Effect of Diet on Vascular Reactivity: An Emerging Marker for Vascular Risk

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New technology for studying vascular activity in vivo has shown that the endothelium plays a critical role in the development of atherosclerosis. The healthy endothelium is a metabolically active tissue that exquisitely regulates vascular tone via release of the powerful vasodilator, nitric oxide. Endothelial integrity reduces cell adhesion, lipid deposition, and other early steps in atherogenesis. There is compelling evidence that endothelial function can be altered within hours of eating certain foods, further affirming the role of dietary factors in the prevention and progression of cardiovascular disease. This article reviews recent work on dietary factors (fatty acids, L-arginine, antioxidants, polyphenols, and folic acid) that alter vascular tone, and critically evaluates two noninvasive measures (flow-mediated dilation and total peripheral resistance) for use in dietary intervention trials.

Introduction

The goal of this paper is to examine the relationship between dietary factors and noninvasive measures of vascular resistance and vascular reactivity for use in controlled clinical studies. I review recent evidence from clinical studies that specific foods and nutritional supplements reduce systemic vascular constriction and promote healthy endothelial function. Although numerous methods have been developed for studying vascular tone in vivo, two noninvasive measures have received the most widespread use and recent attention. These are flow-mediated dilation (FMD) of the brachial artery, a marker of endothelial health and integrity, and, to a lesser extent, total peripheral vascular resistance (TPR), a measure of systemic vascular constriction and an index of myocardial workload (afterload). This review focuses on the use of TPR and FMD as vascular endpoints in studies of dietary interventions.

The Importance of a Healthy Endothelium

Systemic vascular tone is complexly regulated by endocrine and paracrine substances, sympathetic and parasympathetic nervous system activity, and by the activity of the vascular endothelium (Fig. 1). The endothelium is the single cell layer on the surface of the vascular wall; consequently, these cells are in close contact with the blood and blood-borne constituents (platelets, lipids, immune cells, etc). When healthy, the endothelium plays a critical role in regulating vascular tone and in preventing or attenuating several steps in the development of atherosclerotic plaques, including immune and platelet activation, and lipid deposition. With an area of approximately 700 m^2, the vascular endothelium has been called the body's largest endocrine organ [1].

In response to changes in the blood stream, such as shear stress from surges in blood flow or changes in lipid and hormone concentrations, the endothelium secretes powerful vasodilators (*eg*, nitric oxide, prostacyclin) and vasoconstrictors (*eg*, endothelin-1, angiotensin II) onto vascular muscle, thereby regulating its contractile state. Of these vasoactive autocrine factors, nitric oxide has received the most recent attention. In 1980, Furchgott and Zawadzki [2] showed that vascular dilation in response to acetylcholine was critically dependent on the presence of a healthy endothelium. Subsequent studies found that the hypothesized "endothelium-derived relaxing factor" was, in fact, nitric oxide, and that nitric oxide was synthesized within endothelial cells from its precursor, L-arginine. The complex molecular biology underlying endothelial release of constricting and dilating substances has been elegantly reviewed by Luscher and Noll [3] and more recently by Vogel *et al*. [1]. Although great progress has been made in understanding how the endothelium functions under optimal conditions, much more work is needed to develop interventions for restoring endothelial function in patients at high risk for atherosclerosis.

It is now well established that most of the traditional coronary risk factors are associated with impaired endothelial function, and that reductions in the bioavailability of nitric oxide may be a central feature of atherogenesis [3,4]. For example, smoking, hypercholesterolemia, consumption of saturated fat, obesity, diabetes, and elevated homocysteine levels are all associated with impaired endothelial function [1,5]. Perhaps even more

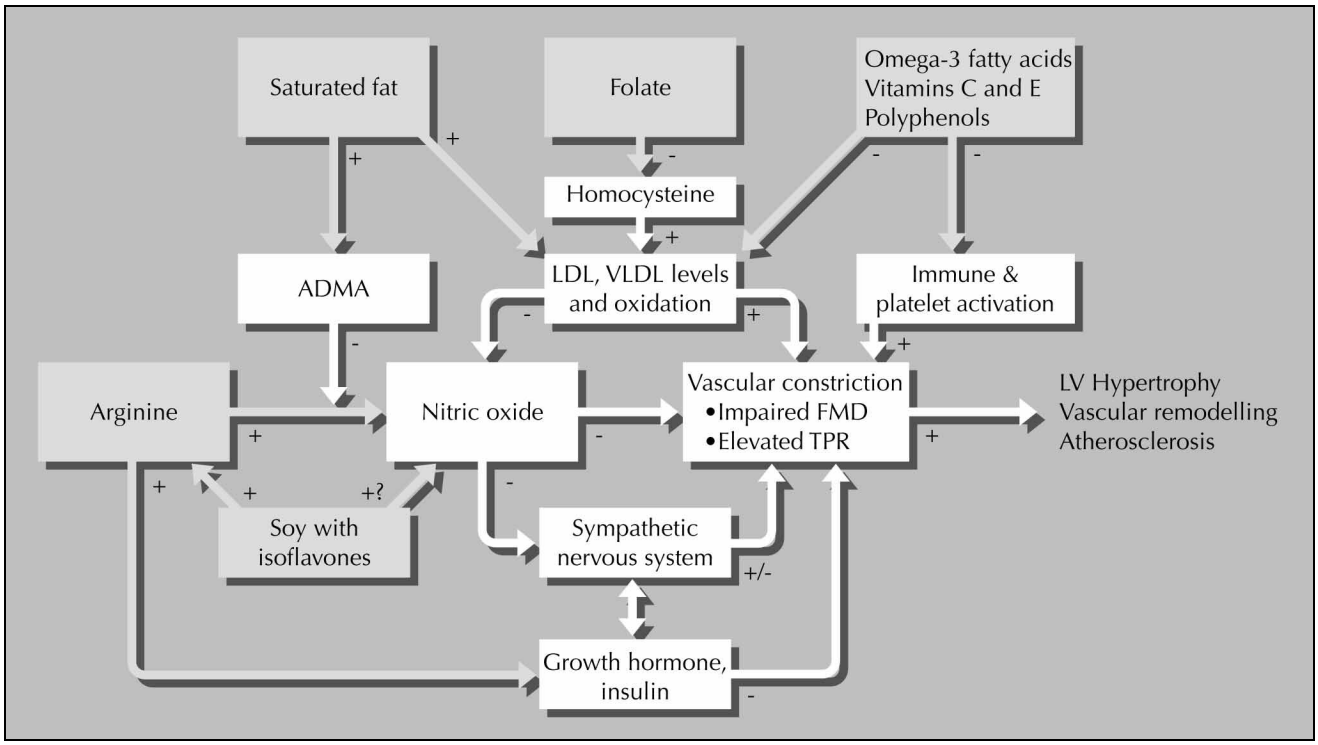

Figure 1. A model of how various dietary factors alter vascular resistance. Vasoactive functional foods and nutrients are in shaded boxes. Potential mechanisms for their vascular effects are shown in white boxes. A *plus sign* indicates an increase, a *minus sign* indicates a decrease. (ADMA—asymmetric dimethylarginine; FMD—flow-mediated dilation; LDL—low-density lipoprotein; LV—left ventricular; TPR—total peripheral resistance; VLDL—very low-density lipoprotein.)

important are studies showing that endothelial dysfunction is reversible following successful treatment of high cholesterol [6] and elevated homocysteine [7]. Whether endothelial dysfunction is truly a prognostic indicator for subsequent development of atherosclerosis is a question that will be explored by Benjamin *et al.* in the Framingham Heart Study (Benjamin, personal communication). In the meantime, it is important to examine whether improvement in endothelial function is a plausible mechanism through which various dietary patterns achieve their beneficial effects. The goal of this review is to critically evaluate the progress to date in the use of nonpharmacologic therapies for improving surrogate markers of vascular health.

Assessment of Endothelial Function In Vivo

In the past, the most common method for studying vascular function involved measuring responses to pharmacologic agents (acetylcholine, angiotensin II, norepinephrine, adrenergic antagonists, etc.) directly infused via an arterial line, or by bathing arterial segments in vasoconstrictive agents and noting their response. However, techniques requiring arterial cannulation are associated with some discomfort and risk, and limit one's ability to study large numbers of patients or assess serial changes in vascular responsiveness. More recently, a noninvasive technique using increased shear stress to stimulate release of nitric oxide and dilation of the brachial artery has been developed to assess endothelial function [8•]. Typically, the stimulus for increased blood flow and shear stress is a brief period of ischemia (4 to 5 minutes) induced by inflation of a blood pressure cuff on the upper or lower arm. The resulting increase in blood flow, termed reactive hyperemia, increases arterial diameter in healthy arteries. Figure 2 shows the change in vessel diameter in a healthy individual, as determined by high-resolution ultrasound imaging. Endothelium-dependent FMD of the brachial artery is quantified as the maximum percent change in arterial diameter (8.4% in this example).

Similar to a glucose tolerance test, measurement of FMD examines dynamic change in a system that is tightly regulated in healthy individuals. Others have reviewed the considerable technical challenges involved in measuring FMD reliably [8•,9]. Briefly, high-resolution ultrasound imaging is used to measure the diameter of the brachial artery before and after induction of reactive hyperemia. Although conceptually simple, the inherent difficulty in conducting these tests is revealed by examining the high resolution and low variability required to detect a response. In my laboratory, average brachial artery diameter ranges from 3 to 6 mm. A large change in diameter during FMD, indicating healthy vessels, is in the range of 8% to 10%. Thus, one must reliably measure differences as small as 0.2 to 0.6 mm. Recent technical advances that improve reliability include 1) high-frequency ultrasound transducers (10 to 15 MHz); 2) the use of automated edge-

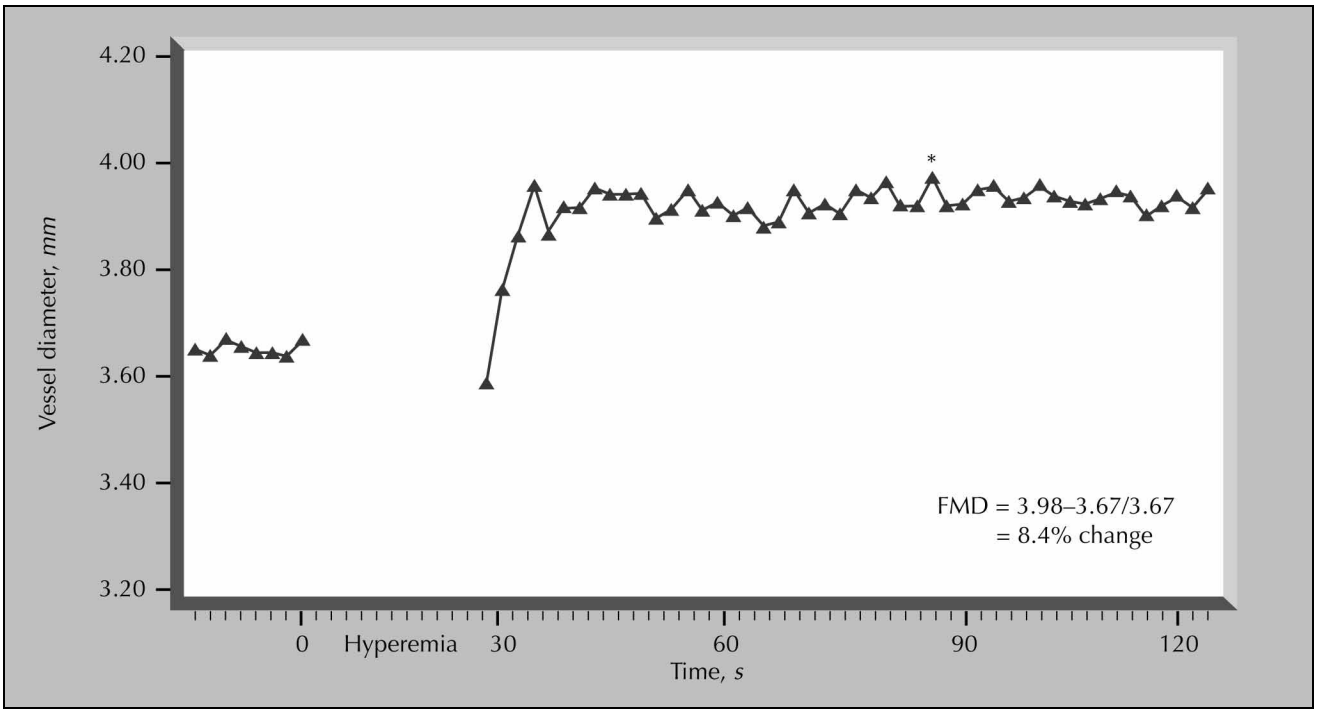

Figure 2. The flow-mediated dilation (FMD) response in a single patient from the Vascular Health Interventions Laboratory. The *y*-axis depicts change in brachial artery diameter following deflation of the ischemic cuff (↑). *Asterisk* indicates that FMD is measured as the percent change in arterial diameter at peak response.

detection software for calculating vessel diameter hundreds of times within each image (*eg*, Brachial Tools Software, Medical Imaging Applications, Iowa City, IA); and 3) simultaneous measurement of blood flow following cuff deflation, to ensure that the stimulus for vasodilation is standardized across testing sessions. Despite the technical challenges associated with this technique, there is tremendous excitement about the sensitivity of FMD, and thus endothelial function, to short-term dietary interventions (Table 1).

Effects of Diet on Vascular Reactivity

Figure 1 shows the hypothesized links between various macronutrients and dietary supplements and the important physiologic systems that regulate vascular resistance. Although controlled studies of single nutrients are important for understanding physiologic mechanisms, it is of great importance to study the effects of longer-term dietary patterns. This model is proposed to encourage the study of foods and nutrients within their dietary context, and to define physiologic systems that may play a role in altering vascular resistance.

Effects of dietary fats on flow-mediated dilation

There is growing evidence that consumption of a single high-fat meal (50 to 105 g of fat) impairs FMD by 45% to 80%. However, it is clear that the type of fat and the other ingredients in the meal are critical determinants of this effect. Ten separate investigations have observed impaired FMD within 2 to 5 hours after a single high-fat meal [10–19], although this effect is not found in all studies [20,21]. Several investigators [15,17] suggest that acute hypertriglyceridemia is responsible for this effect. There is also evidence that oxidative stress [11,16,22] and asymmetric dimethylarginine (ADMA, an endogenous inhibitor of nitric oxide production) [14] play a critical role in impairing endothelial function after a meal (Fig. 1).

Disagreement about the effects of acute fat loading on FMD may arise from the variety of ways in which high-fat meals are prepared. For example, Williams *et al*. [22] reported that previously used cooking fat impaired FMD, whereas the same amount of fresh fat had no effect. Several recent studies have examined whether combinations of foods and nutrients can alter the postprandial response. For example, Vogel *et al*. [13] and Ong *et al*. [17] have shown that olive oil acutely impairs FMD, a finding that seems at odds with the demonstrated vascular benefit of a Mediterranean diet. However, this dietary pattern also increases consumption of antioxidant vitamins, lycopenes, and omega-3 (n-3) fatty acids. The net effect of the Mediterranean diet may result from their interactive effects. In support of this hypothesis, one study showed that consuming the antioxidants vitamin C (1 g) and vitamin E (800 IU) along with the olive oil allowed the patient to retain normal vascular function [13]. Pretreatment with folic acid (10 mg/d for 2 weeks) has also been shown to abolish the acute effects of 50 g of saturated fat on FMD [19]. These findings are exciting because they

Table 1. Vasoactive food components, their sources in the diet, and net effect on endothelial function and vascular resistance

Food component	Source in diet	Net effect on endothelial function and vascular resistance
Fats/fatty acids		
Saturated fat	Fatty meats, full-fat dairy products, hydrogenated fat	Impairment
Oleic acid	Olive oil, avocados, peanuts, other oils	Improvement / impairment
Omega-3 fatty acids	Fish oil, flaxseed oil, canola oil, soybean oil	Improvement
Antioxidants		
Polyphenols	Red wine, grape juice, chocolate, tea	Improvement
Vitamin C	Fruits and vegetables	Improvement
Vitamin E	Nuts, seeds, oil, fish	Improvement
Other		
Folate	Leafy greens, supplements, fortified grains, orange juice	Improvement
Methionine/homocysteine	Red meat and other animal products, may be increased by alcohol and caffeine	Impairment
L-arginine	Plant proteins (soybeans, legumes, nuts)	Improvement
Isoflavones	Soybeans, red clover, flax, alfalfa	Improvement
Dietary patterns		
High carbohydrate	Breads, cereals, fruits, sugared cola	Impairment*
Low-fat diet	Breads, cereals, grains, fruits, vegetables, legumes	Improvement
Mediterranean diet	Olive oil, fish, fruits, vegetables, wine, nuts	Improvement

*Increases total peripheral vascular resistance reactivity only in insulin-resistant adults.

show that FMD is sensitive to dietary factors and that it can be changed rapidly. However, the majority of these studies employ modest sample sizes (n=10 to 50), and are restricted in their focus on the immediate, short-term effects of a few isolated nutrients.

There are few studies of complex dietary patterns that include FMD as an endpoint. In a recent paper, Fuentes *et al.* [23•] reported that 4 weeks of a Mediterranean diet increased FMD by 42% relative to a high-fat control diet. Ryan *et al.* [24] found a similar degree of improvement (56%) in diabetic patients who consumed a high-oleic acid diet for 2 months. Leighton *et al.* [25] and Cuevas *et al.* [26] have shown a significant improvement in FMD with Mediterranean and low-fat diets, and this effect is further augmented when red wine is consumed. Thus, although there is concern that dietary fat can impair vascular function, this must be carefully studied in the context of other foods that are typically consumed at the same time. Because of inconsistent findings on the effects of oleic acid and olive oil on vascular reactivity, it is not included in the model.

There is also evidence that specific fatty acids, particularly those from fish, may improve vascular reactivity. Intense interest in the long chain n-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), resulted from controlled trials showing a substantial reduction in risk of cardiac death with their use [27••]. The largest of these studies, the GISSI trial [28•], randomized over 5000 coronary patients to receive DHA and EPA (850 mg), vitamin E (300 mg), a combination of the two supplements, or placebo for 3.5 years. Risk of cardiovascular death was reduced by 10% to 15% with the fish oil supplement. Although the mechanisms for these effects are not known, the weight of the recent evidence suggests that endothelium-dependent dilation is improved with short-term supplementation of n-3 fatty acids from fish (Table 2).

The majority of these studies [27••,28•,29–32,33•] find improved endothelium-dependent dilation after treatment with fish oil capsules (containing DHA, EPA, or a combination). The two negative findings come from a study of healthy patients [34] and one study that used a very brief treatment period (24 hours) [35]. In one of the few studies that measured brachial artery FMD, Goodfellow *et al.* [36] administered n-3 fatty acids (4 g/d for 4 months) and found that FMD increased from 5% to 12% in their hypercholesterolemic patients. Only one study [33•] has examined the separate contributions of DHA and EPA to the vascular effects of combined fish oil supplements. In a very recent study of hypercholesterolemic adults, Mori *et al.* [33•] showed that 4 g/d of DHA, but not EPA, significantly improved vasodilator response to acetylcholine. Thus, the relative concentrations of the two fatty acids may be of critical importance to their effects on endothelium-dependent dilation. Figure 1 shows three potential mechanisms through which n-3 fatty acids could impact vascular reactivity. These include 1) reductions in atherogenic lipids; 2) improvements in oxidative status; and 3) reductions in immune and platelet activation. It is also likely that these fatty acids regulate endothelial cell function directly, due to their incorporation into the lipid bilayer of cell membranes [32]. There is evidence from human [37] and in vitro studies [38–40] that n-3 fatty acids improve several markers of endothelial dysfunction (*eg*, von Willebrand factor, thrombomodulin, and

Table 2. Randomized, placebo-controlled trials on the effects of omega-3 fatty acids on endothelium-dependent dilation

Study / year	Sample size, *n*	Patient characteristics	Fatty acid preparation	Duration	Vascular assessment method	Results
Chin *et al.* [34] / 1993	22	Healthy males	10 g EPA/DHA, with or without indomethacin	4 wk	Forearm VOP, infusions of ACh, norepinephrine, AgII	Lower constrictive response to AgII and norepinephrine after treatment; no improvement in EDD
Fleischhauer *et al.* [29] / 1993	14	Cardiac transplant patients	5 g EPA/DHA	3 wk	Coronary artery infusion of ACh	Omega-3s restore the vasodilatory response to ACh.
McVeigh *et al.* [30] / 1993	23	Type 2 diabetic patients	3 g EPA/DHA	6 and 18 wk	Forearm VOP, infusion of ACh	Significant improvements in vasodilator response to ACh
Goode *et al.* [32] / 1997	28	HC vs healthy adults	5 g EPA/DHA	12 wk	Arterial biopsy, dilatory response to ACh	Significant improvements in vasodilator response to ACh in HC adults; largest benefit in patients with increased DHA and EPA in erythrocyte membranes.
Kothny *et al.* [35] / 1998	18	Coronary patients	18 g EPA/DHA	1 d	Radial artery FMD	No significant improvements
Goodfellow *et al.* [36] / 2000	30	Healthy, HC adults	4 g EPA/DHA	16 wk	Brachial artery FMD	FMD increases from 5.0% to 12.0% in treated patients
Mori *et al.* [33•] / 2001	40	Overweight, mildly HC men	4g EPA or 4g DHA	6 wk	Forearm VOP, infusion of ACh, with and without NO blocker	DHA (but not EPA) significantly improves EDD and other measures of vasodilation

ACh—acetylcholine; AgII—angiotensin II; DHA—docosahexaenoic acid; EDD—endothelium-dependent dilation; EPA—eicosapentaenoic acid; FMD—flow-mediated dilation; HC—hypercholesterolemic; NO—nitric oxide; VOP—venous occlusion plethysmography.

impaired membrane transport) [27••]. Taken together, it is clear that n-3 fatty acids can improve cardiovascular health in at-risk adults, and that more work is needed to understand their mechanism of action.

L-arginine

A number of studies have reported enhanced brachial artery vasodilation after treatment with the nitric oxide precursor, L-arginine [41•]. The average American consumes 4 to 5 g of L-arginine per day, and red meat is the most frequently consumed source. Clinical studies of L-arginine have shown significant effects when intake is increased three- to fourfold via supplements. For example, Clarkson *et al.* [42] showed that 4 weeks of oral L-arginine supplements (21 g/d) produced a twofold increase in FMD in hypercholesterolemic patients. Doses of 14 to 21 g/d have been shown to improve FMD, although we have observed significant gastrointestinal side effects (*eg*, cramping, gas) at this dosage. Furthermore, the vascular benefits may only be observed in patients with impaired nitric oxide status, such as hypercholesterolemic patients [43] and individuals with established peripheral arterial disease [41•]. L-arginine has also been shown to reduce systemic vascular constriction, perhaps via an increase in insulin and growth hormone; these data are described in the following text. Dietary sources of L-arginine include walnuts, peanuts, soybeans, legumes, and fish protein; L-arginine may be an important mechanism for their demonstrated health benefits. However, no study to date has examined the effects of a diet rich in high-arginine foods on endothelial function.

Antioxidants

Vitamins and food compounds with antioxidant properties have shown great promise in enhancing vascular reactivity and in limiting the effects of fat on blood vessels. Antioxidants that have been shown to improve endothelial function include vitamin E, vitamin C, polyphenolic compounds, and isoflavones. They produce their effects, in part, by reducing oxidation throughout the vascular system. Oxidized lipids (particularly chylomicron remnants, very low-density lipoprotein cholesterol, and low-density lipoprotein cholesterol) are well known for their adverse effects on endothelial function [1], and dietary antioxidants reverse these effects (Fig. 1). In one study, vitamin E (300 IU for 8 weeks) dramatically improved FMD in hypercholesterolemic patients, whether alone or in combination with statin therapy [44]. Chambers *et al.* [45] showed that vitamin C (1 g/d for 1 week) prevented the expected decrease in FMD induced by homocysteine, and others have observed short-term improvements with this antioxidant. However, several investigators question whether these benefits last with longer-term treatment [1,46]. Polyphenolic compounds in red wine [25,26] and purple grape juice [47] have also been shown to improve FMD and oxidative status.

Homocysteine

Of the naturally occurring substances that impair vascular function, homocysteine is one of the most powerful [45]. It is produced from dietary methionine and may be increased by consumption of caffeine and alcohol. Homocysteine has been shown to have pro-oxidant properties, to upregulate thrombotic function, and to attenuate nitric oxide release. In a recent paper, Boger *et al.* [48•] showed that acutely raising homocysteine also produces increases ADMA, an endogenous substance that limits nitric oxide production from L-arginine (Fig. 1). There is a large body of evidence that folic acid supplements significantly reduce homocysteine [49], and that folic acid improves FMD [7].

Although there has been progress in understanding the mechanisms through which a few foods and supplements alter endothelial function, a great deal remains to be learned about the vascular effects of complex dietary patterns that have been shown to reduce blood pressure (*eg*, the Mediterranean diet, the Dietary Approaches to Stop Hypertension diet). A dietary pattern that incorporates fruits and vegetables, fish, plant proteins, and polyphenols may have marked effects on FMD due to the small additive or synergistic effects of the nutrients consumed.

The Importance of Measuring Systemic Vascular Resistance

Flow-mediated dilation is primarily determined by the ability of the endothelial cells to elaborate nitric oxide. In contrast, systemic vascular resistance is determined by multiple factors [50], including arteriolar smooth muscle hypertrophy and the activity of sympathetic vasoconstrictor nerves, parasympathetic vasodilatory nerves, local autocrine factors, baroreceptor activation, and numerous circulating substances produced by the kidneys, heart, and adrenal glands. It has long been known that peripheral vascular constriction is a central feature of established hypertension [50], and that hypertensive patients show exaggerated reactivity to vasoconstrictive tests such as the cold pressor [51]. Total peripheral vascular resistance (TPR) is a measure of systemic vascular constriction, and is an important index of left ventricular afterload. Higher levels of TPR are prospectively associated with adverse changes in myocardial structure, such as increases in left ventricular mass [52]. Individuals with exaggerated vascular responses to laboratory stressors also exhibit endothelial dysfunction [53•] and have greater left ventricular mass [52]. In contrast, FMD response is apparently not an independent predictor of left ventricular mass. For example, Muiesan *et al.* [54] measured FMD and left ventricular geometry in 94 adults with a wide range of resting blood pressure levels. They found no correlation between the magnitude of FMD and the size of the left ventricle. Thus, TPR and FMD provide complementary, but not identical, information about the health of the cardiovascular system.

Total peripheral vascular resistance can be measured noninvasively through the use of impedance cardiography. This technique is commonly used to study the hemodynamic effects of drugs, particularly agents that act on the sympathetic nervous system. We recently reported that 6 months of hormone replacement therapy with either oral or transdermal estrogen lowered TPR by 15% [55]. Reductions in vascular resistance were accompanied by lower levels of norepinephrine and significant reductions in left ventricular mass [56]. New information that sympathetic nervous system activity is attenuated by nitric oxide leaves open the possibility that TPR could be reduced by the same dietary interventions that improve FMD (Fig. 1).

Measurement of Total Peripheral Vascular Resistance

Impedance cardiography was developed in the 1960s for the noninvasive measurement of cardiac output and TPR. Recent interest in vascular resistance has resulted in improved data collection systems and a wealth of data on the effects of dietary changes, exercise training, and pharmacologic treatment on TPR. There are several commercially available impedance cardiographs and software packages for the noninvasive measurement of changes in stroke volume and cardiac output. Surface electrodes are used to record changes in the resistance of the thoracic cavity to a low-voltage current applied to the chest wall. Large, pulsatile changes in resistance are observed across the cardiac cycle. The amplitude and duration of these events can be used to estimate stroke volume via validated equations [57•]. Cardiac output (CO, l/min) is calculated as the product of stroke volume and heart rate. Mean arterial pressure (MAP, mm Hg) is measured simultaneously via an automated device, and allows the calculation of TPR as follows: TPR (dyne-sec x cm^{-5}) = (MAP / CO) x 80. Although some studies have shown very close agreement between impedance-derived estimates of CO and those derived from more invasive techniques [57], it is clear that the greatest strength of impedance cardiography is for the assessment of within-patient change in CO and TPR. McFetridge and Sherwood [57] and Sherwood *et al*. [58] have published excellent reviews on the validity, appropriate use, and important limitations of this technique.

Effects of Diet on Systemic Hemodynamics

Although much recent attention has been focused on dietary factors that alter endothelial function, it is important to note that systemic hemodynamics are also influenced by dietary interventions. In a recent, controlled feeding study [59], we examined vascular and myocardial responses to a high-salt diet (300 mEq of sodium per day) in adults whose blood pressure increased during sodium loading (salt sensitives) versus those who showed no blood pressure response to a high-salt diet (salt resistants).

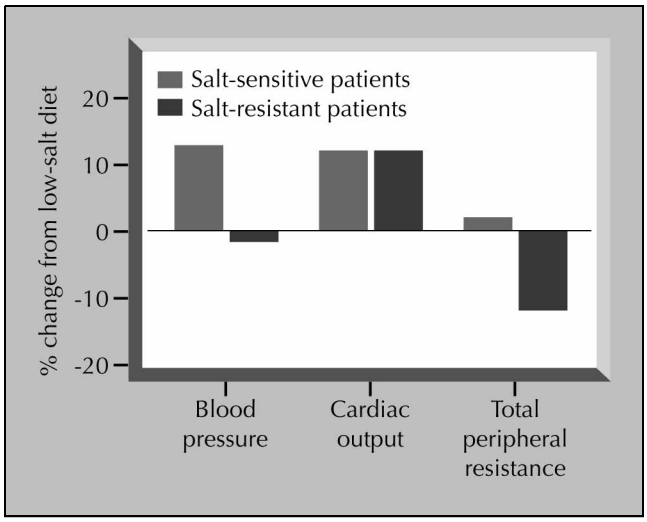

Figure 3. Percentage change in mean arterial blood pressure (BP), cardiac output (CO), and total peripheral resistance (TPR) during a high-salt diet in salt-sensitive (n=34) and salt-resistant (n=33) adults. Although CO significantly increased in both groups, only the salt-resistant group shows the appropriate compensatory change (lower TPR). As a result, they showed no change in BP during sodium loading. (*Adapted from* West *et al*. [59].)

Figure 3 shows that both groups exhibited increases in cardiac output during the high-salt diet. However, only the salt-resistant group showed the appropriate compensatory response (lower TPR). Consequently, their blood pressure was unchanged or actually decreased during sodium loading. When the high-salt diet was accompanied by high potassium (175 mEq/d), the pressor effects of salt were no longer evident [59]. Again, this highlights the importance of studying dietary patterns, rather than single nutrients, in determining the effect of diet on the vasculature.

Nelesen and Dimsdale [60] have shown that degree of insulin resistance is an important moderator of TPR reactivity, and that these effects are most evident during a high-carbohydrate diet. For example, insulin-resistant adults exhibited exaggerated TPR reactivity to behavioral stress tasks, but only during high-carbohydrate intake [60]. Weight-loss programs have also been shown to reduce TPR [61], although it is not clear whether this is an effect of weight loss per se, or real effect of a more nutritious diet. Others have measured TPR to document the acute vascular effects of nutritional supplements. In a series of elegantly designed studies, Bode-Boger *et al*. [62] have shown that infusion of a high dose (30 g) of L-arginine, the precursor for nitric oxide, rapidly reduces TPR, and that this effect is reversed as plasma arginine levels decrease. However, it is not clear whether supraphysiologic levels of L-arginine are required to show this effect, and current studies in my laboratory examine the effects of chronic supplementation with more modest doses of oral arginine on TPR reactivity.

Several studies have examined whether intravenous fatty acids alter TPR reactivity. Battilana *et al*. [63] reported significant increases in blood pressure and TPR reactivity

to mental stress tasks after lipid infusion, and other studies confirm these effects [64]. Although it is tempting to speculate that a high-fat meal would cause a similar increase in TPR reactivity, it is important to note that eating a meal significantly increases cardiac output, while systemic resistance generally drops [65]. This decrease in systemic resistance after a meal (as a result of insulin release) is necessary to supply the digestive organs with proper blood flow. For this reason, we routinely instruct patients to avoid eating from 2 to 4 hours prior to TPR testing.

Although there is promising evidence that systemic vascular constriction is a modifiable coronary risk factor, few studies of dietary interventions have used it. Its relatively low cost, good reliability, and noninvasive methodology make it an attractive technique for studying the effects of dietary changes on systemic vascular resistance.

Conclusions

Although observational studies are often the first source of information about dietary patterns and types of foods that promote vascular health, these findings must be validated in controlled clinical trials to establish whether the food or nutrient is causally involved in plaque development. To the extent that FMD and TPR reactivity are correlated with other markers of coronary disease burden, they provide critical surrogate endpoints for the development of new functional foods, dietary supplements, and dietary patterns. The rapidity with which vascular function can be altered by consumption of specific foods has provided a new way of understanding the interaction of the vascular and metabolic abnormalities that result in atherosclerosis.

Flow-mediated dilation is one candidate marker for measuring endothelial health. Many others have been proposed, including cell adhesion and platelet aggregation factors, as well as general markers of immune activation (cytokines, chemoattractants) and vascular damage (von Willebrand Factor). Thus, the vasodilatory action of the endothelium may be of central importance on its own, or FMD may be a sentinel marker for the many regulatory effects of a healthy endothelium. Blood tests for soluble endothelial markers and indices of immune and platelet activation may be more appropriate tests for endothelial function in larger studies that lack the capability for intensive assessments of each individual's arterial response [8•]. A very recent paper by Brown and Hu [27••] provides an excellent review of the effects of various foods and macronutrients on several blood-derived markers of vascular dysfunction.

Although there is tremendous interest in using FMD in controlled trials, it is important that future investigators understand the technical challenges involved. Given the small magnitude of the response and the tremendous variability in the measurement of FMD, future investigations should report coefficients of variation and test/re-test reliability statistics for their own laboratories. Without this information, it is difficult to know whether null findings are due to the imprecision of the technique or the ineffectiveness of the intervention. Several recent studies have measured vasodilatory response to nitroglycerin, a drug that provides nitric oxide directly to vascular smooth muscle; its effects are not dependent on endothelial health. This control procedure, used as a measure of endothelium-independent dilation, provides additional information about the health of the artery and the resolution of the measurement technique. Regardless of the measurement technique, careful attention must be paid to factors that are known to influence vascular function, including phase of menstrual cycle; use of exogenous hormones; fasting status/levels of insulin; use of substances that reduce cholesterol or act directly on the vessel wall; recent exercise; and the use of alcohol, tobacco, over-the-counter medications; and nutritional supplements.

In conclusion, assessment of vascular reactivity via duplex sonography or impedance cardiography has allowed us to measure changes in vascular function following short-term dietary interventions. These techniques provide complementary, but not identical, information about the mechanisms through which various dietary factors have their beneficial effects. Indeed, systemic vascular resistance is partly determined by the endothelium-dependent dilation that produces the FMD response. TPR may provide a sensitive assay for detecting foods that reduce peripheral vascular constriction and allow regression of left ventricular hypertrophy, itself an important marker of coronary risk. The challenge for future investigators is threefold: 1) to elaborate on the mechanisms through which the dietary factors outlined here alter vascular resistance; 2) to identify other vasoactive foods and supplements; and 3) to establish the requisite dose and combination of foods to achieve vascular health.

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- •• Of major importance

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This is an excellent, detailed review of several techniques for measuring endothelial function. It describes invasive techniques (*eg,* direct infusion of vasoactive drugs), potential blood markers of endothelial dysfunction (nitric oxide metabolites, asymmetric dimethylarginine, endothelin, etc), and the flow-mediated dilation protocol first developed by Celermajer for use as a preclinical test of vascular function.

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