported inverse associations between specific types of alcoholic drinks and IHD. No consistent pattern of a specific type of alcoholic drink (wine, beer, or spirits) reducing the risk of IHD has been confirmed, rather a strong epidemiological accord that all alcoholic drinks are linked with a reduction in risk from IHD, if not consumed in excessive amounts, or binged on. Rimm and colleagues⁶⁴ conducted a systematic review assessing the risk of specific alcoholic drinks on IHD. Focusing on ecological, case-controlled, and cohort studies, they identified 4 investigations that reported an inverse association between wine intake and IHD and mortality,^{68–71}

4 that correlated beer intake and coronary events,^{68,72-74} and 3 with an emphasis on a correlation for spirits.^{2,74,75} A summary of selected major clinical studies reporting the associations between IHD and alcohol consumption, including wine, is presented in Table 4. The 3 largest prospective studies mentioned (N=87526 women; 81825 men and women; 51529 men),^{2,69,71} where the absolute consumption of wine, beer, and spirits would be the greatest, found that the risk of IHD was lower with all 3 types of drinks, with no particular drink emerging as a clear winner.⁶⁴

Table 4. Major Studies Examining the Relationship Between Alcohol Consumption of Wine							
Studies	Sample Size, n	Setting	Study Design	Follow-up	Age Range, y	Alcoholic Drinks Assessed	Incidences
Keil et al ⁶⁷	2084 (1071 men; 1013 women)	Augsburg, Germany	Population-based prospective cohort	8 y	45–64	Wine, beer, and spiritsResults in favor of beer	All-cause mortality: 1.4 (96 men; 45 women)IHD associated mortality: 6.3 men
Grønbaek et al ⁶⁸	24525 (13064 men; 11459 women)	Copenhagen, Denmark	Population-based prospective cohort	257859 person-years	20–98	Wine, beer, and spiritsResults in favor of wine	Total mortality: 4833IHD-associated mortality: 10

Studies	Sample Size, n	Setting	Study Design	Follow-up	Age Range, y	Alcoholic Drinks Assessed	Incidences
Stampfer et al ⁶⁹	87526 women	United States (11 states)	Population-based prospective cohort	334382 person-years	34–59	Wine, beer, and spirits	200 IHD incidences (164 nonfatal MI, 36 deaths)66 ischemic strokes28 subarachnoid hemorrhage
Levantesi et al ⁷⁰	11248 (9601 men; 1647 women)	Italy	Results from a multicenter open-label study	37021 person-years	Cohort not controlled for age	WineResults in favor of wine	889 IHD incidences1 strokes264 sudden cardiac death

Studies	Sample Size, n	Setting	Study Design	Follow-up	Age Range, y	Alcoholic Drinks Assessed	Incidences
Yano et al ⁷²	7705 men	Hawaii	Prospective cohort	8 y	Cohort not controlled for age	Wine, beer, and spirits Results in favor of beer	294 IHD incidences associated mortality: 43136 nonfatal MI, 27 ACI, 88 angina pectoris
Salonen et al ⁷⁵	4063 men	Eastern Finland	Prospective cohort	7 y	30–59	Beer and spirits Results in favor of spirits	209 acute MI, 31 liver cirrhosis or acute pancreatitis, all-cause deaths
Rimm et al ²	51529 men	United States	Prospective cohort	72290 person-years	40–75	Wine, beer, and spirits Results in favor of wine	350 coronary events (164 nonfatal MI, 196 coronary deaths, 12 sudden deaths), 136 CABG or PTCA procedures
ACI indicates acute coronary insufficiency; CABG, coronary artery bypass graft; CI, confidence interval; HR, hazard ratio; IHD, ischemic heart disease; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angiography; and RR, relative risk.							

A prospective observational study by Klatsky and Armstrong⁷¹ found that individuals who preferred wine were at a lower risk of death from IHD, after comparisons with spirits drinkers as a reference

group (RR, 0.7; 95% CI, 0.5–0.9). To support their findings, this group reported an inverse association between IHD risk and frequency of wine consumption, but they were not able to confidently conclude that wine confers a greater protective advantage because of user differences and the inability to control for all potentially confounding variables. A Danish cohort study⁶⁸ assessing the population for all 3 types of alcoholic drinks in a homogeneous setting, where not 1 alcoholic drink predominated, found a significant decrease in mortality, from IHD and all causes, among wine drinkers, at all levels of alcoholic intake. Conversely, light alcohol drinkers who avoided wine had a relatively higher risk of death from IHD than drinkers who consumed wine (RR, 0.76; CI, 0.63–0.92 versus RR, 0.58; CI, 0.47–0.72). Additional evidence of the cardioprotective effects of wine from IHD is provided by the results of the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico)-Prevenzione trial,⁷⁰ which found that moderate wine consumption (≤ 0.5 L/d) was associated with a significant reduction in the risk of cardiovascular events.

Examining the association between beer and IHD, the Honolulu Heart Study, a prospective epidemiological investigation,⁷² found a significant negative correlation between moderate alcohol consumption of beer and death from IHD during a 6-year follow-up period. Such association for wine was not found, but beer was the predominantly consumed drink in the population. Further support for beer is provided by the MONICA (Monitoring Trends and Determinants of Cardiovascular Diseases) Augsburg cohort study,⁶⁷ in which a protective effect from light-to-moderate drinking of alcohol was observed in a predominantly beer-drinking population. Risk reduction from IHD was close to 50%, and the L-shaped relationship was also confirmed.⁶⁷ For spirits, Salonen et al⁷⁵ reported that consumption at least once a week was associated with a reduced risk of acute myocardial infarction among IHD-free men aged 30 to 59 (RR, 0.5; 95% CI, 0.3–0.9). Furthermore, in a study by Rimm et al,² in which spirits were the most commonly consumed drink, found that they were also the most cardioprotective by having a significant inverse association between consumption and risk of IHD ($P=0.0004$).

The Debate Around Light-to-Moderate Intake of Wine and Other Alcoholic Beverages

Although an inverse relationship between alcohol consumption and risk of IHD has been extensively documented in studies using an epidemiological design, there is a debate regarding which of the 3 drinks provides better cardiovascular benefits, or on a broader scale, if alcohol itself can provide protective benefits. One side of the argument is presented by Rehm et al,¹² who argue that alcohol consumption contributes to the global chronic disease burden, and further evidence suggests that high doses of alcohol consumption confer a disadvantage to the heart, especially with an increased risk of arrhythmia, sudden cardiac death, alcoholic cardiomyopathy, and hypertension, among others.⁷⁶ The other side of the argument is presented in the literature on light-to-moderate alcohol consumption, which is in favor of a reduction in IHD and total mortality.³ Yano et al⁷² were successful in showing a strong negative correlation between moderate alcohol consumption and incidence of IHD for all 3 beverages, supporting the idea of the protective benefit of moderate alcohol consumption. The findings of moderate alcohol consumption were further supported by clinical angiography studies⁷⁷ that show a reduction in atherosclerosis and average IHD.

As for wine, Grønbaek⁶⁸ showed that, in a Danish cohort, IHD mortality decreased across levels of stable drinking, and that wine (8–21 drinks/wk) conferred a greater protective effect than the intake of light-to-moderate beer or spirits. In Oakland and San Francisco populations, it was shown that light drinkers were at low risk of IHD, with the greatest reduction observed in older populations.⁷⁸ The investigators further showed that wine preference and its consumption were associated with a significant reduction in cardiovascular death (RR, 0.7; 95% CI, 0.6–0.9; $P=0.01$), with no such correlation observed for beer or spirits.

Significant inverse associations were reported for the 3 alcoholic beverages, especially wine, albeit with limitations, because of their epidemiological and clinical study design. There were populations in whom consumption of a single type of drink prevailed over another, and in most cases, that drink conferred the greater effect. Drinking patterns, lifestyle characteristics, dietary intake, and risk factors varied in the populations studied; hence, these could be potential confounding variables for such associations.⁶⁴ There are methodological inconsistencies present among all studies, and they make it difficult to draw causal interpretations regarding a specific type of alcoholic drink providing cardioprotective effects, but rather support the finding that alcohol intake of all beverages as a whole is linked with a reduction in risk from IHD, if not consumed excessively or binged on. Considering the aforementioned limitations and methodological inconsistencies present in observational and epidemiological studies, one should be cautious when providing general recommendations to the public. Is it time to change our approach to find the answer? Some have suggested doing prospectively controlled, double-blinded randomized clinical trials,¹⁵ but the ethical dilemma of pursuing such an endeavor still needs to be debated within the scientific community. For now, we shall rely on the evidence at present to make an informed decision.

Acute, Sustained, or No Consumption: Which Is Better?

Most epidemiological and population-based literature surrounding alcoholic beverages considers their sustained consumption to be cardioprotective for IHD-associated mortality.⁷⁹ Acute and sustained excessive consumption in the form of binge drinking is associated with arrhythmias (holiday heart syndrome), transient ischemic attack, and sudden cardiac death.⁷⁶ Hence, regulation of dosage is a very important consideration among drinkers. Red wine polyphenols in an acute setting postprandial, however, have been shown to exert antioxidant and anti-inflammatory effects. Covas et al²⁵ reviewed randomized controlled human studies on the effects of sustained wine usage on oxidation. They reported contradictory studies^{80,81} for an increase in plasma antioxidant activity after sustained wine consumption in healthy participants. It must be noted that these studies used parallel and crossover study designs with small sample sizes ($n=78$ and 40 , respectively). The participants were also not followed up throughout their lifetimes, all possible reasons for the reported contradiction.

Is one worse off consuming no wine or any alcoholic drink? From a public health perspective, no, because dependence on alcohol only adds to the growing cohort of alcohol use disorders.¹³ There are studies, however, that have presented compelling evidence for abstinence from alcohol being a risk factor for myocardial infarction⁸² and type 2 diabetes mellitus.⁸³

Dealcoholized Red Wine: Cardioprotection of Wine Polyphenols Beyond Ethanol

It is common to dealcoholize wine, and special attention is paid during the manufacturing process to ensure no loss of polyphenols. With such a drink, effects of polyphenols can be studied in isolation. Oxidative modifications in LDL are thought to be an initiator for the development of atherosclerosis, whereas polyphenols are thought to prevent this oxidative stress and enhance plasma antioxidant capability by being present.⁸⁴ Inhibition of LDL oxidation is one of the proposed mechanisms by which polyphenols delay the onset of atherosclerosis and reduce cardiovascular risk. Furthermore, red wine and components catechins and anthocyanins inhibited *in vitro* LDL oxidation, whereas ethanol and dealcoholized red wine did not affect measured oxidation levels.⁸⁴ These observations were also present in wine with ethanol; here, we confirm the same results in dealcoholized red wine. To evaluate the effects of wine polyphenols and their antioxidant potential, Serafini et al⁸⁵ conducted a human pilot study of patients with IHD and showed that ingestion of dealcoholized red but not white wine at 1-week intervals greatly increased *in vitro* plasma antioxidant capacity, with this effect being credited to red wine polyphenols, which are found in much greater concentrations in red wine than in white.⁸⁵ An investigation by Stein et al⁸⁶ reported that ingestion of purple grape juice (devoid of ethanol) was associated with improved endothelial function via a reduction in susceptibility of LDL-cholesterol to oxidation in 15 adults with IHD.

Red wine polyphenols have also been hypothesized to exert positive effects on flow-mediated vasodilation, which is known to be endothelium-dependent. In the Stein et al⁸⁶ cohort, grape juice was also associated with improved flow-mediated vasodilation of the brachial artery, which would in turn improve endothelial function. Treatment of human umbilical vein endothelial cells with dealcoholized red wine led to a 3.0-fold increase in NO release, and a 2.0-fold increase in human endothelial NO synthase, an enzyme isoform that synthesizes NO.⁸⁷ In addition, dealcoholized red wine polyphenol extract has been found to almost completely reverse the prothrombotic effects of a 2% cholesterol-rich diet by a NO-dependent mechanism.⁸⁸ Red wine polyphenols have displayed potent antioxidant properties *in vivo* for arterial stiffness. Dealcoholized red wine has been acutely demonstrated to reduce arterial stiffness 60 minutes postprandial intake in patients with coronary artery disease, with this reduction attributed to red wine antioxidants.⁸⁹

Alcohol and Cardioprotection: Implications for Causality from Observational Evidence

Much of the evidence regarding alcohol consumption and its beneficial effects on the cardiovascular system derives from observational data. Epidemiological studies have been integral to this discussion, with multiple cohorts with cross-cultural and geographical comparisons reaching similar conclusions. That being said, can one infer causality from epidemiological investigations using an observational-based approach, in which subject bias is inherent with self-reported data?

To determine causation, randomized controlled trials have long been considered the gold standard. However, epidemiological, public health, and clinical research are sometimes barred by ethical concerns, where, for instance, an experimental treatment such as alcohol being investigated for a therapeutic cause may leave participants in harm's way.⁹⁰ Nonetheless, several methods have been developed to make causal inferences from epidemiological research, starting with the seminal set of criteria proposed by Austin Hill, to distinguish causal and correlational associations.⁹¹ Since then, other methods have been developed. Some common ones used today include instrumental variables regression analysis, difference in differences, and regression discontinuity designs.⁹⁰

These methods seek to use a randomized approach, assigning variables to observational data and using mathematical models to associate the effects of a treatment with measured health outcomes. Evaluating for causal inferences in epidemiology is a growing interest, and it is worthwhile to stress that, in cases where longitudinal epidemiological evidence overwhelmingly points in one direction, when a large number of studies are arriving at similar conclusions, when many proposed mechanisms of action are biologically sound and understandable, and when the aforementioned statistical approaches also associate the treatment positively to the health outcome, one can begin to suggest causal links.

Decades of longitudinal data have shown an improvement in cardiovascular risk factors with low-to-moderate alcohol and wine consumption. Very recently, studies based on Mendelian randomization approaches, an instrumental variables analysis using genetic variants as instruments for analysis, have questioned the cardiovascular benefits of alcohol consumption.⁹² In the context of alcohol, Mendelian randomization studies seek to provide evidence of causal relationships and provide a magnitude of the risk associated with lifelong alcohol use by comparing genotypes of interest.⁹² Two large European studies (N=261991; 54604) using a Mendelian randomization approach to explore causal effects of long-term alcohol consumption on IHD and cardiovascular risk factors (body mass index, blood pressure, interleukin, and lipid levels) by assessing variants of the alcohol dehydrogenase (ADH1B and ADH1C) gene reported adverse effects of alcohol consumption on the cardiovascular risk profile and an increased risk of IHD.^{93,94} Further evidence by Yun et al⁹⁵ reports detrimental effects of alcohol consumption on coronary calcification in the Korean population. These studies stand in contrast to the epidemiological investigations.

Public Health Perspectives

Because alcohol is a ubiquitous part of culture, its ever-increasing consumption today because of industrialization and globalization also increases the adverse effects associated with its intake.¹³ Alcohol consumption in excessive amounts can lead to social and personal ramifications not only for individuals, but also for the population as a whole.⁹⁶ Intoxication still remains a major factor in adverse events such as car crashes and domestic violence, both of which are major problems for public health.^{97,98} Rehm et al¹³ explain how some diseases are causally linked to alcohol and would not exist if alcohol was not a contributor to our day-to-day lives. These include alcoholic liver disease, alcohol use disorder, and pancreatitis induced by alcohol. Further analysis by this group on alcohol-associated detriments finds that in 2004, 3.8% of global mortality was alcohol-associated, greater for men (6.3%) than for women (1.1%). They report the cause of these deaths to be from injury, cancer, liver cirrhosis, and cardiovascular diseases. Surprisingly, the authors find that almost all preventable deaths were from the cardiovascular category. However, this does not take away from the fact that, on average, alcohol is detrimentally linked to many diseases and increases the global disease burden.¹³

Recommendations for Wine Consumption

The most comprehensive manual for healthcare professionals is the WHO guide for hazardous and harmful drinking.¹⁸ It provides healthcare professionals a day-to-day guide on dealing with patients who are more inclined to abuse alcohol. Important sections include concepts and terms related to the use and abuse of alcohol, intervention guidelines, 4 zones of risk management, definition of a

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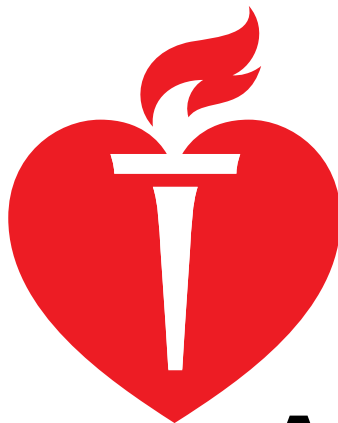
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Red wine: A drink to your heart

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ABSTRACT

Mortality and morbidity are still high in cardiovascular disease (CVD). Myocardial ischemia reperfusion injury leading to myocardial infarction is one of the most frequent causes of the death in humans. Atherosclerosis and generation of reactive oxygen species through oxidative stress is the major risk factor for CVD. From the literature collection, it has been identified that moderate consumption of red wine helps in preventing CVD through several mechanisms, including increasing the high-density lipoprotein cholesterol plasma levels, decreasing platelet aggregation, by antioxidant effects, and by restoration of endothelial function. The aim of this review is to discuss the accumulating evidence that suggests that red wine possesses a diverse range of biological actions and may be beneficial in the prevention of CVD.

Key words: Alcohol, flavonoids, grape juice, polyphenols, resveratrol, wine research

INTRODUCTION

Since ancient times, cardiovascular disease (CVD) has become a known, life-threatening problem for the world. The risk factors and higher mortality from CVD have been proved without doubt from the well-developed countries of Western Europe, North America and East Asia, as well as for the vast majority of developing countries and even the large urban centers of sub-Saharan Africa.^[1] The highest majority of risk factors for this overall mortality are industrial exposure according to their profession, changing dietary habits, lifestyle and increasing obesity. Moreover, tobacco smoking is highly prevalent and risk factors for atherosclerosis tend to occur earlier in life, accounting for earlier presentation of CVD events.^[1]

CVD is a leading cause of mortality and is responsible for one-third of the global deaths. Nearly 85% of the global mortality and disease burden from CVD is borne by low- and middle-income countries. In India, for example, approximately 53% of the CVD deaths are in people younger than 70 years of age; in China, the corresponding figure is 35%. The majority of the estimated 32 million heart attacks and strokes that occur every year are caused by one or more of the following cardiovascular risk factors – hypertension, diabetes, smoking, high levels of blood lipids and physical inactivity – and most of these CVD events are preventable if meaningful action is taken against these risk factors.^[2] The prevalence of coronary artery disease (CAD) in urban North India varies from 7% to 10%^[3,4] compared with 3% in USA.^[5] The CAD rates in South India are two-folds higher than that in North India, with Kerala reporting 14% in urban and 7% in rural Thiruvananthapuram.^[6,7]

A recent report from the World Health Organization (WHO) stated that mortality from CVD in countries of Southern Asia, including India, Pakistan and Bangladesh, is not as high as that of Central Asian countries, but is significantly higher than that of East Asian countries.^[8]

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Large variation, however, likely exists within these countries between the urban and nonurban populations.

Epidemiological studies have shown that consumption of foods and beverages rich in natural polyphenols, including those presented in grape fruit, vegetables, tea or red wine, is associated with lower incidence of CVDs and, especially, with ischemic heart disease.^[9,10] Moderate wine consumption markedly decreased the cardiovascular and cerebrovascular ischemic events, which has been proven by many epidemiological studies. Red wine may exert its effect by different mechanisms, such as the ability to raise the high-density lipoprotein (HDL) levels, to increase the antioxidant plasmatic potential, to improve endothelium-dependent vasodilation and to inhibit platelet aggregation and leukocyte adhesion [Figure 1]. Particularly, nonalcoholized red wine has a protective mechanism due to its active components like polyphenols, quercetin and resveratrol. The protective mechanism of these components was already proven by many human and animal studies.^[11] The present review is aimed at compiling data based on the reported works on red wine and the promising active principles of red wine to prevent CVDs.

RED WINE: A POTENT ANTIOXIDANT

For many years, the emphasis has been on the relationship between serum total cholesterol levels and the risk of CVD. However, the focus has recently shifted to oxidative stress induced by reactive oxygen species (ROS) and nitrogen-reactive species as important key players in the etiology and pathogenesis of various chronic diseases, including CVD.^[12] Antioxidant nutrients are believed to slow down the progression of atherosclerosis due to their ability to inhibit the damaging oxidative processes.^[13,14] Epidemiological and prospective studies have shown that consumption of antioxidant vitamins such as vitamin E

and β -carotene could reduce the risk of CVD.^[15] Clinical trials also suggest a reduced risk of CVD with vitamin E supplementation.^[16] The protective effect of vitamin E can be ascribed to its antioxidant properties. Observations that men and women with CVD show lower levels of circulating antioxidants have led scientists to support the proposed protective role of antioxidants in the prevention and management of CVD.^[13] Red wine-active principles like red wine polyphenols, resveratrol and quercetin have experimental cardioprotective properties^[17] and may counter one of the mechanisms underlying its antioxidant potential. The cardioprotective properties of individual red wine components are discussed below.

Red wine polyphenols

Many research and epidemiological studies have shown that intake of polyphenols as grape juice and red wine is associated with a reduced risk of CVD. The most active polyphenol present in red wine is flavonoids, and it is important due to its putative antioxidant properties. The cardiovascular benefits of red wine flavonoids are explained in the French Paradox phenomenon as well as in the Mediterranean diet.^[10]

Several studies have documented a protective role of moderate wine consumption (15.5–31 g alcohol/day) in both vascular and nonvascular diseases. Different mechanisms may be responsible for these beneficial effects, including increases in the HDL-cholesterol plasma levels, decreased platelet aggregation, antioxidant effects and restoration of endothelial function by flavonoids. Numerous cross-sectional, observational and controlled studies reveal a range of red wine effects on the different aspects related to CVD. In a recent research, it has been reported that red wine elicits different metabolic, autonomic and endothelial responses among individuals with hypercholesterolemia or arterial hypertension and healthy controls.^[18]

Chronic administration of moderate amounts of red wine has been associated with a protective effect on the cardiovascular system.^[19] Impaired endothelium-dependent relaxation in both animals and humans is playing a major role in the development of CVD, such as atherosclerosis and hypertension.^[20] Generation of ROS is one of the factors for endothelium dysfunction, particularly superoxide anions, which reduce the bioavailability of nitric oxide (NO).^[21–24] Although red wine polyphenols have antihypertensive properties, the possibility that they prevent the oxidative stress-induced endothelial dysfunction remains to be determined. In one research, it has been

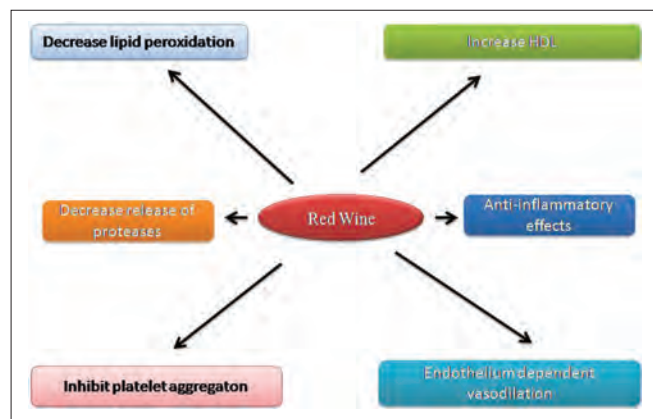


Figure 1: Cardioprotective action of red wine

reported that intake of red wine polyphenols prevents Angiotensin (Ang) II-induced hypertension and endothelial dysfunction. Prevention of vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidase induction and preservation of arterial NO availability during Ang II administration likely contribute to this effect.^[19] Red wine polyphenols and a grape skin extract also reduced the blood pressure in N^G-nitro-L-arginine methyl ester (L-NAME) and desoxycorticosterone acetate (DOCA) salt-induced hypertensive rats.^[25,26]

In another study, it has been reported that chronic administration of resveratrol (a red wine polyphenol) enhances the endothelium-dependent relaxation in spontaneously hypertensive rats, and the protective action might be due to an increase in the bioavailability of NO.^[27] In recent research, by using female spontaneous hypertensive rats, it was reported that chronic administration of red wine polyphenols brings about a reduction in blood pressure and vascular dysfunction through reduction in vascular oxidative stress.^[28]

Inflammation plays a vital role in the pathogenesis of atherosclerosis, which is a known risk factor for CVD. High levels of fibrinogen and C-reactive protein (CRP), both markers of inflammation, are associated with a risk of developing CVD. In one randomized controlled crossover trial, it has been reported that red wine consumption markedly decreases the level of fibrinogen, but it does not have any effect on the CRP level.^[29]

The effects of short-term oral administration of red wine polyphenolic compounds (20 mg/kg/day for 7 days) on the hemodynamics, *ex vivo* cardiac responsiveness and ischemia reperfusion injury were investigated in rats. From this study, it has been concluded that short-term treatment with red wine polyphenols decreases blood pressure and cardiac responsiveness and protects against postischemic infarction via decreased oxidative stress. All the above effects of red wine polyphenols are sensitive to NO synthase inhibition, which implies an involvement of the NO-dependent pathway. This study suggests a basis for the beneficial effects of red wine against CVD.^[9] The same research group already reported that the *in vivo* cardiovascular action of red wine and also the oral administration of red wine polyphenols was able to produce a decrease in blood pressure in normotensive rats.^[9,29] This hemodynamic effect was associated with an enhanced endothelium-dependent relaxation and induction of the expression of inducible NO synthase and cyclooxygenase 2 within the arterial wall. Moreover, red wine polyphenols accelerated the regression of blood pressure and improved the structural

and functional cardiovascular changes, including cardiac fibrosis, in hypertensive rats.^[25,30]

In another study, it has been reported that red wine polyphenolic compounds exert a powerful protective effect on the endothelial cells from the injury caused by carbon tetrachloride (CCl₄). This effect was documented by decreased endothelium, with corresponded to the diminished endothelial cell swelling and detachment evaluated by histology of the vascular intima.^[31] The endothelium-protective effect may be one of the key factors that contribute to the preventive action of red wine on CVDs. Hozumi *et al.*^[32] reported that daily intake of red wine polyphenols may benefit patients with or without CVD by increasing the coronary microcirculation. In patients with CAD, 250 ml of de-alcoholized Greek red wine decreased the arterial stiffness and improved the augmentation index, as derived from arterial wave reflection patterns. A similar dose of de-alcoholized red wine decreased the adverse postsmoking arterial wave reflections and lessened the rise in systolic blood pressure. Brachial artery flow-mediated vasodilation was improved by 250–500 ml of de-alcoholized red wine.^[33]

Several epidemiological studies suggest that moderate alcohol intake, especially red wine, decreases cardiac mortality due to atherosclerosis. The alcohol effect is described by a J curve, suggesting that moderate drinkers may benefit while abstainers and heavy drinkers are at higher risk.^[34] Wine drinkers have higher HDL levels than that of nonwine drinkers. The ingestion of red wine is associated with an increase in the antioxidant activity in the serum, an increase in apolipoprotein A-1 and a decrease in the atherogenic agent lipoprotein (a), mainly due to the presence of flavonoids and stilbenes. It has been further suggested that this increase in the antioxidant activity in patients regularly drinking red wine may be the primary factor inhibiting LDL oxidation, which, in turn, reduces atherosclerotic complications.

RESVERATROL

Interest in this compound has expanded in recent years, when numerous epidemiological studies showed an inverse correlation between red wine consumption and incidence of CVDs. Accumulating evidence indicates that resveratrol may confer a protective action on the cardiovascular system. The cardiovascular benefits of resveratrol may relate to protecting the heart cells from ischemia reperfusion injury, inhibiting platelet aggregation and decreasing plasma triglycerides and cholesterol

accumulation in the aorta. Furthermore, it can also relax the coronary arteries. It seems likely that resveratrol might be partly responsible for the cardiovascular benefits associated with wine consumption.^[30,35] Resveratrol is a potent vasodilator and, in several researches, it has been reported that the vasorelaxant properties of resveratrol might be due to NO-mediated relaxation.^[36] Novakovic *et al.*^[37] reported that resveratrol induces relaxation of the human internal mammary artery (HIMA) rings without endothelium. It seems likely that 4-AP- and margatoxin-sensitive K⁺ channels located in the vascular smooth muscle mediated the relaxation of HIMA produced by resveratrol. In addition, the vasodilator effect of resveratrol through NO-mediated endothelium-dependent relaxation in spontaneous hypertensive rats was also reported.^[27] A separate experiment showed that chronic resveratrol administration enhanced the endothelium-dependent vasodilation in ovariectomized, stroke-prone, spontaneously hypertensive rats.^[38]

Resveratrol shortened the duration of action potential in papillary muscles in normal guinea pig and also decreased the maximal velocity of phase 0 depolarization in partially depolarized papillary muscles. In addition, resveratrol inhibited delayed-after depolarization and triggered activity induced by ouabain and high Ca²⁺ in the papillary muscle of guinea pigs in a dose-dependent manner.^[39-44] In another research, it was found that resveratrol inhibited the spontaneous discharges of neurons in the CA1 area of rat hippocampal slices. These effects were likely due to a decrease of calcium influx.^[45] Zheng *et al.*^[46] reported that resveratrol decreased the intracellular calcium concentration in rat cardiac myocytes. The inhibition of voltage-dependent Ca²⁺ channel and tyrosine kinase and alleviation of Ca²⁺ release from the sarcoplasmic reticulum (SR) are possibly involved in the effects of resveratrol on rat ventricular myocytes. Intake of resveratrol as red wine also increases the production of platelet-dependent NO and, in this way, it decreases the proinflammatory pathway of p38MAPK thus inhibiting ROS production and, ultimately, platelet function. This activity may contribute to the beneficial effects of moderate wine intake on ischemic CVD.^[11]

Resveratrol may exert a protective effect against cell death through many signaling pathways. Hwang *et al.*^[47] reported that resveratrol may exert a protective effect on damage to heart muscle through modulation of the AMP-activated kinase (AMPK) signaling pathway. Resveratrol induced a strong activation of AMPK and inhibited the occurrence of cell death caused by treatment with H₂O₂. Under the same conditions, inhibition of AMPK using dominant

negative AMPK constructs dramatically abolished the effect of resveratrol on cell survival in H₂O₂-treated cardiac muscle cells. These results indicate that resveratrol-induced cell survival is mediated by AMPK in H9c2 cells, and may exert a novel therapeutic effect on oxidative stress induced in cardiac disorders.

Ray *et al.*^[48] reported that resveratrol can ameliorate myocardial ischemia reperfusion injury. In this research, they found that administration of resveratrol to the rat provides cardioprotection by decreasing the oxidative stress generated in the ischemic-reperfused myocardium. The antiischemic effect of resveratrol in another study states that the resveratrol-treated hearts showed better functional recovery at reperfusion and significant vasodilation, but no variation in high-energy phosphates. This suggests that long-term moderate resveratrol consumption could play an important role in late cardioprotective effects.^[49] A preliminary study carried out by the same research group reported that 10 min of resveratrol infusion (10 µM) in Langendorff-perfused rat hearts caused a 40% decrease in the baseline phosphorylation potential without affecting contractility. The level of effluent adenosine was increased by 68%, and paralleled a 50% increase in coronary flow. They suggested that an increase in the adenosine bioavailability is involved in resveratrol-mediated cardioprotection.^[49]

The dose-dependent activity of resveratrol was evaluated by Das *et al.*^[50] by using the ischemic myocardium in rats. The results thus indicate that at, lower doses, resveratrol exerts survival function by upregulating the antiapoptotic and redox proteins Akt and Bcl-2, while at higher doses, it potentiates a death function by downregulating the redox proteins and upregulating the proapoptotic proteins.

In another study, it has been reported that resveratrol prevents leukocyte recruitment and endothelial barrier disruption induced by a number of superoxide-dependent proinflammatory stimuli, including ischemia and reperfusion, hypoxanthine and xanthine oxidase (HX/XO) or platelet activating factor. These salutary effects appear to be related to the antioxidant properties of resveratrol and contribute to the cardioprotective actions associated with the consumption of red wine.^[51]

The protective role of resveratrol in ischemia reperfusion injury is well defined by many researchers. The mitochondrial permeability transition pore (mPTP) opening has been proposed to play an important role in myocardial ischemia/reperfusion injury. The mPTP remains closed during ischemia but opens at the onset of reperfusion, and

modulation of the mPTP opening at early reperfusion can protect the heart from reperfusion injury. Because resveratrol protects the heart through a NO-dependent mechanism, and NO has been demonstrated to prevent the mPTP opening, it is possible that resveratrol could modulate the mPTP opening at reperfusion.^[52]

QUERCETIN

Quercetin is one of the most important flavonoids present in red wine. The antioxidant and protective mechanisms in various ischemic conditions were proved by many researches. It has been reported that quercetin inhibited thrombocyte aggregation^[53] and had an antihypertensive effect through vasodilator action on the vascular smooth muscles.^[54] The studies that focused on the antioxidant efficiency of flavonoids against ischemia/reperfusion (I/R) injury have demonstrated that quercetin possesses robust protective effects in renal, cerebral and hepatic I/R models.^[55-57] Quercetin was also demonstrated to improve the contractile function of the left ventricle in experimental myocardial infarction with subsequent 24-h reperfusion.^[58] Ikizer *et al.* reported that quercetin has the capacity to protect the myocardial tissue against global ischemia and reperfusion injury. In instances where the molecule is administered for the purpose of acute therapy, this cardioprotective effect of a significant degree can be observed, and the protective action might be due to its antioxidant and cytoprotective actions.

CONCLUSION

CVDs are now a current major problem in causing mortality in both Western and developing countries. Oxidative stress associated with atherosclerosis and endothelium-dependent vascular inflammation plays a major role in the development of CVD. Red wine contains antioxidative components like resveratrol, proanthocyanidine, quercetin, etc. and these active components exert protective functions like free radical scavenging effects, decreasing the oxidative stress and reducing the inflammatory atherosclerotic lesion in both animals and humans, which is evident in this review. From these findings, it has been concluded that red wine as a diet supplement might be beneficial for cardiovascular risk factors.

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