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Review Article

The assessment of vascular function during dietary intervention trials in human subjects

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Abstract

The potential to reduce cardiovascular morbidity through dietary modification remains an area of intense clinical and scientific interest. Any putatively beneficial intervention should be tested within a randomised controlled trial which records appropriate endpoints, ideally incident CVD and death. However, the large sample sizes required for these endpoints and associated high costs mean that the majority of dietary intervention research is conducted over short periods among either healthy volunteers or those at only slightly increased risk, with investigators using a diverse range of surrogate measures to estimate arterial health in these studies. The present review identifies commonly employed techniques, discusses the relative merits of each and highlights emerging approaches.

Key words: Endothelium: Pulse wave velocity: Pulse wave analysis: Dietary intervention

Observational evidence linking particular dietary factors with a reduced incidence of cardiovascular morbidity and mortality has been used extensively to support various public health promotion strategies[1,2]. Increasingly, however, investigators are designing randomised controlled trials to confirm whether those diets or food groups may offer vascular protection. While hard clinical endpoints such as myocardial infarction, stroke and death are the ideal in such work, their use would necessitate prohibitively large, prolonged studies. The Dietary Approaches to Stop Hypertension (DASH) trial typifies much of the research in this field in that it recruited mildly hypertensive, but otherwise healthy, volunteers to an 8-week intervention[3]. While DASH was large enough to successfully employ a clinically relevant endpoint (brachial blood pressure), most dietary intervention studies rely on surrogate measures that will sensitively detect much earlier changes in arterial physiology. To underline this point, a recent meta-analysis considered randomised controlled trials which examined the link between flavonoids/flavonoid-rich foods and cardiovascular risk[4]. Of the 133 studies included, none had cardiovascular morbidity or mortality as endpoints.

A range of vascular function methodologies is available to clinical researchers, and the choice for any one study is usually governed by local expertise and experience. However, it is vital that nutrition researchers have a clear understanding of the metric which their chosen technique will generate, and how applicable this is to their overall research question.

Here we consider endothelial vasodilator function, pulse wave mechanic analysis and biomarker measurement in the evaluation of arterial health during nutrition intervention studies conducted among human volunteers.

Endothelial dysfunction

All blood vessels are lined by an active cellular monolayer, known as the endothelium, which is responsible for many vital aspects of vascular homeostasis[5]. Endothelial cells produce a wide range of paracrine mediators, with multiple beneficial actions including anti-thrombotic, anti-platelet,

Abbreviations: CFPWV, carotid-femoral pulse wave velocity; CRP, C-reactive protein; DASH, Dietary Approaches to Stop Hypertension; EMP, endothelial microparticles; EPC, endothelial progenitor cells; FMD, flow-mediated dilatation; ICAM-1, intercellular adhesion molecule-1; PWV, pulse wave velocity; VCAM-1, vascular cell adhesion molecule-1.

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anti-inflammatory and vasodilatory effects[6]. Established cardiovascular risk factors are known to encourage the evolution of an atherosclerotic plaque by unfavourably altering endothelial cell physiology[7]. Thus, assessing the status of endothelial cells *in vivo* through their ability to produce NO, and thus mediate arterial dilatation, is common in cardiovascular research[8]. Provocation of NO production can be either mechanical (flow-mediated) or pharmacological.

Flow-mediated dilatation

Flow-mediated dilatation (FMD) describes arterial dilatation in response to increased intra-luminal shear stress. In humans, this phenomenon has been described using a forearm technique, in which reactive hyperaemia following release of an arm cuff at suprasystolic pressures mediates increased brachial artery diameter[9]. The vasodilatation can be quantified using B-mode ultrasound, and agreed protocols have been published to guide investigators using this procedure[10,11]. Since the technique does not involve needles, it has proven popular with researchers and less daunting for potential volunteers.

A growing body of research has not only demonstrated impaired brachial artery FMD among patients with recognised cardiovascular risk factors[12–14], but also suggests that the measure may serve as an independent prognostic indicator in both high-risk populations[15–17] and healthy volunteers[18].

Brachial FMD has been employed by several groups as an endpoint during diet-related intervention trials. The effect of *n*-3 fatty acids on endothelial function has recently been reviewed[19], as has the effect of fruit polyphenols[20], berries[21] and green tea[22,23] on vascular health, and these reviews include studies using FMD endpoints. In Table 1 [24–34], the intervention studies that have examined the effect of chocolate or cocoa on FMD are shown. Consumption of dark chocolate has been shown to improve brachial FMD, both acutely (six out of seven studies), and chronically (five out of six studies).

While it provides a minimally invasive option for vascular function assessment, the measurement of brachial FMD does rely on considerable skill in ultrasound image acquisition and concerns about this technique's reproducibility have been expressed[35]. De Roos *et al.* report substantial within-subject variability for FMD among healthy volunteers, with a CV estimated at approximately 50 %[36]. Although much better CV figures (7–10 %) have been published by Deanfield's group[37,38], there remains a concern that, when employed in smaller, less experienced centres, the technique's inherent variability precludes adequate study power. In addition, the NO dependency of FMD has been questioned[39], such that this response is better considered a consequence of interplay between competing dilator and constrictor influences[40].

Another potential concern is that not all prospective studies have identified brachial FMD as an independent predictor of cardiovascular morbidity. Fathi *et al.* report no relationship between FMD and the risk of death, acute coronary syndrome or stroke among patients with established risk factors[41]. Austrian investigators note similar findings among patients undergoing coronary angiography[42] while in almost 3000 older adults, brachial artery diameter and FMD proved equally effective predictors of vascular outcome[18]. However, although not always shown to be an independent predictor of cardiovascular events, this does not preclude its use as an endpoint in dietary intervention studies, as long as interpretation of the result is appropriate.

Despite these reservations, brachial FMD is widely regarded as the 'gold standard' technique by which to assess conduit vessel function in cardiovascular research[43]. However, before adopting FMD as the endpoint of choice, trial investigators should consider whether conduit vessel function is the best metric with which to detect any intervention-related effect. The response quantified in standard FMD protocols depends upon forearm ischaemia-induced hyperaemic flow, which is itself a function of reduced vascular resistance. The latter is determined largely by endothelium-dependent microvascular tone and thus measures of reactive hyperaemia have been suggested as novel arterial descriptors[44]. Analysis of brachial ultrasound recordings from over 2000 Framingham volunteers showed that Doppler-derived indices of hyperaemic shear stress correlated better with established cardiovascular risk factors than did FMD[45]. Huang *et al.* have shown that lower hyperaemic flow velocities in the brachial artery following a standard period of forearm ischaemia independently predicted postoperative morbidity and mortality among patients with peripheral arterial disease undergoing elective vascular surgery[46].

It has been speculated that the microvascular dysfunction implied by reduced hyperaemic flow responses may be more sensitive to early atherosclerotic change than are disturbances in conduit artery vasomotion as quantified by FMD[47]. This has particular relevance for the design of dietary intervention trials which often seek to detect subtle changes in healthy volunteers. It would therefore be considered best practice to record FMD and reactive hyperaemia concurrently, as reactive hyperaemia can be measured simultaneously with FMD, using the same equipment.

Pharmacological provocation of endothelium-dependent vasomotion

Where FMD uses mechanical shear stress to provoke arterial endothelial vasodilator production, the local infusion of appropriate agonists can produce a similar effect. While based on well-established physiological and pharmacological principles, this approach does rely on successful arterial puncture.

Furchgott & Zawadzki's description of acetylcholine-mediated endothelium-dependent vasodilatation using isolated arterial segments *in vitro* was soon extended to *in vivo* human work[48]. Within the coronary circulation, direct intra-arterial injection of acetylcholine mediated vasoconstriction among patients with significant atherosclerotic lesions but dilated angiographically normal vessels[49]. Several prospective studies among patients with clinical indications for cardiac catheterisation have established an abnormal coronary response to endothelium-dependent agonists as a powerful independent predictor of cardiovascular morbidity[50–53]. The potential risks associated with invasive cardiac assessment limit this

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Table 1. Effect of cocoa or chocolate interventions on flow-mediated dilatation (FMD): evidence from randomised controlled trials

Study and year	Population	Intervention	Duration	Effect on FMD?	Effect size
Balzer *et al.* (2008)[24]	Diabetic patients	Acute (cocoa containing 371 or 963 mg flavanols) Chronic (cocoa 963 mg flavanols/d)	Acute and chronic (30 d)	Acute ↑ (at both doses) Chronic ↑	Acute +1·8 % (at 2 h; 963 mg flavanols) Chronic +1·0 %
Berry *et al.* (2010)[25]	Overweight or obese men and postmenopausal women	Cocoa containing 701 mg flavanols	Acute	↑	6·1 % (at 2 h). No baseline measure taken. Significantly different from low-flavanol group
Davison *et al.* (2008)[26]	Overweight and obese adults	High-flavanol cocoa (902 mg flavanols/d)	Acute and chronic (12 weeks)	Acute ↑ Chronic ↑	Acute +2·4 % (at 2 h) Chronic +1·6 %
Engler *et al.* (2004)[27]	Healthy adults	High-flavonoid dark chocolate bar (213 mg procyanidins, 46 mg epicatechin)	2 weeks	↑	+1·3 %
Faridi *et al.* (2008)[28]	Healthy adults	Dark chocolate (22 g cocoa powder) Sugar-free cocoa (22 g cocoa powder)	Acute	Dark chocolate ↑ Sugar-free cocoa ↑	Dark chocolate 4·3 % (at 2 h) Sugar-free cocoa 5·7 % (at 2 h)
Farouque *et al.* (2006)[29]	CAD patients	Flavanol-rich chocolate bar and cocoa beverage (total flavanols 444 mg/d)	Acute and chronic (6 weeks)	Acute ↔ Chronic ↔	Acute – Chronic –
Grassi *et al.* (2005)[30]	Essential hypertension patients	100 g dark chocolate/d (containing 88 mg flavanols)	15 d	↑	+1·5 %
Heiss *et al.* (2003)[31]	Out-patients with at least one cardiovascular risk factor	Cocoa drink containing 176 mg flavan-3-ols	Acute and after 2 d supplementation	Single dose ↑ After 2 d ↑	Single dose +2·7 % (at 2 h) After 2 d + 2·9 %
Heiss *et al.* (2007)[32]	Healthy male smokers	Flavanol-rich cocoa drink (918 mg/d)	Acute and chronic (7 d)	Acute ↑ Chronic ↑	Acute +2·4 %; dose-dependent magnitude of 2 h response also demonstrated Chronic +2·9 %
Heiss *et al.* (2010)[33]	CAD patients	High-flavanol cocoa drink containing 750 mg/d	30 d	↑	+3·8 %
Hermann *et al.* (2006)[34]	Male smokers	40 g dark chocolate	Acute	↑	+2·6 %

↑, Significant increase; CAD, coronary artery disease; ↔, no significant change.

technique's applicability to trials conducted in healthy volunteers. However, the forearm is an accessible and relatively safe vascular bed, in which pharmacological challenges analogous to those described for epicardial vessels can be performed.

While coronary procedures have relied upon quantitative angiography, forearm studies mainly employ venous occlusion plethysmography to measure the efficacy of intra-brachial vasodilators. Concordant, proportionate responses to acetylcholine have been described in synchronously infused coronary and brachial arteries[54,55]. Poor forearm dilator responses to acetylcholine have been shown to predict increased rates of cardiovascular morbidity among hypertensive volunteers[56] and patients with coronary artery disease[57,58].

It is important to appreciate that, when infused into the brachial artery, vasoactive agents exert their influence on forearm blood flow by modulating small vessel tone and this technique is thus an assessment of microvascular function[59].

Forearm blood flow response to locally infused endothelium-dependent vasodilators has been used as an endpoint in several dietary intervention trials. Healthy adults who consumed a Mediterranean-style diet for 6 weeks showed significant improvements in endothelium-dependent forearm hyperaemia[60,61]; however, Ambring *et al.* subsequently reported negative findings for a similar 4-week intervention conducted among younger volunteers using the same endpoint[61]. Investigators have also employed this technique to document the deleterious effects of increasing salt consumption. It was shown that 5 d of salt loading significantly reduced forearm blood flow responses to intra-brachial acetylcholine[62]. Our group recently reported a significant dose-dependent relationship between increased dietary fruit and vegetable consumption and improved microvascular endothelial function, as quantified using this method[63].

However, the applicability of forearm blood flow manipulation through local vasodilator infusion to large, multi-centre clinical trials is limited by its reliance on arterial puncture, and the prospect of needle insertion is likely to prove inherently unattractive to many.

The most serious risks of brachial artery cannulation include occlusion of the artery leading to limb ischaemia, and median nerve damage due to direct trauma or compression by haematoma or infection. In reality, complications are rare, and usually involve minor bruising or local discomfort that resolve quickly, without intervention. It is, however, essential that this procedure is performed by an experienced researcher (with a background in vascular access and aseptic techniques) such as an intensivist, cardiologist or surgeon. This therefore restricts the use of this methodology to research centres with access to the aforementioned skilled operators, and also limits its use to smaller studies. The requirement for needle insertion may also deter participants, and those on oral anticoagulation or with significantly increased BMI must be excluded[59,64,65].

An alternative, less invasive, approach to pharmacological manipulation of the microvascular endothelium involves transdermal drug delivery by iontophoresis[66]. A small electrical current is applied to the forearm and mediates movement of polar drugs such as acetylcholine into cutaneous vessels. Changes in skin blood flow are then quantified using laser Doppler flowmetry[67]. In a recent study, a strong correlation between this measure of skin microvasculature and FMD of the brachial artery was reported[68]. Therefore, this may offer an alternative endpoint for future intervention trials, although the clinical relevance is, as yet, less established than for other methods.

A small number of trials have already used this endpoint, including a trial of fruit and vegetable purée-based drinks (a trend towards an effect shown on this endpoint in both acute and chronic settings)[69], a trial of a green tea polyphenol extract (no effect in the tested chronic setting)[70], a study of acute fish oil consumption (effect on endpoint demonstrated)[71] and a chronic study of weight reduction and exercise (no effect demonstrated)[72]. A recent study of orange juice demonstrated an acute postprandial effect of orange juice or hesperidin consumption on microvascular reactivity, but no effect on fasting reactivity after 4 weeks of consumption[73].

Non-invasive assessment using pulse wave mechanics and pulse contour analysis

A range of commercially available devices offers clinical investigators the opportunity to rapidly acquire arterial descriptors, usually by non-invasively recording pulse pressure tracings through a device, which then computes one or more output variables. While user friendly and therefore popular, these techniques rely on important biomechanical assumptions which often complicate their applicability and interpretation.

Translating intermittent ejection of blood from the heart to smooth end-organ perfusion is a complex biomechanical process that relies on optimal ventricular–vascular coupling. A variety of mechanical arterial descriptors has been used to quantify unfavourable disease-related changes in this interaction[74]. Since these parameters are derived largely from non-invasive techniques, they have proved popular surrogate endpoints during intervention trials in cardiovascular medicine[43]. Popular methods include measuring pressure pulse wave velocity (PWV) across a particular arterial segment and calculating indices of vascular compliance by mathematical pulse contour analysis. The effects of dietary and nutrient interventions on these endpoints have recently been systematically reviewed[75].

Pulse wave velocity

The velocity with which pressure pulse waves travel along an arterial segment can be mathematically related to that vessel's mechanical properties, by either the Moens–Kortweg or Bramwell–Hill equations[76]. These formulae predict that pressure pulse waves will travel faster in less distensible arteries and thus PWV is a commonly cited descriptor of 'arterial stiffness'[77]. A pressure transducer or tonometer is used to detect passage of the pulse wave between two anatomical locations. This can be done sequentially[78] or simultaneously[79], with gating to a contemporaneously recorded electrocardiogram. The carotid and femoral arteries are commonly chosen tonometry sites, as this allows estimation of aortic PWV.

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Prospective data are now available to suggest that carotid-femoral PWV (CFPWV) is an independent predictor of cardiovascular morbidity among healthy individuals[80,81]. While tonometry at the radial site is much more convenient for both volunteer and investigator, carotid-radial PWV did not predict coronary events or strokes among a group of patients with end-stage renal failure in whom CFPWV did have independent prognostic value[82]. This finding reflects structural distinctions between muscular forearm arteries and larger elastic vessels such as the aorta where medial cells have ectodermal rather than mesodermal origins[83].

As the intra-luminal pressure within an artery increases, progressively more collagen fibres are recruited and thus its mechanical characteristics change[84]. An intervention which reduces blood pressure is, therefore, also likely to reduce PWV independent of any pleiotropic effect on the arterial wall[85]. This is an important caveat to the interpretation of any trial which employs PWV as an endpoint. Several recent trials have reported significant reductions in CFPWV following interventions including weight reduction[86], DASH[87], low carbohydrate[88] and low-glycaemic index[89] diets. However, in each case, significant blood pressure reductions are also recorded. Similarly, in a Na-loading study among hypertensive volunteers, changes in CFPWV were positively correlated with changes in brachial blood pressure[90], whilst 6 months' supplementation with conjugated linoleic acid[91] or 2 weeks of dietary salt restriction[92] failed to alter either blood pressure or CFPWV. However, an isoflavone intervention over 6 weeks did mediate significantly slower CFPWV in healthy volunteers without 24 h ambulatory blood pressure reduction[93].

In summary, if an intervention mediates a significant effect on arterial blood pressure, a concordant effect on PWV will be observed. This does not imply an alteration of vascular structure or function and can be predicted from arterial physiology.

Before choosing it as endpoint, investigators should postulate a mechanism by which their proposed intervention could change CFPWV. Through an *in vitro* model, impairment of local endothelial NO production has been shown to increase PWV across predefined arterial segments[94,95]. Furthermore, brachial artery FMD has been independently associated with CFPWV in cross-sectional technique comparison studies among healthy volunteers[96] and patients with isolated systolic hypertension[97]. A significant, positive correlation between carotid-radial PWV and microvascular function in the forearm has also been described[98].

However, during the relatively brief trials that typify much human dietary intervention work it is questionable whether subtle alterations in levels of an endothelium-derived paracrine mediator could significantly change CFPWV which principally reflects aortic medial elastin:collagen ratios. Thus, this parameter is not ideal for use in short-term studies which aim to evaluate the possible endothelial effects of an intervention.

Pulse contour analysis

Palpation and analysis of the radial pulse as a means by which to assess systemic arterial health is a long-established practice in cardiovascular medicine[99]. Algorithm-based reconstruction of the aortic pressure pulse from tonometer-derived radial waveforms has fuelled renewed interest in this approach[85]. A commercially available device has been widely used in cardiovascular research to estimate the aortic augmentation index, a measure of arterial wave reflection[100,101]. Since it is non-invasive, requires minimal training and generates an easily interpretable numeric output, this technique has proved popular in cardiovascular research.

Among patients undergoing cardiac catheterisation, those in the highest quartile of augmentation index had significantly more coronary disease[102]. Similarly, patients with end-stage renal failure who had higher augmentation indices were more likely to die during an 8-year follow-up period[103]. However, a recent review of arterial stiffness by The Framingham Heart Study found that whilst a higher aortic PWV was associated with a 48 % increase in cardiovascular events, the aortic augmentation index, central pulse pressure and pulse pressure amplification showed no such correlation[104]. Like PWV, the aortic augmentation index is dependent on distending arterial blood pressure[105,106], but additional variables, including height[107] and heart rate[108], must be factored in to its interpretation.

A number of nutrition intervention studies have used pulse contour analysis as an intermediate measure of vascular function. In an acute feeding study, food and water, but not water alone, reduced the aortic augmentation index 2 h after consumption[109]. This finding is confounded by concomitant decreases in arterial blood pressure and could be argued to reflect mean blood pressure change and therefore altered vessel haemodynamics, rather than an intrinsic alteration of vascular function. After 8 weeks, both low-fat and low-carbohydrate hypoenergetic diets significantly decreased brachial blood pressure among overweight volunteers, but only the former mediated a significant reduction in aortic augmentation[110].

Again, investigators should have a clear hypothesis about the mechanism through which their intervention is likely to alter pulse wave morphology before choosing aortic augmentation as the endpoint. It has been shown that pharmacological manipulation of systemic endothelial NO production significantly changes aortic pressure pulse wave morphology and thus the augmentation index[96]. In a situation analogous to the intra-brachial infusion of vasodilators and subsequent forearm blood flow measurement, systemic salbutamol (endothelium-dependent) and nitroglycerine (endothelium-independent) are administered with resulting changes in tonometry-derived aortic augmentation quantified to arrive at a 'global endothelial function index'[96]. Such an approach may prove more sensitive to altered vascular health than resting measurements of aortic augmentation.

Biomarkers of vascular function

Biomarker measurement remains a popular endpoint in clinical research, as it is minimally invasive and samples can be stored for future analysis. A wide variety of biochemical species has been employed to quantify inflammation, oxidative stress, endothelial activation and arterial injury. Since no single 'gold

standard' measure has emerged, it is common practice during dietary research trials to employ a panel of such markers.

Biochemical measures

Since atherosclerosis is characterised by ongoing vascular inflammation, the acute-phase reactant C-reactive protein (CRP) has been proposed as a useful tool for improving disease prediction models[111]. A high-sensitivity assay is used to accurately measure the lower CRP concentrations encountered among healthy individuals. Recent data suggest that statin-mediated reductions in CRP are associated with lower rates of cardiovascular morbidity[112].

The evidence regarding dietary interventions and CRP is equivocal. For example, as summarised in Table 2 [113–128], only five out of sixteen fruit and vegetable randomised controlled trials (including Mediterranean diet and DASH trials, as fruit and vegetables are key components of these diets) have demonstrated a lowering in CRP levels. The duration of these five studies varied from 4 weeks in three studies, to 3 months in one study and 2 years in another. Of the studies, two were juice-based, one was a carotenoid-rich FV intervention and two were Mediterranean diet interventions. We refer the reader to a review by Giugliano *et al.*[129] for a broader discussion of the association between diet and inflammation.

In mediating leucocytic infiltration of the arterial intima, glycoprotein membrane components such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) promote oxidative stress and ongoing arterial inflammation[130]. Activated endothelial cells are thought to shed these molecules into the circulation, allowing levels of their soluble form to be quantified as an estimate of ongoing arterial injury[131]. Adipocytes also express ICAM-1, and the soluble form of ICAM-1 is elevated in obese patients, being expressed in the stromal-vascular fraction of adipose tissue[132]. This is likely to contribute to the link between obesity and inflammatory complications such as atherosclerosis.

Supplementation with isoflavones for 6 weeks has been reported to significantly reduce serum levels of VCAM-1 in healthy subjects[93], while additional dietary α-linolenic acid had a similar effect among dyslipidaemic male patients[133]. Over a 24-month period, daily consumption of the phytoestrogen genistein significantly reduced circulating levels of both ICAM-1 and VCAM-1[134]. While additional cherry consumption for 4 weeks lowered CRP levels among healthy volunteers, it had no effect on ICAM-1 concentrations[135] and similar negative results have been noted for brief fruit and vegetable interventions[125].

Oxidation both contributes to and follows on from the continuous cycle of low-grade vascular inflammation which characterises atherosclerotic arterial degeneration[136]. Markers of systemic oxidative stress in biological fluids have long been suggested as surrogates for vascular injury, and the isoprostanes, derived from non-enzymic arachidonic acid peroxidation, are commonly measured[137]. A dietary intervention study has reported that urinary levels of 8-iso-$PGF_{2\alpha}$ were significantly reduced among healthy women consuming nine or ten portions of fruit and vegetables daily for 8 weeks[138]. However, investigators have largely reported negative findings when this endpoint has been employed to study the effects of black tea[139], a Mediterranean diet[61], five or six portions of fruit and vegetables daily[98,138] and pure dietary flavonoids[140].

Other potential biomarkers of the atherosclerotic process include the enzyme lipoprotein-associated phospholipase A_2[141,142], tissue plasminogen activator[143,144] and plasminogen activator inhibitor-1[145].

Novel biochemical approaches

The array of biomarkers available and lack of an agreed 'gold standard' often prohibits rational study design. To quantify the biological effects of an 'anti-inflammatory mix' dietary supplement among obese volunteers, Bakker *et al.* employed a novel 'nutrigenomics' approach[146]. This involved measurement and integrated analysis of 120 plasma proteins, 274 plasma metabolites and peripheral blood cell transcriptomes. While such a comprehensive approach is labour intensive and statistically complex, it may represent a valuable means by which to define more subtle intervention-related changes.

More recently, circulating endothelial microparticles (EMP), endothelial progenitor cells and endothelial cells have also been used as indices of vascular health.

Endothelial microparticles. The endothelium is responsible for a diverse range of functions, including regulation of vascular vasomotor activity, coagulation activity, anti-inflammatory status, and therefore a comprehensive assessment of endothelial function or dysfunction is difficult, with available methods usually only providing information on one separate aspect of endothelial function. It has been proposed recently that EMP may fulfil the role of a more universal marker of vascular health[147]. EMP are small non-nucleated phospholipid vesicles shed from injured endothelial cells in response to pro-inflammatory stimuli and vascular injury. They affect endothelial NO synthesis[148,149], diminishing acetylcholine-induced vasorelaxation and NO production by endothelial cells *in vitro*[150], correlate with markers of inflammation[147], and have pro-coagulant potential[147,151–153].

A number of studies have examined EMP in relation to vascular damage and CVD risk. A significant increase in EMP has been shown in patients with CHD, the metabolic syndrome, diabetes and heart failure[154–158]. A recent study by Wang *et al.* has shown that EMP count is associated with systolic blood pressure, being elevated even in patients with mild hypertension, and was also associated with arterial stiffness[159]. Wang *et al.* suggest that EMP may be a useful parameter for monitoring the process of vascular repair in hypertensive subjects, whilst the accompanying Editorial calls for further studies to determine whether EMP quantification might be a useful marker of endothelial dysfunction[147]. Their use in dietary intervention research has been limited to date, but circulating microparticle concentrations are known to increase after ingestion of a fatty meal[160], whilst a recent paper has shown that following a Mediterranean diet for 4 weeks in older subjects significantly decreased total microparticle, EMP and apoptotic EMP concentrations

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Table 2. Effect of fruit and vegetable (FV) interventions on C-reactive protein (CRP): evidence from randomised controlled trials

Study and year	Population	Intervention	Duration	Effect on CRP
Berry *et al.* (2010)[113]	Pre-hypertension	Cross-over: control level FV containing 15 mmol K per d or an additional 20 or 40 mmol K per d provided as FV or 40 mmol K per d as potassium citrate capsules	6 weeks (5-week washout)	↔
Blum *et al.* (2007)[114]	Healthy adults	300 g tomatoes *v.* usual diet with tomatoes prohibited	4 weeks	↔
Dalgard *et al.* (2009)[115]	Peripheral arterial disease	Cross-over: orange and blackcurrant juice (500 ml per d) and vitamin E (15 mg); juice + placebo; sugar drink + vitamin E; sugar drink + placebo	4 weeks (4-week washout)	↓*
Erlinger *et al.* (2003)[116]	Hypertension	Control *v.* DASH diet (rich in FV, about nine servings per d)	12 weeks	↔
Esposito *et al.* (2004)[117]	Metabolic syndrome	Mediterranean diet *v.* prudent diet	2 years	↓
Freese *et al.* (2004)[118]	Healthy adults	810 g VBA + rich linoleic acid; 196 g VBA + rich linoleic acid; 810 g VBA + rich oleic acid; 196 g VBA + rich oleic acid	6 weeks	↔
Jin *et al.* (2010)[119]	Healthy adults	Placebo; FV juice powder concentrate; FV powder concentrate with added berry powders	60 d	↔
Karlsen *et al.* (2010)[120]	≥ One risk factor for CVD	Bilberry juice *v.* water	4 weeks	↓
Lehtonen *et al.* (2010)[121]	Healthy adults (female)	Lifestyle intervention *v.* lifestyle intervention with berry products (equalling 1138 g berries per week +3·5 g berry oils per week)	20 weeks	↔
McCall *et al.* (2010)[122]	Hypertension	Dose–response: 1 *v.* 3 *v.* 6 portions FV per d	8 weeks	↔
Mena *et al.* (2009)[123]	High-risk CVD	Low-fat diet *v.* Mediterranean diet with olive oil *v.* Mediterranean diet with nuts	3 months	↓†
Michalsen *et al.* (2006)[124]	CAD	Mediterranean diet *v.* written advice-only group	1 year	↔
Paterson *et al.* (2006)[125]	Healthy adults	Cross-over: carotenoid-rich or control vegetable soups and beverages	4 weeks (10-week washout)	↔
Rallidis *et al.* (2009)[126]	Abdominal obesity	Mediterranean diet *v.* Mediterranean diet with dietetic supervision	2 months	↔
Stull *et al.* (2010)[127]	Obese, non-diabetic	Smoothie containing 22·5 g blueberry bioactives twice daily *v.* smoothie of equal nutritional value without added blueberry actives	6 weeks	↔
Watzl *et al.* (2005)[128]	Non-smoking men	Dose–response: 2 *v.* 5 *v.* 8 servings per d carotenoid-rich FV	4 weeks	↓

↔, No significant change; ↓, significant decrease; DASH, Dietary Approaches to Stop Hypertension; VBA, vegetables, berries and apples; CAD, coronary artery disease.
*Orange and blackcurrant juice reduced CRP relative to sugar drink.
†CRP decreased only after Mediterranean diet with olive oil.

when compared with a SFA-rich diet or a low-fat, high-carbohydrate diet[161]. They therefore potentially offer a novel and informative endpoint.

Endothelial progenitor cells. Asahara *et al.* isolated and characterised a circulating angioblast which had the potential to form endothelial cells *in vitro*, thus allowing subsequent quantitative flow cytometry in samples of peripheral blood[162]. Endothelial progenitor cells (EPC) have a constitutive vasoreparative function, but after acute vascular damage, such as stroke or myocardial infarction, these cells are mobilised into peripheral blood where they participate in endothelial repair, regenerative processes and neovascularisation[163,164]. It has been proposed that assessment of endothelial progenitor cells offers a dynamic, integrated index of systemic vascular damage and, as such, will offer more insight than any currently available biochemical markers[165]. While a wide range of EPC subsets have been identified, most clinical studies have concentrated on $CD34^+$ populations isolated from peripheral blood. An inverse relationship between circulating $CD34^+$ EPC and cardiovascular risk factors has been demonstrated in both healthy subjects and patients with CVD[166,167], whilst circulating EPC count may also act as a prognostic biomarker, being associated with worse outcome in patients with suspected or confirmed coronary artery disease[168]. A recent study has shown that circulating $CD34^+$ cells are inversely associated with obesity, and that weight loss results in an increase in circulating progenitor cells, including EPC[169].

Among healthy volunteers, short-term dietary interventions with green tea[170], vegetables[171], red wine[172] and the Mediterranean diet[161] have all been shown to significantly increase circulating concentrations of endothelial progenitor cells. Assessing EPC numbers and function may also be informative, as increasing red wine consumption in healthy volunteers has recently been shown to increase endothelial progenitor cell migration and proliferation and to decrease the extent of apoptosis[173], whilst a high-flavanol intervention has been shown to increase EPC number, but had no effect on their function (measured as ability to survive, differentiate, proliferate and to migrate)[33].

Circulating endothelial cells. Circulating endothelial cells are also recognised as markers of endothelial damage, and have been shown to be increased in acute coronary syndromes, heart failure, stroke and diabetes mellitus[174]. Although no dietary intervention studies have examined effects on numbers of circulating endothelial cells, they may be an informative target in future studies.

Considerations when choosing a method

Investigators should first consider the mechanism through which they propose that the intervention will act, alongside the locally available resources and skills. A decision tree outlining the main considerations and choices is shown in Fig. 1. A panel of approaches, assessing both conduit vessels and the microvasculature, would be most appropriate where

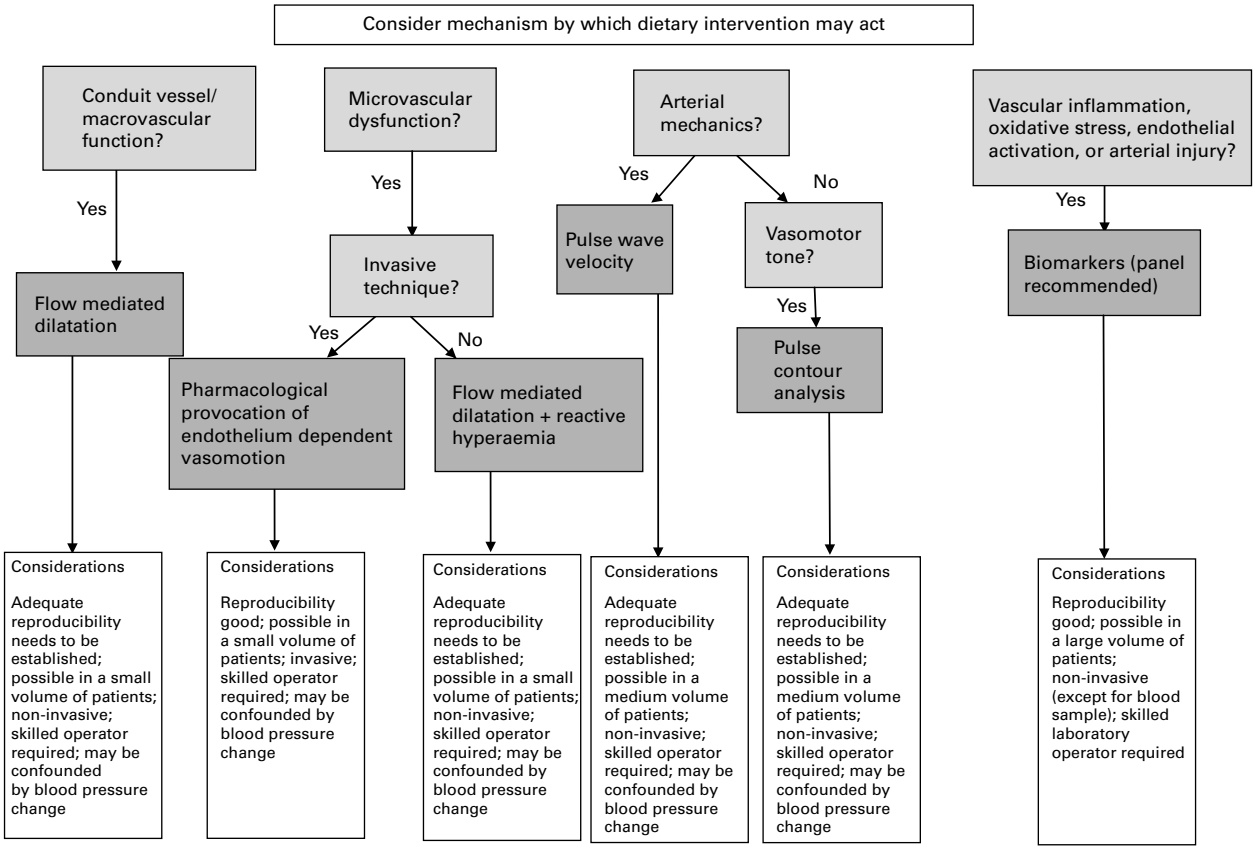

Fig. 1. Decision tree when considering method of vascular function assessment.

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possible. Recent studies using different vascular function methodologies, although also with variation in study designs and populations, have demonstrated contrasting results, despite similar interventions[63,113], and such a panel of endpoints may well have been informative in explaining these differing results. The choice of method may depend on whether investigators propose and are testing an acute or chronic effect of the dietary intervention. Investigators should also consider whether effects of the intervention are likely to be demonstrated in the fasting or postprandial state, as a recent study of *n*-3 fatty acids has demonstrated effects on postprandial macro- and microvascular function, but no effect on fasting measures[175].

The confounding effects of blood pressure must be considered when interpreting study results. Changes in blood pressure, and therefore blood flow, will also cause changes in the FMD of a conduit artery, and which do not necessarily reflect a change in the endothelial function of that vessel[10]. Forearm blood flow and PWV will also be affected by changes in blood pressure. In summary, if an intervention mediates a significant change in arterial blood pressure, a concordant effect on other vascular assessments will be observed. This does not imply an alteration of vascular structure or function and can be predicted from arterial physiology.

Conclusions

A wide variety of techniques are employed to provide surrogate vascular endpoints for short-term dietary trials in human subjects. FMD of the brachial artery and aortic PWV examine conduit and large elastic vessel properties respectively, and therefore do not estimate arterial function at a microvascular level where the bulk of endothelial cells are found.

The mechanism through which an investigator believes their intervention will act, combined with the resources and skill set of the investigating team, will most probably influence the choice of assessment method. At present no single, all-encompassing vascular function test exists, and perhaps a more useful approach would be to combine several methods to comprehensively assess arterial function and mechanics at multiple sites.

Potentially useful emerging techniques include the analysis of post-ischaemic arterial waveforms during FMD determination, quantifying pulse contour changes in response to vasodilator challenges and measuring circulating endothelial progenitor cell and microparticle concentrations.

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References

1. He FJ, Nowson CA & MacGregor GA (2006) Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet* **367**, 320–326.
2. Trichopoulou A, Costacou T, Bamia C, *et al.* (2003) Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* **348**, 2599–2608.
3. Appel LJ, Moore TJ, Obarzanek E, *et al.* (1997) A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* **336**, 1117–1124.
4. Hooper L, Kroon PA, Rimm EB, *et al.* (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **88**, 38–50.
5. Félétou M & Vanhoutte PM (2006) Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *Am J Physiol Heart Circ Physiol* **291**, H985–H1002.
6. Davignon J & Ganz P (2004) Role of endothelial dysfunction in atherosclerosis. *Circulation* **109**, Suppl. 1, 27–32.
7. Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* **352**, 1685–1695.
8. Tousoulis D, Antoniades C & Stefanadis C (2005) Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart* **91**, 553–558.
9. Celermajer DS, Sorensen KE, Gooch VM, *et al.* (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* **340**, 1111–1115.
10. Corretti MC, Anderson TJ, Benjamin EJ, *et al.* (2002) Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* **39**, 257–265.
11. Deanfield J, Donald A, Ferri C, *et al.* (2005) Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens* **23**, 7–17.
12. Lekakis J, Papamichael C, Vemmos C, *et al.* (1997) Effect of acute cigarette smoking on endothelium-dependent brachial artery dilatation in healthy individuals. *Am J Cardiol* **79**, 529–531.
13. Schnell GB, Robertson A, Houston D, *et al.* (1999) Impaired brachial artery endothelial function is not predicted by elevated triglycerides. *J Am Coll Cardiol* **33**, 2038–2043.
14. Evans M, Anderson RA, Graham J, *et al.* (2000) Ciprofibrate therapy improves endothelial function and reduces postprandial lipemia and oxidative stress in type 2 diabetes mellitus. *Circulation* **101**, 1773–1779.
15. Brevetti G, Silvestro A, Schiano V, *et al.* (2003) Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. *Circulation* **108**, 2093–2098.
16. Gokce N, Keaney JF Jr, Hunter LM, *et al.* (2002) Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* **105**, 1567–1572.
17. Patti G, Pasceri V, Melfi R, *et al.* (2005) Impaired flow-mediated dilation and risk of restenosis in patients undergoing coronary stent implantation. *Circulation* **111**, 70–75.
18. Yeboah J, Crouse JR, Hsu FC, *et al.* (2007) Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation* **115**, 2390–2397.

British Journal of Nutrition

19. Egert S & Stehle P (2011) Impact of *n*-3 fatty acids on endothelial function: results from human intervention studies. *Curr Opin Nutr Metab Care* **14**, 121–131.
20. Chong MF, Macdonald R & Lovegrove JA (2010) Fruit polyphenols and CVD risk: a review of human intervention studies. *Br J Nutr* **104**, S28–S39.
21. Basu A, Rhone M & Lyons TJ (2010) Berries: emerging impact on cardiovascular health. *Nutr Rev* **68**, 168–177.
22. Moore RJ, Jackson KG & Minihane AM (2009) Green tea (*Camellia sinensis*) catechins and vascular function. *Br J Nutr* **102**, 1790–1802.
23. Wolfram S (2007) Effects of green tea and EGCG on cardiovascular and metabolic health. *J Am Coll Nutr* **26**, 373S–388S.
24. Balzer J, Rassaf T, Heiss C, *et al.* (2008) Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients. *J Am Coll Cardiol* **51**, 2141–2149.
25. Berry NM, Davison K, Coates AM, *et al.* (2010) Impact of cocoa flavanol consumption on blood pressure responsiveness to exercise. *Br J Nutr* **103**, 1480–1484.
26. Davison K, Coates AM, Buckley JD, *et al.* (2008) Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. *Int J Obes* **32**, 1289–1296.
27. Engler MB, Engler MM, Chen YV, *et al.* (2004) Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J Am Coll Nutr* **23**, 197–204.
28. Faridi Z, Njike VY, Dutta S, *et al.* (2008) Acute dark chocolate and cocoa ingestion and endothelial function: a randomised controlled crossover trial. *Am J Clin Nutr* **88**, 58–63.
29. Farouque HMO, Leung M, Hope SA, *et al.* (2006) Acute and chronic effects of flavanol-rich cocoa on vascular function in subjects with coronary artery disease: a randomised double-blind placebo-controlled study. *Clin Sci* **111**, 71–80.
30. Grassi D, Necozione S, Lippi C, *et al.* (2005) Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension* **46**, 398–405.
31. Heiss C, Dejam A, Kleinbongard P, *et al.* (2003) Vascular effects of cocoa rich in flavan-3-ols. *JAMA* **290**, 1030–1031.
32. Heiss C, Finis D, Kleinbongard P, *et al.* (2007) Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. *J Cardiovasc Pharmacol* **49**, 74–80.
33. Heiss C, Jahn S, Taylor M, *et al.* (2010) Improvement of endothelial function with dietary flavanols is associated with mobilisation of circulating angiogenic cells in patients with coronary artery disease. *J Am Coll Cardiol* **56**, 218–224.
34. Hermann F, Spieker LE, Ruschitzka F, *et al.* (2006) Dark chocolate improves endothelial and platelet function. *Heart* **92**, 119–120.
35. Sanderson P, Sattar N, Olthof M, *et al.* (2004) Dietary lipids and vascular function: UK Food Standards Agency workshop report. *Br J Nutr* **91**, 491–500.
36. De Roos NM, Bots ML, Schouten EG, *et al.* (2003) Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. *Ultrasound Med Biol* **29**, 401–406.
37. Donald AE, Charakida M, Cole TJ, *et al.* (2006) Non-invasive assessment of endothelial function: which technique? *J Am Coll Cardiol* **48**, 1846–1850.
38. Donald AE, Halcox JP, Charakida M, *et al.* (2008) Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. *J Am Coll Cardiol* **51**, 1959–1964.
39. Tschakovsky ME & Pyke KE (2005) Counterpoint: flow-mediated dilation does not reflect nitric oxide-mediated endothelial function. *J Appl Physiol* **99**, 1235–1237.
40. Mullen MJ, Kharbanda RK, Cross J, *et al.* (2001) Heterogenous nature of flow-mediated dilatation in human conduit arteries *in vivo*: relevance to endothelial dysfunction in hypercholesterolemia. *Circ Res* **88**, 145–151.
41. Fathi R, Haluska B, Isbel N, *et al.* (2004) The relative importance of vascular structure and function in predicting cardiovascular events. *J Am Coll Cardiol* **43**, 616–623.
42. Frick M, Suessenbacher A, Alber HF, *et al.* (2005) Prognostic value of brachial artery endothelial function and wall thickness. *J Am Coll Cardiol* **46**, 1006–1010.
43. Deanfield JE, Halcox JP & Rabelink TJ (2007) Endothelial function and dysfunction: testing and clinical relevance. *Circulation* **115**, 1285–1295.
44. Widlansky ME (2009) Shear stress and flow-mediated dilation: all shear responses are not created equally. *Am J Physiol Heart Circ Physiol* **296**, H31–H32.
45. Mitchell GF, Parise H, Vita JA, *et al.* (2004) Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. *Hypertension* **44**, 134–139.
46. Huang AL, Silver AE, Shvenke E, *et al.* (2007) Predictive value of reactive hyperemia for cardiovascular events in patients with peripheral arterial disease undergoing vascular surgery. *Arterioscler Thromb Vasc Biol* **27**, 2113–2119.
47. Philpott A & Anderson TJ (2007) Reactive hyperemia and cardiovascular risk. *Arterioscler Thromb Vasc Biol* **27**, 2065–2067.
48. Furchgott RF & Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**, 373–376.
49. Ludmer PL, Selwyn AP, Shook TL, *et al.* (1986) Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* **315**, 1046–1051.
50. Al Suwaidi J, Higano ST, Holmes DR Jr, *et al.* (2001) Obesity is independently associated with coronary endothelial dysfunction in patients with normal or mildly diseased coronary arteries. *J Am Coll Cardiol* **37**, 1523–1528.
51. Schachinger V, Britten MB & Zeiher AM (2000) Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* **101**, 1899–1906.
52. Halcox JP, Schenke WH, Zalos G, *et al.* (2002) Prognostic value of coronary vascular endothelial dysfunction. *Circulation* **106**, 653–658.
53. Targonski PV, Bonetti PO, Pumper GM, *et al.* (2003) Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. *Circulation* **107**, 2805–2809.
54. Hirooka Y, Imaizumi T, Tagawa T, *et al.* (1994) Effects of L-arginine on impaired acetylcholine-induced and ischemic vasodilation of the forearm in patients with heart failure. *Circulation* **90**, 658–668.
55. Tagawa T, Mohri M, Tagawa H, *et al.* (1997) Role of nitric oxide in substance P-induced vasodilation differs between the coronary and forearm circulation in humans. *J Cardiovasc Pharmacol* **29**, 546–553.
56. Perticone F, Ceravolo R, Pujia A, *et al.* (2001) Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* **104**, 191–196.
57. Heitzer T, Schlinzig T, Krohn K, *et al.* (2001) Endothelial dysfunction, oxidative stress, and risk of cardiovascular

NS British Journal of Nutrition

events in patients with coronary artery disease. *Circulation* **104**, 2673–2678.
58. Fichtlscherer S, Breuer S & Zeiher AM (2004) Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the "vulnerable" patient. *Circulation* **110**, 1926–1932.
59. Wilkinson IB & Webb DJ (2001) Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol* **52**, 631–646.
60. Singh N, Graves J, Taylor PD, *et al.* (2002) Effects of a 'healthy' diet and of acute and long-term vitamin C on vascular function in healthy older subjects. *Cardiovasc Res* **56**, 118–125.
61. Ambring A, Friberg P, Axelsen M, *et al.* (2004) Effects of a Mediterranean-inspired diet on blood lipids, vascular function and oxidative stress in healthy subjects. *Clin Sci* **106**, 519–525.
62. Tzemos N, Lim PO, Wong S, *et al.* (2008) Adverse cardiovascular effects of acute salt loading in young normotensive individuals. *Hypertension* **51**, 1525–1530.
63. McCall DO, McGartland CP, McKinley MC, *et al.* (2009) Dietary intake of fruits and vegetables improves microvascular function in hypertensive subjects in a dose-dependent manner. *Circulation* **119**, 2153–2160.
64. Benjamin N, Calver A, Collier J, *et al.* (1995) Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertension* **25**, 918–923.
65. Kennedy AM, Grocott M, Schwartz MS, *et al.* (1997) Median nerve injury: an underrecognised complication of brachial artery cardiac catheterisation? *J Neurol Neurosurg Psychiatry* **63**, 542–546.
66. Morris SJ & Shore AC (1996) Skin blood flow responses to the iontophoresis of acetylcholine and sodium nitroprusside in man: possible mechanisms. *J Physiol (Lond)* **496**, 531–542.
67. Kvandal P, Stefanovska A, Veber M, *et al.* (2003) Regulation of human cutaneous circulation evaluated by laser Doppler flowmetry, iontophoresis, and spectral analysis: importance of nitric oxide and prostaglandines. *Microvasc Res* **65**, 160–171.
68. Debbabi H, Bonnin P, Ducluzeau PH, *et al.* (2010) Noninvasive assessment of endothelial function in the skin microcirculation. *Am J Hypertens* **23**, 541–546.
69. George TW, Niwat C, Waroonphan S, *et al.* (2009) Effects of chronic and acute consumption of fruit- and vegetable-puree-based drinks on vasodilation, risk factors for CVD and the response as a result of the *eNOS* G298T polymorphism. *Proc Nutr Soc* **68**, 148–161.
70. Frank J, George TW, Lodge JK, *et al.* (2009) Daily consumption of an aqueous green tea extract supplement does not impair liver function or alter cardiovascular disease risk biomarkers in healthy men. *J Nutr* **139**, 58–62.
71. Armah CK, Jackson KG, Doman I, *et al.* (2008) Fish oil fatty acids improve postprandial vascular reactivity in healthy men. *Clin Sci* **114**, 679–686.
72. Hamdy O, Ledbury S, Mullooly C, *et al.* (2003) Lifestyle modification improves endothelial function in obese subjects with the insulin resistance syndrome. *Diabetes Care* **26**, 2119–2125.
73. Morand C, Dubray C, Milenkovic D, *et al.* (2011) Hesperidin contributes to the vascular protective effects of orange juice: a randomised crossover study in healthy volunteers. *Am J Clin Nutr* **93**, 73–80.
74. Hamilton PK, Lockhart CJ, Quinn CE, *et al.* (2007) Arterial stiffness: clinical relevance, measurement and treatment. *Clin Sci* **113**, 157–170.
75. Pase MP, Grima NA & Sarris J (2011) The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. *Am J Clin Nutr* **93**, 446–454.
76. Oliver JJ & Webb DJ (2003) Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol* **23**, 554–566.
77. Hughes SM, Dixon LJ & McVeigh GE (2004) Arterial stiffness and pulse wave velocity: problems with terminology. *Circulation* **109**, e3.
78. Schillaci G, Pirro M, Vaudo G, *et al.* (2005) Metabolic syndrome is associated with aortic stiffness in untreated essential hypertension. *Hypertension* **45**, 1078–1082.
79. Laurent S, Katsahian S, Fassot C, *et al.* (2003) Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke* **34**, 1203–1206.
80. Mattace-Raso FU, van der Cammen TJ, Hofman A, *et al.* (2006) Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* **113**, 657–663.
81. Willum-Hansen T, Staessen JA, Torp-Pedersen C, *et al.* (2006) Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* **113**, 664–670.
82. Pannier B, Guerin AP, Marchais SJ, *et al.* (2005) Stiffness of capacitive and conduit arteries: prognostic significance for end-stage renal disease patients. *Hypertension* **45**, 592–596.
83. Safar ME & Laurent P (2003) Pulse pressure and arterial stiffness in rats: comparison with humans. *Am J Physiol Heart Circ Physiol* **285**, H1363–H1369.
84. Payne RA & Webb DJ (2006) Arterial blood pressure and stiffness in hypertension: is arterial structure important? *Hypertension* **48**, 366–367.
85. O'Rourke MF & Nichols WW (2005) Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. *Hypertension* **45**, 652–658.
86. Dengo AL, Dennis EA, Orr JS, *et al.* (2010) Arterial destiffening with weight loss in overweight and obese middle-aged and older adults. *Hypertension* **55**, 855–861.
87. Blumenthal JA, Babyak MA, Hinderliter A, *et al.* (2010) Effects of the DASH diet alone and in combination with exercise and weight loss on blood pressure and cardiovascular biomarkers in men and women with high blood pressure: the ENCORE study. *Arch Intern Med* **170**, 126–135.
88. Keogh JB, Brinkworth GD, Noakes M, *et al.* (2008) Effects of weight loss from a very-low-carbohydrate diet on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity. *Am J Clin Nutr* **87**, 567–576.
89. Philippou E, Bovill-Taylor C, Rajkumar C, *et al.* (2009) Preliminary report: the effect of a 6-month dietary glycemic index manipulation in addition to healthy eating advice and weight loss on arterial compliance and 24-hour ambulatory blood pressure in men: a pilot study. *Metabolism* **58**, 1703–1708.
90. Todd AS, Macginley RJ, Schollum JB, *et al.* (2010) Dietary salt loading impairs arterial vascular reactivity. *Am J Clin Nutr* **91**, 557–564.
91. Sluijs I, Plantinga Y, de Roos B, *et al.* (2010) Dietary supplementation with *cis*-9,*trans*-11 conjugated linoleic acid and aortic stiffness in overweight and obese adults. *Am J Clin Nutr* **91**, 175–183.
92. Dickinson KM, Keogh JB & Clifton PM (2009) Effects of a low-salt diet on flow-mediated dilatation in humans. *Am J Clin Nutr* **89**, 485–490.
93. Teede HJ, McGrath BP, DeSilva L, *et al.* (2003) Isoflavones reduce arterial stiffness: a placebo-controlled study in

NS British Journal of Nutrition

men and postmenopausal women. *Arterioscler Thromb Vasc Biol* **23**, 1066–1071.
94. Wilkinson IB, MacCallum H, Cockcroft JR, *et al.* (2002) Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity *in vivo*. *Br J Clin Pharmacol* **53**, 189–192.
95. Schmitt M, Avolio A, Qasem A, *et al.* (2005) Basal NO locally modulates human iliac artery function *in vivo*. *Hypertension* **46**, 227–231.
96. McEniery CM, Wallace S, Mackenzie IS, *et al.* (2006) Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension* **48**, 602–608.
97. Wallace SM, Yasmin, McEniery CM, *et al.* (2007) Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. *Hypertension* **50**, 228–233.
98. McCall DO, McGartland CP, Woodside JV, *et al.* (2010) The relationship between microvascular endothelial function and carotid-radial pulse wave velocity in patients with mild hypertension. *Clin Exp Hypertens* **32**, 474–479.
99. Oparil S & Izzo JL Jr (2006) Pulsology rediscovered: commentary on the Conduit Artery Function Evaluation (CAFE) study. *Circulation* **113**, 1162–1163.
100. Karamanoglu M, O'Rourke MF, Avolio AP, *et al.* (1993) An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J* **14**, 160–167.
101. Chen CH, Nevo E, Fetics B, *et al.* (1997) Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation* **95**, 1827–1836.
102. Weber T, Auer J, O'Rourke MF, *et al.* (2004) Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation* **109**, 184–189.
103. London GM, Blacher J, Pannier B, *et al.* (2001) Arterial wave reflections and survival in end-stage renal failure. *Hypertension* **38**, 434–438.
104. Mitchell GF, Hwang S-J, Ramachandran RS, *et al.* (2010) Arterial stiffness and cardiovascular events – The Framingham Heart Study. *Circulation* **121**, 505–511.
105. Booth AD, Wallace S, McEniery CM, *et al.* (2004) Inflammation and arterial stiffness in systemic vasculitis: a model of vascular inflammation. *Arthritis Rheum* **50**, 581–588.
106. Dart AM, Gatzka CD, Cameron JD, *et al.* (2004) Large artery stiffness is not related to plasma cholesterol in older subjects with hypertension. *Arterioscler Thromb Vasc Biol* **24**, 962–968.
107. Yasmin & Brown MJ (1999) Similarities and differences between augmentation index and pulse wave velocity in the assessment of arterial stiffness. *QJM* **92**, 595–600.
108. Wilkinson IB, MacCallum H, Flint L, *et al.* (2000) The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol (Lond)* **525**, 263–270.
109. Ahuja KD, Robertson IK & Ball MJ (2009) Acute effects of food on postprandial blood pressure and measures of arterial stiffness in healthy humans. *Am J Clin Nutr* **90**, 298–303.
110. Bradley U, Spence M, Courtney CH, *et al.* (2009) Low-fat versus low-carbohydrate weight reduction diets: effects on weight loss, insulin resistance, and cardiovascular risk: a randomized control trial. *Diabetes* **58**, 2741–2748.
111. Albert MA & Ridker PM (2006) C-reactive protein as a risk predictor: do race/ethnicity and gender make a difference? *Circulation* **114**, e67–e74.
112. Ridker PM, Danielson E, Fonseca FA, *et al.* (2008) Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* **359**, 2195–2207.
113. Berry SE, Mulla UZ, Chowienczyk PJ, *et al.* (2010) Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. *Br J Nutr* **104**, 1839–1847.
114. Blum A, Monir M, Khazim K, *et al.* (2007) Tomato-rich (Mediterranean) diet does not modify inflammatory markers. *Clin Invest Med* **30**, E70–E74.
115. Dalgard C, Nielsen F, Morrow JD, *et al.* (2009) Supplementation with orange and blackcurrant juice, but not vitamin E, improves inflammatory markers in patients with peripheral arterial disease. *Br J Nutr* **101**, 263–269.
116. Erlinger TP, Miller ER, Charleston J, *et al.* (2003) Inflammation modifies the effects of a reduced-fat low-cholesterol diet on lipids. Results from the DASH-sodium trial. *Circulation* **108**, 150–154.
117. Esposito K, Marfella R, Ciotola M, *et al.* (2004) Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* **292**, 1440–1446.
118. Freese R, Vaarala O, Turpeinen AM, *et al.* (2004) No difference in platelet activation or inflammation markers after diets rich or poor in vegetables, berries and apples in healthy subjects. *Eur J Nutr* **43**, 175–182.
119. Jin Y, Cui X, Singh UP, *et al.* (2010) Systemic inflammatory load in humans is suppressed by consumption of two formulations of dried, encapsulated juice concentrate. *Mol Nutr Food Res* **54**, 1506–1514.
120. Karlsen A, Paur I, Bohn SK, *et al.* (2010) Bilberry juice modulates plasma concentration of NF-κB related inflammatory markers in subjects at increased risk of CVD. *Eur J Nutr* **49**, 345–355.
121. Lehtonen H-M, Suomela J-P, Tahvonen R, *et al.* (2010) Berry meals and risk factors associated with metabolic syndrome. *Eur J Clin Nutr* **64**, 614–621.
122. McCall DO, McGartland CP, McKinley MC, *et al.* (2010) The effect of increased dietary fruit and vegetable consumption on endothelial activation, inflammation and oxidative stress in hypertensive volunteers. *Nutr Metab Cardiovasc Dis* (Epublication ahead of print version 12 April 2010).
123. Mena M-P, Sacanella E, Vazquez-Agell M, *et al.* (2009) Inhibition of circulating immune cell activation: a molecular anti-inflammatory effect of the Mediterranean diet. *Am J Clin Nutr* **89**, 248–256.
124. Michalsen A, Lehmann N, Pithan C, *et al.* (2006) Mediterranean diet has no effect on markers of inflammation and metabolic risk factors in patients with coronary artery disease. *Eur J Clin Nutr* **60**, 478–485.
125. Paterson E, Gordon MH, Niwat C, *et al.* (2006) Supplementation with fruit and vegetable soups and beverages increases plasma concentrations but does not alter markers of oxidative stress or cardiovascular risk factors. *J Nutr* **136**, 2849–2855.
126. Rallidis LS, Lekakis J, Kolomvotsou A, *et al.* (2009) Close adherence to a Mediterranean diet improves endothelial function in subjects with abdominal obesity. *Am J Clin Nutr* **90**, 263–268.
127. Stull AJ, Cash KC, Johnson WD, *et al.* (2010) Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. *J Nutr* **140**, 1764–1768.
128. Watzl B, Kulling SE, Moseneder J, *et al.* (2005) A 4-wk intervention with high intake of carotenoid-rich vegetables and

NS British Journal of Nutrition

fruit reduces plasma C-reactive protein in healthy, non-smoking men. *Am J Clin Nutr* **82**, 1052–1058.
129. Giugliano D, Ceriello A & Esposito K. (2006) The effect of diet on inflammation: emphasis on the metabolic syndrome. *J Am Coll Cardiol* **48**, 677–685.
130. Ferrario CM & Strawn WB (2006) Role of the renin–angiotensin–aldosterone system and proinflammatory mediators in cardiovascular disease. *Am J Cardiol* **98**, 121–128.
131. Hwang SJ, Ballantyne CM, Sharrett AR, *et al.* (1997) Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. *Circulation* **96**, 4219–4225.
132. Brake DK, O'Brian Smith E, Mersmann H, *et al.* (2006) ICAM-1 expression in adipose tissue: effects of diet-induced obesity in mice. *Am J Physiol* **291**, C1232–C1239.
133. Rallidis LS, Paschos G, Papaioannou ML, *et al.* (2004) The effect of diet enriched with α-linolenic acid on soluble cellular adhesion molecules in dyslipidaemic patients. *Atherosclerosis* **174**, 127–132.
134. Atteritano M, Marini H, Minutoli L, *et al.* (2007) Effects of the phytoestrogen genistein on some predictors of cardiovascular risk in osteopenic, postmenopausal women: a two-year randomized, double-blind, placebo-controlled study. *J Clin Endocrinol Metab* **92**, 3068–3075.
135. Kelley DS, Rasooly R, Jacob RA, *et al.* (2006) Consumption of Bing sweet cherries lowers circulating concentrations of inflammation markers in healthy men and women. *J Nutr* **136**, 981–986.
136. Chisolm GM & Steinberg D (2000) The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med* **28**, 1815–1826.
137. Roberts LJ & Morrow JD (2000) Measurement of F-isoprostanes as an index of oxidative stress *in vivo*. *Free Radic Biol Med* **28**, 505–513.
138. Thompson HJ, Heimendinger J, Sedlacek S, *et al.* (2005) 8-Isoprostane F2α excretion is reduced in women by increased vegetable and fruit intake. *Am J Clin Nutr* **82**, 768–776.
139. O'Reilly JD, Mallet AI, McAnlis GT, *et al.* (2001) Consumption of flavonoids in onions and black tea: lack of effect on F2-isoprostanes and autoantibodies to oxidized LDL in healthy humans. *Am J Clin Nutr* **73**, 1040–1044.
140. Loke WM, Hodgson JM, Proudfoot JM, *et al.* (2008) Pure dietary flavonoids quercetin and (−)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr* **88**, 1018–1025.
141. Zalewski A & Macphee C (2005) Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology and possible therapeutic target. *Arterioscler Thromb Vasc Biol* **25**, 923–931.
142. Packard CJ, O'Reilly DSJ, Caslake MJ, *et al.* (2000) Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. *N Engl J Med* **343**, 1148–1155.
143. Thogerson AM, Jansson J, Boman K, *et al.* (1998) High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* **98**, 2241–2247.
144. Thompson SG, Kienast J, Pyke SD, *et al.* (1995) Haemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med* **332**, 635–641.
145. Hamsten A, de Faire U, Walldius G, *et al.* (1987) Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* **ii**, 3–9.
146. Bakker GC, van Erk MJ, Pellis L, *et al.* (2010) An antiinflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. *Am J Clin Nutr* **91**, 1044–1059.
147. Shantsila E (2009) Endothelial microparticles: a universal marker of vascular health? *J Hum Hypertens* **23**, 359–361.
148. Amabile N, Guerin AP, Leroyer A, *et al.* (2005) Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. *J Am Soc Nephrol* **16**, 3381–3388.
149. Boulanger CM, Scoazec A, Ebrahimian T, *et al.* (2001) Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation* **104**, 2649–2652.
150. Brodsky SV, Malinowski K, Golightly M, *et al.* (2002) Plasminogen activator inhibitor-1 promotes formation of endothelial microparticles with procoagulant potential. *Circulation* **106**, 2372–2378.
151. Mallat Z, Benamer H, Hugel B, *et al.* (2000) Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation* **101**, 841–843.
152. Heloire F, Weill B, Weber S, *et al.* (2003) Aggregates of endothelial microparticles and platelets circulate in peripheral blood. Variations during stable coronary disease and acute myocardial infarction. *Thromb Res* **110**, 173–180.
153. Jimenez JJ, Jy W, Mauro LM, *et al.* (2003) Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. *Thromb Res* **109**, 175–180.
154. Mallat Z, Hugel B, Ohan J, *et al.* (1999) Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques: a role for apoptosis in plaque thrombogenicity. *Circulation* **99**, 348–353.
155. Bernal-Mizrachi L, Jy W, Fierro C, *et al.* (2004) Endothelial microparticles correlate with high-risk angiographic lesions in acute coronary syndromes. *Int J Cardiol* **97**, 439–446.
156. Koga H, Sugiyama S, Kugiyama K, *et al.* (2005) Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol* **45**, 1622–1630.
157. Arteaga RB, Chirinos JA, Soriano AO, *et al.* (2006) Endothelial microparticles and platelet and leukocyte activation in patients with the metabolic syndrome. *Am J Cardiol* **98**, 70–74.
158. Garcia S, Chirinos J, Jiminez J, *et al.* (2005) Phenotypic assessment of endothelial microparticles in patients with heart failure and after heart transplantation: switch from cell activation to apoptosis. *J Heart Lung Transplant* **24**, 2184–2189.
159. Wang JM, Su C, Wang YJ, *et al.* (2009) Elevated circulating endothelial microparticles and brachial-ankle pulse wave velocity in well-controlled hypertensive patients. *J Hum Hypertens* **23**, 307–315.
160. Harrison M, Murphy RP, O'Connor PL, *et al.* (2009) The endothelial microparticle response to a high fat meal is not attenuated by prior exercise. *Eur J Appl Physiol* **106**, 555–562.
161. Marin C, Ramirez R, Delgado-Lista J, *et al.* (2011) Mediterranean diet reduces endothelial damage and improves the

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regenerative capacity of the endothelium. *Am J Clin Nutr* **93**, 267–274.
162. Asahara T, Murohara T, Sullivan A, *et al.* (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* **275**, 964–967.
163. Shintani S, Murohara T, Ikeda H, *et al.* (2001) Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* **103**, 2776–2779.
164. Wojakowski W, Tendera M, Michalowska A, *et al.* (2004) Mobilization of CD34/CXCR4+, CD34/CD117+, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. *Circulation* **110**, 3213–3220.
165. Werner N & Nickenig G (2006) Influence of cardiovascular risk factors on endothelial progenitor cells: limitations for therapy? *Arterioscler Thromb Vasc Biol* **26**, 257–266.
166. Vasa M, Fichtlscherer S, Aicher A, *et al.* (2001) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* **89**, 1–7.
167. Hill JM, Zalos G, Halcox JP, *et al.* (2003) Circulating endothelial progenitor cells, vascular function, cardiovascular risk. *N Engl J Med* **348**, 593–600.
168. Werner N, Kosiol S, Schiegl T, *et al.* (2005) Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* **353**, 999–1007.
169. Müller-Ehmsen J, Braun D, Schneider T, *et al.* (2008) Decreased number of circulating progenitor cells in obesity: beneficial effects of weight reduction. *Eur Heart J* **29**, 1560–1568.
170. Kim W, Jeong MH, Cho SH, *et al.* (2006) Effect of green tea consumption on endothelial function and circulating endothelial progenitor cells in chronic smokers. *Circ J* **70**, 1052–1057.
171. Mano R, Ishida A, Ohya Y, *et al.* (2009) Dietary intervention with Okinawan vegetables increased circulating endothelial progenitor cells in healthy young women. *Atherosclerosis* **204**, 544–548.
172. Huang PH, Chen YH, Tsai HY, *et al.* (2010) Intake of red wine increases the number and functional capacity of circulating endothelial progenitor cells by enhancing nitric oxide bioavailability. *Arterioscler Thromb Vasc Biol* **30**, 869–877.
173. Hamed S, Alshiek J, Aharon A, *et al.* (2010) Red wine consumption improves *in vitro* migration of endothelial progenitor cells in young, healthy individuals. *Am J Clin Nutr* **92**, 161–169.
174. Boof CJ, Lip GYH & Blann AD (2006) Circulating endothelial cells in cardiovascular disease. *J Am Coll Cardiol* **48**, 1538–1547.
175. Stirban A, Nandrean S, Gotting C, *et al.* (2010) Effects of *n*-3 fatty acids on macro- and microvascular function in subjects with type 2 diabetes mellitus. *Am J Clin Nutr* **91**, 808–813.

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