Effects of dietary polyphenols on gene expression in human vascular endothelial cells

Sonja K. Nicholson[*], Gregory A. Tucker and John M. Brameld
Division of Nutritional Sciences, University of Nottingham, School of Biosciences, Loughborough LE12 5RD, UK

Previous studies have shown that consumption of fruit and vegetables plays a role in preventing the onset of CVD. These beneficial effects have been linked to the presence of polyphenolic compounds in plant-derived foods and their antioxidant capacity. It has been hypothesised that polyphenols may also have a direct effect on vascular endothelial cell growth and the expression of genes involved in angiogenesis and other roles of the endothelium. Previous studies in this area have tended to use concentrations of polyphenols that are supraphysiological (1–100 μM). The effects of more physiological concentrations (0·1 μM) of various individual polyphenols on gene expression were therefore investigated in cultured human umbilical vein endothelial cells (HUVEC) using both microarray and quantitative RT–PCR methodologies. Treatment of HUVEC with ferulic acid, quercetin or resveratrol (0·1 μM) resulted in changes to gene expression that for the three treatments amounted to significant (>2-fold) down-regulation of the expression of 363 genes and significant (>2-fold) up-regulation of 233 genes of the 10 000 genes present on the microarray. The majority of these genes were affected by resveratrol. Quantitative RT–PCR studies indicated that resveratrol (0·1 μM) significantly increased the expression of the gene encoding endothelial NO synthase (eNOS), which synthesises the vasodilator molecule NO, and both resveratrol and quercetin decreased expression of the potent vasoconstrictor, endothelin-1 (ET-1), while ferulic acid had no effect. The effects of resveratrol (0·1 μM) were also investigated when HUVEC were under oxidative stress following treatment with H_2O_2 (0–50 μM), which dose-dependently increased expression of eNOS and ET-1. Resveratrol stimulated eNOS mRNA in the absence of H_2O_2 and still allowed the increase with H_2O_2, but the effects were not additive. In contrast, resveratrol blocked the stimulatory effect of H_2O_2 on ET-1 expression. Hence, resveratrol has potent effects at a physiological concentration (0·1 μM) that would be expected to result in vasodilation and therefore help reduce blood pressure and the risk of CVD.

Polyphenols: Human umbilical vein endothelial cells: Gene expression

In the UK there was a dramatic increase in the incidence rates of CVD such as CHD and atherosclerosis during the 20th century up to the end of the 1970s[1]. Although the numbers of deaths from these diseases are currently decreasing within the UK population, CVD are still the main cause of death in the UK, and the incidence rates of CVD are on the increase worldwide. In 2005 CVD was the main cause of morbidity in the UK, resulting in 208 229 deaths, with CHD accounting for 48·5% of these cases[1]. Thus, efforts are being made to identify methods for both the prevention and the treatment of CVD to further reduce the rate of incidence of these diseases within both the UK population and worldwide.

CVD refers to a group of diseases affecting the heart and/or blood vessels. These diseases usually result from arterial damage, therefore the symptoms and treatment of these diseases are dependent on the set of arteries affected[2]. The three types of arteries predominantly affected by CVD include coronary arteries (CHD), cerebral arteries (stroke) and peripheral arteries (tissue death and gangrene)[2]. CHD and stroke are the two most important CVD and are responsible for the greatest number of deaths among the UK population[1]. CHD begins with inflammation of the wall of blood vessels, which then narrow, leading to angina pectoris[3]. In later stages of this disease blood clots obstruct arteries and severe myocardial

---

ischaemia results in a myocardial infarction (heart attack). In extreme cases of CHD heart failure may occur as a result of deteriorated strength of the heart muscle to pump blood around the body[3].

There are also other illnesses that may develop in association with these three main CVD, such as hypertension, cardiomegaly, cardiomyopathy and cardiac arrest[1]. There are numerous risk factors that predispose individuals to CVD, including obesity, high blood pressure, age, gender, tobacco smoking and diabetes mellitus[4]. Treatments for CVD are dependent on the type of disease, but effective treatments always include prevention of risk factors such as low-fat, low-energy and low-Na diets, reduction in alcohol intake and regular cardiovascular exercise[4].

The inclusion of plant products such as fruit and vegetables in the diet has long been associated with numerous health benefits, which has led the Department of Health to recommend the intake of five portions of fruit and vegetables daily[5]. Early studies addressing the issue of prevention methods for CVD have shown that the consumption of fruit and vegetables plays a role in preventing the onset of CVD such as atherosclerosis, IHD and CHD[6,7]. Epidemiological studies have also shown an association between the intake of teas, particularly green teas (*Camillia sinensis*), and a decrease in CHD[8,9]. There is also evidence that moderate consumption of other plant-based products such as wine provide cardiovascular protection against disease and offer antiproliferative, anti-inflammatory and antioxidant activity *in vitro* and *in vivo*[9].

Early assumptions

The beneficial effects of fruit and vegetables on reducing CVD onset and in CVD treatment were originally attributed to the presence of the antioxidant vitamins C and E, which were believed to act as free radical scavengers. However, many intervention studies have failed to find any association between the beneficial effects observed with fruit and vegetable intake and vitamins C and E[10]. Other studies that have looked at combined supplements of the antioxidant vitamins C and E and β-carotene have also found insufficient correlations between the intake of these vitamins and reduced CVD[11].

Thus, researchers began to look at other compounds present in plants that may be responsible for the beneficial effects of fruit and vegetables on CVD. Numerous compounds such as vitamins, amino acids, polyphenols, alkaloids and lectins present in plant foods have been found to have health-beneficial properties. It was suggested that polyphenols present in plants are the main components responsible for the cardiovascular health benefits conveyed by fruit and vegetables, initially because of their antioxidant properties. Further study has now demonstrated that in addition to their antioxidant properties polyphenols have antiangiogenic and antiproliferative activity, inhibit inflammatory mediators and down regulate the expression of transcription factors and genes involved in hypertension[12].

Polyphenolic compounds

Polyphenols are natural products found in a diverse range of plants that arise through one of two metabolic pathways: the 'shikimate pathway', which provides phenylpropanoids, hydroxycinnamic acids and coumarins; the 'polyketide pathway' (acetate pathway)[12]. They are defined chemically as substances that have an aromatic ring with a hydroxyl substituent, including esters and glycosides (functional derivatives). Most polyphenols possess two or three hydroxyl substituents, and are derived from a common dihydric or trihydric polyphenolic compound[13]. Thus, because of the diverse range of their chemical structures polyphenols are grouped into different classes, depending on the number of phenol rings they possess and the type and number of structural elements binding the phenolic rings together[14]. The classes include simple phenolic acids (e.g. ferulic and gallic acid; Fig. 1) and stilbenes (e.g. resveratrol; Fig. 1) and more complex chalcones (e.g. phloridzin; Fig. 1) and flavonoids, with the latter being split into seven subclasses that include flavonols (e.g. quercetin; Fig. 1), flavanols (e.g. epigallocatechin gallate; Fig. 1), flavones, flavanones, flavanonols, isoflavones and anthocyanins[12].

Polyphenols thought to provide health benefits include resveratrol, quercetin, ferulic acid, epicatechin gallate and epigallocatechin gallate, all of which belong to different classes of polyphenol (Fig. 1). Resveratrol is found in the skin of red grapes, wine, apples (*Malus domestica*), peanuts (*Arachis hypogaea*), blueberries (*Vaccinium* spp.) and cranberries (*Vaccinium oxycoccus*), and has been shown to provide antiangiogenic, anti-inflammatory and antiviral properties, as well as inducing cell death by apoptosis[15]. Quercetin is found in apples, tea, onions (*Allium cepa*), citrus fruit, broccoli (*Brassica oleracea*) and cherries (*Prunus avium and Prunus cerasus*), and has been shown to possess anti-inflammatory, antioxidant and antitumour properties[16]. Quercetin has also been suggested to act as a mast cell inhibitor, which may be useful in the treatment of chronic prostatitis[17]. Ferulic acid, a hydroxycinnamic acid, is found in cereal grains such as rice, wheat and oats, and in coffee (*Coffea canephora* and *Coffea arabica*) beans, apples, artichoke (*Cynara scolymus*), peanuts, oranges (*Citrus sinensis*) and pineapples (*Ananas comosus*). Ferulic acid is reported to display pro-apoptopic effects in cancer cells, along with antitumour activity against breast cancer[18]. It is also a powerful antioxidant, and may scavenge oxygen free radicals, provide protection of DNA against oxidative damage and increase intracellular cAMP and cGMP[19]. Epigallocatechin gallate is a major component of green tea and other teas, and also cocoa (*Theobroma cacao*) beans. Epigallocatechin gallate has been shown to interfere with numerous enzyme systems and can protect DNA from UV damage, and provide protection against breast cancer and other cancers[20]. Epigallocatechin gallate may also act as a nutritional chemopreventive agent for the treatment of cancer, atherosclerosis and neurodegenerative diseases. It selectively induces apoptosis in human carcinoma cell lines[21] and is thought to be involved in the suppression of vascular endothelial growth factor and angiogenesis[22].
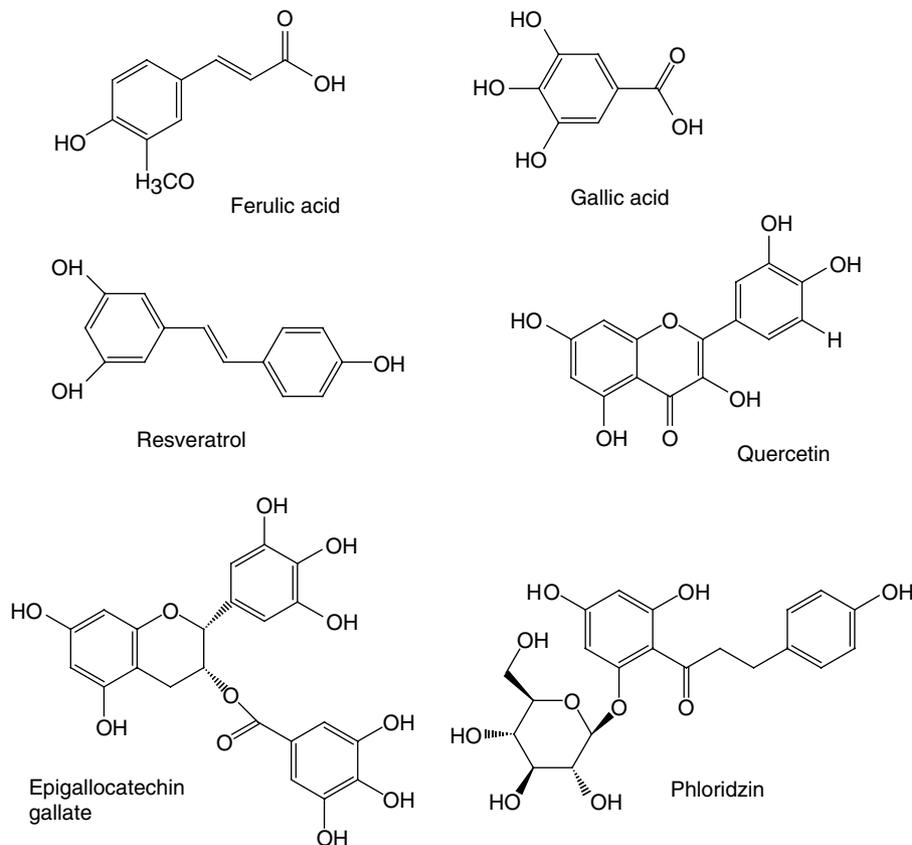
Fig. 1. Chemical structures of selected polyphenols.

The mechanism of action for the beneficial effects of polyphenols on CVD was previously thought to be associated with their antioxidant activity[23]. However, many polyphenols are metabolised in the body and these metabolites have much lower antioxidant capacities than their parent compounds, suggesting that antioxidant activity may not be their only mechanism of action[12]. Polyphenols present in red wine have been reported to reduce the incidence of CVD through protection against oxidative damage and reduced expression of transcription factors responsible for the activation, induction or control of genes involved in vasoconstriction.

Vascular endothelial cells

Vascular endothelial cells are flat elongated cells that contain a single nucleus and have a width of 1–20 μm and a depth of 0·2–20 μm[24]. Endothelial cells are the only cell type known to contain Weibel palade bodies, in addition to the other cytoplasmic organelles common to most cells including the golgi and mitochondria[25]. Weibel palade bodies are the site of storage of Von Willebrand factor, a glycoprotein carrier of clotting factor VIII, which is required for the maintenance of haemostasis through the conversion of prothrombin to thrombin[26]. The endothelium is composed of a single layer of endothelial cells joined together by one of three types of intercellular junctions (tight, gap and close junctions) determined by the distance between adjacent cells[25]. The endothelium has an internal coating of anionic biopolymers, the glycocalyx, and forms the inner lining of blood vessels[27]. The functions of the endothelium include:

1. acting as a semi-permeable barrier for the exchange of macromolecules, fluid and electrolytes between intracellular and extracellular spaces[24].
2. site of synthesis of vasoactive substances such as endothelial NO synthase (eNOS), prostacyclin and endothelin-1 (ET-1), which lead to relaxation (eNOS and prostacyclin) or constriction (ET-1) of smooth muscle cells, thereby regulating blood pressure.
3. maintenance of blood coagulation and the inflammatory response, through the transport of clotting factor VIII and the secretion of endothelium-derived hyperpolarising factor, in response to alterations in blood flow-rate. It is also the site of production of adhesion molecules to capture and transport circulating leucocytes to affected tissues[28].
4. initiation site for formation of new blood vessels, a process termed 'angiogenesis', which is required for normal tissue growth and wound repair; uncontrolled angiogenesis is a major component of tumour growth, with prevention of angiogenesis being an important target for cancer drugs[24].

Vascular endothelial genes and cardiovascular health

The expression of specific genes in vascular endothelial cells plays an important role in the functions of the

endothelium, and therefore cardiovascular health, through the effects on the formation of new blood vessels during angiogenesis or the regulation of blood pressure[29]. For example, vascular endothelial growth factor and pigment epithelium-derived factor are proteins produced by endothelial cells that stimulate and inhibit angiogenesis respectively. Vascular endothelial growth factor is an important signalling protein secreted by the endothelium for the induction of vascular permeability and the initiation of the processes of angiogenesis and vasculogenesis[30].

Other genes expressed by vascular endothelial cells are involved in the synthesis of vasoactive substances, including eNOS and ET-1. The membrane-bound enzyme eNOS cleaves NO from L-arginine in response to stress exerted on the endothelium by blood flow and stimulants such as acetylcholine and Ca–calmodulin complexes. NO is a soluble lypophilic gas that is freely diffusible and produced continuously by the endothelium[26]. It diffuses into vascular smooth muscle cells to react with the haem group in guanylyl cyclase, leading to the formation of cGMP and relaxation of vascular endothelial cells[28]. Endothelial-derived NO also inhibits atherogenesis by reducing the expression of genes such as monocyte chemoattractant protein-1, P-selectin and the adhesion molecules CD11 + CD18[31].

ET-1, the main isoform of the peptide endothelin, is synthesised as a preprohormone consisting of 212 amino acids (preproET-1), which is post-translationally cleaved to produce the active vasoconstrictor peptide ET-1 (consisting of twenty-one amino acids). ET-1 predominantly binds to endothelin A and endothelin B receptors on smooth muscle cells resulting in the vasoconstriction of smooth muscle. However, ET-1 can also bind to endothelin B receptors on neighbouring endothelial cells to cause vasodilation by increasing NO production[32].

Bioavailability and effects of polyphenols on gene expression

Polyphenolic compounds have been shown to have a wide range of effects on the expression of numerous genes within the human body, as determined by both *in vitro* and *in vivo* experiments[33,34]. Polyphenols such as resveratrol and quercetin, both found in abundant quantities in red wine, are thought to be responsible for the health-beneficial effects of red wine, also termed 'the French paradox'[35].

However, studies of the bioavailability and blood concentrations of polyphenols indicate that many of the published *in vitro* studies showing health benefits have used concentrations (1–100 μM) much higher than those achievable in the body. For example, plasma concentrations of resveratrol are approximately 20 nM and peak plasma levels of resveratrol and its metabolites are approximately 2 μM following consumption by six healthy patients of approximately 25 mg resveratrol in a suspension of simple syrup followed by 250 ml water[36].

Hence, *in vitro* experiments at Nottingham using cultured human umbilical vein endothelial cells (HUVEC) have studied a more-physiologically-relevant concentration of 0·1 μM, followed by measurement of gene expression by
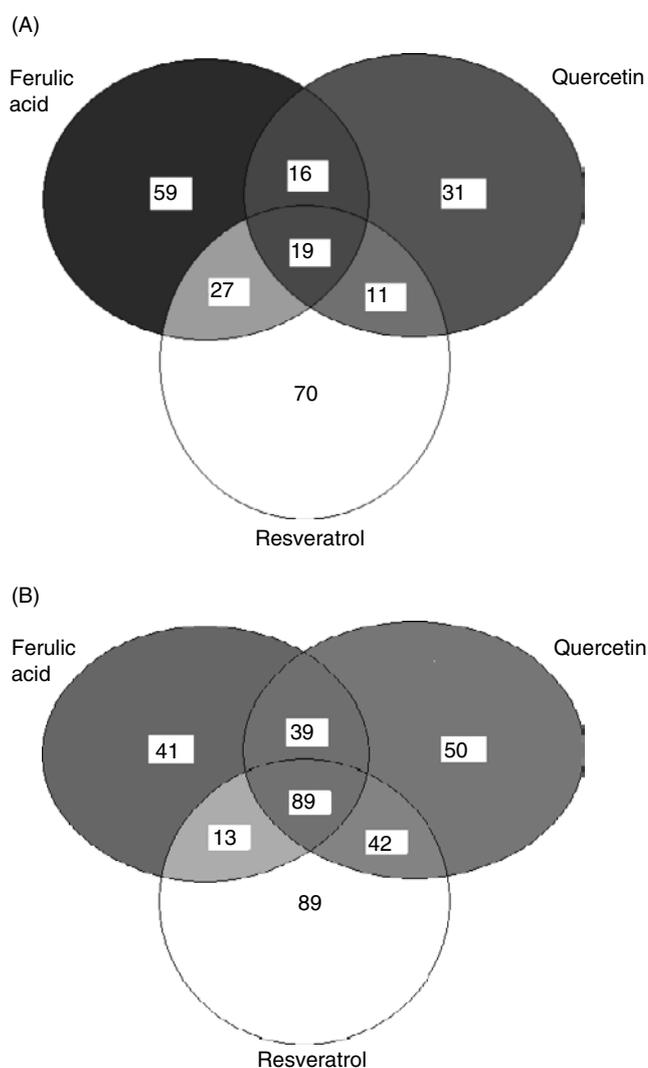
Fig. 2. In human umbilical vein endothelial cells treated with 0·1 μM-polyphenol (ferulic acid, quercetin or resveratrol) for 24 h at 37°C a total of 233 genes were up regulated (A) and 363 genes were down regulated (B).

microarray and quantitative RT–PCR. The microarray study used in-house printed 10K human slides to investigate the effects of ferulic acid, quercetin and resveratrol (all at 0·1 μM) on gene expression in HUVEC. The results show that overall these three polyphenolic compounds cause a significant (>2-fold) increase in the expression of 233 genes (2 % of the genes; Fig. 2(A)) and a decrease in expression of 363 genes (approximately 4 % of the genes; Fig. 2(B)). The majority of genes affected were found to respond to treatment with resveratrol, with fewer genes being affected only by quercetin or ferulic acid. Previous studies have also reported the ability of red wine polyphenols, particularly resveratrol, to alter gene expression in rat aortas[33].

Bioinformatic analyses of the genes up regulated or down regulated by treatment with the individual polyphenols have indicated that most of the genes are involved in transport, enzyme activity, cell signalling or transcription, accounting for 46 % of the 233 genes significantly up

Table 1. Effects of treatment with three individual polyphenols at a physiologically-relevant concentration (0·1 μM) on the expression of two candidate genes involved in the regulation of blood pressure in cultured human umbilical vein endothelial cells after treatment for 24 h relative to control

	RT–PCR data†		
	Ferulic acid	Quercetin	Resveratrol
Endothelial NO synthase	↔	↔	↑ 1·62 × *
Endothelin-1	↔	↓2·57 × **	↓ 2·62 × **

↔, No effect; ↓, decreased expression; ↑, increased expression.
*$P<0.05$, ** $P<0.001$ (Dunnett's test).
†All RT–PCR treatments were normalised to glyceraldehyde-3-phosphate dehydrogenase.

regulated and 30 % of the 363 genes significantly down regulated (SK Nicholson, GA Tucker and JB Brameld, unpublished results). Encouragingly, because preliminary studies have confirmed that polyphenol treatment at 0·1 μM does not lead to increased cell death, very few of the genes significantly up regulated or down regulated (2 % and approximately 4 % respectively) are involved in DNA repair or apoptosis. Microarray analysis has shown that resveratrol is the only polyphenol of the three tested that reduces ET-1 gene expression by ≥ 2-fold. As indicated earlier, ET-1 is a potent vasoconstrictor and is also involved in angiogenesis[32].

Further studies using more quantitative measures of gene expression (real-time PCR, Taqman) have examined the effects of physiological concentrations (0·1 μM) of polyphenols on the expression of three candidate genes involved in the regulation of blood pressure and endothelial function (eNOS, ET-1 and vascular endothelial growth factor). Resveratrol (0·1 μM) was found to significantly increase expression of eNOS in HUVEC following 24 h treatment. However, ferulic acid and quercetin at equimolar concentrations have no effect (Table 1; SK Nicholson, GA Tucker and JB Brameld, unpublished results). A previous study has also found that resveratol at concentrations of 1–33 μM increases the expression of eNOS in HUVEC-derived hy 926 cells, but equimolar concentrations of ferulic acid have no effect[31]. Red wine extracts containing polyphenols have also been reported to increase mRNA and protein expression of eNOS[37].

Other genes involved in blood pressure regulation and in cardiovascular health such as ET-1 are also affected by polyphenols and polyphenolic-containing foods. Transcription and synthesis of ET-1 are significantly decreased in bovine aorta endothelial cells treated with a red wine extract (6·25–25 μg/ml) containing polyphenols[38]. The present authors' studies have found that expression of ET-1 mRNA in HUVEC decreases by 2·62-fold and 2·57-fold following 24 h treatment with 0·1 μM resveratrol and quercetin respectively. However, ferulic acid at 0·1 μM has no effect on ET-1 mRNA expression (Table 1; SK Nicholson, GA Tucker and JB Brameld. unpublished results).

Several other polyphenolic compounds have also been tested for their effects on these three genes, including caffeic acid, genistein, phloretin and phloridzin, but none of these compounds were shown to have any significant effect (SK Nicholson, GA Tucker and JB Brameld, unpublished results).

Effects of polyphenols on basal and stress-induced gene expression

Oxidative stress is known to elevate the expression of certain genes in several cell types including endothelial cells (HUVEC) and smooth muscle cells[39]. Different methods designed to put cells under oxidative stress or to elevate gene expression by the addition of a stimulating agent include cyclic strain methods[39], treatment with H_2O_2[39] and the addition of angiotensin II[40].

It has been shown that expression of eNOS and ET-1 mRNA in HUVEC increases in a dose-dependent manner following H_2O_2 (0–50 μM) treatment (SK Nicholson, GA Tucker and JB Brameld, unpublished results). Maximal responses relative to the control are 2·4- and 2·2-fold for eNOS and ET-1 mRNA respectively at 50 μM-H_2O_2. The addition of 0·1 μM-resveratrol increases eNOS to a similar extent (1·98-fold) but is not additive to the effects of the H_2O_2 treatment. On the other hand, resveratrol blocks the stimulatory effects of H_2O_2 on ET-1 mRNA expression (SK Nicholson, GA Tucker and JB Brameld, unpublished results). Previously published studies have found that resveratol (at 100 μM) blocks the stimulatory effect of H_2O_2 (25 μM) on ET-1 mRNA expression in HUVEC; while concentrations of 1–100 μM-resveratrol reduce ET-1 mRNA expression elevated following cyclic strain[39]. Other studies have also shown that resveratrol (1–100 μM) has a similar effect in reducing elevated ET-1 expression in rat aorta smooth muscle cells induced by angiotensin II[40]. Thus, the ability of resveratrol to reduce elevated gene expression is not specific to one cell type, but importantly has now been demonstrated at a more-physiologically-relevant concentration.

Conclusion

In the present author's studies resveratrol has been found to be the only polyphenol that significantly affects both eNOS and ET-1 expression in endothelial cells at a potentially physiological concentration (0·1 μM). The ability of resveratrol to increase the expression of the gene encoding eNOS (the enzyme synthesising the vasodilator NO) and reduce expression of the gene encoding the vasoconstrictor ET-1 would be expected to result in a potent vasodilation and thereby reduce blood pressure. Of the polyphenols tested to date, only quercetin has any similar effects to resveratrol, in that it appears to reduce ET-1 mRNA expression, but this finding needs to be confirmed. The reasons for the potency of resveratrol and the mechanisms involved in its action remain unclear. Importantly, resveratrol, at a physiological concentration, has been found to have beneficial effects on endothelial gene expression, and these effects are still apparent in cells under oxidative stress. Hence, resveratrol may be an important component of the diet in terms of reducing blood pressure and risk of CVD.

References

1. Allender S, Peto V, Scarborough P, Boxer A & Rayner M (2007) *Coronary Heart Disease Statistics*. London: BHF.

2. Vander A, Sherman J & Luciano D (1998) *Human Physiology: The Mechanisms of Body Function*, 7th ed., pp. 444--448. Columbus, OH: McGraw-Hill.

3. Guyton AC & Hall JE (2006) *Textbook of Medical Physiology*, 9th ed., pp. 252–253. Philadelphia, PA: Elsevier Inc.

4. De Backer G, Ambrosionie E, Borch-Johnsen K *et al.* (2003) European guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J* **24**, 1601–1610.

5. Department of Health (2003) 5 A DAY health benefits. http://www.dh.gov.uk/en/Policyandguidance/Healthandsocialcaretopics/FiveADay/FiveADaygeneralinformation/DH_4002343

6. Brat P, Georgé S, Bellamy A, Du Chaffaut L, Scalbert A, Mennen L, Arnault N & Amiot MJ (2006) Daily polyphenol intake in France from fruit and vegetables. *J Nutr* **136**, 2368–2373.

7. Hertog MG, Feskens EJ & Kromhout D (1997) Antioxidant flavonols and coronary heart disease risk. *Lancet* **349**, 699.

8. Zhao B, Guo Q & Xin W (2001) Free radical scavenging by green tea polyphenols. In *Flavonoids and Other Polyphenols. Methods in Enzymology*, vol. 335, pp. 217–231 [L Packer and H Sies, editors]. London: Academic Press.

9. Manach C, Mazur A & Scalbert A (2005) Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol* **16**, 77–84.

10. Muntwyler J, Hennekens CH, Manson JE, Buring JE & Gaziano JM (2002) Vitamin supplement use in a low-risk population of US male physicians and subsequent cardiovascular mortality. *Arch Int Med* **162**, 1472–1476.

11. Heart Protection Study Collaborative Group (2002) MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomized placebo-controlled trial. *Lancet* **360**, 23–33.

12. Hakkinen S (2000) Flavonols and phenolic acids in berries. PhD Thesis, Kuopio University, Finland.

13. Dey PM & Harborne JB (editors) (1989) *Methods in Plant Biochemistry*, vol. 1: *Plant Phenolics*, p. 3. London: Academic Press.

14. Manach C, Scalbert A, Morand C, Remesy C & Jimenez L (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**, 727–747.

15. Signorelli P & Ghidoni R (2005) Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. *J Nutr Biochem* **16**, 449–466.

16. Yance DR & Sagar SM (2006) Targeting angiogenesis with integrative cancer therapies. *Integrative Cancer Therapies* **5**, 9–29.

17. Shoskes DA, Zeitlin SI, Shahed A & Rajfer J (1999) Quercetin in men with category III chronic prostatitis: a preliminary prospective, double blind, placebo-controlled trial. *Urology* **54**, 960–963.

18. Lee YS (2005) Role of NADPH oxidase-mediated generation of reactive oxygen species in the mechanism of apoptosis induced by phenolic acids in HepG2 human hepatoma cells. *Arch Pharm Res* **28**, 1183–1189.

19. Sudheer A, Muthukumaran S, Kalpana C, Srinivasan M & Menon VP (2007) Protective effect of ferulic acid on nicotine-induced DNA damage and cellular changes in cultured rat peripheral blood lymphocytes: A comparison with *N*-acetylcysteine. *Toxicol In Vitro* **21**, 576–585.

20. Guo S, Yang S, Taylor C & Sonenshein GE (2005) Green tea polyphenol epigallocatechin-3 gallate (EGCG) affects gene expression of breast cancer cells transformed by the carcinogen 7,12-dimethylbenz[*a*]anthracene. *J Nutr* **135**, 2978S–2986S.

21. Borska S, Gebarowska E, Wysocka T, Drag-Zalesińska M & Zabel M (2003) Induction of apoptosis by EGCG in selected tumour cell lines *in vitro*. *Folia Histochem Cytobiol* **41**, 229–232.

22. Tosetti F, Ferrari N, De Flora S & Albini A (2002) Angioprevention: angiogenesis is a common and key target for cancer chemoprevention. *FASEB Journal* **16**, 2–14.

23. Zern TL and Fernandez ML (2005) Cardioprotective effects of dietary polyphenols. *J Nutr* **135**, 2291–2294.

24. Klabunde RE (2005) *Cardiovascular Physiology Concepts*. Philadelphia, PA: Lippencott, Williams and Wilkins.

25. Vickers J (1995) Endothelial cells and platelet function. PhD Thesis, University of Nottingham.

26. Fox SI (2004) *Human Physiology*, 9th ed. Maidenhead, Berks.: McGraw-Hill Higher Education.

27. Levick JR (editor) (2004) *An Introduction to Cardiovascular Physiology*, 4th ed., pp. 131–132. London: Arnold Publishing.

28. Sherwood L (2003) *Fundamentals of Physiology; A Human Perspective*, 3rd ed. London: Thomson, Brooks, Cole.

29. Ambra R, Rimbach G, de Pascual Teresa S, Fuchs D, Wenzel U, Daniel H & Virgili F (2006) Genistein affects the expression of genes involved in blood pressure regulation and angiogenesis in primary human endothelial cells. *Nutr Metab Cardiovasc Dis* **16**, 35–43.

30. Oak MH, Chataigneau M, Keravis T, Chataigneau T, Beretz A, Andriantsitohaina R, Stoclet JC, Chang SJ & Schini-Kerth VB (2003) Red wine polyphenolic compounds inhibit vascular endothelial growth factor expression in vascular smooth muscle cells by preventing the activation of the p38 mitogen-activated protein kinase pathway. *Arterioscler Thromb Vasc Biol* **23**, 1001–1007.

31. Wallerath T, Li H, Godtel-Ambrust U, Schwarz PM & Forstermann U (2005) A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase. *Nitric Oxide* **12**, 97–104.

32. Fagan KA, McMurthy IF & Rodman DM (2001) Role of endothelin-1 in lung disease. *Respir Res* **2**, 90–101.

33. Diebolt M, Bucher B & Andriantsitohaina R (2001) Wine polyphenols decrease blood pressure, improve NO vasodilation, and induce gene expression. *Hypertension* **38**, 159–165.

34. Noé V, Peñuelas S, Lamuela-Raventós RM, Permanyer J, Ciudad CJ & Izquierdo-Pulido M (2004) Epicatechin and a Cocoa Polyphenolic Extract Modulate Gene Expression in Human Caco-2 Cells. *J Nutr* **134**, 2509–2516.

35. Iijima K, Yoshizumi M & Ouchi Y (2003) Effects of red wine polyphenols on vascular smooth muscle cells function-molecular mechanisms of the 'French paradox'. *Mech Ageing Dev* **123**, 1033–1039.

36. Walle T, Hsieh F, DeLegge MH, Oatis JE & Walle UK (2004) High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos* **32**, 1377–1382.

37. Wallerath T, Poleo D, Li H & Forstermann U (2003) Red wine increases the expression of human endothelial nitric oxide synthase: a mechanism that may contribute to its beneficial cardiovascular effects. *J Am Coll Cardiol* **41**, 471–478.

38. Corder R, Douthwaite JA, Lees DM, Khan NQ, Viseu Dos Santos AC, Wood EG & Carrier MJ (2001) Health: Endothelin-1 synthesis reduced by red wine. *Nature* **414**, 863–864.

39. Liu JC, Chen JJ, Chan P, Cheng CF & Cheng TH (2003) Inhibition of cyclic strain-induced endothelin-1 gene expression by resveratrol. *Hypertension* **42**, 1198–1205.

40. Chao HH, Juan SH, Liu JC, Yang HY, Yang E, Cheng TH & Shyu KG (2005) Resveratrol inhibits angiotensin II-induced endothelin-1 gene expression and subsequent proliferation in rat aortic smooth muscle cells. *Eur J Pharmacol* **515**, 1–9.