

Polyphenols and Microvascular Function in Humans: A Systematic Review

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ABSTRACT (graphical abstract included)

Background: Polyphenol-rich dietary sources are acknowledged to have potential cardiovascular health benefits, particularly in reducing cardiovascular disease risk. **Methods:** This systematic review sought to determine the effect of polyphenol-rich foods and beverages upon microvascular function, which is of considerable importance in its contribution towards the pathophysiology of microvascular-related complications but also in the future development of (macro-vessel) cardiovascular disease. **Results:** Overall, consumption of polyphenol-rich foods and beverages demonstrate improved microvascular function, although this is dependent upon the polyphenol source, the dose of the product, the duration of consumption and the population group studied. Most subgroups reviewed suggest an overall beneficial effect on microvascular function, particularly grape-derived products, cocoa, tea, pine bark and Rutaceae aurantiae. Other groups remain equivocal and require further study due to the limited research performed to date. **Conclusion:** Polyphenols are abundant in the human diet and this systematic review demonstrates that they are an inexpensive, non-pharmacological approach for improving cardiovascular health in currently healthy individuals and in populations with microvascular dysfunction.

INTRODUCTION

The phrase ‘you are what you eat’ refers to the notion that you can contribute to a healthy body and mind by eating the right foods, such as fruits and vegetables, and consuming others in moderation which are, among others, rich in empty calories, saturated fat and salt. How food impacts upon health remains, however, largely obscure. We have reviewed, therefore, the evidence for one specific dietary molecular subgroup, polyphenols, found in many fruits and vegetables and their effect upon microvascular function contributing to cardiovascular health and beyond.

Structure and Function of the Cardiovascular System

The cardiovascular system is a complex, interrelated organ system that is composed of the heart, vasculature and blood which together serve three primary functions: transportation, homeostasis and protection. The cardiovascular system forms an efficient delivery network for cellular nutrition and removal of metabolic waste products, gaseous exchange and hormonal transport, in addition to being a key regulator of homeostasis for body temperature, pH balance, hydration and blood pressure [1]. In order to achieve these vital functions, however, the cardiovascular system must closely interact with other major systems, particularly the respiratory, neural, endocrine, digestive, skeletal, urinary and integumentary systems. In this

regard, the vascular tree, particularly the microvasculature, is integral in providing an extensive transport network of vessels to serve each of these systems as a site to facilitate exchange of macromolecules and fluids, in addition to responding to various mechanical forces and chemical signals concerned with homeostasis and protection [2].

Microvasculature

The microvasculature encompasses the smallest resistance vessels (<150µm in diameter) embedded within all human tissues and organs, consisting of terminal arterioles, capillaries and venules [3, 4]. Pre-capillary resistance vessels regulate local blood perfusion according to local metabolic demand, via neural, myogenic, metabolic and flow-induced (chemically-mediated) mechanisms. Vasodilators associated with neural pathways are primarily acetylcholine (ACh), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and substance P, with vasoconstriction achieved following sympathetic activation [4]. The vascular smooth muscle cells within the vessel wall (tunica media) closely interact with the endothelial monolayer which is innervated by autonomic nerves and local chemical mediators such as nitric oxide (NO).

Optimal microvascular function is essential for organ systems to function effectively, from the brain, eyes, kidneys, heart, muscle, adipose tissue to the skin. The cerebral microcirculation serves a pivotal role in homeostatic control to enable adequate central nervous system function. Precise regulation is required due to the brain's high metabolic rate and its limited capacity for energy storage, which is achieved through a combination of complex endothelial, myogenic, metabolic and neural regulatory mechanisms [5, 6]. Blood flow regulation in the human eye is complex and serves to maintain adequate eye health. Two vascular systems supply the eye; the retinal vessels supply the retina and prelaminar portion of the optic nerve and the ciliary vessels supply the rest of the eye [7]. Ocular blood flow is governed by local vascular control mechanisms, in addition to neural, systemic, endocrine and paracrine factors. The kidney is another highly complex organ that requires a well-regulated oxygen supply to meet a high energy demand. However, the kidney is vulnerable to hypoxaemic injury so the microvasculature is critical in ensuring a well regulated and maintained oxygen supply [8, 9]. The coronary microcirculation is responsible for ensuring that the cardiac muscle receives adequate oxygen and nutrition to perform its function as the engine of the human body [10]. Furthermore, the microvasculature needs to respond efficiently to local metabolic demand, particularly during times of cardiac stress such as physical exertion. These local metabolic demands are also true of the skeletal muscle microvasculature. Adequate perfusion is required

for tissue metabolism and maintenance of muscle mass [11, 12]. Adipose tissue requires adequate blood flow to and from adipocytes for lipid deposition and its utilisation as an energy source [13]. The microvasculature in the skin has been studied extensively, due to its relative ease of accessibility. It is a highly specialised vascular network that is organised into two plexuses in the dermis; superficial and deep layers which run parallel to the surface of the skin, with the majority of vessels located in the superficial plexus [14]. Skin blood flow is primarily concerned with temperature regulation and to a lesser extent, fulfilling nutritive demands. Non-glabrous skin of the limbs, head and trunk is largely governed by dual sympathetic neural control through noradrenergic vasoconstrictor and cholinergic vasodilator mechanisms [14, 15], in addition to local factors such as endothelial release of NO.

Microvascular Dysfunction

The microcirculation continues to receive widespread attention for its contribution towards overt cardiovascular disease and type 2 diabetes [16-19]. Considered a transitional stage between diet-induced obesity and insulin resistance [20, 21], microvascular dysfunction is characterised by structural and functional changes to the microvasculature that largely arise from impaired endothelial function. A healthy endothelium is essential for maintaining overall vascular health. As a major regulator of vascular homeostasis, the endothelial monolayer is crucial in maintaining the balance between vasodilation and vasoconstriction [22]. Endothelium-derived chemical mediators are central to this regulated equilibrium, with any disturbance leading to endothelial dysfunction and the associated pathological processes of cardiovascular and metabolic diseases [22-24]. Microvascular dysfunction often precedes microvascular complications and (macro) vascular dysfunction in larger vessels that subsequently leads to pathological interactions and the development of both small and large vessel disease [25], such as atherosclerosis and other plaque-related problems including stenosis and ischaemic vascular disease.

Many diseases arising from microvascular dysfunction share similar pathological pathways relating to inflammatory processes, endothelial dysfunction and altered glucose metabolism. Therefore, dysfunction is often present in multiple organs concurrently. Without intending to be fully comprehensive, the following examples illustrate the importance of a functional microcirculation. Microvascular degeneration arising from advancing age and other cardiovascular risk factors is associated with cerebrovascular disease, such as stroke arising from microbleeds [26] and Alzheimer's disease [27]. Furthermore, cerebral hypoperfusion leads to increased systemic blood pressure as a compensatory mechanism to ensure that the

brain tissue receives enough oxygen and nutrients in order to function adequately [28]. This compensation can lead to the development of systemic cardiovascular disease, such as essential hypertension. Impaired renal microcirculation leading to hypoxaemia is associated with acute and chronic kidney disease [9, 29]. Altered function in the retinal microvessels can lead to the development of retinopathy, with the retinal vessels also providing a biological model to detect the early manifestation of hypertension [30]. Coronary microvascular dysfunction is associated with changes such as reduced coronary flow reserve that is present in diseases including arterial hypertension, hypertrophic cardiomyopathy and diabetes mellitus [31]. The skeletal muscle is critical in regulating nutrient metabolism, and microvascular dysfunction leads to changes in glucose metabolism that arise from insulin resistant pathways [32]. Such changes in the microvasculature in various organs are closely related to the mechanisms underlying many cardiometabolic diseases.

Microvascular Function – Measurement Techniques

Several methods of measuring microvascular function (Figure 1) have been developed due to continued clinical interest in the role of microvessels, particularly since microvascular beds are thought to exhibit detectable changes in endothelial (dys)function earlier than macrovessels [3, 22, 24, 33] and can provide an insight into the mechanisms underlying these disease states. The cutaneous microcirculation is an easily accessible vascular bed that may be used as a surrogate for generalised vascular function [34]. Various approaches have been used to measure skin blood flow (SkBF), such as, venous occlusion plethysmography (VOP), laser Doppler flowmetry (LDF), videomicroscopy, laser Doppler perfusion imaging (LDPI) and laser speckle contrast imaging (LSCI) [14, 35], and typically involve measures being taken from limited regions due to the heterogeneity of the microvascular anatomy and underlying control mechanisms [14]. The most commonly used techniques for assessing microvascular function are briefly described below.

Venous occlusion plethysmography (VOP). This technique has been used extensively to determine whole limb (e.g., muscle and skin) blood flow based upon the rationale that the (subdiastolic) occlusion of venous outflow from an extremity (forearm/leg) induces a change in its volume that corresponds proportionally with continued arterial inflow [36]. Blood flow is calculated according to limb volume change by displacement or circumference change [14].

Laser Doppler flowmetry (LDF). Routinely used to study microvascular function, LDF is sensitive in detecting changes in skin perfusion over a period of time and in response to

transient pharmacological and non-pharmacological stimuli, including local thermal hyperaemia (LTH), post-occlusive reactive hyperaemia (PORH) and iontophoresis of vasodilators [37, 38]. LDF is based upon the reflection, scatter and Doppler shift of laser emitted photons in detecting moving red blood cells [37]. The magnitude and frequency of the Doppler shifted photons and the change in wavelength is related to the concentration and velocity of blood cells [14, 37]. LDF is superior to other measures of SkBF due to its continuous signal, excellent temporal resolution and high spatial resolution [14]. Depending on the depth utilized, LDF can be applied to many tissues including the skin, intestine, bone and muscle.

Laser Doppler perfusion imaging (LDPI). This technique is based on light scattering in tissue as in LDF, but rather than being a single-point measurement covering $\sim 1 \text{ mm}^2$, it combines several single measurements taken over a tissue surface to produce a single image of overall microcirculation covering up to 2500 mm^2 [35, 39]. A movable scanning mirror directs the laser beam to the measurement sites, but this is a time-consuming process that requires processing of vast amounts of raw data; typically, 4,096 measurement sites recorded over a 4-min period are required for a single perfusion image [35, 40]. LDPI is most frequently used in evaluating cutaneous function, largely to assess various wounds and ulcers, rather than as an index of microvascular function *per se*. The poor temporal resolution makes this technique less able to assess changes in skin perfusion that occur across shorter time frames, such as during (local) heating, exercise or drug infusions.

Laser speckle contrast imaging (LSCI). Laser speckle is generated when tissue is illuminated by laser light. This speckle pattern changes when blood cells move within the region of interest and creates blurring of the captured image; higher levels of movement (fast flow) produce a more blurred image that reduces the contrast in the region, with lower levels of movement (low flow) corresponding with an increase in contrast [35, 41]. The advantage of LSCI over LDPI is that LSCI is a near-instant measure of the whole tissue, rather than scanning multiple times. Video frame rate blood flow images (up to 25 per second) enable immediate tracking of fast transient changes in blood flow over a surface area from $5 \times 7 \text{ mm}$ to $15 \times 20 \text{ cm}$, to a depth of approximately 150-300 micron [35, 41]. LSCI is a usual method for evaluating cutaneous microcirculation and has also been used to assess intraoperative cerebral microcirculation, as well as retinal perfusion.

Videomicroscopy. Direct visualisation of the microcirculation is achieved using orthogonal polarisation spectral (OPS) imaging and, more recently, sidestream darkfield (SDF) imaging [35]. These techniques illuminate the tissue via polarised light from an external or integrated light source, respectively, and allow the measurement of vessel diameter, vessel density and red blood cell velocity. Videomicroscopy has been used to study microvascular blood flow in the sublingual mucosa, in addition to the gingiva, skin, liver and conjunctiva [35, 42].

Capillary microscopy. This technique is a simple, non-invasive method of assessing microvascular function of the nailfold capillaries, including their morphology, density and blood velocity, and is useful in detecting microangiopathies such as Raynaud's phenomenon and hypertension [43, 44].

Additional methodologies can also be used to investigate microvascular function in other organs. Assessment of cerebral perfusion is difficult due to the natural skull barrier. Several non-invasive techniques have been developed including single photon emission computed tomography (SPECT), positron emission tomography (PET), dynamic perfusion computed tomography (PCT) and arterial spin-labelling (ASL) functional magnetic resonance imaging (fMRI) that can examine regional micro- through to macrovascular cerebral perfusion. Renal perfusion may be assessed via blood oxygen level-dependent (BOLD) MRI which gives an index of tissue oxygenation (pO_2) [45], with alternative methods including ASL fMRI and PET scans [46]. The retinal microvasculature is assessed through advanced imaging techniques focusing on static structural features and dynamic retinal analysis related to functional measures (e.g. flow), which have been reviewed comprehensively by Liew and colleagues (2008). Coronary microvascular function is assessed via invasive methods such as intracoronary Doppler and non-invasive methods such as transthoracic Doppler echocardiography (TTDE) which allows calculation of the coronary flow reserve and can be used as an index of coronary microvascular dysfunction [31, 48].

Modifiable Risk Factors

It is important to recognise pre-disease as a window of opportunity to improve endothelial and overall microvascular function to prevent/limit progression towards clinical pathology. Therapeutic strategies include a variety of drug therapies that target improved microvascular function through structural modifications. Current non-pharmacological strategies for preventing the progression of microvascular dysfunction in pre-diseased states are primarily

focused on controlling modifiable risk factors, particularly related to lifestyle modification. Physical inactivity and obesity are considered two of the most important risk factors for the development of cardiometabolic disorders. Sedentariness and excessive caloric intake through altered dietary structure promote a positive energy balance that increase central adiposity and body weight [49]. Dietary components also have profound effects upon microvascular health. Salt intake has been linked to hypertension arising via dysfunction due to microvascular rarefaction [50]. Modest reductions in salt intake have demonstrated both functional and structural improvements to capillary rarefaction [51][51, 52]. Excessive dietary protein is linked to renal damage through hyperfiltration [53], although the current scientific evidence remains equivocal [54]. However, other dietary components are suggested to exert a positive influence upon cardiovascular health, such as fruits and vegetables, spices, omega-3-fatty acids and low-fat dairy products [55]. As the deleterious effects of sedentary lifestyles have become more apparent and the beneficial effects of certain dietary components receive wider scientific recognition, dietary interventions have received greater attention as inexpensive tools to combat the ever-increasing global burden of cardiovascular disease.

Dietary Intervention: The Role of Polyphenols

The Mediterranean diet is widely reputed to exert a protective effect upon cardiometabolic health, largely achieved via mechanisms related to its richness in plant-derived products, such as olive oil, fruits and vegetables, which contain bioactive compounds known as polyphenols [56]. These naturally occurring compounds are the most abundant antioxidant in the human diet; to date, in excess of 8,000 polyphenolic compounds have been identified [57]. Polyphenols can be broadly categorised into four subclasses (Figure 2) according to their phenolic ring structure and ring-binding elements: flavonoids, phenolic acids, lignans and stilbenes [57, 58].

Flavonoids account for the greatest proportion of polyphenols (60%) and are characterised by two or more aromatic rings bound together by a 3-carbon bridge forming an oxygenated heterocycle, with the degree of oxidation generating further subclasses, in ascending order of oxidation: flavanols (often called catechins), flavanones, flavones, isoflavones, flavonols and anthocyanins (Figure 2) [59, 60]. Hydroxylation and conjugation patterns of the aromatic rings further characterises the individual flavonoids and isoflavones within these subclasses [60]. Phenolic acids are the second most abundant polyphenol subclass, accounting for 30% of

dietary polyphenols [59]. These can be categorised as hydroxybenzoic acids, as found in tea, and hydroxycinnamic acids which are found in coffee, cinnamon and fruits such as blueberries, apples and plums [57, 61]. Lignans are diphenolic compounds formed of 2 phenylpropane units and are found in fruits, legumes, cereals and grains [61]. Several lignans are regarded as phytoestrogens, such as secoisolariciresinol [57] which is found in linseed (flax), the richest sources of lignans. Stilbenes are the final of the four polyphenol subclasses and are characterised by two phenyl moieties connected by a two-carbon methylene bridge. Low quantities of stilbenes occur in the human diet, with resveratrol and pterostilbene considered the main ones. Resveratrol is the most widely known of the two and is found in grapes and its derivative red wine, as well as blueberries, cranberries and peanuts. Pterostilbene is also found in grapes and blueberries, and is suggested to have superior bioavailability compared to resveratrol, in addition to neuroprotective properties in age-related diseases [62].

Epidemiological research has explored the effects of a variety of polyphenol-rich foods upon cardiovascular health, with beneficial effects observed for glycaemic control [63, 64] and macrovessel endothelial (dys)function [65]. Furthermore, a negative correlation has been identified between the consumption of polyphenol-rich dietary products and cardiovascular disease incidence [66-68]. As the microcirculation represents an important vascular bed in the pathogenesis of many cardiometabolic diseases, it is important to review the current literature to determine the effect of polyphenol-rich foods and beverages upon microvascular function in several of organ systems. Therefore, in this chapter, we will perform a systematic review pertaining to the literature that examined the impact of polyphenols on microvascular function.

METHODS

Search strategy

The PubMed database was searched for studies in the English language between 1945 and June 2016 involving human subjects. A combination of medical subject heading (MeSH) search terms were used (microcirc* OR microvasc* OR microvessels OR capillar* OR arterioles OR venules OR iontophoresis OR blood flow OR perfusion OR vasodila* OR laser Doppler OR videocapillaroscopy OR laser speckle OR NIRS OR plethysmography) AND (polyphenol* OR flavonoid* OR catechin* OR flavanol* OR flavan-3-ols OR flavonol* OR isoflavone* OR flavanone* OR flavone* OR anthocyanidin* OR proanthocyanidin* OR epicatechin OR EGCG OR quercetin OR kaempferol OR myricetin OR resveratrol) AND (human OR subjects OR volunteers OR patients OR males OR females).

Inclusion of studies

A total of 2567 studies were identified through the PubMed database search. Three independent investigators (K.W., R.D. and D.L.) reviewed the studies using a systematic hierarchy of exclusion criteria: *in vitro*, review article, not polyphenols, not microcirculation, not human and ex vivo (see Figure 3). A total of 2399 abstracts were excluded, with 168 full-text articles going forward to the next stage. Studies were excluded according to the same exclusion criteria as for the abstracts, in addition to the following: if the contribution of microcirculation to the vascular measurement could not be determined (e.g. pulse form analysis or fMRI), no full paper was available, duplicate paper, not oral administration of polyphenols and data was unavailable for inclusion (requested from the authors but no response received). A total of 55 studies fulfilled all review criteria and the extracted data are shown in Tables 1 to 9, in which the tested polyphenols are aggregated per dietary source or listed as pure polyphenols.

RESULTS AND DISCUSSION

For many years polyphenol-rich dietary sources have been acknowledged to have potential cardiovascular health benefits, particularly in reducing cardiovascular disease risk [69-72]. Also, in the 1950s citrus polyphenols, at that time called 'vitamin P', were shown to affect capillary fragility in rabbit ears [73]. Nevertheless, the research on polyphenols improving microvascular function in different vascular beds and linking that to a (cardiovascular) benefit is still in its infancy, reflected by the fact that 50% of the eligible studies were published in the last decade (Tables 1-9). The first seriously studied polyphenol-rich product was Daflon, a flavonoid fraction of Rutaceae aurantiae containing the flavone diosmin and flavanone hesperidin, followed by the procyanidin-rich pine bark extract Pycnogenol. These two products have paved the way for other polyphenol-rich dietary products, such as olive oil, soy, berries, grape, wine, tea and cocoa to be studied in relation to microvascular function. Most clinical studies have focused on the skin microcirculation, which may probably be explained by its relatively easy accessibility. In the following sections the different sources of polyphenols tested on microvascular function are summarised in separate sections in alphabetical order.

Berries (insert table 1 near this text)

Berries are rich in polyphenols and contain phenolic acids, stilbenes and a variety of flavonoid subclasses, of which they are richest in anthocyanins. Limited studies have examined the effect of berries upon microvascular function (Table 1). In a randomised, double-blind, crossover, placebo-controlled acute study in a healthy population, a 20% blackcurrant juice

drink, likely to represent the dose consumed in a normal diet, did not demonstrate any immediate improvement in cutaneous endothelium-dependent and endothelium-independent vascular reactivity [74] assessed via acetylcholine (ACh) and sodium nitroprusside (SNP) iontophoresis with laser Doppler imaging (LDI). These results suggest that berries do not have any acute effect upon microvascular function in healthy volunteers, although further investigation is warranted given the lack of research to date.

Individuals with non-medicated retinal vasculopathy, who typically exhibit impaired microvascular health, demonstrated significant improvements in ocular blood flow following chronic (6-months) supplementation with 160mg/day bilberry extract [75] compared to a control group receiving standard management only. Two standardised bilberry extracts were administered in a parallel group design, each containing 36% anthocyanins, but with one extract containing the full range of non-anthocyanin components as well. Both intervention groups demonstrated improvement in basal retinal blood flow velocity measured by high resolution colour Doppler imaging. Supplementation with the full range of non-anthocyanin components further improved retinal blood flow when compared to standard management. These studies may suggest that the impact of berries on microvascular health differs between healthy vs diseased, with a potential impact in a diseased population with *a priori* impaired vascular health. Although this fits with several previous observations of benefits of dietary interventions in populations at risk, we need to be careful since differences in methodological design between studies and the lack of properly designed, longitudinal studies prevent strong conclusions regarding the impact of berries on microvascular function.

Cocoa (insert table 2 near this text)

Consumption of a flavanol-rich cocoa beverage versus a low flavanol control beverage increased skin blood flow in healthy females when measured by LDF and spectroscopy [76]. Similarly, Heinrich and colleagues (2006) observed increased cutaneous blood flow after 6- and 12-weeks consumption of high- versus low-flavanol cocoa. A randomised, parallel-group study comparing the effects of 900mg daily cocoa flavanol consumption in healthy young and elderly male individuals demonstrated an increase in microvascular function for both groups, measured by forearm strain gauge plethysmography and reactive hyperaemia LDPI [78]. These observations in healthy subjects could not be replicated in those with cardiovascular disease and/or risk. Indeed, microvascular function was not affected 2-hours following consumption of flavonoid-rich dark chocolate in individuals with symptomatic peripheral artery disease when compared to a cocoa-free white chocolate control [79]. Also, prolonged use of

flavanol-rich cocoa treatment (2- or 6-weeks) in a randomized controlled trial did not significantly affect microvascular function in individuals with essential hypertension [80] or coronary artery disease [81]. Although this might suggest that flavanol-rich cocoa is more effective in improving microvascular function in healthy individuals, variability in assessments of the primary outcomes and/or the polyphenol dose may (at least partly) have contributed to these differences.

In addition to skin blood flow, the effects of polyphenol-rich cocoa have been investigated on microvascular function in other organs. In agreement with the data derived from the skin, retinal vessel diameter dilation was observed 2h post cocoa-rich chocolate in individuals with glaucoma, but not in age-matched controls [82]. Furthermore, increased renal perfusion was observed in healthy individuals 2-hours post-consumption of cocoa-rich dark chocolate (70% cocoa) [83]. In studies that utilised MRI techniques, 5 days consumption of flavanol-rich cocoa (150mg daily) demonstrated increased CBF measured by BOLD-MRI in young, healthy males following a cognitive task [84]. Similar beneficial chronic effects of cocoa on regional cerebral perfusion (assessed via fMRI) were reported by Brickman et al. [85]. Furthermore, using arterial spin labelling fMRI, Lampert and colleagues (2015) reported increases in regional cerebral perfusion 2-hours following consumption of a high-flavanol versus low-flavanol beverage in healthy, older (55-65) males. Current research, therefore, suggests that flavonoid-rich cocoa positively affects microvascular function in healthy individuals, in skin as well as in other tissues, whilst such effects were not observed in patient populations. Nonetheless, the lack of long-term studies examining the effects of flavonoid-rich cocoa prevents strong conclusions on the impact of cocoa on microvascular health.

Daflon (insert table 3 near this text)

Daflon is a micronized flavonoid fraction of *Rutaceae aurantiae* (citrus family) consisting of 90% diosmin and 10% hesperidin. Some uncontrolled observational studies in chronic venous insufficiency patients have suggested beneficial effects of Daflon (1000 mg per day) on the cutaneous and capillary microcirculation [87, 88]. In randomised and placebo controlled studies improvements in lower limb venous distensibility and capacitance (via venous occlusion plethysmography) occurred in chronic venous insufficiency patients after chronic Daflon supplementation (1-week – 2-months) [89, 90]. Similar beneficial effects were also evident in healthy populations and/or healthy legs of patients with unilateral venous insufficiency [89, 91]. Galley and colleagues (1993) observed increased forearm capillary conductance in older individuals with symptomatic capillary fragility following both 4- and 6-

weeks supplementation with S5682 flavonoid fraction, consisting of diosmin and hesperidin, compared to placebo control. Beneficial effects of Daflon have also been reported after acute administration of 1000mg in randomised and placebo controlled studies [89]. Overall, these results indicate a positive effect of Daflon on the peripheral microcirculation, particularly in individuals with compromised peripheral circulation. Whether Daflon also affects other patient populations prone to microcirculatory disorders is currently unknown.

Grape and Wine (insert table 4 near this text)

Polyphenol-rich grape are amongst the most frequently examined polyphenol-rich food products, and appear to improve microvascular function in the majority of studies. This observation matched with macrovessels, since red wine is associated with improved endothelial function in macrovessels [92, 93]. Regarding the microcirculation in healthy volunteers, it was found that daily consumption of grape juice for 3-weeks in a single arm trial of male, adult triathletes was associated with improved functional capillary density although no control trial was included [94]. Furthermore, a single arm trial in healthy women observed a borderline significant increase in forearm endothelium-dependent dilation ($P=0.06$), whilst a significant improvement in endothelium-independent vasodilation ($P<0.01$) was found following 3-weeks daily supplementation with red wine [95]. However, an acute single dose of red wine did not demonstrate any effect on forearm blood flow. In agreement with healthy subjects, a previous randomised, double-blind, placebo-controlled study of obese, male smokers, supplementation with grape seeds found improved skin blood flow at 4-weeks (but not at 8-weeks) compared to the control group [96]. In addition, the grape-derived product red vine leaf enhanced basal malleolar skin blood flow (Laser Doppler flowmetry) following 6-weeks daily 360mg supplementation in a randomised, double-blind, cross over, placebo-controlled study in individuals with chronic venous insufficiency [97]. Beneficial chronic effects of 16 weeks thrice daily grape juice on regional cerebral perfusion (assessed via fMRI) in older adults with mild cognitive impairment were reported by Krikorian and colleagues [98]. Despite these encouraging effects of grapes (products), further research is required to better understand their acute and longitudinal effects on microvascular function in both pathological and healthy populations.

Olive Oil (insert table 5 near this text)

The Mediterranean diet is associated with improved cardiovascular health and a major constituent of this diet is olive oil, which is rich in phenolic compounds [99]. In a study of older

(53-68yrs) hypercholesterolaemic individuals, no difference was observed in digital microvascular function between acute ingestion of PPR olive oil and low phenol control olive oil [100]. In contrast, a two month randomised, double-blind, crossover study comparing the effects of polyphenol-rich (PPR) and polyphenol-free (PPF) olive oil in a young, female, mildly hypertensive population, demonstrated an increase in reactive hyperaemia following the PPR diet compared to baseline, whereas no change was observed after the PPF diet [101]. This study adopted a 30mg/day dose, which is consistent with the quantity consumed as part of a normal diet. To date, no studies have been performed in healthy individuals. Taken together, whilst acute ingestion of olive oil has limited effects, regular consumption of olive oil is associated with improved microvascular health.

Pine Bark (insert table 6 near this text)

Nishioka and colleagues (2007) observed an increased FBF response to ACh following 2-months of daily supplementation with Pycnogenol, a standardised extract from French maritime pine bark (*Pinus pinaster Ait*), in healthy males compared to placebo. Pycnogenol has been extensively studied in the treatment and prevention of venous microangiopathy, particularly chronic venous insufficiency (CVI) [103, 104]. In a randomised three-group design, individuals with severe CVI underwent assessment of cutaneous LDF derived resting flux in the perimalleolar region 8-weeks following a daily intervention of capsular Pycnogenol 150mg/day, Pycnogenol 150mg/day with compression stockings or compression stockings only [103]. Microvascular function improved following regular consumption of Pycnogenol, and Pycnogenol combined with compression stockings. These findings are supported by several further studies by the same research group that were undertaken in similar patient groups where improved cutaneous microvascular function was observed following 8-weeks supplementation with 150mg/day Pycnogenol versus placebo [105], 4-weeks supplementation versus placebo in individuals with severe diabetic microangiopathy [106] and 8-weeks of either 150mg/day or 300mg/day Pycnogenol [107]. A further study by Hu and colleagues (2015) recently observed that 12-weeks supplementation with 150mg/day oral Pycnogenol increased basal LDF flux in pre-clinical, borderline hypertensive, hyperlipidemic and hyperglycemic individuals. Another research group also observed a beneficial effect of Pycnogenol on skin blood flow after 6-weeks in individuals with diabetic microangiopathy [109], essential hypertension [110] and CVI [111]. Collectively, these studies suggest that Pycnogenol demonstrates increased skin blood flow in a range of patient populations observed by several research groups.

The microvascular benefits of Pycnogenol supplementation are not confined to the skin. Pycnogenol consumed daily for 6-months had a beneficial effect on blood velocity of the cochlear artery in individuals with Meniere's disease and moderate tinnitus [112]. Individuals with diabetic retinopathy and other vascular retinal diseases demonstrated improved retinal vascularisation and reduced endothelial permeability following a 2-month randomised, double-blind, placebo-controlled, parallel group study where individuals received 150mg/day Pycnogenol [113]. Retinal blood flow was also increased following 3-months of Pycnogenol versus placebo in individuals with type 2 diabetes mellitus and moderate diabetic retinopathy [114].

Studies have also explored the potential clinical benefits of oral consumption of Pycnogenol combined with topical application of Pycnogenol powder. In individuals with severe CVI, Belcaro and colleagues (2005) observed a significant difference in the transcutaneous partial pressure of oxygen (PO₂) and partial pressure of carbon dioxide (PCO₂) in the 6-week oral and oral combined with topical Pycnogenol groups versus control, with better venous ulcer healing in the combined group. A similar outcome was observed in a subsequent 6-week study in individuals with severe diabetic microangiopathy performed by the same research group [109], suggesting that oral and topical Pycnogenol could be a very useful treatment for individuals with microangiopathy. These observations in (pre-)clinical trials suggest that pine bark represents a useful non-pharmacological therapeutic treatment for improving microvascular function with potential clinical benefit.

Pure Polyphenol Compounds (insert table 7 near this text)

The number of studies that have examined the effects of pure polyphenol compounds on microvascular function is limited. Most studies have evaluated the effects of resveratrol. From the four studies conducted to date, resveratrol has demonstrated no acute improvements in cerebral microvascular function compared to placebo control [115, 116], with the exception of an acute increase in total haemoglobin in the pre-frontal cortex measured by NIRS [117]. However, the same study observed no significant difference after four weeks supplementation. Gliemann and colleagues (2014) showed actually that training-induced angiogenesis of skeletal muscle capillaries was limited following resveratrol supplementation. In conclusion, there is insufficient evidence to suggest that resveratrol beneficially affects the microcirculation. Each of the four resveratrol studies were performed in a healthy population so the effects of resveratrol in a pathological population remain unknown.

In line with resveratrol, other pure polyphenol compounds were unable to increase cerebral perfusion. Epigallocatechin gallate, the most abundant flavonoid in green tea, was not associated with any increase in cerebral blood flow in the prefrontal cortex following either a 135mg or 270mg dose in an acute study of young, healthy adults [119]. The 135mg dose was associated with reduced CBF following cognitive task performance which highlights how observing microvascular changes under a challenged condition can confound results and make interpretation of findings more difficult, particularly when there is limited research examining the effects in baseline conditions. When examining pure polyphenol compounds on peripheral vascular beds, dispersible hesperetin, an aglycone of citrus flavonoids, demonstrated a beneficial effect upon cutaneous microvascular function in a study of young, healthy females with cold sensitivity [120]. Overall, there appears to be mixed evidence for improvements in microvascular function following supplementation with pure polyphenol compounds. As several studies have so far been undertaken by a single research group, it would be advantageous for multiple research centres to address this gap in our current knowledge of the microvascular consequences of pure polyphenol supplementation.

Soy (insert table 8 near this text)

Three studies have examined the effect of soy in post- and peri-menopausal populations. These groups were chosen since isoflavones within the soy bean have a similar chemical structure to the estrogen hormone estradiol [121]. Various measures of microvascular function have been used to examine the effect of soy, with Wong and colleagues (2012) observing no significant difference in forearm blood flow when measured via strain-gauge plethysmography and peak reactive hyperaemia after 80mg daily supplementation with soy tablets for 6-weeks. Similarly, no differences were observed for laser Doppler velocimetry [122] or forearm venous occlusion plethysmography response to ACh or SNP [123] after 55mg daily soy capsules for 6-weeks and 80mg daily soy tablets for 5-10-weeks, respectively. It seems relevant that these observations were made in patient groups, who all demonstrated impaired vascular health prior to the start of the intervention. This provides further support that soy unlikely has benefits in improving microvascular health.

Tea (insert table 9 near this text)

Macrovascular function is generally improved following the consumption of polyphenol-rich tea. In agreement with larger vessels, some evidence now also supports the benefits of acute

as well as chronic tea consumption on skin blood flow. Fuchs and colleagues (2016) recently observed the ability of tea to prevent the increase in postprandial vascular resistance of forearm microvessels within 3-hours following 100ml black tea in a randomised, cross-over study in obese, insulin-resistant males. Heinrich and colleagues (2011) performed an acute, double-blind trial in 15 healthy females who were randomised into 3 groups, each of whom received a single dose of capsular green tea extract (0.5, 1.0 or 2.0g). Skin blood flow after all three doses increased at 15-30-min with no difference between doses. A further acute pilot study by Miller and colleagues (2011) investigated the effect of capsular green tea extract (836mg green tea catechins) on laser Doppler responses to ACh and SNP iontophoresis in healthy, non-smoking, overweight individuals, but found no impact of tea. Overall, these studies on the acute effects of tea suggest that flavonoid-rich tea has the capacity to acutely increase microvascular function. In line with these observations, a 12-week double-blind, placebo-controlled study in 60 middle-aged, healthy females observed increased forearm cutaneous blood flow at both the midpoint and end of the 12-week intervention for green tea consumption [125]. Oxygen saturation of haemoglobin was also increased at 6- and 12-weeks following daily consumption of the green tea beverage. However, regular consumption of capsular green tea for 3-weeks was not associated with any difference in endothelium-dependent or endothelium-independent microvascular reactivity in a double-blind, placebo-controlled parallel study in healthy males [127]. Furthermore, Vidyasagar and colleagues (2013) observed no effect of black tea on cerebral blood flow 2-hours after consumption in healthy males (assessed by fMRI).

The nature of the intervention product may be responsible for some of the differences in microvascular function observed in these studies of healthy individuals. Studies that used a beverage seem to show a benefit in improved microvascular function, whilst such effects were not found when using a capsular form of tea. This may be due to the capsular shell material hampering bioavailability, as cellulose based shells and fillers are known to have a negative interaction with polyphenols [129]. To support this idea, gelatin capsules without a cellulose filler demonstrate more positive outcomes. However, precise details are scarce regarding the shell and filler materials of capsules used in the studies discussed, so it is difficult to establish whether this may be a causal factor for the apparent differences between studies and their intervention products.

The microvascular effects of green tea have also been examined in individuals with increased cardiovascular disease risk. Smoking is associated with impaired endothelial function, even

when individuals are otherwise healthy [130, 131]. With this in mind, Oyama and colleagues (2010) investigated whether daily consumption of green tea catechins for 2-weeks demonstrated any effect on forearm blood flow (FBF) responses to ACh and SNP in healthy, male smokers. Individuals were divided into three groups receiving daily green tea catechins: control (0mg), medium-dose (80mg) or high-dose (580mg). FBF was assessed acutely (2h), at the mid-stage (day-7) and at the end of the trial period (day-14). The acute response to ACh was increased for the high-dose group, but no difference was observed for the medium-dose or control groups. No significant acute response to SNP was observed for any group. Similar to the acute responses, chronic consumption of green tea catechins was associated with significantly increased FBF responses to ACh for the high-dose group, but not for the medium-dose or control groups and no difference was observed for any group in response to SNP. These findings suggest an endothelium-dependent vasodilatory response for high-dose green tea catechins, both acutely and following regular consumption. This improvement in endothelial function is encouraging for green tea having a positive effect upon cardiovascular health in at-risk populations. Further studies are required to explore these effects further.

Summary Discussion

(insert table 10 near this text)

This systematic review highlights the effects of polyphenol-rich dietary sources on microvascular function. Due to the wide range of microvascular networks and various polyphenolic dietary sources, it has previously been difficult to establish any conclusions on this topic. Our robust methodology, systematically reviewing all publications on polyphenols and microcirculation, has enabled us to provide valuable insight into this important area of research and to identify where future research is required. Whilst it is difficult to determine the effects of some subgroups due to limited clinical studies, flawed statistics and mixed evidence likely related to small sample sizes and variable measurement techniques, precluding a more definitive indicator of overall microvascular effects, the review suggests that some polyphenol sources do improve microvascular function (see Table 10). Consistent improvement in microvascular function were observed with Pycnogenol and Daflon. Promising effects were observed for cocoa (in healthy subjects), black and green tea (consumed as beverage) and grape products (grape juice and red wine). These results suggest that different subtypes of polyphenols may benefit the microcirculation. The number of studies conducted with isolated pure polyphenols is too limited indeed to declare superiority in efficacy of one polyphenol (subtype) over another. Larger, well designed studies are required to further our collective knowledge of the microvascular effects of dietary polyphenols in all subgroups.

Limitations

Currently there is insufficient data for a single measure of microvascular function to enable us to perform a meta-analysis which would provide a more precise analysis of the microvascular effects of polyphenols. A further limitation of this review is that there is an absence of analysis to determine the potential mechanisms underlying any beneficial effects of polyphenol-rich dietary sources upon microvascular function. However, this is beyond the scope and original aims of the review.

Conclusion

Overall, consumption of polyphenol-rich foods and beverages demonstrate improved microvascular function, although this is dependent upon the polyphenol source, the dose of the product, the duration of consumption and the population group studied. Most subgroups reviewed suggest an overall beneficial effect on microvascular function, particularly grape-derived products, cocoa, tea, pine bark and Daflon. Other groups (berries, olive oil, soy and pure polyphenol compounds) remain equivocal and require further study due to the limited research performed to date. Polyphenols are abundant in the human diet and this systematic review demonstrates that they are an inexpensive, non-pharmacological approach for improving cardiovascular health in currently healthy individuals and in populations with microvascular dysfunction. It can be expected that such improvements may contribute to lower risk for the development of future cardiovascular disease.

Conflict of Interest: R. Draijer is an employee of Unilever. Unilever markets green and black teas.

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FIGURE LEGENDS

Graphical Abstract.

Figure 1. An overview of the techniques used to measure microvascular function.

Figure 2. Dietary polyphenol subclasses, their basic chemical structure and typical dietary sources.

Figure 3. A flowchart of the methodology used for identifying studies and screening for their eligibility in the systematic review.

Graphical Abstract

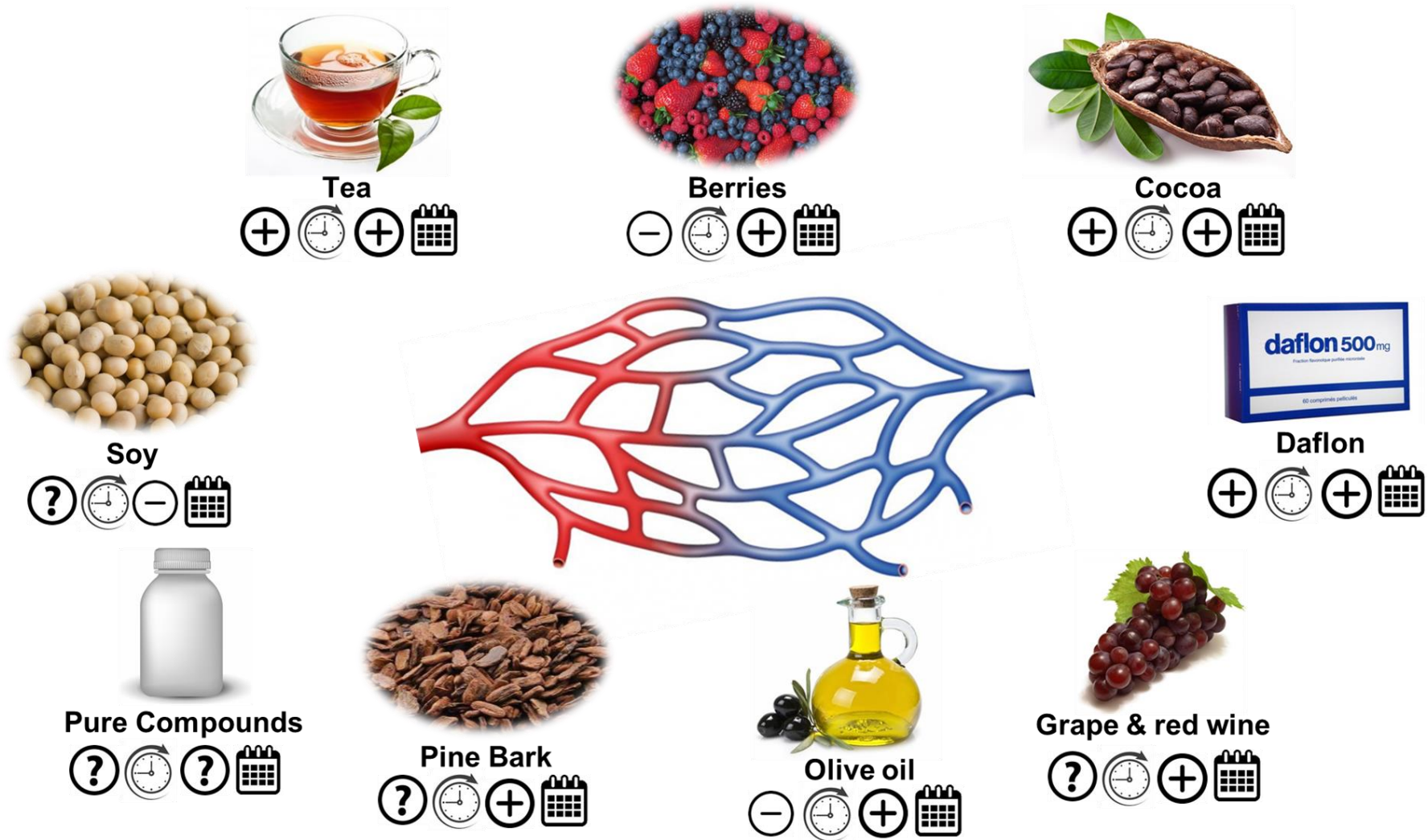


Figure 1

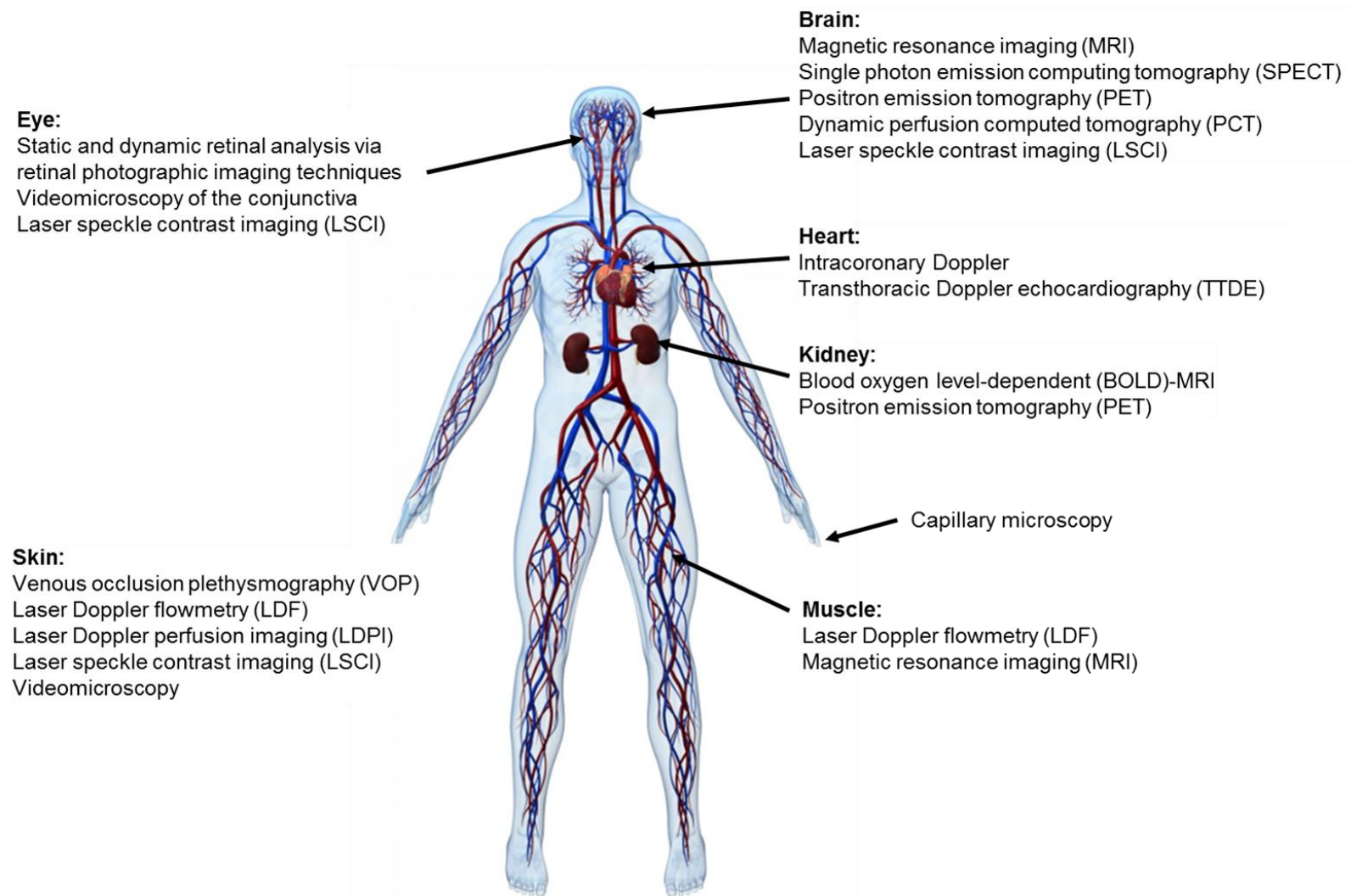


Figure 2

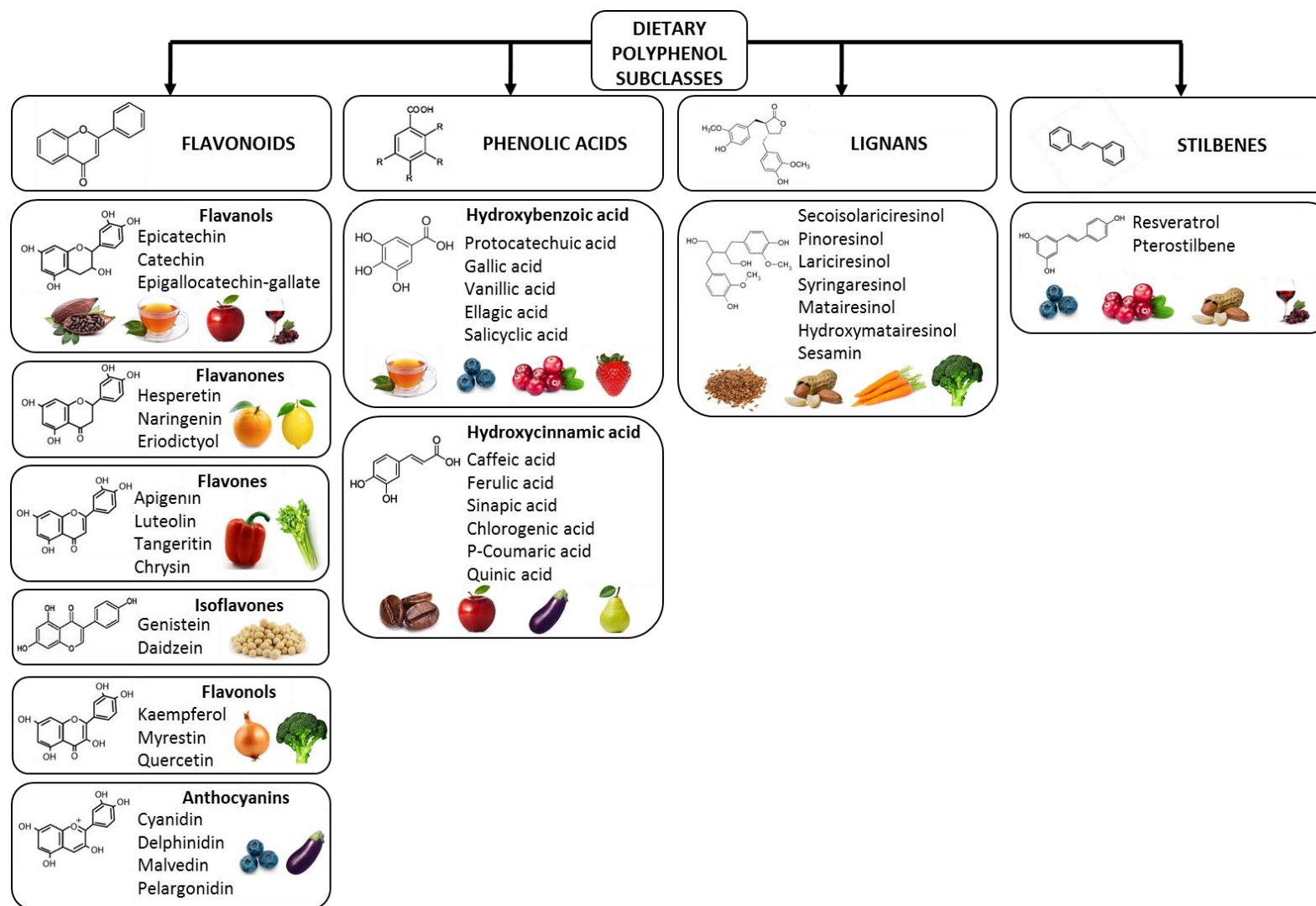


Figure 3

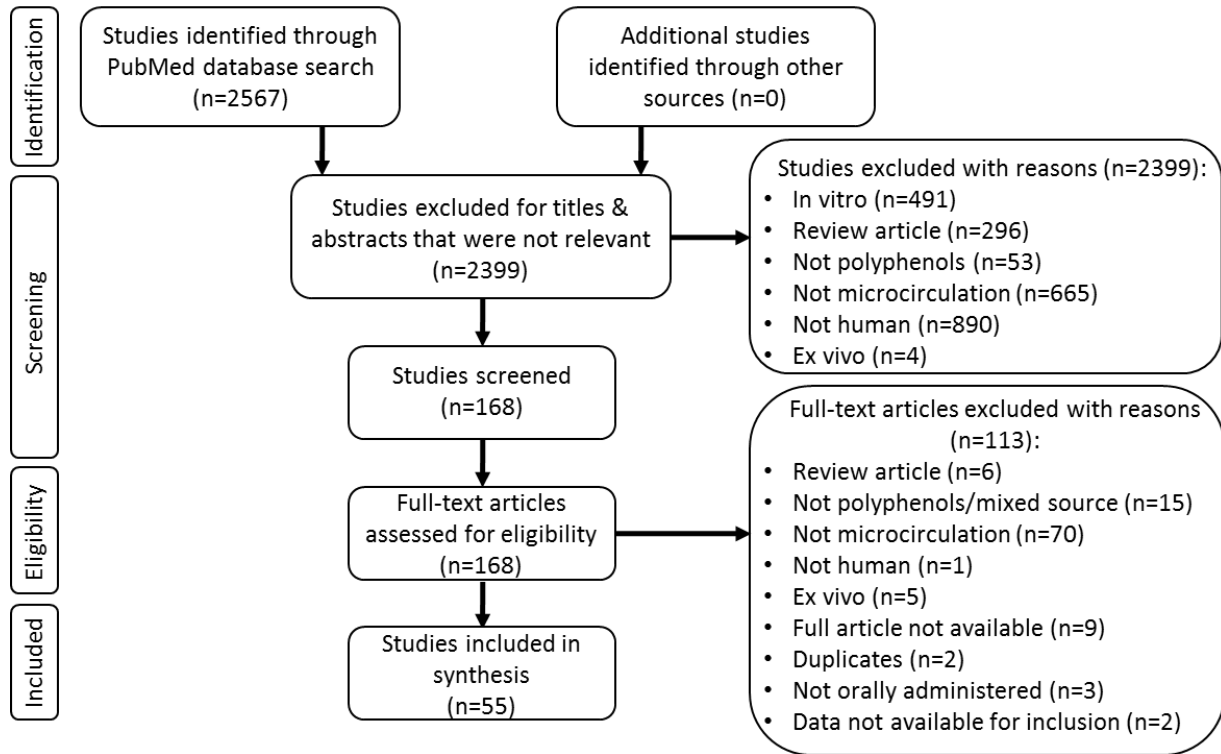


Table Legends

Table 1. Berries: characteristics of the studies included for review.

Table 2. Cocoa: characteristics of the studies included for review.

Table 3. Daflon: characteristics of the studies included for review.

Table 4. Grape and Wine: characteristics of the studies included for review.

Table 5. Olive Oil: characteristics of the studies included for review.

Table 6. Pine Bark: characteristics of the studies included for review.

Table 7. Pure Polyphenol Compounds: characteristics of the studies included for review.

Table 8. Soy: characteristics of the studies included for review.

Table 9. Tea: characteristics of the studies included for review.

Table 10. Summary of polyphenol sources and their acute and chronic effectiveness in improving microvascular function.

Table 1: Berries.

Author	Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[75]	Mirtoselect (Mi) & generic bilberry extract (GBE) (both 36% anthocyanins)	160 mg/day bilberry extract (both Mirtoselect & generic bilberry extract with 36% anthocyanins)	No supplement	Diabetics with retinopathy 2 groups: Hyperflow & ischemic n=140 Mi: n=47, 44y; GBE: n=55, 44y; C: n=38, 45y	Open-label, registry, supplement study	6 months	Retina High resolution colour Doppler imaging central retinal artery	Peak systolic/diastolic flow velocity (cm/s) Hyperflow: Mi: 24±2/12±1* GBE: 28±3/16±3 Ischemic: Mi: 23±2/14±2* GBE: 19±2/11±1* *p<0.05 vs Control,	Peak systolic/diastolic flow velocity (cm/s) Hyperflow: 28±3/15±3 Ischemic: 17±2/12±1
[74]	Blackcurrant juice drink	250 ml drink (20% blackcurrant)	250 ml drink	H n=20, 44.6y 45% M	R, C, DB (4 week washout)	Acute (0-2h)	Skin (site not specified). Vascular reactivity, LDI, ACh, SNP	(PU) ACh: 52±149 SNP: 251±273 (ns vs. Control)	(PU) ACh: 189±129 SNP: 209±472

¹ H, healthy; M, males; ² C, crossover trial; DB, double blind; R, randomised; ³ ACh, acetylcholine; LDI, laser Doppler imaging; SNP, sodium nitroprusside; ⁴ cm/s, centimetres per second; ns, not significant; PU, perfusion units.

Table 2: Cocoa.

Author	Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[78]	Cocoa drink (450 mg flavanols)	900 mg daily	Nutrient matched cocoa-free drink (0 mg flavanols)	H, NS Young: n=22, 26y (<35y), 100% M + Elderly: n=20, 60yrs (50-80y), 100% M	R, DB, P	2 weeks + Acute (1h)	Skin: FBF, VOP, RH, LDPI	<u>Young</u> Acute, chronic, acute-on chronic: FBF (ml/100ml*min) 1.8±0.1, 1.8±0.1, 1.8±0.1 FBF max (ml/100ml*min) 18.2±1.0, 18.9±0.8, 18.9±1.1 LDPI (PU) 40±1, 39±1, 40±1 LDPI max (PU) 292±15, 307±14, 298±17 <u>Elderly</u> Acute, chronic, acute on chronic: FBF (ml/100ml*min) 1.2±0.1, 1.1±0.1, 1.1±0.0 FBF max (ml/100ml*min) 12.3±1.5 [^] , 14.0±1.4 [^] , 13.9±1.1 [^] LDPI (PU) 39±0, 40±1, 39±1 LDPI max (PU) 200±14 [^] , 214±12 [^] , 213±12 [^] [^] p<0.05 vs Young for same time point and intervention group *p<0.05 vs Control in same group	<u>Young</u> Acute, chronic, acute-on chronic: FBF (ml/100ml*min) 1.5±0.1, 1.5±0.1, 1.4±0.1 FBF max (ml/100ml*min) 14.3±1.8, 13.7±1.7, 13.8±1.5 LDPI (PU) 42±1, 42±1, 39±1 LDPI max (PU) 270±17, 260±22, 258±17 <u>Elderly</u> Acute, chronic, acute on chronic: FBF (ml/100ml*min) 0.9±0.1, 0.8±0.0, 0.9±0.0 FBF max (ml/100ml*min) 10.8±1.5 [^] , 11.6±1.7 [^] , 11.0±1.5 [^] LDPI (PU) 44±0, 39±1, 40±1 LDPI max (PU) 186±8 [^] , 178±5 [^] , 180±5 [^] [^] p<0.05 vs Young for same time point and intervention group
[79]	Flavonoid-rich dark chocolate	50 g	Cocoa-free white chocolate (50 g)	Symptomatic PAD (Fontaine stage II) n=21, 66.9y 17% M	R, SB (investigator), C	Acute: 2h 7-day washout	Laser Doppler: RH	Perfusion (AU) 0.31 (0.25-0.55) (ns) Biological zero (AU) 0.14 (0.12-0.16) (ns) Time to peak perfusion (s) 25 (10-48) (ns) Peak perfusion (AU) 1.18 (0.70-2.27) (ns) Recovery time (s) 136 (107-172) (ns)	Perfusion (AU) 0.41 (0.24-0.51) (ns) Biological zero (AU) 0.15 (0.09-0.19)* Time to peak perfusion (s) 26 (18-42) (ns) Peak perfusion (AU) 1.24 (0.85-1.79) (ns) Recovery time (s) 140 (104-165) (ns) *p=0.01 within group difference
[82]	Polyphenol-rich dark chocolate	40 g dark chocolate	White chocolate	Glaucoma n=60 (30 + 30 age-matched controls) 65y	R	Acute (2h)	Retina: diameter – static & dynamic vessel analysis (right eye only)	Glaucoma patients CRAE (µm) 169±9 (ns) CRVE (µm) 192±15 (ns) AV ratio 0.88±0.07 (ns) Dilation arterioles (%)	Glaucoma patients CRAE (µm) 171±18 CRVE (µm) 206±15 AV ratio 0.83±0.06 Dilation arterioles (%)

								3.3±1.9 (ns) Constriction arterioles (%) 0.4±1.3 (ns) Dilation venules (%) 5.0±2.1 (ns) Age-matched controls CRAE (μm) 179±13 (ns) CRVE (μm) 205±17 (ns) AV ratio 0.87±0.06 (ns) Dilation arterioles (%) 3.5±1.8 (ns) Constriction arterioles (%) -0.6±1.1 (ns) Dilation venules (%) 4.2±1.4* *p=0.01 vs Control	3.0±1.7 Constriction arterioles (%) -0.8±1.3 Dilation venules (%) 4.2±1.8 Age-matched controls CRAE (μm) 183±12 CRVE (μm) 208±20 AV ratio 0.88±0.07 Dilation arterioles (%) 2.6±1.9 Constriction arterioles (%) -0.3±1.2 Dilation venules (%) 3.4±1.9
[83]	Cocoa rich dark chocolate	1 g dark chocolate (70% cocoa) per kg body weight (50-80 mg polyphenols)	Flavonoid poor white chocolate (4% cocoa)	H, preserved kidney function n=10, 35y 30% M	CO, pilot study	Acute (2h)	Renal tissue oxygenation: BOLD fMRI. Low R2* estimate of high pO ₂ in renal tissue	Medullary R2* Baseline vs 2 h (s) 29.6±1.3 vs 28.2±1.3 (p=0.04) Cortical R2* not changed	Medullary R2* Baseline vs 2 h (s) 29.0±2.1 vs 28.9±1.7 (ns) Cortical R2* not changed
[80]	Flavanol-rich cocoa drink	~900 mg/day	Isocaloric, nutrient matched flavanol-poor cocoa drink (~28 mg/day)	H, mild-to-moderate hypertension n=20, 51y 40% M	R, DB, C	2 weeks per intervention (1 week washout)	Forearm skeletal muscle capillary recruitment / blood flow: microbubble contrast-enhanced US	Capillary blood flow (AU) Baseline vs cocoa 0.45±0.06 vs 0.51±0.07 (ns) Steady state insulin infusion (AU) 0.76±0.09 vs 0.74±0.09 (ns) Insulin-induced change from baseline (%) 123±35 vs 77±24 (ns)	Capillary blood flow (AU) Baseline vs placebo 0.45±0.06 vs 0.68±0.23 Steady state insulin infusion (AU) 0.76±0.09 vs 0.67±0.11 Insulin-induced change from baseline (%) 123±35 vs 309±220
[76]	High flavanol cocoa drink	100 ml drink (329 mg flavanols)	Low flavanol cocoa drink (27 mg flavanols)	H n=10, 0% M	C	Acute (0-6h)	Skin (1mm depth): LDF & spectroscopy	High flavanol: Baseline vs 2h (AU) 30.08±11.40 vs 49.26±9.26* *p<0.05 compared to low flavanol group	Low flavanol: Baseline vs 2h (AU) 23.14±17.02 vs 23.40±10.74
[77]	High flavanol cocoa	329 mg/day (61 mg/day epicatechin + 20 mg/day catechin)	Low flavanol cocoa 27 mg/day (6.6 mg/day epicatechin +1.6 mg/day catechin)	H, type II normal skin n=24, 18-65y, 0% M	R, DB, P	12 weeks	Skin: cutaneous (1mm) + subcutaneous (7-8mm). O2C system (LDF + tissue spectrometry)	Baseline (AU): Cutaneous 16±7 Sub-c 133±57 6weeks (AU): Cutaneous 24±12*^ Sub-c 155±61* 12weeks (AU): Cutaneous 32±16*^ Sub-c 183±66*^ *p<0.05 vs wk 0	Baseline (AU): Cutaneous 17±9 Sub-c 144±45 6weeks (AU): Cutaneous 17±6 Sub-c 134±50 12weeks (AU): Cutaneous 16±6 Sub-c 131±47

								[^] p<0.05 vs Control	
[84]	Flavanol-rich cocoa drink	1 drink/day (172 mg flavanols per drink)	Low flavanol cocoa drink (13 mg flavanols per drink)	H n=16 18-30y 100% M	DB, CB	5 days	Cerebral: fMRI BOLD responses to cognitive task	(% change from baseline) DLPFC: 3.0±0.2 PC: 2.5±0.2 ACC: 2.1±0.3	(% change from baseline) DLPFC: 2.3±0.2 PC: 2.1±0.3 ACC: 1.7±0.1
[81]	Flavanol-rich chocolate bar + cocoa beverage	444 mg/day total flavanols	Isocaloric placebo bar + beverage (19.6 mg/day total flavanols)	CAD n=40, 61y 75% M	R, DB, placebo-controlled	6 weeks	Forearm blood flow: VOP 2-min wrist exercise + 5-min ischemia protocols	(mL/min) Exercise Resting FBF: 2.2±0.2 Peak FHBF: 24.9±2.2 (ns) Ischemia Resting FBF: 2.5±0.2 Peak RHBF: 30.4±1.7 (ns)	(mL/min) Exercise Resting FBF: 2.5±0.3 Peak FHBF: 23.1±1.8 Ischemia Resting FBF: 2.8±0.4 Peak RHBF: 28.1±1.8

¹ CAD, coronary artery disease; H, healthy; M, male; NS, non-smokers; PAD; peripheral artery disease; ² C, crossover trial; CB; counterbalanced; DB, double blind; P, parallel arms; R, randomised; SB, single blind; ³ BOLD, blood oxygenation level-dependent; FBF, forearm blood flow; fMRI, functional magnetic resonance imaging; LDF, laser Doppler flowmetry; LDPI, laser Doppler perfusion imaging; pO₂, partial pressure of oxygen; RH, reactive hyperemia; US, ultrasonography; VOP, venous occlusion plethysmography; ⁴ ACC, anterior cingulate cortex; AU, arbitrary units; AVR, arterio-venous ratio; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; DLPFC, dorsolateral prefrontal cortex; FBF, forearm blood flow; mL/min, FHBF, forearm hyperaemic blood flow; ns, not significant; PC, parietal cortex; PU, perfusion units; RHBF, reactive hyperaemic blood flow; s, seconds.

Table 3: Daflon.

Author	Intervention Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[133]	Daflon	500 mg twice daily	None	mild to moderate CVI n= 28, 56±13 y, 46% M	Observational	6 months (pre vs post)	Air plethysmography	Mean calf circumference (cm) 37.0 ±4.3 vs 36.4 ±4.3 (p<0.001) Venous filling index (mL/s) 3.7 ±3.5 vs 3.4 ±2.5 (ns) Ejection fraction (%) 54.5±15.9 vs 57.7±19.7 (ns) Residual volume fraction (%) 41.4±19.2 vs 39.4±24.2 (ns)	N/A
[134]	Daflon	500 mg twice daily	None	CVI: +/- venous reflux n= 3,075 46 y, 16% M	Observational	6 months (pre vs post)	Leg circumference	With venous reflux (cm) 27.99 vs 26.51 (p=0.0001) Without venous reflux (cm) 28.61 vs 27.28 (p=0.0001)	N/A
[135]	Daflon	500 mg twice daily	Placebo	Diabetics (18 Type 1 and 22 Type 2) 49±15 y 53% M	R, DB, P	30-42 days (0 vs 30-42 days)	Capillary filtration of albumin (upper arm)	(%) 19.8±8.5 vs 6.7±8.9 (p<0.001) (p<0.015 vs Control Post)	(%) 17.8±7.0 vs 13.6±9.8 (p=0.046)
[136]	Daflon (500 mg tablets)	500 mg 1000 mg 2000 mg	None	Mild CVI n= 104 44±13 y 4 % M	R, DB, P	90 days (0, 28 and 90 days)	Lower limb LDV, % change from day 0 (baseline, orthostasis, venoarteriolar reflex flux)	Change in baseline (%) (day 90) 0.00±0.48 (500 mg) 0.08±0.66 (1000 mg) -0.34±1.02 (2000 mg) (all ns) Change in orthostatic flux (%) (day 90) -0.08±0.24 (500 mg) -0.01±0.32 (1000 mg) -0.15±0.55 (2000 mg) (all ns) Change in venoarteriolar reflex (%) (day 90) 5.72±17.14 (500 mg) 2.75±21.77 (1000 mg) 1.07±15.40 (2000 mg) (all ns)	N/A
[88]	Daflon	2 x 500 mg (50 mg hesperidin) daily	None	CVI n= 25 55±16 y 19% M	Observational	28 days (1 and 28 days) and 14 day washout	Ankle skin dynamic capillaroscopy: RBC velocity and intravenous injection	Day 1 vs 28 Basal RBC velocity (mm/sec)	N/A

							of vital dye for venous appearance and disappearance	0.26±0.14 vs 0.35±0.11 (p=0.024) Washout: 0.33±0.16 Appearance time dye (s) 23.1±7.8 vs 23.7±10.2 (ns) Disappearance time dye (min) 26.1±3.4 vs 26.0±3.6 (ns)	
[90]	Daflon	2 x 500 mg per day	Nonmicronized diosmin	CVI n= 88 42±12 vs 42±11 y 30 vs 45% M	R, DB, P	60 days (0 and 60 days)	VOP Max increase venous volume, time to reach max, max volume to void, total time emptying, venous distensibility	Venous volume (mL/100mL) 3.31±0.91 vs 2.60±0.72 (p<0.01; p=0.003 vs Control) Time max (s) 200.5±41.3 vs 168.3±32.1 (p<0.01; ns vs Control) Max volume to void (mL/100mL/min) 61.9±20.3 vs 59.3±16.4 (ns; ns vs Control) Total time emptying (s) 107.5±87.2 vs 95.3±62.9 (ns; ns vs. Control) Venous distensibility (dV/dP) 0.06±0.00 vs 0.04±0.00 (p<0.01; p<0.001 vs Control)	Venous volume (mL/100mL) 3.17±1.00 vs 2.88±0.94 (p<0.01) Time max (s) 188.5±51.6 vs 165.3±44.3 (p<0.01) Max volume to void (mL/100mL/min) 62.7±17.4 vs 63.3±19.4 (ns) Total time emptying (s) 102.3±65.7 vs 93.7±46.9 (ns) Venous distensibility (dV/dP) 0.06±0.00 vs 0.05±0.00 (p<0.01)
[137]	S 5682 flavonoid fraction (diosmin & hesperidin)	1000 mg per day micronized diosmin (90%) & hesperidin (10%)	Placebo, not specified	Symptomatic capillary fragility n=100 67y, 42% M	R, DB, P	6 weeks	Forearm. Capillary resistance (negative pressure induced no. of petechiae)	(mmHg) 4 wks 219±10 6 wks 261±12 (both p<0.001 vs Control)	(mmHg) 4 wks 159±8 6 wks 163±9
[89]	Daflon	500 mg	Placebo	Unilateral Post-thrombotic Syndrome (diseased leg vs healthy leg) n= 20	R, DB, C	Acute (2 hours)	VOP Venous capacitance (Hmax 50) Venous distensibility (ΔV) Venous emptying (Total emptying time (Tt), Emptying time of 1 st half of Hmax (T50), Emptying time of 2 nd half of Hmax (T2p)) Hmax 50	<u>Healthy leg</u> Hmax 50: -1.9% (p<0.001 vs Control) ΔVmax 40: -2.1% ΔVmax 50: -1.6% ΔVmax 60: -1.9% All p<0.001 vs. Control T _i : -1.5s (p=0.004 vs Control) T ₅₀ : -1 s (ns vs Control) T _{2p} : -1.5 s (p=0.005 vs Control)	Healthy leg Hmax 50: +0.6% ΔVmax 40: -0.4% ΔVmax 50: 0.0% ΔVmax 60: +0.4% T _i : +0.3s T ₅₀ : +0.3s T _{2p} : +0.2 s

							Vmax 50	<u>Diseased Leg</u> Hmax 50: (-2.4% p<0.001 vs control) ΔVmax 40: -1.2% (p=0.013 vs Control) ΔVmax 50: -1.6% ΔVmax 60: -1.9% Both p<0.001 vs Control T _i : -1.7s (p<0.001 vs. Control) T ₅₀ : -0.5 s (ns) T _{2p} : -1.8 s (p=0.001 vs Control) Venous Distensibility 3.59 ΔVmax 50 at +2 hours (p<0.01 vs Control)	<u>Diseased Leg</u> Hmax 50: +0.4% ΔVmax 40: +0.2% ΔVmax 50: 0.0% ΔVmax 60: +0.6% T _i : +0.5s T ₅₀ : +0.4s T _{2p} : +0.6 s Venous Distensibility ΔVmax 50: 3.68
	Daflon	500 mg and 500 mg per day	Placebo	H n=10	R, DB, C	0-24 hours and 1 week	VOP	3.55 ΔVmax 50 at +2 hours after 1 week (p<0.01 vs Control)	3.67 ΔVmax 50 at +2 hours after 1 week
	Daflon	500 mg	Placebo	Venous insufficiency (females) Functional venous insufficiency Pregnant Post-thrombotic syndrome n= 20	R, DB, C	Acute (1 and 2 hours)	VOP	2 hours Mean venous capacitance (Hmax _{mm}) 26.3±0.5 vs 24.3±0.4 (p<0.01) 29.9±0.5 vs 27.9±0.5 (p<0.01) 30.4±1.0 vs 27.8±1.0 (p<0.01)	Not reported Not reported Not reported
	Daflon	500 mg per day	Placebo	Functional chronic venous insufficiency n=18	R, DB, P	30 and 60 days	VOP	60 vs 0 days Mean venous capacitance (Hmax _{mm}) 30.2 vs 29.4 (p<0.001) Venous emptying (s) 28.1 vs 27.6 T _{ts} (ns) 2.39 vs 2.39 T ₅₀ (ns) 25.7 vs 25.2 T _{2ps} (p<0.01) Mean change in ankle perimeter (cm) 24.31±0.49 vs 23.91±0.46 (p<0.001)	60 vs 0 days Mean venous capacitance (Hmax _{mm}) 30.5 vs 30.4 (ns) Venous emptying (s) 27.7 vs 27.8 T _{ts} (ns) 2.39 vs 2.39 T ₅₀ (ns) 25.3 vs 25.4 T _{ts} (ns) Mean change in ankle perimeter (cm) 24.80±0.30 vs 24.81±0.31 (ns)
[87]	Daflon	2 x 500 mg daily	None	Diabetics with microcirculatory disorders (7 with	Observational	1 month (6 months after treatment	Capillary filtration of albumin (upper arm)	Day 0 vs day 28 (%) 12.8±1.6 v -1.4±0.5 (p<0.05 vs Day 0)	N/A

				retinopathy, 7 with peripheral neuropathy, 6 with HT) n= 13, 55±3 y, 31% M		cessation in 10)		6 months post (%) 8.4±1.8 (ns vs day 0)	
[138]	Daflon tablets (450 mg diosmine and 50 mg hesperidin)	2 x 500 mg daily	None	n= 18 patients (n=11 varicose veins, n=7 venous ulcers)	Observational	3-4 weeks	Photo-plethysmography; Skin Perfusion Pressure	(% of arterial BP) Varicose veins 86.5±12.3 vs 91.3±13.3 Venous ulcers 68.4±7.2 vs 80.2±4.6 No statistics reported	N/A
[91]	Daflon	2 x 500 mg (50 mg hesperidin) Acute and daily for 7 days	Placebo	H n= 10 32±1 y 0% M	R, DB, C	1-24 hours at Day 1, 8	VOP (calf) Venous capacitance (Hmax 50) Venous distensibility (ΔV) Venous emptying (Total emptying time (Tt), Emptying time of 1 st half of Hmax (T50), Emptying time of 2 nd half of Hmax (T2p)) Hmax 50 Vmax 50	Day 1 No data reported Day 8 Baseline, 2h, 24h Hmax 50: 30.6, 30.0, 30.4 (all p<0.05 vs Control) Vmax 50: 36.2, 35.5, 36.1 (all p<0.05 vs Control)	Day 8 Baseline, 2h, 24h Hmax 50: 31.1, 31.1, 31.1 Vmax 50: 36.7, 36.7, 36.7
[139]	Daflon	2 x 500 mg daily	idiopathic cyclic oedema	n= 30	R, DB	6 weeks	Capillary filtration of albumin (upper arm)	(%) 18.0±2.4 vs 5.5±2.5 (p=0.02 vs Control)	(%) 14.8±1.4 vs 12.5±2.6

¹ CVI, chronic venous insufficiency; H, healthy; M, male; ² C, crossover trial; DB, double blind; P, parallel arms; R, randomised; SB, single blind; ³ LDV, laser Doppler velocimetry; RBC, red blood cell; VOP, venous occlusion plethysmography; WO, wash-out; ⁴ BP, blood pressure; ns, not significant.

Table 4. Grape Products.

Author	Intervention Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Micro-circulation Target ³	Outcome Measures ⁴	
								Active	Control
[96]	Grape seeds (monomeric & oligomeric flavanols)	200 mg/day	Microcrystalline cellulose	Non-obese, S (≥10 cigarettes/d for 5+ y) n= 28 47y 100% M	R, DB, P	8 weeks	Skin, LDF ED ₅₀ ACh, ACh dose required for 50% of max response	(units not stated) Baseline 5 (2–7) (ns) 4wks 4 (3–6)(p<0.05) 8wks 4 (2–6) (ns)	(units not stated) Baseline 5 (0–7) 4wks 5 (0–8) 8wks 3 (0–6)
[94]	Grape juice	300 ml juice, 1600 mg/day	Not included	Adult triathletes n=10 34y 100% M	Single arm	3 weeks	Nailfold videocapillaroscopy. Functional capillary density	(n/mm ²) Baseline vs 3wks 10.7 ± 2.3 vs 14.9 ± 2.7 (p<0.05)	N/A
[95]	Red wine	660 ml wine, approx. 220 mg (acute) and 660 mg/day (3 weeks)	None	H n=18 22y 0% M	Single arm	Acute (1h) & 3 weeks	Forearm blood flow ACh, SNP	(ml/min/100 ml) Baseline vs 1h resting FBF 3.3±0.4 vs 5.6±0.7 (p< 0.01) 3 wks change from baseline ACh response: 2.6±1.3 (p=0.06) SNP response: 1.9±0.6 (p<0.01)	N/A
[97]	Red vine leaf, AS 195	360 mg	Included, not defined	CVI patients n=71 67y 23% M	R, DB, C	6 weeks	Skin of ankle, LDF	(AU; change from baseline) +241.8±18.7 (p<0.0001)	(AU) -41.0±18.7

¹ CVI, chronic venous insufficiency; H, healthy; M, males; S, smokers; ² C, crossover trial; DB, double blind; P, parallel arms; R, randomised; ³ ACh, acetylcholine; LDF, laser Doppler flowmetry; SNP, sodium nitroprusside; ⁴ ns, non-significant; AU, arbitrary units.

Table 5. Olive Oil.

Author	Intervention Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[101]	PP-rich olive oil	~30 mg/day	PP-free olive oil	EBP, S1H n=24 26y (24-27) 0% M	DB, R, C	2 months (4 week washout)	Forearm IRH	(PU) Baseline: 1084±266 Change from baseline: +345±386 (p<0.001 vs. Control)	(PU) Baseline: 1084±266 Change from baseline: +36±367
[100]	Phenol-rich olive oil (400 ppm)	400 ppm	Low phenol olive oil (80 ppm)	HC n=21 59y (53-68) 23.8% M	R, S, C	Acute (0h, 2h, 4h)	Skin: palmar surface of second finger of dominant hand. IRH.	(% increase in IRH AU) 0h: 199±30 (ns) 2h: 361±60 (p<0.018 vs Control) 4h: 353±59 (0<0.039 vs Control)	(% increase in IRH AU) 0h: 253±37 2h: 237±34 4h: 260±37

¹ EBP, elevated normal blood pressure; HC, hypercholesterolaemic; M, males; S1H, stage 1 essential hypertension; ² C, crossover trial; DB, double blind; R, randomised; S, sequential; ³ IRH, ischaemic reactive hyperaemia; ⁴ AU, arbitrary units; ns, non-significant; PU, perfusion units.

Table 6: Pine Bark.

Author	Intervention Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[108]	Pycnogenol oral capsules	150 mg/day	No placebo given	BH, HL, HG n=93 Active n=49 Control n=43 45y 51% M	Pilot registry study	12 weeks	Skin, finger. LDF, SGP, RH	Week 12 LDF (% increase in baseline flux) 24.7±4.2* SGP (% increase in baseline volume flow) 21.3±3.2* (*p<0.05 vs Control)	Week 12 LDF (% increase in baseline flux) 13.5±3.0 SGP (% increase in baseline volume flow) 15.8±3.1
[112]	Pycnogenol oral capsules	150 mg/day	No placebo given	Meniere's disease, moderate tinnitus >2 wks n=107 Active n=55 Control n=52 46y 50% M	Self-selection of group	6 months	Cochlea. Doppler flow velocity of cochlear artery	6 months (cm/s) Most affected ear: Systolic 21.2±1.1* Diastolic 14.1±1.2* Less affected ear: Systolic 23.4±1.0* Diastolic 16.3±1.0* (*p<0.05 vs Control)	6 months (cm/s) Most affected ear: Systolic 16.1±2.2 Diastolic 7.9±1.2 Less affected ear: Systolic 21.3±1.1 Diastolic 11.5±1.4
[103]	Pycnogenol oral capsules	150 mg/day (P) or	Compression stockings only (S)	Severe CVI n=98	R, P	8 weeks	Skin, ankle. LDF, capillary filtration as the rate of ankle	Week 8 LDF (RF): P: 1.6±0.8*; P+S: 1.3±0.6^	Week 8 LDF (RF): 2.4±0.9

		150mg/d + compression stockings (P+S)		P: n=33 P+S n=34 S n=31 48y 54% M			swelling via SGP, transcutaneous pO ₂ & pCO ₂	SGP (mL/min/100mL tissue): P: 1.5±0.6*; P+S: 1.3±0.6^ pO ₂ (mmHg): P: 50.1±4.2*; P+S: 51.1±5.1^ (*p<0.05 vs Control) (^p<0.05 difference vs both other groups)	SGP (mL/min/100mL tissue): 2.0±0.8 pO ₂ (mmHg): 46.1±4.2
[114]	Pycnogenol oral capsules	150 mg/day	Placebo	Type 2 DM (>4y), EDR (moderate) n=46 Active n=24 Control n=22 52y 63% M	R, DB, P	3 months	Retina. Laser Doppler flow velocity of central retinal artery (mild/moderate retinal oedema)	3 months (cm/s) Retinal oedema Mild: 48±4* Moderate: 44±3* (*p<0.05 vs Control)	3 months (cm/s) Retinal oedema Mild: 36±3 Moderate: 36±3*^
[102]	Pycnogenol oral capsules	180 mg/day	Placebo	H n=16 22y 100% M	R, DB, P	2 weeks	Skin, forearm. SGP, ACh, SNP	(mL/min/100mL tissue) Baseline vs 2 weeks ACh 15µg/min 12.72±4.90 vs 18.68±3.97* SNP 1.5µg/min 16.33±4.72 vs 15.97±4.36 (*p<0.05 vs Control)	(mL/min/100mL tissue) Baseline vs 2 weeks ACh 15µg/min 15.29±7.47 vs 17.04±9.22 (ns) SNP 1.5µg/min 15.97±4.36 vs 17.66±4.84
[105]	Pycnogenol oral capsules	150 mg/day	No treatment group	Severe CVI n=39 (21/18) 53y 52% M	P	8 weeks	Skin, ankle. LDF, capillary filtration as the rate of ankle swelling via SGP	Week 8 LDF (RF): 1.93* SGP (mL/min/100mL tissue): 1.68* (*p<0.05 vs Control)	Week 8 LDF (RF): 3.00 SGP (mL/min/100mL tissue): 2.15
[106]	Pycnogenol oral capsules	150 mg/day	Placebo capsules 150mg/d	Severe diabetic microangiopathy n=60 59y (55-68) 60% M	P	4 weeks	Skin, dorsum of foot; LDF, capillary filtration as the rate of ankle swelling via SGP	Week 4 LDF (RF): 2.20±0.30* SGP (mL/min/100mL tissue): 1.86±0.12* *p<0.05 vs Control	Week 4 LDF (RF): 3.24±0.40 SGP (mL/min/100mL tissue): 2.22±0.07
[107]	Pycnogenol oral capsules	150 mg/day (LD) or 300 mg/day (HD) 1000 mg/day	-	CVI, venous hypertension, ankle oedema P150 n=24, 53y, 58% M P300 n=20, 48y, 60% M	P	8 weeks	Ankle. LDF, capillary filtration as the rate of ankle swelling via SGP, pO ₂ & pCO ₂	Baseline vs week 8 LDF (RF): LD: 3.21±0.10 vs 1.90±0.08* HD 3.40±0.10 vs 2.10±0.01* SGP (mL/min/100mL tissue): LD: 2.38±0.20 vs. 1.58±0.11*	N/A

								HD: 2.43±0.21 vs. 1.70±0.10* pO ₂ (mmHg): LD: 46±5 vs 54±3* HD: 43±3 vs 49±3* pCO ₂ (mmHg): LD 32±2 vs. 27±3* HD 32±2 vs 27±1* (*p<0.05 baseline vs week 8)	
[109]	Pycnogenol oral capsules Pycnogenol oral capsules + topical application of powder	150 mg (50 mg x 3 per day) 150 mg/day + 100 mg topically	Control – no medical treatment	Diabetic, severe microangiopathy n=22, 54y 50% M Oral n=6, 54y, 38% M Oral/t n=8, 54y, 38% M Control n=8, 52y, 50% M	P	6 weeks	Skin, lower limb. LDF, transcutaneous PO ₂ & PCO ₂	Pycnogenol week 6 PO ₂ (mmHg): 55±4 PCO ₂ (mmHg): 29±2 LDF (PU): 2.1±1.0 Pycnogenol oral + topical week 6 PO ₂ (mmHg): 58±3* PCO ₂ (mmHg): 27±3 LDF (PU): 2.0±0.7 (*p<0.05 with other groups)	Week 6 PO ₂ (mmHg): 48±3 PCO ₂ (mmHg): 30±3 (ns)
[110]	Pycnogenol oral capsules	150 mg (50 mg x 3 per day)	Placebo, equivalent dose	Essential hypertension, distal oedema, on ACE inhibitors or Nifedipine n=53, 48y 55% M	P	8 weeks	Foot. Volumetric capillary filtration via SGP (mL/min per 100 cm ³)	Week 8 (mL/min/100cm ³ tissue) ACE: 1.56±0.23 Nifedipine: 1.61±0.20 (ns vs Control)	Week 8 (mL/min/100cm ³ tissue) ACE: 2.44±0.30 Nifedipine: 2.48±0.30
[111]	Pycnogenol oral capsules Pycnogenol oral + topical	150 mg (50 mg x 3 per day) 150 mg/day + 100 mg topically every 2 days	Control	Severe CVI AVP >55mgHg n=16, 57y 50% M	P	6 weeks	Transcutaneous PO ₂ & PCO ₂	Pycnogenol week 6 PO ₂ (mmHg): 55±4* PCO ₂ (mmHg): 29±2* Pycnogenol oral + topical PO ₂ (mmHg): 58±3* PCO ₂ (mmHg): 27±3* (ns vs Control)	Week 6 PO ₂ (mmHg): 48±1 PCO ₂ (mmHg): 29±3
[113]	Pycnogenol	150 mg/day	Placebo	Vascular retinal disorders n=40, 56y 40% M Active n=30, 60y, 40% M Control n=10, 53y, 40% M	R, DB, P	2 months	Retina. Fluoroangiography, electroretinogram (ERG)	Fluoroangiography: Right: 1.30±0.18 Left: 1.37±0.18 ERG right: 0.73±0.14 ERG left: 0.57±0.12 (ns vs. Control)	Fluoroangiography: Right: 1.30±0.21 Left: 1.40±0.22 ERG right: 0.70±0.26 ERG left: 1.00±0.29

¹ ACE, angiotensin-converting-enzyme inhibitor; BH, borderline hypertensive; CVI, chronic venous insufficiency; DM, diabetes mellitus; EDR, early diabetic retinopathy; H, healthy; HG, hyperglycemic; HL, hyperlipidemic; M, males; ² DB, double blind; P, parallel arms; R, randomised; ³ ACh, acetylcholine; LDF, laser Doppler flowmetry; pCO₂, partial pressure of carbon dioxide; pO₂, partial pressure of oxygen, RH, reactive hyperaemia; SGP, strain gauge plethysmography; SNP, sodium nitroprusside; ⁴ ns, not significant; RAS, rate of ankle swelling; RF, resting flux.

Table 7: Pure Polyphenol Compounds.

Author	Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[117]	Resveratrol	500 mg trans-resveratrol	Methyl-cellulose	H n=39 20.5y 18% M	R, DB, P	Acute (38 min) & 4 weeks	Change in CBF prefrontal cortex, NIRS	Total-Hb (μM) Acute: 1.57±0.24* 4 weeks: 1.04±0.32 (ns) (*p<0.01 vs Control)	Total-Hb (μM) Acute: 0.80±0.29 4 weeks: 0.79±0.30
[117]	Resveratrol and resveratrol + piperine	250 mg trans-resveratrol	Inert placebo	H n=23 21y 17% M	R, DB, P	Acute (38 min)	Change in CBF prefrontal cortex, NIRS	Total-Hb (μM) -1.58±0.54 (ns)	Total-Hb (μM) -1.36±0.62
[118]	Resveratrol with physical activity (PA)	250 mg trans-resveratrol	Placebo, not specified	H, physically inactive n=43 65y 100% M	R, DB, P	8 weeks	Skeletal muscle capillary-to-fiber ratio	Baseline vs week 8 1.6±0.1 vs 1.7±0.1 (ns)	Baseline vs week 8 1.5±0.2 vs 1.8±0.1 (p<0.005)
[116]	Resveratrol	250 mg and 500 mg trans-resveratrol	Inert placebo	H	R, DB, CO	Acute (45 min)	Change in CBF prefrontal cortex, NIRS	Total-Hb (μM) 250 mg: 1.09±0.81 (ns) 500 mg: 1.06±0.81 (ns)	Total-Hb (μM) 0.77±0.47
[120]	Hesperetin	100 ml water with water-dispersible hesperetin 17 and 170 mg	Water	H, cold sensitivity n=10 18-22y 0% M	R, DB, CO	Acute	Annular finger of the left hand, laser-Doppler	(ml/min/100g) 17 mg: -6.9±7.1* 170 mg: -5.9±7.7^ (*p<0.0001 vs Control) (^p<0.0001 vs 17 mg)	(ml/min/100g) -35.7±8.7
[119]	EGCG	135 and 270mg	Inert placebo	H n=27 22y 41% M	R, DB, CO	Acute (45 min)	Change in CBF prefrontal cortex, NIRS	Total-Hb (μM) 135 mg: -0.82±0.33 (ns) 270 mg: -0.03±0.38 (ns)	Total-Hb (μM) -0.13±0.40

¹ H, healthy adults; M, males; ² C, crossover trial; DB, double blind; P, parallel arms; R, randomised; ³ CBF, cerebral blood flow; NIRS, near-infrared spectroscopy; ⁴ Hb, haemoglobin; ns, not-significant.

Table 8: Soy.

Author	Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[140]	Soy hypocotyl isoflavones (>95% glycosides)	2 tablets daily (80 mg) (40.51 mg aglycone/tablet)	Placebo (<1 mg aglycone/tablet)	PM, EBP n=24 (12+12) 55.6y (40-60y) 0% M	R, DB, P	6 weeks	FBF; SGP/ PRH	Week 6 (mL/min/100g tissue) FBF: 1.55±0.70 (ns) PRH: 17.0±8.1 (ns)	Week 6 (mL/min/100g tissue) FBF: 1.92±0.55 PRH: 22.3±8.0
[122]	Soy phytoestrogens (capsules: genistin & daidzin)	55mg daily	Placebo capsules	H, PM n=22 58.5y 0% M	R, DB, C (6 week washout)	6 weeks	Skin: LDV, ischaemia	6 weeks (AUC) 15537.6±13681.1 (ns) AUC response 20924.0±10617.4 (ns) AUC ratio 2.5±2.4 (ns)	6 weeks (AUC) 13250.4±13523.4 AUC response 17490.1±11492.5 AUC ratio 2.5±2.4
[123]	Soy isoflavone tablet	80 mg daily (45 mg Genistein)	Placebo tablet	H, PM, peri-menopausal n=21 54y 0% M	R, C	5-10 weeks	Skin; forearm VOP, ACh, SNP, ischaemia	Basal flow (mL/min/100mL): 3.75±1.42 (% change) ACh37µg/min: 86.6±4.4 (ns) SNP16µg/min: 68.9±12.6 (ns) Ischaemia 87.6±5.1 (ns)	Basal flow (mL/min/100mL): 3.31±1.35 (% change) ACh37µg/min: 84.8±7.3 SNP16µg/min: 69.5±9.2 Ischaemia 87.3±4.6

¹ EBP, elevated normal blood pressure; H, healthy; M, males; PM, post-menopausal; ² C, crossover trial; DB, double blind; P, parallel arms; R, randomised; ³ ACh, acetylcholine; FBF, forearm blood flow; LDV, laser Doppler velocimetry; PRH, peak reactive hyperaemia; SGP, strain-gauge plethysmography; SNP, sodium nitroprusside; VOP, venous occlusion plethysmography; ⁴ AUC, area under the curve; ns, non-significant.

Table 9: Tea.

Author	Intervention Product	Polyphenol Dose	Control	Population ¹	Study Design ²	Study Duration	Microcirculation Target ³	Outcome Measures ⁴	
								Active	Control
[124]	Flavonoid-rich black tea beverage	100 ml drink (228.1 mg polyphenols)	100ml water	O, IR n=16, 61y 100% M	R, SB, C	3 hours (2-7 day washout)	Forearm NIRS & VOP, leg VOP	Vascular resistance; LSMeans over all time points VOP arm (mmHg/min/100ml/ml): 48 (ns) VOP leg (mmHg/min/100ml/ml): 38 (p=0.04) NIRS arm (AU): 84* (p<0.002 vs Control)	Vascular resistance; LSMeans over all time points VOP arm (mmHg/min/100ml/ml): 50 VOP leg (mmHg/min/100ml/ml): 40 NIRS arm (AU): 112
[126]	Green tea extract	836 mg green tea catechins	-	H, NS, OW n=20, 70% M Homozygous COMT genotype (AA: n=10; GG: n=10)	Pilot	Acute (0-480 min)	Skin: LDI iontophoresis, ACh, SNP	Change from baseline (AUC) for combined genotypes ACh: -1547±723 (ns) SNP: -1728±895 (ns)	Change from baseline (AUC) for combined genotypes ACh: -1024±413 (ns) SNP: -1344±748 (ns)
[125]	Green tea beverage	1402 mg catechins/day	Constituent matched beverage	H, normal type II skin n=60 (30 per group) 40-65y, 0% M	R, DB, P	12 weeks (measures 0, 6, 12 weeks)	Skin (1mm depth): LDF & oxygen saturation via O2C system	0, 6, 12 weeks LDF (AU): 9.5±4.3, 13.3±6.0, 12.3±5.2 0-6 wks % change: +40* 0-12 wks % change: +29* O ₂ saturation (%) 29.7±17.5, 38.2±21.1, 39.7±20.2 0-6wks % change: +29* 0-12wks % change: +34* (*p<0.05 vs Control)	0, 6, 12 weeks LDF (AU): 11.4±4.6, 10.6±4.5, 11.5±4.3 0-6 wks % change: -7.0 0-12 wks %change: +0.9 O ₂ saturation (%) 25.2±20.0, 23.2±16.7, 24.3±17.8 0-6wks % change: -7.9 0-12wks % change: -3.6
	Green tea extract	Single dose (0.5, 1.0, 2.0 g)	-	n=15 0% M	R, DB, P	Acute (0-240 min)	Skin (1mm depth): LDF, O ₂ C system	(AU) Baseline, peak, 2h 0.5g: 5.0±2.8, 9.0±4.0, 5.1±2.0 1.0g: 6.0±2.8, 9.9±3.3, 6.0±2.0 2.0g: 4.9±1.1, 10.9±4.6, 6.1±2.2 (ns)	N/A
[132]	Green tea beverage – medium and high dose groups	Medium dose: 80 mg/day High dose: 580 mg/day	Catechin-free beverage (0 mg)	H, S n=30, 35y, M: 37y H: 35y, CF: 35y 100% M	R, P	1, 2 weeks + acute (2h)	Skin: forearm VOP, ACh, SNP	(ml/min*100ml tissue) Acute Medium dose: ACh: 12.62±1.21 SNP: 8.86±0.39 High dose:	(ml/min*100ml tissue) Acute ACh: 11.74±1.16 SNP: 11.74±1.16

								ACh: 14.96±0.98* SNP: 9.96±0.08 Days 7 and 14 Medium dose: ACh: 12.00±1.43, 13.43±1.14 SNP: 8.64±0.60, 14 9.14±0.50 High dose: ACh: 15.54±0.95*, 16.89±0.68* SNP: 10.32±0.49, 10.22±0.49 (*p<0.01 vs pre-treatment)	Days 7 and 14 ACh: 11.88±1.01, 12.02±1.31 SNP: 9.23±0.31, 8.92±0.73
[127]	Green tea extract gelatin capsules (384mg, containing 119mg polyphenols)	6 capsules/day (714 mg/day polyphenols)	Caffeine matched gelatin capsules	H n=33 (17/16), 41y, 100% M	DB, P	3 weeks	Skin: forearm LDI, ACh, SNP	(AU) Baseline vs 3 weeks ACh: 3576±2060 vs 3221 ± 1751 (ns) SNP: 4094 ± 2165 vs 4399 ± 2428 (ns)	(AU) Baseline vs 3 weeks ACh: 3016±1517 vs 3216±1557 SNP 4368±2161 vs 4552±2318

¹ COMT, catechol-*O*-methyltransferase; H, healthy; IR, insulin resistant (>5.55mmol/l); M, males; NS, non-smokers; O, obese (BMI>30 kg/m²); OW, overweight (BMI 25-32kg/m²); S, smokers; ² C, crossover trial; DB, double blind; P, parallel arms; R, randomised; SB, single blind; ³ ACh, acetylcholine; LDF, laser Doppler flowmetry; LDI, laser Doppler iontophoresis; NIRS, near infra-red spectroscopy; SNP, sodium nitroprusside; VOP, venous occlusion plethysmography; ⁴ AU, arbitrary units; AUC, area under the curve; LSMeans, least-squares means; ns, not significant.

Table 10: Summary of polyphenol sources and their acute and chronic effectiveness in improving microvascular function.

Polyphenol Source	Outcome	
	Acute	Chronic
Berries	Not effective	Effective (retinopathy)
Cocoa	Effective	Effective
Daflon	Effective	Effective
Grape and wine	Equivocal	Effective
Olive oil	Not effective	Effective
Pine bark	Unknown	Effective
Pure compounds	Equivocal	Equivocal
Soy	Unknown	Not effective
Tea	Effective	Effective