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

Keywords
Tea, Cocoa, Polyphenols, Endothelial function, Cardiovascular diseases
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 1. Classification of phenolic compounds

<table>
<thead>
<tr>
<th>Polyphenols</th>
<th>Substances</th>
<th>Representative compounds</th>
<th>Food sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Flavones</td>
<td>Quercetin</td>
<td>Vegetables, legumes, soy milk</td>
</tr>
<tr>
<td></td>
<td>Flavones</td>
<td>Kaempferol</td>
<td>Vegetables, kale, broccoli, tomatoes</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>Myricetin</td>
<td>Beverages: red wine, green tea, black tea, grape juice</td>
</tr>
<tr>
<td></td>
<td>Anthocyanids</td>
<td>Pelargonidin</td>
<td>Fruits: black grapes</td>
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<td></td>
<td></td>
<td>Cyanidin</td>
<td>Beverages: red wine, grape juice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diphidrin</td>
<td>Beverages: red wine, grape juice</td>
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<tr>
<td></td>
<td></td>
<td>Petunidin</td>
<td>Beverages: red wine, grape juice</td>
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<tr>
<td></td>
<td></td>
<td>Malvidin</td>
<td>Beverages: red wine, grape juice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flavanoids</td>
<td>Cocoa-derived products</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catechin</td>
<td>Fruits: apples, apricots, cherries, grapes, peaches, blackberries</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Procyanidins</td>
<td>Beverages: green tea, black tea, red wine, grape juice, elder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Procyanidins</td>
<td>Fruits: apples, peaches, persimmons, apples, pears, bananas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Procyanidins</td>
<td>Beverages: red wine, elder, tea, beer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proanthocyanidins</td>
<td>Beverages: Coffee</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proanthocyanidins</td>
<td>Fruits: nectarines, strawberries, blackberries, prunes, pomegranates, persimmon, walnuts, hazelnuts</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
<td>Beverages: red wine</td>
<td>Fruits: nuts</td>
</tr>
<tr>
<td></td>
<td>Lignans</td>
<td>Beverages: nuts</td>
<td>Fruits: fuitsseeds</td>
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<td></td>
<td>Curcumin</td>
<td>Beverages: Spices</td>
<td>Food spices</td>
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Table 2. Natural products and its effects on endothelial-dependent vasodilatation

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<th>Intervention Study</th>
<th>Treatment</th>
<th>Summary of the findings</th>
<th>References</th>
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<td><strong>Meta-analysis</strong></td>
<td>Tea (500 mg) compared to placebo</td>
<td>Increased FMD by 2.6% compared to placebo</td>
<td>Ras et al., 2011</td>
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<tr>
<td>Healthy individuals (n=64)</td>
<td>Green tea (5 g), caffeine (125 mg) or hot water</td>
<td>Increased FMD with green tea, and not affected by caffeine or hot water intake</td>
<td>Alecxantis et al., 2006</td>
</tr>
<tr>
<td>Healthy smokers (n=30)</td>
<td>Green tea (90 mg/d) for 2 weeks</td>
<td>Improved FMD</td>
<td>Nagy et al., 2004; Kim et al., 2006</td>
</tr>
<tr>
<td>Patients with coronary artery disease (n=50)</td>
<td>Short term effect: black tea (450 mg) for 2 hours</td>
<td>Improved endothelial-dependent FMD</td>
<td>Duffy et al., 2001</td>
</tr>
<tr>
<td>Patients with CAD (n=15)</td>
<td>Green tea (5 g/d) for 4 weeks</td>
<td>Improved FMD</td>
<td>Park et al., 2010</td>
</tr>
<tr>
<td>Healthy men (n=15)</td>
<td>5 treatments with twice daily intakes of black tea (0, 100, 200, 400 and 800 mg) for 1 week</td>
<td>Improved FMD</td>
<td>Oussar et al., 2009</td>
</tr>
<tr>
<td>Healthy women (n=21)</td>
<td>Green and black tea</td>
<td>Increased FMD</td>
<td>Joehmann et al., 2008</td>
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<tr>
<td><strong>Experimental in vivo study</strong></td>
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<tr>
<td>Isolated rat aorta</td>
<td>Green and black tea</td>
<td>Improved endothelium-mediated vasodilatation</td>
<td>Joehmann et al., 2008</td>
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<td>Cultured endothelial cells</td>
<td>Green and black tea</td>
<td>Prevented eNOS activity and NO bioavailability</td>
<td>Lorenz et al., 2004; Ameur et al., 2004; Steenstra et al., 2019</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Derivatives of tea flavonoids (EC, ECG, EGC, EGC2)</td>
<td>Dose-dependent increased NO production</td>
<td>Peracchi et al., 2008</td>
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<tr>
<td>Diabetic rats</td>
<td>Green tea</td>
<td>Decreased uncoupling of eNOS and increased NO production</td>
<td>Faris et al., 2012</td>
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<td><strong>Group and wine</strong></td>
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<tr>
<td><strong>Intervention study</strong></td>
<td></td>
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<tr>
<td>Meta-analysis of healthy and CAD patients (n=64)</td>
<td>Grape polyphenols</td>
<td>Improved FMD in both groups</td>
<td>Li et al., 2013</td>
</tr>
<tr>
<td>Patients with CAD (n=15)</td>
<td>Grape juice (4 ml/kg)</td>
<td>Improved FMD</td>
<td>Dian et al., 1999</td>
</tr>
<tr>
<td>Healthy individuals (n=15)</td>
<td>De-caffeinated red wine (250 ml) for 1 hour</td>
<td>Improved FMD</td>
<td>Aghdasi et al., 2000</td>
</tr>
<tr>
<td>Patients with CAD (n=15)</td>
<td>Regular red wine or de-caffeinated red wine (250 ml)</td>
<td>FMD higher with the consumption of de-caffeinated red wine compared to regular red wine</td>
<td>Hatami et al., 2004</td>
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<tr>
<td><strong>Experimental in vitro study</strong></td>
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<tr>
<td>Rat frenal artery</td>
<td>Provinol</td>
<td>Increased endothelial-dependent vasodilatation by stimulated NOS activity concomitantly and scavenged free radicals, which led to the enhancement of NO bioavailability</td>
<td>Zerebe et al., 2003</td>
</tr>
<tr>
<td>SHR (left ventricle, aorta and kidney tissues)</td>
<td>Alkaline red wine extract</td>
<td>Increased in NOS and SOD activities</td>
<td>Kondratov et al., 2012</td>
</tr>
<tr>
<td>Aorta of SHR</td>
<td>Oral treatment with red wine polyphenols (40 mg/kg) for 5 weeks</td>
<td>Improved endothelial function and reduced vascular oxidative stress</td>
<td>Lopez-Guerrero et al., 2011</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Resveratrol</td>
<td>Increased eNOS mRNA and protein expression and NO synthesis</td>
<td>Waltenh et al., 2003</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Non-alcoholic wine extract</td>
<td>Activated estrogen receptors, enhanced expression and activity of eNOS, increased NOS synthesis</td>
<td>Serremini et al., 2014</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Non-alcoholic wine extract</td>
<td>Enhanced eNOS expression, increased NO synthesis</td>
<td>Rathi et al., 2007</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Non-alcoholic wine extract</td>
<td>Enhanced eNOS expression, increased NO</td>
<td>Leibert et al., 2002</td>
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<td>MVEGS</td>
<td>Resveratrol</td>
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<td>Activated estrogen receptors and FFA synthesis in</td>
<td>Takahashi and</td>
<td>Nakashima, 2012</td>
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<td>endothelial cells. Increased eNOS mRNA and</td>
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<td>protein expressions, promoting NO release</td>
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<tr>
<th>Cultured endothelial cell</th>
<th>Wine extract</th>
<th>Akt-kinase, 2010</th>
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<tbody>
<tr>
<td>Activated estrogen receptor alpha</td>
<td>Akt-kinase, 2010</td>
<td>Phosphorylation of Akt and eNOS, leading to NO synthesis</td>
</tr>
</tbody>
</table>

**Cocoa**

**Intervention study**

**Healthy adults**
- 6.6 g dark chocolate bar (213 mg procyanidin, 46 mg epicatechin) daily for 16 weeks
  - Improved endothelium-dependent FMD and increased NO release
  - Improved plasma epicatechin concentration
  - Eisler et al., 2004

**Healthy and hypermenorrheic patients (n=14)**
- 2.5 g dark chocolate bar (25 g cocoa powder) vs. cocoa-free placebo for 16 weeks
  - Reduced blood pressure and increased NO release
  - Teuber et al., 2007

**Healthy adults (n=18)**
- High-flavanol cocoa drink (617 mg) vs. low-flavanol cocoa drink (57 mg)
  - Improved FMD and increased NO release
  - Schmied et al., 2006

**Healthy women (n=10)**
- 27 g dark chocolate and sugar-free cocoa beverage (614 mg flavanoids) vs. low-flavanol chocolate bar and cocoa-free beverage (3 mg flavanoids) for 4 weeks
  - Improved vasodilation and reduced arterial stiffness
  - West et al., 2014

**Hypermenorrheic patients**
- Flavanol-rich dark chocolate vs. white chocolate
  - Improved FMD
  - Chassa et al., 2008

**Diabetic patients**
- Single ingestion of flavanol-rich cocoa
  - Increased FMD and reversed vascular dysfunction
  - Bauer et al., 2008

**Patients with at least 1 cardiovascular risk factor**
- Cocoa drink (100 ml) containing 178 mg flavanoids
  - Increased NO bioavailability in human plasma and improved FMD
  - Khan et al., 2003

**Healthy individuals (n=7)**
- Flavanol-rich cocoa beverage for 5 days
  - Improved NO-dependent FMD
  - Fisher and Holloszy, 2000

**Experimental in vitro study**

**Isolated rabbit aorta**
- Prostaglandins
  - Increased endothelium-dependent vasodilation mediated by activation of eNOS
  - Akram et al., 2000

**Cultured human endothelial cells**
- Cocoa-flavanols
  - Reduced arachidonic acid (AA) mRNA expression, suggesting that cocoa-flavanols may contribute to the regulation of eNOS activity and NO and cGMP substrate supply
  - Schumaker et al., 2008

**Cultured human endothelial cells**
- Cocoa-derived epicatechin
  - Increased NO concentration via inhibition of NOS1 oxidation
  - Steffen et al., 2007

**Soy**

**Intervention study**

**Meta-analysis (n=6)**
- Soy isoflavones
  - Increased FMD in subjects with low baseline FMD levels
  - Li et al., 2010

**Meta-analysis (n=17)**
- Soy isoflavones
  - Improved endothelial function
  - Beavers et al., 2012

**Healthy postmenopausal women (n=18)**
- Soy isoflavones (40 g soy protein and 69 mg isoflavones) for 4 weeks
  - Increased FMD
  - Coe et al., 2003

**Healthy postmenopausal women (n=22)**
- Soy isoflavones enriched low-fat meal (80 mg isoflavones) for 4 weeks
  - Increased FMD
  - Hall et al., 2008
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 (BH₄) 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 cytosol 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 exert cardioprotective benefit is through its 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 oz) (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, procyanidins 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 NO 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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