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Mini Review

Plant-derived foods containing polyphenols with endothelial protective effects

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Abstract

Cardiovascular disease (CVD) is the leading cause of death and disability in the world. The primary cause of CVD is development of atherosclerosis resulting from chronic inflammation and endothelial dysfunction. Indeed, endothelial dysfunction is considered to be the earliest stage in the process of atherosclerosis development. There is great interest in discovering strategies to inhibit endothelial dysfunction and atherosclerosis progression. The role of plant constituents routinely consumed have attracted much attention as preventive health approaches due to their availability and perceived safety. Accumulating studies suggest that constituents present in tea, grape, cocoa, soy and pomegranate are associated with reduced risks of CVD. In this review, we discuss the potential of the above mentioned dietary ingredients to improve endothelial function *in vivo* and *in vitro.*

Introduction

Endothelium, the inner monolayer of the blood vessel, regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, inflammatory activity as well as cell proliferation. Alterations to these functions lead to endothelial dysfunction (Vanhoutte *et al*., 2009). Endothelial dysfunction has been considered to be an early event of pathophysiologic importance in the atherosclerotic process. Endothelial dysfunction is associated with most forms of cardiovascular diseases (CVD) such as hypertension, coronary artery disease (CAD), chronic heart failure and peripheral artery disease. The hallmark of endothelial dysfunction is reduced endothelial nitric oxide synthase (eNOS) expression and/or impaired nitric oxide (NO) availability (Felaco *et al*., 2001; Mokhtar *et al*., 2013). In blood vessels, NO is synthesized by the eNOS enzyme in endothelial cells and diffuses into vascular smooth muscle cells, leading to vasodilatation. NO is a major anti-atherogenic factor due to a number of vasoprotective effects; thus decreased NO availability in the vasculature is likely to promote the progression of vascular diseases. Thus improved NO bioavailability would be a promising step in the therapy and prevention of cardiovascular disorders.

Polyphenols are naturally occurring compounds found largely in fruits, vegetables, cereals and beverages. Polyphenols is the subject of increasing scientific interest because of their possible beneficial effects on human health (Pandey and Rizvi, 2009). Polyphenol molecules and components typically carry several hydroxyl groups; and more than 4000 to 7000 varieties are present in plants. Polyphenols can be categorized into flavonoids and non-flavonoids. The flavonoid group has a phenyl chroman frame (C6-C3-C6) and based on differences in side-chain structures can be classified into 6 subclasses: flavones, isoflavones, flavanones, flavonols, anthocyanidins and flavanols. Typical non-flavonoids include phenolic acids, tannins, curcumins and resveratrol (Table 1) (Habauzit and Morand, 2012; Del Rio *et al*., 2013; Yamagata *et al.*, 2015).

This paper aimed to review available evidence on use of polyphenol-containing foods (tea, grapes, cocoa, soy and pomegranate) on endothelial function in humans. Possible mechanistic principles involving the effects of these dietary ingredients on endothelial function are also discussed using experimental and in vitro studies. A table summarising the effects of these natural products on endothelial function are given as Table 2.

Black and green tea

Intervention study

Tea, a product made up from the leaf and bud of the plant Camellia sinensis, is the second most widely

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Table 1. Classification of phenolic compounds

Polyphenols	Classes	Representative compounds	Food sources
Flavonoids	Flavones	Apigenin	Vegetables: Parsley, celery, sweet peppers
		Luteolin	
	Isoflavones	Genistein	Legumes: Soybeans
		Daidzein	Processes foods: miso, tofu, tempeh, soy milk
	Flavanones	Hesperetin	Fruits: citrus fruits (orange, grapefruit, lemon)
		Naringenin	Beverages: citrus juices
		Eriodictyol	
	Flavonols	Quercetin	Fruits: apples, apricots, plums, cranberries, strawberries, grapes
		Kaempferol	Vegetables: kale, onions, broccoli, tomatoes
		Myricetin	Beverages: red wine, green tea, black tea, grape juice
	Anthocyanidins	Pelargonidin	Fruits: black grapes
		Cyanidin	Beverages: red wine, grape juice
		Delphinidin	
		Petunidin	
		Malvidin	
	Flavanols	Catechin	Cocoa-derived products
			Fruits: apples, apricots, cherries, grapes, peaches, blackberries
			Beverages: green tea, black tea, red wine, grape juice, cider
		Proanthocyanidins	Fruits: grapes, peaches, persimmons, apples, pears, berries
			Beverages: red wine, cider, tea, beer
Non-flavonoids	Phenolic acids	Hydroxybenzoic acids	Fruits: raspberries, strawberries, blackberries, pomegranate, persimmon, walnuts, hazelnuts
		Hydroxycinnamic acids	Beverages: Coffee
	Resveratrol		Beverages: red wine
			Fruits: nuts
	Lignans		Fruits: flaxseeds
	Curcumins		Food: spices

consumed drink in the world after water. Tea is a rich source of polyphenolic compounds, particularly flavonoids. The major flavonoids present in green tea are catechin (under the subclass of flavanols) such as epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). In black tea, the major flavonoids present are polymerized catechins such as theflavins and thearubigens (McKay and Blumberg, 2002; Cabrera *et al*., 2006; Chacko *et al*., 2010; Khan and Mukhtar, 2013; Fuchs *et al*., 2014).

A number of studies have investigated the effects of black and green tea, or tea flavonoids on flow-mediated dilatation (FMD) of the human brachial arteries. FMD represents endothelium-dependent relaxation of the brachial artery, mediated via release of NO. Improvement in FMD has been observed in human trials with the consumption of green or black teas (Duffy *et al*., 2001; Nagaya *et al*., 2004; Hodgson 2006; Kim *et al*., 2006; Alexopoulos *et al*., 2008; Jochmann *et al*., 2008; Grassi *et al*., 2009). The effect of green tea consumption on FMD has been studied in 14 healthy individuals who took either six grams of green tea, 125 milligrams of caffeine or hot water. This study showed that FMD increased significantly with green tea consumption, but was not affected by caffeine or hot water (Alexopoulos *et al*., 2008). In a randomized study of 66 patients with pre-existing CAD, acute (450 millilitres for two hours) and chronic consumption (900 ml/day for four weeks) of black tea resulted in significant improvements in endothelium-dependent FMD (Duffy *et al*., 2001). Consumption of green tea (eight g/day) for two weeks also improved FMD in young healthy smokers (Nagaya *et al*., 2004; Kim *et al*., 2006). In patients with chronic kidney disease, consumption of green tea (five g/day) for four weeks improved FMD (Park *et al*., 2010). In a cross-over study performed in 19 healthy men, twice daily intake of black tea (0, 100, 200, 400 and 800 mg/day) for a period of one week increased FMD and decreased peripheral arterial stiffness in a dose-dependent manner (Grassi *et al*., 2009). Another study in 21 healthy women showed a significant increase in FMD after two hours of

Table 2. Natural products and its effects on endothelial-dependent vasodilatation

	Treatments	Summary of the findings	References
Black and Green tea			
Intervention study			
Meta-analyses (n=9)	Tea (500 ml) compared to placebo	Increased FMD by 2.6% compared to placebo	Ras *et al.*, 2011
Healthy individuals (n=14)	Green tea (6 g), caffeine (125 mg) or hot water	Increased FMD with green tea, and not affected by caffeine or hot water intakes	Alexopoulos *et al.*, 2008
Healthy smokers (n=20)	Green tea (5g/day) for 2 weeks	Improved FMD	Nagaya *et al.*, 2004; Kim *et al.*, 2006
Patients with coronary artery disease (n=66)	Short term effect : black tea (450 ml) for 2 hours Long term effect: black tea (900 ml/day) for 2 weeks	Improved endothelial-dependent FMD	Duffy *et al.*, 2001
Patients with CAD	Green tea (5 g/day) for 4 weeks	Improved FMD	Park *et al.*, 2010
Healthy men (n=19)	5 treatments with twice daily intakes of black tea (0, 100, 200, 400 and 800 mg) for 1 week	Tea dose-dependently increased FMD and reduced peripheral arterial stiffness	Grassi *et al.*, 2009
Healthy women (n=21)	Green and black tea	Increased FMD	Jochmann *et al.*, 2008
Experimental/in vitro study			
Isolated rat aorta	Green and black tea	Improved endothelium-mediated vasodilatation	Jochmann *et al.*, 2008
Cultured endothelial cells	Green and black tea	Promoted eNOS activity and NO bioavailability	Lorenz *et al.*, 2004; Anter *et al.*, 2004; Siamwala *et al.*, 2013
HUVECs	Derivatives of tea flavanols (EC, ECG, EGC, EGCG)	Dose-dependent increased NO production	Persson *et al.*, 2006
Diabetic rats	Green tea	Decreased uncoupling of eNOS and increased NO production	Faria *et al.*, 2012
Grape and wine			
Intervention study			
Meta-analyses of healthy and patients with (n=9)	Grape polyphenols	Improved FMD in both groups	Li *et al.*, 2013
Patients with CAD (n=15)	Grape juice (4 ml/kg)	Improved FMD	Stein *et al.*, 1999
Healthy individuals (n=12)	De-alcoholized red wine (250 ml) for 1 hour	Improved FMD	Agewall *et al.*, 2000
Patients with CAD (n=15)	Regular red wine or de-alcoholized red wine (250 ml)	FMD higher with the consumption of de-alcoholized red wine compared to regular red wine	Karatzi *et al.*, 2004
Experimental/in vitro study			
Rat femoral artery	Provinol	Increased endothelium-dependent vasodilatation by stimulated NOS activity concomitantly and scavenged free radicals, which led to the enhancement of NO bioavailability	Zenebe *et al.*, 2003
SHR (left ventricle, aorta and kidney tissues)	Alibernet red wine extract (24.2 mg/kg/day) for 3 weeks	Increased in NOS and SOD activities	Kondrashov *et al.*, 2012
Aorta of SHR	Oral treatment with red wine polyphenols (40 mg/kg) for 5 weeks	Improved endothelial function and reduced vascular oxidative stress	Lopez-Sepulveda *et al.*, 2011
HUVECs	Resveratrol	Increased eNOS mRNA and protein expression and NO synthesis	Wallerath *et al.*, 2003
HUVECs	Non-alcoholic wine extracts	Activated estrogen receptors, enhanced expression and activity of eNOS, increased NO synthesis	Simoncini *et al.*, 2011
HUVECs	Non-alcoholic wine extracts	Enhanced eNOS expression, increased NO synthesis	Rathel *et al.*, 2007
HUVECs	Non-alcoholic wine extracts	Enhanced eNOS expression, increased NO	Leikert *et al.*, 2002

		synthesis	
HUVECs	Resveratrol	Activated estrogen receptors and PPARα in endothelial cells, increased eNOS mRNA and protein expressions, promoting NO production	Takahashi and Nakashima, 2012
Cultured endothelial cell	Wine extract	Activated estrogen receptor alpha on endothelial cells and recruitment of the mitogen-activated protein kinase ERK 1/2 and phosphatidylinositol-3-OH kinase/Akt pathways, resulted into the phosphorylation and activation of the eNOS, leading to NO synthesis	Chalopin *et al.*, 2010
Cocoa			
Intervention study			
Healthy adults	Flavanoid-rich dark chocolate bar (213 mg procyanidins, 46 mg epicatechin) vs. low-flavanoid dark chocolate bars (46 g, 1.6 o.z)	Improved endothelium-dependent FMD and increased plasma epicatechin concentration	Engler *et al.*, 2004
Pre-hypertensive and hypertensive patients (n=44)	6.3 g dark chocolate (30 mg polyphenol) vs. polyphenol free white chocolate; daily for 18 weeks	Reduced blood pressure and increased NO metabolites	Taubert *et al.*, 2007
Overweight adults (n=15)	Phase 1: solid dark chocolate bar (22 g cocoa powder) vs. cocoa-free placebo bar (0 mg cocoa powder) Phase 2: sugar-free cocoa or sugared cocoa (22 g cocoa powder) or placebo (0 g cocoa powder)	Improved FMD and reduced blood pressure	Faridi *et al.*, 2008
Healthy subjects (n=16)	High-flavanol cocoa drink (917 mg) vs. low-flavanol cocoa drink (37 mg)	Improved FMD and increased NO metabolites	Schroeter *et al.*, 2006
Overweight women (n=30)	37 g dark chocolate and sugar-free cocoa beverage (814 mg flavanol) vs. low flavanol chocolate bar and cocoa-free beverage (3 mg flavanol) for 4 weeks	Improved vasodilatation and reduced arterial stiffness	West *et al.*, 2014
Hypertensive patients	Flavanol-rich dark chocolate vs. white chocolate	Improved FMD	Grassi *et al.*, 2005
Diabetes patients	Single ingestion of flavanol-rich cocoa	Increased FMD and reversed vascular dysfunction	Balzer *et al.*, 2008
Patients with at least 1 cardiovascular risk factor	Cocoa drink (100 ml) contained 176 mg flavanols)	Increased NO bioactivity in human plasma and improved FMD	Heiss *et al.*, 2003
Healthy individuals (n=27)	Flavanol-rich cocoa beverage for 5 days	Improved NO-dependent FMD	Fisher and Hollenberg, 2006
Experimental/in vitro study			
Isolated rabbit aorta	Procyanidins	Increased endothelium-dependent vasodilatation mediated by activation of eNOS	Karim *et al.*, 2000
Cultured human endothelial cells	Cocoa-flavanols	Reduced arginase mRNA expression, suggested that cocoa-flavanols may contribute to the regulation of L-arginine concentration and NOS substrate supply	Schnorr *et al.*, 2008
Cultured human endothelial cells	Cocoa-derived epicatechin	Increased NO concentration via inhibition of NADPH oxidase	Steffen *et al.*, 2007
Soy			
Intervention study			
Meta-analyses postmenopausal women (n=9)	Soy-isoflavones	Increased FMD in subjects with low baseline FMD levels, but not in high baseline FMD levels	Li *et al.*, 2010
Meta-analyses (n=17)	Soy isoflavones	Improved endothelial function	Beavers *et al.*, 2012
Healthy postmenopausal women with hypercholesterolemia (n=18)	Soy isoflavones (40 g soy protein and 80 mg isoflavones) for 4 weeks	Increased FMD	Cuevas *et al.*, 2003
Healthy postmenopausal women (n=22)	Soy isoflavons enriched low-fat meal (80 mg)	Increased FMD	Hall *et al.*, 2008

Healthy postmenopausal women (n=57)	Soy isoflavones tablet (60 mg) vs. control tablets for 6 months	Improved endothelium-dependent FMD	Colacurci *et al.*, 2005
Healthy postmenopausal women (n=182)	Soy isoflavons tablet (100 mg) vs. control tablets for 12 months	Reduced oxidative stress, but no improvement in endothelial function	Pusparini *et al.*, 2013
Hypercholesterolemia men (n=20)	Soy protein	Improved endothelial function	Yildirir et al., 2001
Renal transplant patients	Soy protein (25 g/day) for 5 weeks	Improved FMD. The effects disappeared after soy withdrawal	Cupisti et al., 2007
Experimentalin vitro study			
Aorta of ovariectomized rats	Soy-isoflavones (5 mg/kg) for 4 weeks	Improved endothelium-dependent vasodilatation	Catania et al., 2002
Coronary artery of atherosclerotic female monkeys	Soy-based diet (low vs. high isoflavones) for 6 months	Enhanced coronary vascular reactivity	Honore et al., 1997
Rat aorta	Soy isoflavones	Increased antioxidant, increased eNOS expression and improved endothelium-dependent vasodilatation	Mann et al., 2006
Pomegranate			
Intervention study			
Hypertensive men (n=13)	Pomegranate juice (150 ml) for 4 hours	Reduced SBP and DBP and increased trend in FMD	Asgary et al., 2013
Hypertensive patients (n=21)	Receive either natural pomegranate juice (150 ml/day) or water (150 ml) as placebo	Reduced SBP and DBP but not FMD Reduced serum levels of VCAM-1 and elevated E-selectin	Asgary et al., 2014
Adolescent with metabolic syndrome (n=30)	Pomegranate juice (240/ml/day) for 30 days	Improved FMD	Hasheni *et al.*, 2010; Faria and Calhau, 2011, Kelishadi *et al.*, 2011
Experimentalin vitro study			
Human coronary artery endothelial cells	Pomegranate juice and fruit extract	Increased eNOS expression	De Nigris *et al.*, 2007
Bovine pulmonary artery	Pomegranate juice	Protected NO against oxidative damage	Ignarro et al, 2006

CAD, coronary artery disease; DBP, diastolic blood pressure; eNOS, endothelial nitric oxide synthase; EC, epicatechin; ECG, epicatechin-3-gallate; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate;FMD, flow-mediated dilatation; HUVEC, human umbilical vein endothelial cells; NADPH, nicotinamide adenine dinucleotide phosphate ; NO, nitric oxide; PPARα, peroxisome proliferator-activated receptor α; RCT, randomized controlled trial; SBP, systolic blood pressure; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase

green and black tea consumption (Jochmann *et al.*, 2008). Meta-analysis from nine human intervention studies illustrated that moderate consumption of tea substantially enhanced FMD, in which the overall increase in FMD with daily dose of 500 ml of tea (2-3 cups) compared to placebo was 2.6% of the arterial diameter (Ras *et al.*, 2011).

Experimental / In vitro *study*

The underlying molecular mechanisms for tea-induced effects on endothelial function may be due to its direct effect on the NO system. It has been demonstrated that both black and green teas promoted both endothelial NOS activity and NO bioavailability in cultured endothelial cells (Anter *et al.*, 2004; Lorenz *et al.*, 2004; Jochmann *et al.*, 2008; Siamwala *et al.*, 2013). In addition, incubation of human endothelial cells with four derivatives of tea flavanols; EC, ECG, EGC, and EGCG showed dose-dependent increases in NO production (Persson *et al.*, 2006). In diabetic rats, treatment with green tea improved uncoupling of eNOS, thus increased NO bioavailability (Faria *et al.*, 2012). Uncoupling of eNOS is characterized by a reduction in tetrahydrobiopterin (BH_4) levels and

a decrease in the eNOS dimer-to-monomer ratio. BH_4 is a critical cofactor for the production of NO. When BH_4 is limited, eNOS becomes uncoupled, and superoxide ion is produced instead of NO. Thus BH_4 availability is essential for normal endothelial function. Hence, Faria *et al.* (2012) demonstrated that green tea reversed diabetes-induced reduction of BH_4 levels, decreased eNOS uncoupling, leading to increased NO production.

Grape and/ or wine

Intervention study

Recently, it has been suggested that grape polyphenols including epicatechin, catechin, quercetin, gallic acid and resveratrol have vasculoprotective effects and can improve endothelial function. In CAD patients, consumption for 14 days of four ml/kg grape juice improved FMD (Stein *et al*., 1999). Similarly, Agewall *et al.* (2000) demonstrated improvement of FMD one hour after consumption of de-alcoholized red wine (250 ml) among healthy volunteers (Agewall *et al*., 2000). Karatzi *et al*. (2004) assessed the acute effects of 250 ml of either red wine or de-alcoholized red wine consumption on FMD in 15 males with CAD. FMD was shown to be higher following the consumption of de-alcoholized red wine compared to regular red wine, suggesting that the beneficial effects may be attributed to the presence of polyphenols in wine without the presence of alcohol (Karatzi *et al*., 2004). Meta-analysis of nine studies revealed that intake of grape polyphenols increased FMD levels in both healthy and subjects with high cardiovascular risk (smoker and CAD); the increase in FMD appeared to be much more obvious in the latter subject groups. The effect of grape polyphenols on FMD in healthy subjects was observed at 30 minutes after ingestion; the effect was delayed in subjects with high cardiovascular (CVS) risk, which was at 60 minutes after ingestion. The difference in timing of the acute effects of grape polyphenols between the two groups may be due to impaired endothelial function in the high CVS risk group. This is supported by the observation that baseline FMD in the group with high CVS risk ranged from 2.6% to 5.65%, which were lower than in the healthy group (5.4% to 7.4%) (Li *et al*., 2013).

Experimental / In vitro *study*

In vitro and animal studies have indicated that grape polyphenols enhance eNOS activity and increase NO production in endothelial cells. The compounds obtained by purification of the non-alcoholic fraction of wine enhanced eNOS mRNA and protein expression and stimulated the synthesis of NO in human endothelial cells (Leikert *et al*., 2002; Wallerath *et al*., 2003; Rathel *et al*., 2007; Simoncini *et al*., 2011). Simoncini *et al.* (2011) reported that the compounds derived from wine enhanced expression of eNOS in human endothelial cells, indicating that the wine extract acts at the transcriptional level. Isometric study using rat femoral artery reported that the red wine polyphenol, Provinol elicited endothelium-dependent relaxation by stimulating NOS activity concomitantly with scavenging free radicals, which led to the enhancement of NO bioavailability (Zenebe *et al*., 2003). In spontaneously hypertensive rats (SHR), treatment with Alibernet red wine extract (24.2 mg/kg/day) for three weeks contributed to an increase in NOS and superoxide dismutase (SOD) activities in left ventricle, aorta and kidney tissues (Kondrashov *et al*., 2012).

It has been established that the compounds contained in wine behave like estrogens. Wine extract induces NO synthesis in vascular endothelial cells through the activation of estrogen receptors expressed in vascular cells. These estrogen receptors play important vascular regulatory actions. Indeed, similar to estradiol, exposure of endothelial cells to wine polyphenols activates estrogen receptor alpha. The activation of estrogen receptors leads to the recruitment of the mitogen-activated protein kinase ERK 1/2 and phosphatidylinositol-3-OH kinase/Akt pathways at the cell membrane or within the cell's cytosoplasm resulting in the eNOS activation that eventually increases NO production (Chalopin *et al*., 2010). Indeed, similar observations have been demonstrated in a study using resveratrol treatment, a polyphenol present in wine. Repeated treatment with resveratrol for five days increased eNOS mRNA and protein expression in cultured human endothelial cells, possibly by activating the estrogen receptors and PPARα in endothelial cells (Takahashi and Nakashima, 2012). Another mechanism by which grape polyphenols can exerts it cardioprotective benefit is through it antioxidant effects. In a study using aorta from female SHR, treatment with red wine polyphenols (40 mg/kg for five weeks) improved endothelial function which was associated with reduced vascular oxidative stress (Lopez-Sepulveda *et al*., 2011).

Cocoa

Intervention study

Cocoa is derived from the seeds of the fruit from Theobroma cacao tree. Human trials suggested that cocoa or chocolate consumption were correlated

with improvement in NO-mediated FMD and increased plasma or urine NO-derived species (S-nitrosothiols). Faridi *et al.* (2008) showed that acute ingestion of solid dark chocolate (22 g cocoa powder) and liquid cocoa improved FMD in 45 overweight adults (Faridi *et al*., 2008). Recently, West *et al.* demonstrated enhanced vasodilatation in both conduit and resistance arteries of overweight women after consumption of high-flavanol cocoa drink (814 mg/day) and dark chocolate (37 g/day) (West *et al*., 2014). Ingestion of high-flavanol cocoa drink (917 mg of flavonols) increased NO metabolites in plasma and urine of healthy subjects, and this was associated with improvement of FMD (Schroeter *et al.*, 2006). It has been demonstrated that administration of a high flavonoid-cocoa drink transiently improved NO-dependent FMD in the presence of pre-existing CVS risk, including hypertension and diabetes (Heiss *et al*., 2003; Grassi *et al.*, 2005; Balzer *et al*., 2008). In pre-hypertensive and hypertensive patients, consumption of 6.3 g of chocolate (30 mg of polyphenols) daily for 18 weeks showed increased plasma NO metabolites (Taubert *et al*., 2007). In healthy individuals, two weeks ingestion of flavanoid-rich dark chocolate bar (213 mg procyanidins, 46 mg epicatechin) improved FMD compared to ingestion of low-flavanoid dark chocolate bars (46 g, 1.6 o.z) (Engler *et al.*, 2004). Similarly, consumption of cocoa-derived beverage over five days in 27 healthy volunteers improved NO-dependent vasodilatation (Fisher *et al.*, 2006). A meta-analysis of 11 chronic and 11 acute clinical studies suggested improvement in FMD after acute and chronic ingestion of chocolate (Hooper *et al*., 2012).

Experimental / In vitro *study*

Animal study showed that treatment of rabbit aorta with the cocoa extract, procyanidin caused endothelium-dependent vasodilatation, which was likely dependent on the activation of eNOS and increased NO production (Karim *et al*., 2000). Increased NO bioavailability is dependent on the availability of the substrate for eNOS, L–arginine. In mammals, arginase catalyzes the conversion of L-arginine to urea. Vascular arginase competes with eNOS for their common substrate L-arginine, and this may impair NO production. Schnorr *et al.* (2008) demonstrated that treatment with cocoa-flavanols decreased arginase activity in humans and reduced arginase mRNA expression in cultured human endothelial cells. This finding suggested that cocoa-flavanols may contribute to the regulation of L-arginine concentration and NOS substrate supply (Schnorr *et al*., 2008). Besides their effects on eNOS expression and activity, cocoa flavanols also exert antioxidant effects *in vitro*. Treatment of cultured human endothelial cells with cocoa-derived epicatechin increased NO concentration via inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is an enzyme that catalyzes the production of superoxide anion (Steffen *et al.*, 2007).

Soy

Intervention studies

There is a growing interest on the effects of soy isoflavones on endothelial dysfunction in animals and humans. Human trial in healthy, postmenopausal women with hypercholesterolemia showed that four weeks treatment with soy protein (40 g soy protein powder; 80 mg isoflavones) increased FMD indicating the improvement in endothelial function (Cuevas *et al*., 2003). Hall *et al*. (2008) demonstrated that ingestion of isoflavone-enriched low-fat meal increased endothelium-dependent vasodilatation in postmenopausal women (Hall *et al.*, 2008). In another study on healthy postmenopausal women, treatment with soy isoflavone tablets (30 mg genistin and 30 mg daidzein) for 6 months showed significant improvement in endothelium-dependent vasodilatation among these women (Colacurci *et al*., 2005). Recently, a 12 months human trial was conducted among 182 Indonesian postmenopausal women to determine the effect of 100 mg/day soy isoflavone tablets on vascular endothelial function. This study showed a reduction in oxidative stress through lowering of malondialdehyde (MDA) concentration, which is a marker of lipid oxidation. However there was no improvement in vascular endothelial function observed (Pusparini *et al.*, 2013). The different results obtained between Pusparini *et al.* (2013) and other clinical studies may be due to few factors such as including duration and dosage of soy isoflavones, as well as subjects' characteristics. The subjects in the study by Pusparini *et al.* (2013) consumed relatively high daily soy isoflavones (100 mg/day) for a year, whereas other human trials that showed significant results only consumed low soy isoflavone for shorter durations. In addition, the study by Pusparini *et al.* (2013) was performed in an Asian country, whereas the other studies were conducted in Western countries, suggesting that ethnic influences may affect the different outcomes. Yildirir *et al.* (2001) also reported improvement in endothelial function in 20 males with hypercholesterolemia after soy ingestion (Yildirir *et al*., 2001). In patients with renal transplant, soy protein diet consumption

(25 g/day) for five weeks improved FMD and the effects disappeared after soy withdrawal, suggesting that the improvement was dependent on soy intake (Cupisti *et al.*, 2007). A meta-analysis of nine human trials found that soy isoflavones increased FMD in postmenopausal women with low baseline, but not in high baseline FMD levels (Li *et al*., 2010). Recently, a Bayesian meta-analysis by Beavers *et al.* (2012) accumulated evidence from 17 human trials and revealed that exposure to soy isoflavones improved endothelial function as measured by FMD (Beavers *et al*., 2012). The difference between these two meta-analysis were that the first analysis omitted studies which included men and used soy protein, while the latter expands the study inclusion criteria to accommodate a larger sample size without regard to gender.

Experimental / In vitro *study*

The vascular effects of soy isoflavones were often studied in animal models which have reduced levels of circulating estrogens and eNOS activity, such as ovariectomized animals. It was demonstrated that diet enriched in soy isoflavones improved acetylcholine-induced endothelium-dependent vasodilatation in aorta from ovariectomized rats (Catania *et al*., 2002) and coronary arteries from atherosclerotic female monkeys (Honore *et al*., 1997). Besides, a diet rich in soy isoflavones also induces increase in antioxidant and eNOS expression in animal aortas, leading to improved endothelium-dependent vasodilatation and reduced blood pressure (BP) (Mahn *et al*., 2005).

Pomegranate

Intervention study

Pomegranate (*Punica granatum* L. Punicaceae) is a seeded or granular apple, a delicious fruit consumed worldwide. Pomegranate contains substantial amounts of phenolic compounds, including flavonoids and hydrolysable tannins especially punicalagin. Most studies have demonstrated antioxidant, anticancer and anti-inflammatory properties of pomegranate (Faria and Calhau, 2011; Ismail *et al.*, 2012). Although several studies have demonstrated cardioprotective role of pomegranate extracts such as attenuation of atherosclerosis development and reduction of in BP (Aviram and Dornfeld, 2001; Aviram *et al.*, 2004), there seemed to be very scarce literature that reports the effect of pomegranate on endothelium-mediated vasodilatation.

In hypertensive patients, ingestion of pomegranate juice (150 ml/day) for two weeks reduced BP and showed increasing trends in FMD (Asgary *et al*., 2013; Asgary *et al*., 2014). Furthermore, two studies demonstrated that consumption of pomegranate juice (240 ml/day) for 30 days improved FMD in 30 adolescents with metabolic syndrome (Hashemi *et al*., 2010; Kelishadi *et al*., 2011).

Experimental / In vitro *study*

The principal mechanisms of action of pomegranate juice on vasodilatation may be due to enhanced expression of eNOS protein and NO production. Treatment with pomegranate juice increased eNOS protein expression in cultured human endothelial cells and carotid arteries of hypercholesterolemic mice (De Nigris *et al*., 2007). Besides, pomegranate juice also possesses potent antioxidant activity. Pomegranate juice has been shown to protect NO against oxidative damage, thus resulting in increased bioavailability of NO in bovine pulmonary artery (Ignarro *et al*., 2006).

Conclusion

Endothelial dysfunction is the precursor and early marker in the development and progression of atherosclerosis. There is increasing evidence showing that several plant-derived foods taken in the diet influences endothelial NO production and improves endothelial function. Thus, consumption of these natural products as dietary supplements may serve as a preventive measure to prevent vasculopathy. They may also serve as an adjunct to routine conventional treatments in patients with CVD.

Despite progress seen in the field of natural products and their cardioprotective effects, further research is still needed, especially in terms of phytochemical analyses of these active extracts and their pharmacological activities. In addition, more detailed work needs to be performed on the synergistic effects of plant constituents, to understand whether it can exert maximum therapeutic efficacy individually or in combination with other plant constituents. As most natural products have not been scientifically evaluated, the information of their efficacy, safety and potential interaction with conventionally used drugs are limited. Thus, research aimed to fill the current gaps in knowledge about potential harm and effectiveness of the plant-derived foods is needed to translate their applications into the clinical setting.

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