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Review Article

Endothelial dysfunction as a risk factor for cardiovascular disease; its modulations by phyto-ingredients and implication in better cardiovascular health

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Abstract

Nitric oxide (NO), produced by endothelial nitric-oxide synthase (eNOS), is recognized as a central anti-inflammatory and anti-atherogenic principle in the vasculature. Decreased availability of NO in the vasculature promotes the progression of cardiovascular diseases. Epidemiological and clinical studies have demonstrated that natural products, in daily diet or phytomedical preparations, may improve vascular function by enhancing NO production and bioavailability. Phyto-ingredients in tea, cocoa, pomegranate and soy are known to positively influence eNOS activity and/or endothelial function, *in vitro* and *in vivo*. In this review, we discuss the common pathways modulating endothelial NO production to provide a basis for subsequent mechanistic discussions. We herein also discuss about the mechanism and clinical efficacy of the mentioned dietary ingredients. In summary, there is increasing evidence that several single natural products and plant extracts influence endothelial NO production. Identification of such compounds and characterization of their cellular actions may increase our knowledge of the regulation of endothelial NO production and could provide valuable clues for the prevention or treatment of cardiovascular diseases.

INTRODUCTION

The endothelium is an anatomic and functional interface between the circulating blood and the vascular wall [1]. Endothelial cells lining the endothelium efflux a number of factors which are involved in the regulation of coagulation, platelet activation, vascular permeability, inflammation, vascular smooth muscle cell (VSMC) contraction, proliferation and migration [2]. Deleterious alterations of endothelium, both structurally and functionally results in endothelial dysfunction, characterized by reduction of the bioavailability of vasodilators, particularly nitric oxide (NO), and also would increase endothelium-derived contracting factors like endothelin-1, thromboxanes and serotonins [3, 4]. The resulting imbalance between vaso-dilatory and vaso-constricting factors leads to an impairment of endothelium-dependent vasodilation, a functional characteristic of endothelial dysfunction. This represents a key early step in the development of atherosclerosis which would finally lead to plaque progression and occurrence of atherosclerotic complications [5, 6], thus serving as a marker of an unfavourable cardiovascular prognosis.

PATHOPHYSIOLOGY OF ENDOTHELIAL DYSFUNCTION

The vasomotor component of endothelium can be assessed by the response of the vasculature to certain stimuli that normally evoke increased blood flow whenever needed. A strong inverse association between vessel-flexibility and adverse cardiovascular outcomes was reported in a meta-analysis by Bairey Merz *et al* [7] that included 15 published reports evaluating cardiovascular risk and endothelial dysfunction assessed by coronary or peripheral tone. The results suggested an approximately 10-fold increase in relative

risk for cardiovascular events during follow-up among subjects with endothelial dysfunction (patients with atherosclerosis). Atherosclerotic blood vessels are highly susceptible to the development of vasospasm *in vivo* [8] and are hyper-reactive to contractile agonist's *in vitro* [5]. NO accounts majorly for the biological activity of endothelium-derived relaxing factor as demonstrated in artery segment, which loses endothelium-dependent relaxation upon NO inhibition [9].

Numerous *in vitro* studies confirmed the defect in the endothelial NO signalling pathway in isolated atherosclerotic blood vessels in rabbits [10], pigs [11], rats [12], primates [3] and humans [10], where basal as well as stimulated NO release appeared to be affected. Catheterization-based studies in patients with coronary artery disease also demonstrated the impairment of endothelium dependent coronary vasodilatation to acetylcholine [13] or increased flow [10] particularly at atherosclerosis-prone branch points [14]. The deterioration of endothelium-dependent vasodilatation is an early event, as it can be observed in patients with typical angina or cardiac risk factors [13].

Risk factors of endothelial injury include oxidized low-density lipoprotein (LDL), cholesterol, toxins, including the by-products of cigarette smoking and pollution, hyperglycemia and hyper-homocysteinemia [15]. These risk factors lead to endothelial (vascular) inflammation [2]. Inflamed endothelium leads to infiltration of circulating monocytes and macrophages towards the intima of the vessel wall, and these tissue macrophages act as scavenger cells, taking up LDL cholesterol and forming the characteristic foam cell of early atherosclerosis. These activated macrophages produce numerous factors that are injurious to the endothelium. The decrease in the availability of nitric oxide also is associated with increased platelet adhesion, increased plasminogen activator inhibitor, decreased plasminogen activator, decreased thrombomodulin, and alterations in heparin sulfate proteoglycans [16]. The consequences include a procoagulant milieu and enhanced platelet thrombus formation. Endothelial injury is subjected to repair mechanism, wherein the endothelium may restore its vasodilatory property or conversely, due to progressive trauma, might lead to dysfunctional endothelium as depicted in Fig.1.

MOLECULAR BASIS OF NITRIC OXIDE SIGNALING

Endothelium derived NO is majorly produced by Type-III (endothelial) NOS. Nitric oxide production in endothelial cells (ECs) is a complex event, stimulated by a variety of mechanical forces such as shear stress [10], and humoral factors ranging from growth factors to peptide hormones. Shear mediated NO production is mostly regulated by cardiac pumping and cannot be modulated by nutritional intervention. Analogously, receptor mediated agonist stimulation can be modulated by nutritional intervention. This dual mechanism of vasculature is to maintain homeostatic regulation of blood flow. Shear mediated dilation supplies essential nutrients and oxygen under homeostatic condition; while agonist mediated responses are generated under the influence of augmented psychological or physical requirement. Physiological agonists in circulation include Ach (acetylcholine), VEGF (vascular endothelial growth factor), bradykinin, estrogen and sphingosine-1-phosphate (S1P) [17]. Nutritional modulation involves binding of phyto-chemical (agonist) to receptors; thereby potentiating eNOS mediated NO response. Thus understanding receptor mediated NO regulation becomes important, prior to nutritional modulation. Agonist interaction with cell surface receptors, trigger series of signalling events. Endothelial NOS is an acylated peripheral membrane protein [18], which is targeted to endothelial plasmalemmal caveolae through an interaction with the caveolae structural protein caveolin-1 (Cav1) [19]. Cav1 inhibition of eNOS is relieved by calmodulin (CaM), which causes dissociation of eNOS from caveolin [20]. This regulatory mechanism is further modified by heat shock protein-90 (HSP90) [21], which bind to eNOS and facilitates displacement of Cav1 by CaM. In addition to these protein interactions that modulate CaM binding, other cellular signaling cascades also regulate eNOS activity. Physiologically, endothelial cells are exposed to the hemodynamic forces of blood including laminar shear stress. Shear stress, via G-proteins activate several signal transduction pathways, including the phosphatidyl-inositol 3-kinase (PI3K) [22], phosphoinositide-dependent kinase (PDK) [22] and adenylate cyclase (AC) via cyclic adenosine monophosphate (cAMP) [23], leading to eNOS activation by phosphorylation of serine residues (S617 and S1179 for Akt, and S635 and S1177/79 for PKA), which promote eNOS activation. Additional stimuli, such as VEGF, estrogen, S1P and bradykinin (Bdk), bind to their cognate receptors (RTKs, VEGF-R, ESR, EDG and Bdk-R) and stimulate PI3K/Akt [24]. However, they also activate phospholipase C (PLC)-γ and phosphatidylinositol 4,5-bisphosphate (PIP2) to increase cytoplasmic calcium and diacylglycerol (DAG) levels [24]. The increase in cytoplasmic calcium levels activates CaM, which binds to the canonical CaM-binding domain in eNOS to promote the alignment of the oxygenase and reductase domains of eNOS, leading to efficient NO synthesis. In addition, CaM activates CaMKII (CaM kinase-II), which phosphorylates eNOS on S1179. Increases in DAG levels activate protein kinase C (PKC) pathway, which may negatively regulate eNOS [17].

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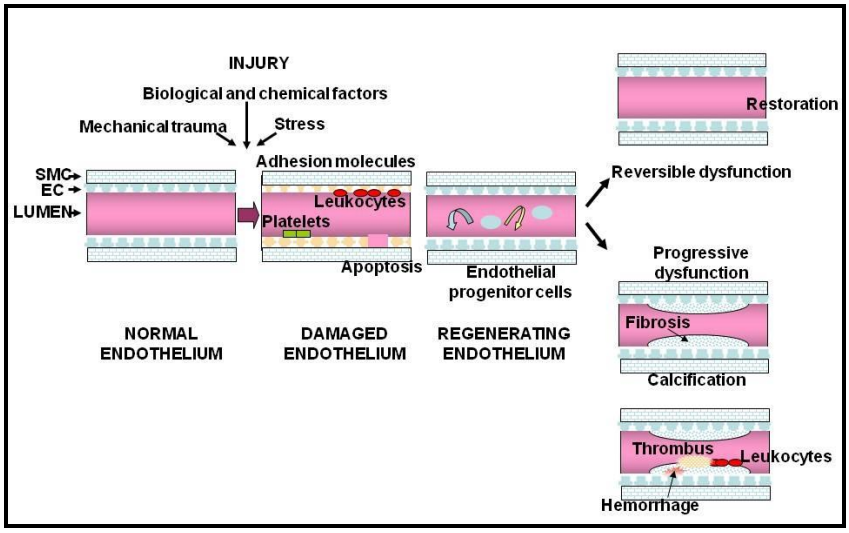

Figure 1.
Endothelial injury and repair outcome.
Physiological and life style changes increases biological risk factors in circulation. Increased risk factors triggers inflammatory event in endothelium, which leads to endothelial apoptosis, smooth muscle cell activation increased adhesion molecule expression in endothelium. Consecutive loss of endothelial cells results in hyper-inflammatory status, finally leading to endothelial damage. Enhanced leukocyte adhesion leads to pro-atherogenic state, finally progressing towards thrombus formation. Circulating endothelial progenitor cells, restores damage by replenishing endothelial cells. NO produced by endothelial cells acts as anti-inflammatory and anti-atherogenic, reducing inflammatory status, and thus restoring functional endothelium.

Metabolic stress triggering the breakdown of ATP stimulates AMP kinase (AMPK) to phosphorylate eNOS on S1179. [24]. Phosphorylation of this residue by protein kinase A (PKA) is also associated with increased enzyme activity. Other proteins which are associated with increased eNOS activity or NO release are dynamin-2 (a GTP-binding protein) and porin, which co-localize and directly interact with eNOS. Their interactions with eNOS are stimulated by intracellular Ca^{2+} and lead to eNOS activation. Efficient supply with substrate during all this is ensured by localization of the arginine transporter cationic amino acid transporter-1 (CAT1) in caveolae and its direct interaction with eNOS [25].

Myristoylation of eNOS occurs co-translationally and targets eNOS to cellular membranes, where eNOS is then palmitoylated. These processes promote eNOS association with cell membranes and are essential for linking upstream signal transduction pathways to eNOS activity in cells. N-myristoylated and palmitoylated membrane-bound eNOS associates with the caveolae coat protein Cav1 and with HSP90. C-terminal HSP70-interacting protein (CHIP) interacts with both HSP70 and HSP90, and negatively regulates eNOS trafficking into the Golgi complex. By contrast, nitric oxide synthase-interacting protein (NOSIP) and nitric oxide synthase traffic inducer (NOSTRIN) negatively regulates eNOS localization in the plasma membrane. Acute activation of eNOS in blood vessels in response to agonists such as Ach or Bdk results in the activation of the soluble guanylyl cyclase (sGC) in smooth muscle cells, production of cyclic guanosine monophosphate (cGMP) and degradation of cyclin-A. An increase in intracellular cGMP levels affect vascular tone by decreasing the intracellular concentration of free Ca^{2+} by cyclic-nucleotide gated (CNG) ion channel modulation as well as by activating protein kinase G (PGK) and phosphorylating HSP20, which regulate force by binding to thin filaments and inhibit cross-bridge cycling [25]. Nitrosylation of caspase-3 and caspase-8 inactivates the protein which leads to inhibition of apoptosis.

CELLULAR AND MOLECULAR BASIS OF NITRIC OXIDE MODULATION IN DYSFUNCTIONAL ENDOTHELIUM

It is evident from various investigations that, life style modification, diet, physical inactivity leads to cardiovascular disease (CVD), which results from complications such as diabetes, hypertension and obesity as depicted in Table 1. The biochemical and cellular modulation of NO that are associated with endothelial dysfunction are summarized as follows:

(1) NADPH is required for proper NO generation. Conditions like hyperglycemia may lead to intracellular changes in the redox state with activation of PKC resulting in depletion of the cellular NADPH pool [26].

(2) Over expression of growth factors [27] has also been implicated as a link between diabetes and proliferation of both ECs and VSM, possibly promoting neovascularization. Levels of these growth factors are increased in diabetic models, but the temporal sequence is not well defined and, therefore, these issues require further investigation.

(3) Conditions like chronic hyperglycemia lead to nonenzymatic glycation of proteins and macromolecules, resulting in changes in the properties of protein and DNA. Independent of chronic effects of hyperglycemia, acute glucose exposure dilates arteries with intrinsic tone and impairs cerebrovascular reactivity to changes in intravascular pressure via an endothelium-mediated mechanism that involves NO and prostaglandins [28].

Table 1. Risk factor for cardiovascular disease from 'Syndrome of Obesity and Risk Factors for Cardiovascular Disease Study' (SOFT Study) (N = 1,007)

Parameter	Prevalance of CVD (95% CI)	RR (95%CI)	P value
Age (years)			< 0.001
18-49	1.5 (0.7-3.3)	1	
50-64	8.6 (4.9-14.9)	6.1 (2.2-16.7)	
65-90	23.1 (18.5-28.4)	17.6 (7.5-41.5)	
Skin colour			0.13
White	5.5 (3.9-7.7)	1	
Non-white	8.3 (5.6-12.2)	1.5 (0.9-2.7)	
Physical exercise			0.002
Yes	4.8 (3.4-6.8)	1	
No	6.8 (5-9.2)	1.4 (0.7-2.6)	
Obesity			< 0.001
No	4.5 (3.3-6.2)	1	
Yes	12.2 (8.6-16.9)	2.8 (1.8-4.4)	
Diabetes melitus			< 0.001
No	5.2 (3.9-7)	1	
Yes	19.6 (13.0-28.5)	4.1 (2.3-7)	
Hypertension			< 0.001
No	1.1 (0.6-2)	1	
Yes	18.9 (14.6-24)	14.1 (7.8-25.5)	
Phytochemical intake			0.3
Yes	4.9 (3-7.9)	1	
No	6.8 (5-9.2)	1.4 (0.7-2.6)	

Data extrapolated from Fuchs *et al* [33]. The data indicate increase in prevalance of CVD risk with age, physical inactivity, obesity and stress. Dietery intervention with phytochemicals results in reduced risk of CVD, suggesting supression of CVD risk factor with phytochemical intervension.

(4) Hyperglycemia increases the flux of glucose through the glycolytic pathway, increasing *de novo* synthesis of DAG [29]. Increased DAG has been shown to occur in both ECs and vascular smooth muscle (VSM) cells, leading to increased PKC activity. Both DAG and PKC are important intracellular signaling molecules involved in wide variety of cellular responses, including modulating vasoconstriction [29]. Excessive PKC activation leads to eNOS uncoupling, which will lead to superoxide production, resulting in NO quenching [17, 26]. Excessive PKC activation also leads to increased levels of asymmetric dimethyl arginine (ADMA), which acts as competitive substrate analogue and inhibit eNOS activity, further reducing NO production in endothelial cells.

(5) The endothelium is very susceptible to damage by oxidative stress. High levels of fatty acids and hyperglycemia have both been shown to induce an increased level of oxidation of phospholipids as well as proteins, leading to endothelial dysfunction in type 2 diabetes (D2M) [10].

(6) Diminished NO production and decreased fibrinolytic activity lead to ameliorated levels of plasminogen activator inhibitor-1 (PAI-1) in the plasma of D2M patients, which is associated with pro-thrombotic state [30]. In addition to decreased fibrinolysis, the diabetic state is also associated with an increase in the activation of the coagulation cascade by various mechanisms such as non-enzymatic glycation, formation of advanced glycosylation end-products (AGE) and decreased heparin sulfate synthesis.

(7) Inflammatory marker, tumor necrosis factor (TNF) has been implicated as a link between insulin resistance, diabetes, and endothelial dysfunction [31]. Increased expression of this factor in human obesity supports the hypothesis that elevated TNF induces insulin resistance. TNF also can induce the synthesis of other cytokines, which alone or in concert with others, may alter endothelial function [32].

MODULATION OF ENDOTHELIAL NITRIC OXIDE BY PLANT DERIVED PRODUCTS

Epidemiological and clinical studies have demonstrated that a growing list of natural products, as components of the daily diet or phytomedical preparations, may improve vascular function by enhancing NO bioavailability.

Grapes and red wine

Epidemiologic data clearly indicate a significant negative correlation of moderate wine consumption with the risk of cardiovascular events [34]. These beneficial effects are mainly attributed to the red wine polyphenol (RWP) fraction [35], which induces eNOS expression and causes a sustained increase in endothelial NO production [36]. Upregulation of eNOS is probably based on synergistic mechanisms between the different polyphenolic components [36]. Red wine polyphenol has been shown to improve endothelial function in various animal models of hypertension [37, 38]. Mechanistic investigations revealed a partial dependence on calcium influx into the endothelium, triggering NO production presumably via binding of CaM to eNOS and promoted phosphorylation of eNOS at Ser1177 via the PI3K/Akt-pathway, resulting in the convergence of two independent pathways of rapid eNOS activation [39].

Rapid activation of eNOS and endothelium-dependent vasodilation has also been demonstrated for grape juice (*in vitro* and *in vivo*) [40, 37], red grape polyphenol extract (*in vivo*) [37] and a grape skin extract (*in vitro* and *in vivo*) [41], Grape seed extracts are especially rich in oligomeric procyanidins (OPC) and are able to promote endothelium-dependent dilation of aortic rings *ex vivo* [41]. Mechanistically, one can discriminate

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between a long-term effect based on upregulation of eNOS gene expression, and acute actions on the endothelium due to rapid eNOS activation via increases in intracellular calcium and/or activation of the PI3K/Akt-pathway. All these data support the well-established cardiovascular benefit associated with moderate red wine consumption (Table 2).

Green tea and black tea

Tea is strongly linked to cardiovascular health [42] (Table 3). Several human studies have shown the beneficial effects of tea consumption on CVDs especially against myocardial infarction, stroke [42, 43], and coronary heart diseases [42]. The key ingredient promoting vascular benefit includes class of catechins [44], thearubigins and theaflavins [45].These ingredients influence NO production by promoting endothelial nitric oxide synthase activation by phosphorylation at Serine 1177 position. The signalling event leading to phosporlyation at ser1177 include p38 MAPK mediated activation of PI3K/Akt pathway [46], Src kinase/ERK1/2 [46], and PKA [48].

Clinical studies have been carried out in healthy as well as diseased population and have investigated the effect of tea and tea flavonoids on endothelial function. Meta-analysis carried out by Arab *et al* [43] suggests individual consuming >3 cups of tea per day has 21% lower risk of stroke than individual consuming less than 1 cup of tea per day. Meta-analysis by Peters *et al* [49] based on 10 cohort studies and 7 case-control studies conducted between years 1972 to 2000, reported 11% reduction in myocardial infarction incidence rate with 3 cups per day (711 ml) tea consumption. The study by Kim *et al* [50] showed green tea consumption for 2 weeks significantly improves flow-mediated dilation (FMD). Besides improvement in FMD, this study also showed amelioration of circulatory endothelial progenitor cells (EPCs). Jochmann *et al* [51] showed that both green and black teas are equally effective in improving endothelial function, *in vivo* and *in vitro*. A study by Duffy *et al* [52] showed acute and chronic tea consumption improved endothelium-dependent FMD of brachial artery in patients with coronary artery disease.

Addition of milk to a tea is a common practice. Lorenz *et al* [52] showed addition of milk to the tea abolishes the beneficial effects of tea. The FMD measurement was carried out in 16 post menopausal women after 2 hours of consumption of water, tea alone and tea along with 10% milk. Consumption of black tea with 10% milk suppressed the FMD as compared to tea without milk. The endothelium-independent vasodilation was, however, not changed. In *in vitro* endothelial cell based models, tea polyphenols were found to bind polyphenols. This led to a decrease in the concentration of catechins without affecting the gallic acid and caffeine levels [54, 55]. Such effects, however, may not be valid in physiological conditions of stomach and intestine where such bindings are expected to break-down. Indeed in a simulated conditions of human digestion (stomach and intestine), the polyphenol-protein complexes were broken down releasing amino-acid and free polyphenols [55].

Cocoa

Cocoa drinks made from cocoa (*Theobroma cacao*) powder are flavanol-enriched beverages, which have received considerable attention in recent years [56]. In a double-blind study, Fisher *et al* [57] demonstrated that a cocoa drink induced vasodilation via increased endothelial NO production after five days of regular consumption in healthy humans. Follow-up investigations revealed this effect to be mediated in part by (-)-epicatechin (EC) [44]. Similarly, daily consumption of flavanol-rich, but not of a flavanol-poor, cocoa drink for two weeks improved insulin-mediated vasodilation in hypertensive patients [58]. In a randomized, placebo-controlled study in healthy men pure (-)-epicatechin acutely elevated plasma and urinary nitrite levels, suggesting activation of the eNOS system [59]. Consumption of flavonoid-rich dark chocolate bars over a period of two weeks improved endothelium-dependent vasodilation, whereas low-flavonoid chocolate bars had no effect [60]. Karim *et al* [61] studied the effect of isolated cocoa procyanidins using rabbit aortic rings. Interestingly they found that only oligomeric procyanidins (tetramers or bigger) elicited endothelium-dependent vasodilation and increased eNOS activity [61]. This finding is in agreement with similar observations regarding vascular reactivity of OPC found in red wine, grape seeds and hawthorn [62].

Soy isoflavones

Epidemiological data from Eastern countries suggest that regular dietary uptake of soy products may decrease cardiovascular risk (Fig.2). This is mainly attributed to soy isoflavones, which exert well described beneficial effects on plasma lipid and lipoprotein levels [63]. Soy isoflavones are able to modulate endothelial NO production by mimicking hormonal effects of estrogens [64].

A diet rich in soy isoflavones improved endothelium-dependent vasodilation in ovariectomised rats [65] and enhanced the vascular response to acetylcholine in atherosclerotic female rhesus monkeys [66]. Mahn *et al* [67] explored the long-term effects of a diet enriched in soy protein and isoflavones in rats; they found that after ten months, soy-consuming animals expressed increased amounts of eNOS in aortas and showed improved endothelium-dependent vasodilation.

Pomegranate

Pomegranate (*Punica granatum)*, contains high levels of polyphenols, especially of the hydrolysable tannin punicalagin. Pomegranate juice has been shown to increase eNOS expression in human coronary artery endothelial cells (HCAEC) and to decrease atherosclerosis development in hyper-cholesterolemic mice [68]. In another study, using bovine pulmonary artery endothelial cells, however, eNOS expression and catalytic activity remained unaltered by pomegranate juice. Instead, it seemed that NO was protected from oxidative degradation via the scavenging of superoxides [69]. A pomegranate fruit extract displayed a similar range of effects, increasing eNOS expression and NO release in cultured human coronary aortic endothelial cells [69], as well as *in vivo* in the vasculature of diabetic rats [69]. Taken together, it appears that pomegranate polyphenols exert positive effects on the endothelium, mainly by increasing eNOS expression and by protecting NO from degradation via the scavenging of superoxide.

RECOMMENDATIONS

Even though, human being is born healthy, life-style modification and age increases the probability to acquire cardiovascular risk factors. Undoubtedly, endothelium derived NO has a critical role in the maintenance and repair of the vasculature, and a decrease in bioavailable NO is linked to adverse outcomes as predicted by the epidemiological and follows up studies. Although benefit of endothelium derived NO cannot be linked in healthy subjects, but its role in compromised subject is crucial, especially in case of diabetes.

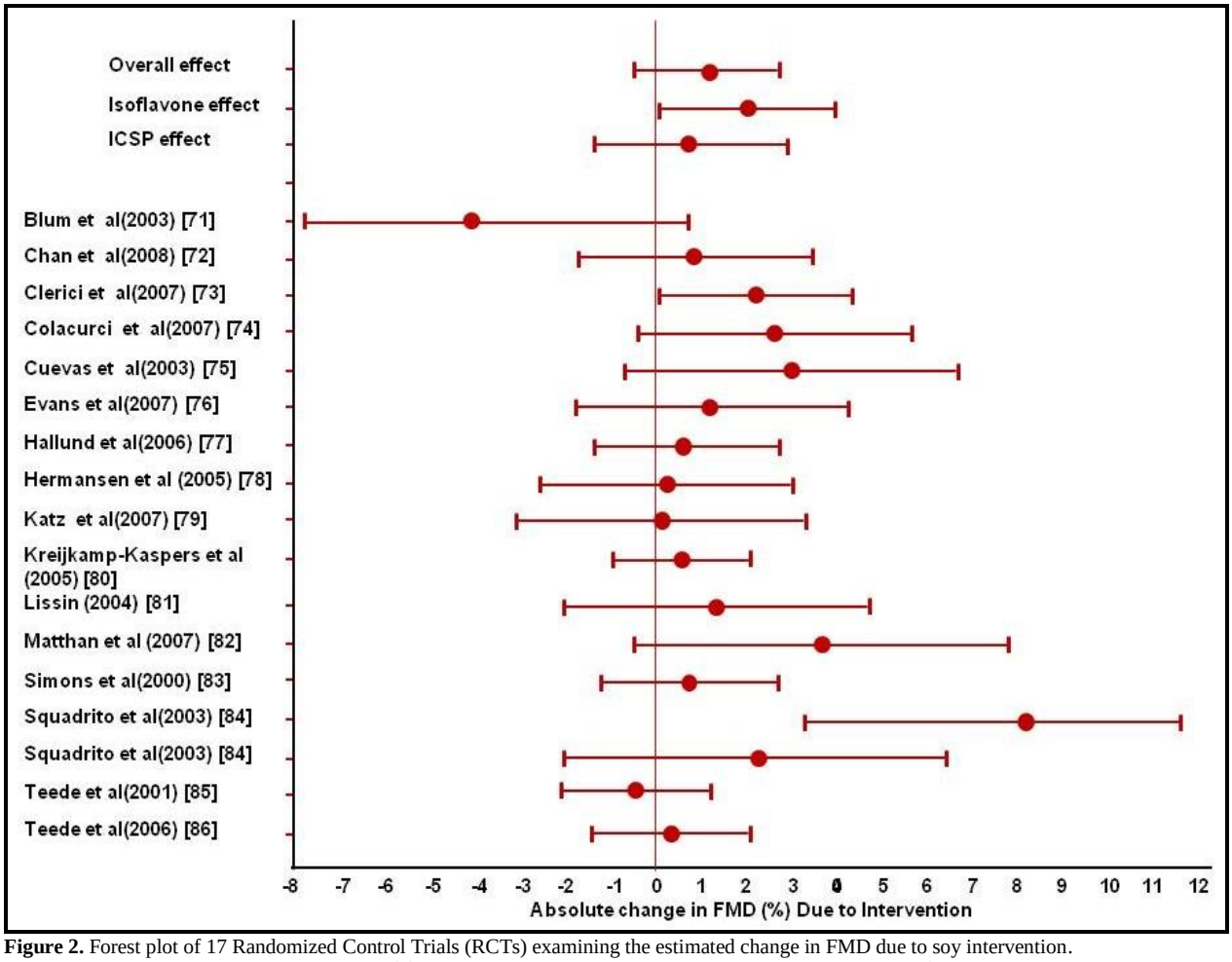

Figure 2. Forest plot of 17 Randomized Control Trials (RCTs) examining the estimated change in FMD due to soy intervention.
Flow-mediated dilation (FMD) is a measure of functional endothelium. Nutritional interventions with soy isoflavones results in enhanced FMD, indicative of functional endothelium (Beavers *et al*, 2010) [87]. The pooled data of RCTs indicate overall >2% increase in FMD. This increase in FMD can be extrapolated to 11% reduction in incidence of stroke (Arab *et al*, 2009) [43].

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Table 2. Studies investigating the effect of red-wine or its constituents on FMD

Study	Subjects	Beverage	Dose	Outcome
Agewall *et al* [88]	12 healthy	Red wine *vs* dealcoholized red wine	250 ml	Dealcoholised red wine increases FMD, red wine causes no effect.
Hashimoto *et al* [89]	11 healthy	Red wine *vs* dealcoholised red wine, *vs* vodka, *vs* water	500 ml	Dealcoholised red wine increases FMD at 30 and 120 min, red wine increases FMD at 120 min, vodka decreases FMD at 30 and 120 min, water causes no effects.
Karatzi *et al* [90]	15 patients with CAD	Red wine *vs* dealcoholised red wine	250 ml	Dealcoholised red wine increases and red wine decreases FMD at 60 min post ingestion.
Whelan *et al* [91]	14 patients with CAD	Red wine *vs* white wine	4 ml/kg	Both red and white wine increased FMD at 360 min post-ingestion.
Lekakis *et al* [92]	30 patients with CAD	Red grape polyphenol extract in water *vs* water	600 mg	Red grape polyphenol extract increased FMD at 60 min.
Hijmering *et al* [93]	20 healthy	Red wine *vs* isocaloric low polyphenolic drink	3 units	Red wine and low polyphenolic drink decreased FMD at 90 min post ingestion.
Boban *et al* [94]	9 healthy	Red wine vs dealcoholised red wine, *vs* polyphenol stripped red wine, *vs* ethanol solution, *vs* water	3 ml/kg	Red wine increased FMD 60 min after ingestion. No other beverage caused significant effect on FMD.
Bau *et al* [95]	100 healthy	Alcoholic *vs* non-alcoholic drink	60 g alcohol	Alcohol decreased FMD at 4 h post ingestion.
Vlachopoulos *et al* [96]	12 healthy	Alcohol diluted in grapefruit juice *vs* grapefruit juice	1 ounce alcohol	No effect on FMD by either drink.
Magyar *et al* [97]	40 post infraction	10 mg reservetrol	10 mg	Significant FMD benefit at 120 min.

Table 3. Studies evaluating effect of Black/Green tea (flavanoids or polyphenols) on FMD benefit

Study	Population	Flavonoids/ PP dose	Tea	Timing	Parameter	P *vs* control
Duffy *et al* [52]	CVD patients	477 mg fla	Black	2 hours	FMD	P < 0.001
		873 mg fla		4 weeks		P = 0.002
		1350 mg fla		4weeks/ 2hours		P = 0.003
Hodgson *et al* [98]	Healthy	-	Black	4weeks/ 2hours	FMD	P = 0.008
Hirata *et al* [99]	Healthy	762 mg PP/655 mg fla	Black	2 hours	CFVR	P = 0.002
Hodgson *et al* [100]	CVD patients	900 mg PP/ 774 mg fla	Black	4 hours	FMD	P = 0.02
		900 mg PP/ 774 mg fla			FMD	**P > 0.05**
Lorenz *et al* [53]	Healthy post-menopousal women	500	Black	2 hours	FMD	P < 0.01
		500	Black + 10% milk		FMD	**P > 0.05**
Kim *et al* [50]	Healthy smokers	729 mg catechins	Green	2 weeks / 8 hours	FMD	P < 0.001
Nagaya *et al* [101]	Healthy smokers	400 ml	Green	2 hours	FBF	P < 0.001
Jochmann *et al* [51]	Healthy post-menopousal women	500 ml	Black	2 hours	FMD	P < 0.001
		500 ml	Green		FMD	P < 0.001
Tinahones *et al* [102]	Healthy women	375 mg catechol	Green	5 weeks	FMD	P = 0.02
Alexopoulos *et al* [103]	Healthy individuals	450 ml	Green	30, 60, 120 min	FMD	P = 0.02
Grassi *et al* [104]	Healthy males	100 mg fla	Black	1 week	FMD	P = 0.02
		200 mg fla				P = 0.01
		400 mg fla				P = 0.002
		800 mg fla				P < 0.001

Literature evidence suggests that, this compromised state of endothelium can be reverted to gain its normal functionality, either by modulation by nutraceutical diet or physical activity. NO is a key factor determining the state of functionality of the endothelium. Dietary intake of nutraceutical ingredients are reported to modulate endothelial NO production, either by interfering with NO signalling or by scavenging NO dissipating free radicals. There is increasing evidence that several single natural products and plant extracts derived from foodstuffs, drinks and also herbal medicines influence endothelial NO production. Despite large efforts and progress in the field, there is still room for further research activities, especially in terms of phytochemical analysis of active extracts and regarding the mode of action of identified single natural products. The understanding of the latter may increase our knowledge about eNOS regulation and provide valuable strategies for the prevention of cardiovascular disease.

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COMPETING INTERESTS

None

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