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Plant-Based Diet Reverses Vascular Endothelial Dysfunction in Patients with Peripheral Arterial Disease

Peter H. Lin, MD, Debra Leslie, MPH, RDN, Mary Levine, RD, Garth Davis, MD, & Caldwell B. Esselstyn, Jr, MD

Abstract

OBJECTIVE: Peripheral arterial disease (PAD) is characterized by impaired arterial circulation to the extremities caused in part by atherosclerosis. This study examined the effect of a plant-based diet (PBD) on vascular function in PAD patients.

METHODS: Patients with PAD were randomized to plant-based dietary intervention (PBD group, $n = 24$) or no specific dietary advice (control group, $n = 28$). Biochemical parameters, including lipid profile and inflammatory biomarkers, and nitric oxide were measured at baseline and 4 months after dietary intervention. Vascular function including brachial artery flow-mediated vasodilation (FMD), carotid intima-media thickness (IMT), carotid-femoral pulse wave velocity (PWV), and brachial-ankle PWV were measured at baseline and 4 months after dietary intervention.

RESULTS: Biochemical parameters were similar at baseline between the 2 groups. There was no change in any of the biochemical parameters in the control group at 4 months. However, patients in the PBD group had a significant improvement in lipid profile, including total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and apolipoprotein A1 (Apo-A1) levels. Greater nitric oxide and reduced high-sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6) levels were found in the PBD group at 4 months, whereas there were no changes in the control group. At baseline, FMD was similar between the 2 groups. After 4 months, PBD participants showed significant endothelial function improvement in FMD response and arterial stiffness response, with increased carotid-femoral and brachial-ankle PWV compared to the control group.

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CONCLUSIONS: A plant-based diet improves vascular endothelial function in PAD patients following 4 months of dietary intervention. This dietary intervention can result in decreased serum cholesterol and inflammatory biomarkers, which may further enhance vascular endothelial function.

KEYWORDS: Plant-based diet; Vascular endothelial function; Flow-mediated dilation; Brachial artery reactivity test

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Lin, MD,[a,b] Leslie, MPH, RDN,[c] Levine, RD,[d] Davis, MD,[e] & Esselstyn, Jr, MD[f]

Affiliations

[a]Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX

[b]University Vascular Associates, Los Angeles, CA

[c]Department of Nutritional Sciences, California State University Los Angeles, Los Angeles, CA

[d]Department of Dietetics and Nutrition, California State University Channel Islands, Camarillo, CA

[e]Department of Surgery, University of Texas Health Science Center at Houston, TX; Mission Health, Asheville, NC

[f]Cleveland Clinic Wellness Institute, Cleveland, OH

Corresponding Author

Peter H. Lin, MD
Baylor College of Medicine,
Houston, TX 77030
Email: plin@bcm.edu

Introduction

Cardiovascular disease (CVD), a condition predominantly caused by atherosclerosis, is the leading cause of morbidity and mortality worldwide. Peripheral arterial disease (PAD), a subset of CVD, occurs when the atherosclerosis progresses to compromise the lower extremity circulation resulting in ischemic symptoms. Although atherosclerosis has been generally regarded as a disease of developed or affluent countries, recent evidence shows a progressive rise in the prevalence of CVD in developing countries where an epidemiological shift of disease prevalence patterns from infectious illnesses to atherosclerotic disease has occurred. Management of CVD, with an emphasis on disease prevention, will undoubtedly play an increasing vital role in the health care system around the world.

There have been significant pharmacological advances in past decades to lower the incidence of CVD including the advent of cardioprotective drugs such as platelet-inhibiting antithrombotics, cholesterol-lowering statins, and angiotensin-converting enzyme inhibitors. Similar advances have also occurred with improved interventional strategies to reduce the morbidity and mortality associated with CVD including drug-eluting stents, plaque-removing atherectomy devices, and bioprosthetic bypass graft conduits. A recent practice guideline published by the American College of Cardiology and American Heart Association highlighted the role of lifestyle modification (LM), including dietary intervention, as a major contributor in the reduction of CVD-related mortality.[1,2,3] Epidemiological study suggests that more than half of the decline in CVD-related deaths in the United States from 1980 to 2000 could be attributed to cardiovascular risk factor management achieved through a combination of dietary interventions and pharmacotherapies.[3,4,5]

Endothelial dysfunction, as reflected by the impaired arterial vasodilatory capacity, represents one of the pathogenic mechanisms linking atherosclerosis and cardiovascular mortality. The ability of arteries to dilate in response to stimuli is a significant indicator of underlying vascular endothelial function and associated CVD.[6,7] Factors modulating vasodilatory response include the release of vasoactive compounds such as nitric oxide (NO) from the endothelium and vascular compliance. In healthy individuals, a major mechanism responsible for vasodilation is the hyperemic-stimulated release of NO from the endothelium, resulting in vascular smooth muscle relaxation with subsequent vasodilation.[6]

Vascular endothelial function can be assessed using a non-invasive technique to determine brachial artery reactivity. A high-resolution ultrasound is used to measure changes in brachial artery diameter due to flow-mediated dilation (FMD) from increased endogenous production of endothelium-derived NO. Reduced FMD has been described as a reliable indicator of vascular endothelial dysfunction, as well as presence of underlying CVD risk factors and related diseases.[8] Recent studies have similarly

shown that arterial pulse wave velocity (PWV), which is a non-invasive evaluation of arterial stiffness, is a reliable indicator of vascular function.[9,10] While numerous studies have documented the benefit of dietary intervention in the reduction of CVD-related sequelae, limited data are available regarding whether the beneficial effect of dietary intervention is reflected in vascular endothelial function. This study was therefore conducted to assess the effects of a plant-based diet (PBD) on vascular endothelial function as assessed by FMD and PWV in patients with peripheral arterial disease.

Methods

This was an open-label, randomized parallel-group study in which 66 adult patients with PAD were recruited. Inclusion criteria for PAD included an ankle-brachial index (ABI) of 0.90 or less at baseline. Those with an ABI greater than 0.90 at baseline were eligible if a non-invasive vascular ultrasound demonstrated PAD, a lower extremity angiography showed arterial stenosis of 70% or greater, or medical records documented prior revascularization of lower extremities.[11] Additionally, subjects had to be willing and able to maintain an online food journal using smartphone-based dietary apps. All subjects screened in this study were recruited from the first author's clinical practice, which predominantly consists of patients with PAD. Once enrolled, all participants underwent baseline evaluation, after which they were randomized to either dietary advice for a plant-based diet (PBD group) or no specific dietary advice (control group). The dietary intervention was continued for 4 months, at the end of which a follow-up evaluation was performed. The study was registered on ClinicalTrials.gov before commencement (NCT03798938). The study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. Appropriate approval by the Institutional Review Board/Ethics Committee was obtained for the study, and all of the study participants provided written informed consent.

Baseline Evaluation

All participants underwent baseline clinical evaluation, biochemical testing, carotid intima-media thickness (IMT) assessment, and vascular endothelial function assessment with brachial artery FMD and PWV before treatment group randomization. Clinical evaluation included detailed history regarding the presence or absence of cardiovascular risk factors, duration of these risk factors, and PAD-related symptoms. Physical examination included measurement of height, weight, waist circumference, blood pressure (BP), and examination of the cardiovascular system. BP was measured in the right arm, with the participant in supine position, using a standard sphygmomanometer. Biochemical investigations included fasting and two-hour postprandial blood glucose; fasting lipid profile including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c); apolipoprotein A1 (Apo-A1); and apolipoprotein B (Apo-B). Nitric oxide, superoxide dismutase, and inflammatory biomarkers including high-sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), IL-7, and IL-18 were also analyzed.

Study Randomization

Once enrolled in the study, participants were randomized unblinded to either a PBD group or control group using a computer-generated random number for simple randomization. The PBD group was given dietary advice by a certified dietitian and a specially trained research nurse. These participants were advised to follow a whole-food, plant-based diet consisting of vegetables, fruits, legumes, whole grains, nuts, seeds, herbs, and spices, which can be consumed in countless combinations. Food components of the PBD with recommended daily servings are shown in Table 1.

Table 1. Food groups of plant-based diet with recommended servings per day

Food group	Recommended serving per day
Fruits, all types	2–4 servings (1 serving = 1 medium piece or 1/2 cup)
Vegetables, all types	ad libitum
Whole grains (e.g., brown rice, quinoa, oats)	6–11 servings (1 serving = 1/2 cup cooked or 1 slice whole grain bread)
Legume (beans, lentils, peas, soy foods)	2–3 servings (1 serving = 1/2 cup cooked)
Leafy green vegetables (e.g., broccoli, cabbage, lettuce)	2–3 servings (1 serving = 1/2 cup raw or 1/2 cup cooked)
Seeds (e.g., chia, flax, hemp seeds)	1–2 tablespoons
Nuts (e.g., almonds, pecans, walnuts)	1–2 ounces
Fortified plant milks (e.g., almond, soy, rice)	Optional, 2–3 cups
Fresh herbs and spices	Optional, ad libitum

This PBD recommendation is in accordance with the guideline endorsed by the United States Department of Agriculture, the American Heart Association, and the American Institute for Cancer Research.[1] Additionally, PBD participants were also encouraged to avoid processed foods and animal products. In contrast, the control group was offered booklets about heart-healthy diets without specific dietary advice. All study participants were instructed to maintain a food diary using web-based dietary journal apps whereby a smartphone camera was used to photograph their meals. Information related to the online food journal was monitored by the study coordinator, and dietary counseling was provided when necessary. Both groups were encouraged to engage in regular physical activity with low-impact aerobic exercise such as walking at least 3 times per week. Patients from both groups were evaluated monthly in an outpatient clinic.

FMD Assessment

Endothelial function based on brachial artery FMD assessment was performed based on previously published reports.[6,8] A brachial artery FMD assessment was performed using a 7.5-MHz linear transducer with an ultrasound scanning unit (Philips HP Sonos 5500, Andover, MA). Participants were not required to fast, but they were asked not to take their morning medication on the day of evaluation. Every participant had the examination performed at the same time of day at baseline and at 4 months. Smoking was prohibited for at least 8 hours before the test and all vasoactive drugs, such as nitrates, were withheld for a period of 48 hours prior to the test. For the FMD assessment, the participant was placed in the supine position and a standard sphygmomanometer cuff was placed on the right arm. The right brachial artery was then imaged in the antecubital fossa and its diameter measured at end-diastole using electrocardiographic (EKG) gating. This was followed by brachial artery occlusion with a sphygmomanometer cuff inflation to at least 50 mm Hg above systolic BP for 5 minutes. The cuff was then deflated to allow rapid inflow of blood to the forearm. The brachial artery diameter was measured again at 1 minute after pressure cuff deflation. The brachial artery measurements were performed based on the recorded videos using a computer workstation (Echopac version 6.2, GE Wingmed Ultrasound, Horten, Norway). FMD was calculated for each subject as percentage increase in the diameter of the brachial artery

from baseline during the condition of increased flow. A lower FMD value indicated impaired endothelial function.

PWV Assessment

PWV is defined as the velocity at which the pressure waves, generated by the systolic contraction of the heart, propagate along the arterial tree. The evaluation of PWV provides complementary information about the elastic properties of the arterial system. The higher PWV corresponds to lower vessel distensibility and compliance and, therefore, to higher arterial stiffness. The PWV assessment was performed using a noninvasive device (SphygmoCor, AtCor Medical, Sydney, Australia) which has been validated with a high degree of reproducibility for this purpose.[9,10] The device is based on an oscillometric method and records arterial pressure waveforms noninvasively. EKG-gated pressure waveforms are recorded simultaneously from both arms and both ankles. From these pressure waveforms, in-built software automatically calculates PWV for different vascular segments. After 10 minutes of rest in the supine position, 4 BP cuffs, which were connected to the SphygmoCor device, were placed around both arms and both ankles. These BP cuffs carried oscillometric sensors to record pressure waveforms from the underlying arteries. EKG electrodes were applied on wrists and ankles to allow for EKG gating. The machine then automatically inflated and deflated all the cuffs simultaneously, while recording pressure waveforms from all 4 sites. From these pressure waveforms, carotid-femoral PWV and right and left brachial-ankle PWV were calculated and used for analysis. A higher value of PWV indicated greater arterial stiffness in the corresponding vascular segment.

Carotid IMT Measurement

Carotid IMT analysis was performed using carotid ultrasonography,[12] as the resulting measurement represents systemic atherosclerosis and risk for future cardiovascular events. The IMT measurements were obtained with the patient in the supine position with the head rotated to face the opposite side of examination. Common carotid artery images were obtained to measure IMT by using 3 different angle views for each vessel. At least 3 IMT points were measured in the near and far walls in the most thickened area of each vessel. IMT was measured by manual technique using electronic calipers, and the maximum IMT value was selected for each angle. An IMT greater than 0.9 mm was considered an abnormal finding due to atherosclerosis. All personnel who performed the non-invasive evaluations including carotid IMT, PWV, and FMD assessments were blinded to the patient's group assignment.

Statistical Analysis

Continuous data are expressed as mean values ± SD. Comparisons between the groups were carried out using Student's unpaired *t* test or c2 test, as appropriate. The within-group changes between baseline and the 4-month visits were assessed using Student's paired *t* test. Two-way analysis of variance (ANOVA) was used for determining the effect of dietary intervention on the study parameters. A *P*-value < 0.05 was considered statistically significant. All statistical analyses were done using SPSS for Windows (SPSS Inc, Chicago, IL).

Results

Sixty-six participants were randomized following an assessment of 157 patients. The overall mean age of these 66 patients was 62.5 ± 12.4 years (range 56 to 75 years). Thirty-three patients were randomized to the PBD group, and 33 patients were randomized to the control group (Figure 1).

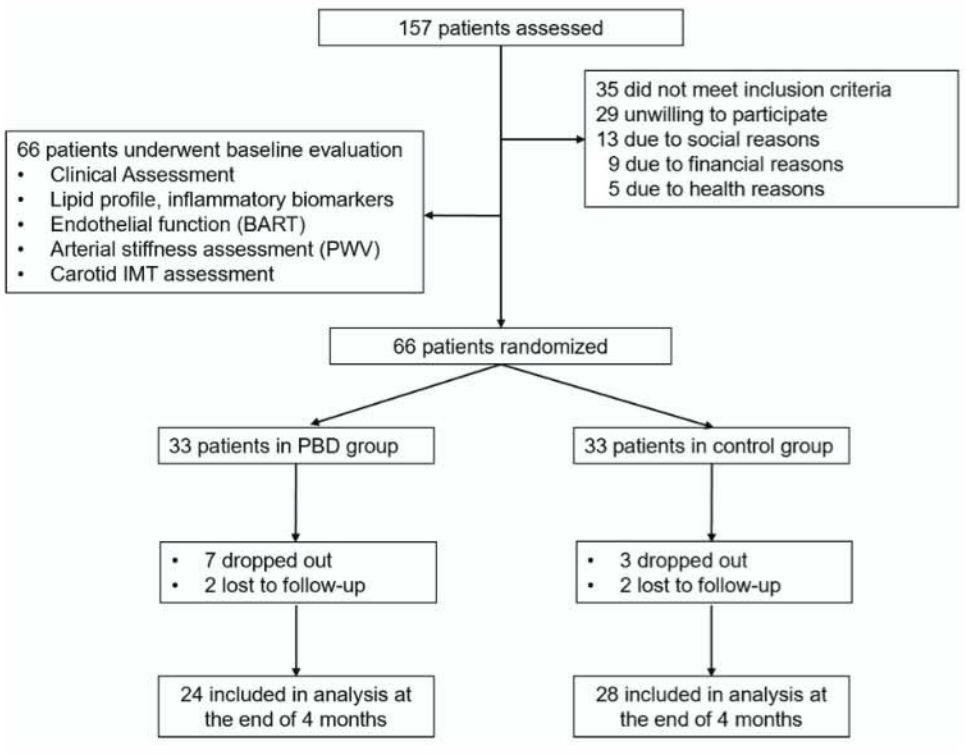

Figure 1. Study design

Nine subjects in the PBD group and 5 subjects in the control group were unable to complete the study. As a result, 24 patients in the PBD group and 28 patients in the control group who completed the study formed the basis of analysis. The baseline clinical and biochemical characteristics were similar between the 2 groups (Table 2).

Table 2. Baseline clinical and laboratory values in the PBD and control groups

	PBD group ($n=33$)	Control group ($n=33$)	*P* value
Age (year)	63.4 ± 11.8	61.6 ± 13.3	0.24
Male, *n* (%)	25 (74%)	23 (72%)	0.16
Weight (pounds)	184 ± 16	178 ± 22	0.43
Body mass index	27.6 ± 4.6	27.9 ± 5.2	0.25
Waist circumference (in)	37.5 ± 3.4	35.6 ± 2.6	0.32
Hypertension, *n* (%)	19 (56%)	15 (45%)	0.57
Diabetes mellitus, *n* (%)	16 (47%)	14 (44%)	0.62
ABI	0.86 ± 0.08	0.88 ± 0.07	0.78
Blood pressure			
Systolic BP (mm Hg)	135 ± 14	138 ± 16	0.45
Diastolic BP (mm Hg)	79 ± 11	75 ± 10	0.38
Cytokine biomarkers			
hs-CRP (mg/L)	2.9 ± 2.4	2.3 ± 2.1	0.32
IL-6 (pg/mL)	2.2 ± 1.9	2.0 ± 1.9	0.45
IL-7 (pg/mL)	2.6 ± 1.7	2.5 ± 1.9	0.65
IL-18 (pg/mL)	168± 45	172 ± 48	0.37
Nitric oxide (umol/L)	6.87 ± 4.6	6.73 ± 5.1	0.45
Superoxide dismutase (SAU/mg)	2.45 ± 0.2	2.35 ± 0.3	0.53

Table 2. (continued)

	PBD group ($n=33$)	Control group ($n=33$)	*P* value
Lipid profile			
Total cholesterol (mg/dL)	234.6 ± 35.4	227.3 ± 31.5	0.19
Triglycerides (mg/dL)	176.6 ± 54.7	164.3 ± 65.8	0.21
HDL cholesterol (mg/dL)	47.3 ± 12.3	42.5 ± 14.7	0.27
LDL cholesterol (mg/dL)	146.8 ± 24.6	138.6 ± 27.4	0.38
Apo-A1 (mg/dL)	127.5 ± 23.5	121.4 ± 26.6	0.26
Apo-B (mg/dL)	98.4 ± 24.1	96.3 ± 27.3	0.57
Total/HDL cholesterol ratio	5.4 ± 1.3	5.6 ± 1.7	0.36
Apo-A1/Apo-B ratio	0.87 ± 0.21	0.83 ± 0.14	0.47

Notes: ABI, ankle-brachial index; Apo, apolipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; IL, interleukin; LDL, low-density lipoprotein; PAD, peripheral arterial disease; PBD, plant-based diet; SAU, specific activity unit.

No difference in epidemiological, biochemical, or lipid profile was detected between the PBD and control group. Vascular endothelial function, based on brachial artery FMD, and arterial stiffness parameter, based on PWV assessment, did not differ between the 2 groups at baseline. (Table 3).

Table 3. Baseline carotid IMT, endothelial function, and arterial stiffness parameters in the PBD and control groups

	PBD group ($n=33$)	Control group ($n=33$)	*P* value
Brachial artery diameter (mm)	4.23 ± 0.43	4.19 ± 0.38	0.84
Brachial artery FMD (%)	6.36 ± 4.31	7.54 ± 4.63	0.24
Carotid-femoral PWV (cm/sec)	1025.8 ± 185.5	958.5 ± 167.3	0.65
Brachial-ankle PWV right (cm/sec)	1254.2 ± 186.3	1231.2 ± 186.4	0.73
Brachial-ankle PWV left (cm/sec)	1284.5 ± 143.6	1268.4 ± 158.3	0.64
Brachial-ankle PWV mean (cm/sec)	1276.4 ± 175.5	1247.5 ± 182.4	0.72
Carotid IMT (mm)	0.77 ± 0.18	0.73 ± 0.15	0.68

Notes: FMD, flow-mediated dilation; IMT, intima-media thickness; PBD, plant-based diet; PWV, pulse wave velocity.

At 4 months, participants in the PBD group had a significant decrease in total cholesterol (235.7 ± 21.6 mg/dL vs. 176.3 ± 17.4 mg/dL, $p=0.03$), triglycerides (186.7 ± 45.3 mg/dL vs. 148.7 ± 21.6 mg/dL, $p=0.02$), LDL cholesterol (152.3 ± 27.4 mg/dL vs. 124.6 ± 23.5 mg/dL, $p=0.03$), and total/HDL ratio (5.3 ± 1.6 vs. 3.5 ± 1.7 $P=0.01$) compared to baseline (Table 4). There was an increase of Apo-A1 in the PBD group (128.3 ± 23.6 mg/dL vs. 135.2 ± 24.5 mg/dL; $p=0.04$). Significant reduction in inflammatory biomarkers including hs-CRP (2.9 ± 1.4 mg/L vs. 1.3 ± 0.3 mg/L, $p=0.02$) and IL-6 (2.6 ± 1.4 mg/L vs. 1.4 ± 0.5 mg/L, $p=0.03$) with increased NO production (6.86 ± 4.5 umol/L vs. 15.32 ± 6.42 umol/L, p = 0.01) was observed in the PBD group at 4 months. In contrast, no difference in biochemical parameters or inflammatory biomarkers between baseline and 4 months was noted in the control group (Table 4). Two-way ANOVA revealed significant treatment effects of PBD dietary intervention on total cholesterol ($p=0.04$), triglycerides ($p=0.03$), total/HDL-C ratio ($p=0.03$), hs-CRP ($p=0.03$), IL-6 ($p=0.04$), and NO ($p=0.02$) compared to the control group (Table 4).

Table 4. Comparison of clinical and laboratory values in the PBD and control groups before and after intervention

	PBD group ($n=24$)			Control group ($n=28$)			*P* value for between-group comparison at 4 months
	Baseline	4 months	*P* value	Baseline	4 months	*P* value	
Weight (pounds)	182±14	178±17	0.46	179±25	176±28	0.78	0.45
Body mass index	27.3±4.2	26.3±3.7	0.24	28.5±6.3	27.3±5.1	0.56	0.54
Waist circumference (in)	37.1±3.2	36.2±4.8	0.37	35.8±2.7	36.3±2.8	0.64	0.24
Hypertension, *n* (%)	14 (58%)	9 (38%)	0.04	14 (50%)	14 (50%)	1.0	0.07
Hypercholesterolemia, *n* (%)	15 (63%)	10 (42%)	0.04	17 (61%)	18 (64%)	1.0	0.06
Diabetes mellitus, *n* (%)	13 (54%)	11 (46%)	0.21	12 (43%)	13 (46%)	0.78	0.34
ABI	0.85±0.08	0.91±0.06	0.05	0.86±0.07	0.88±0.06	0.23	0.88
Blood pressure							
Systolic BP (mm Hg)	138±13	125±11	0.07	137.2±15	135.3±17	0.54	0.46
Diastolic BP (mm Hg)	85±13	72±9	0.04	74±12	76±14	0.74	0.06
Cytokine biomarkers							
hs-CRP (mg/L)	2.9±1.4	1.3±0.3	0.02	2.4±2.3	2.3±2.1	0.75	0.03
IL-6 (pg/mL)	2.6±1.4	1.4±0.5	0.03	2.1±1.7	2.2±1.8	0.57	0.04
IL-7 (pg/mL)	2.67±1.8	2.31±1.5	0.54	2.4±1.7	2.6±1.9	0.48	0.45
IL-18 (pg/mL)	169±49	142±37	0.43	174±47	178±53	0.52	0.43
Nitric oxide (umol/L)	6.86±4.5	15.32±6.42	0.01	6.32±3.7	6.45±5.1	0.48	0.02
Superoxide dismutase	2.46±0.2	2.37±0.3	0.65	2.42±0.3	2.39±0.4	0.63	0.52
Lipid profile							
Total cholesterol (mg/dL)	235.7±21.6	176.3.±17.4	0.03	227.1±32.5	231.3±35.3	0.32	0.04
Triglycerides (mg/dL)	186.7±45.3	148.7±21.6	0.02	181.7±48.5	178.6±52.4	0.25	0.03
HDL cholesterol (mg/dL)	48.3±13.7	53.4±17.4	0.18	45.8±14.6	46.3±15.2	0.46	0.29
LDL cholesterol (mg/dL)	152.3±27.4	124.6±23.5	0.03	138.2±27.6	143.±31.6	0.38	0.22
Apo-A1 (mg/dL)	128.3±23.6	135.2±24.5	0.04	123.5±22.4	125.6±24.9	0.68	0.43
Apo-B (mg/dL)	98.6±25.1	84.5±14.3	0.27	96.3±27.7	97.4±32.5	0.73	0.46
Total/HDL cholesterol ratio	5.3±1.6	3.5±1.7	0.01	5.8±1.9	5.6±1.5	0.63	0.03
Apo-A1/Apo-B ratio	0.88±0.23	0.92±0.31	0.47	0.85±0.16	0.84±0.14	0.82	0.43

Notes: ABI, ankle-brachial index; Apo, apolipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; IL, interleukin; LDL, low-density lipoprotein; PBD, plant-based diet.

Comparison of vascular endothelial function based on brachial artery FMD and arterial stiffness parameter based on PWV assessment following dietary intervention is displayed in Table 5.

Table 5. Comparison of carotid IMT, endothelial function, and arterial stiffness parameters in the PBD and control groups before and after intervention

	PBD group ($n=24$)			Control group ($n=28$)			*P* value for between-group comparison at 4 months
	Baseline	4 months	*P* value	Baseline	4 months	*P* value	
Brachial artery diameter (mm)	4.21 ±0.45	4.23 ±0.47	0.84	4.24 ±0.38	4.19 ±0.53	0.65	0.45
Brachial artery FMD (%)	6.33 ±4.21	8.54 ±4.15	0.02	7.56 ±4.61	7.31 ±4.73	0.43	0.04
Carotid-femoral PWV (cm/sec)	1067.4 ±187.6	986.4 ±145.2	0.03	954.2 ±182.3	976.4 ±163.4	0.64	0.03
Brachial-ankle PWV right (cm/sec)	1289.3 ±194.6	1273.4 ±179.5	0.07	1246.7 ±187.2	1235.6 ±184.2	0.21	0.22
Brachial-ankle PWV left (cm/sec)	1287.3 ±152.6	1205.4 ±147.8	0.03	1268.6 ±159.4	1275.4 ±164.7	0.47	0.04
Brachial-ankle PWV mean (cm/sec)	1273.9 ±176.8	1232.5 ±165.4	0.04	1248.2 ±186.3	1256.8 ±184.2	0.37	0.04
Carotid IMT (mm)	0.78 ±0.14	0.75 ±0.18	0.07	0.77 ±0.16	0.79 ±0.21	0.24	0.34

Notes: FMD, flow-mediated dilation; IMT, intima-media thickness; PBD, plant-based diet; PWV, pulse wave velocity.

Participants in the PBD group had significant improvement of brachial artery FMD (6.33 ± 4.21% increase vs. 8.54 ± 4.15 % increase, $p = 0.02$), carotid-femoral PWV (1067.4 ± 187.6 cm/sec vs. 986.4 ± 145.2 cm/sec, $p = 0.03$), left brachial-ankle PWV (1287.3 ± 152.6 cm/sec vs. 1205.4 ± 147.8 cm/sec, $p = 0.03$), and mean brachial-ankle PWV (1273.9 ± 176.8 cm/sec vs. 1232.5 ± 165.4 cm/sec, $p = 0.04$) compared to baseline at 4 months. The control group showed no difference in brachial artery FMD or PWV parameters at 4 months. As a result, there was a significant treatment effect of PBD dietary intervention on brachial artery FMD, carotid-femoral PWV, and brachial-ankle PWV ($p = 0.04$, 0.03, and 0.04, respectively) compared to control based on two-way ANOVA. No difference in carotid IMT was noted in either group after 4 months.

Discussion

Despite significant advances in recent decades in pharmacological, endovascular, and surgical therapies to reduce CVD-related morbidity and mortality, dietary intervention has only recently received a emphasis as the first-line therapeutic modality in the management of CVD. Healthcare providers typically are more likely to recommend pharmacological or procedural-based treatment rather than dietary intervention, as the latter treatment is often regarded as more difficult to implement because it may be an integral part of individual, family, and cultural identity. Lifestyle modification with dietary intervention can address the root cause of CVD by modulating risk factors contributing to it. In this study, we found that plant-based dietary intervention can improve vascular endothelial dysfunction as assessed by FMD and PWV in patients with PAD.

Plant-based foods are generally rich in fiber and phytonutrients, two critical food components in human health. Plant-based fiber, found in nearly all fruits and vegetables, provides important cellular nutrient for the gastrointestinal, cardiovascular, and immune systems.[13,14] Phytonutrients represents a category of nutrients including glucosinolates, carotenoids, and flavonoids, which work synergistically to reduce inflammation and oxidation, thereby providing protection from disease initiation and progression.[14,15,16] The health benefits of PBD in reversing endothelial dysfunction in

our study are likely the consequence of regular consumption of plant-based fibers and phytonutrients as well as the avoidance of harmful substances found in highly processed foods and animal products. Clinical evidence shows that animal products contain health-damaging saturated fats, heme iron, N-glycolylneuraminic acid, carnitine, and chemical contaminants that form when flesh is cooked, such as polycyclic aromatic hydrocarbons, heterocyclic amines, and advanced glycation end products. These substances can trigger a wide array of enzymatic cascades leading to inflammation in the digestive and cardiovascular systems.[17,18] Similarly, nutritional studies have shown highly processed foods encompass a class of commercially produced items made with refined ingredients including oils, salts, sugars, and other food additives that can trigger cellular inflammatory and carcinogenic responses.[13,19,20,21] Our study, consistent with other reports of plant-based dietary interventions to modulate CVD outcomes,[22,23,24] adds to the current body of literature regarding the health benefit of a dietary strategy rich in plant-based nutrients and devoid of proinflammatory ingredients derived from animal products and processed foods.

Our study showed impaired vascular endothelial function is common in patients with PAD, an observation well described in other reports in patients with underlying cardiovascular morbidities.[10,25] The endothelium represents the largest endocrine organ in the human body, as it maintains vascular homeostasis via anti-inflammatory, antithrombotic, and vasodilatory functions. Healthy endothelium is influenced by laminar shear stress, which enhances the expression and activity of endothelial NO synthase resulting in NO production. Nitric oxide inhibits smooth muscle cell proliferation and stimulates cellular relaxation by promoting cyclic guanosine monophosphate production, thus resulting in vasodilatation. It also exerts anti-inflammatory effects that are capable of inactivating reactive oxygen species such as hydrogen peroxide, a molecule with well recognized proinflammatory effects.[26] In patients with PAD, atherosclerosis can lead to arterial luminal obstruction resulting in the reduction of shear stress, a phenomenon that can trigger the activation of leukocytes and enhances the expression of adhesion molecules. These reactions can lead to the infiltration of inflammatory cells into the vessel wall resulting in the production of inflammatory mediators such as chemokines, cytokines, and proteases to further establish an environment that promotes cellular inflammation.[26,27,28] Previous studies have shown upregulated inflammatory cytokine levels in patients with PAD with arterial thrombosis, underscoring a pathogenic relationship between acute inflammation, endothelial dysfunction, and thrombus formation.[25,29,30] Endothelial dysfunction has also been demonstrated in patients with CVD and associated co-morbidities.[10,25] The results of our study are consistent with these observations, as patients with PAD showed endothelial dysfunction with enhanced inflammatory response due in part to the suppressed FMD and elevated cytokine biomarkers. Importantly, our report represents the first study to demonstrate that dietary intervention can reverse endothelial dysfunction and ameliorate arterial stiffness in patients with PAD. While the precise mechanism responsible for the reversal of vascular endothelial dysfunction remains elusive in our dietary cohorts, we postulate a plant-based nutrition with strict avoidance of processed foods and animal products can reduce cellular inflammation, which results in improvement of vascular function.

Our results showed a reversal of vascular endothelial dysfunction following a 4-month course of plant-based diet in patients with PAD utilizing FMD and PWV assessment, which are well-described and widely used techniques for analyzing vascular function. Researchers have previously examined the effect of various dietary strategies on endothelial function and inflammatory response in patients with CVD.[22,24,31] The

dietary intervention that has received the most research focus is the Mediterranean diet, which consists primarily of plant-based foods with occasional meat consumption. Several studies have reported improvement of endothelial function in patients with CVD risk factors, including metabolic syndrome and diabetes, following this dietary strategy.[16,32,33] Vogel et al. analyzed the effect of the Mediterranean diet on postprandial FMD and noted the benefit in brachial vasoreactivity appears to be related to the antioxidant-rich plant-based foods, including fruits and vegetables and their derivatives such as vinegar, and omega-3-rich fish and canola oils.[34] Sondergaard et al. analyzed brachial artery vasoreactivity by randomizing 131 patients with ischemic heart disease to either Mediterranean diet intervention or no dietary advice. After 12 months of dietary intervention, the diet group showed a significantly improved FMD of 8.62% compared to 5.7% in the control group ($P < 0.01$). Those treated with dietary intervention similarly experienced improvement of their serum cholesterol parameters.[24] A single-blinded trial that randomized 180 patients with metabolic syndrome to a Mediterranean-style diet versus no specific diet advice showed significantly improved brachial artery FMD, reduced inflammatory biomarkers, and reversal of insulin resistance following 2 years of dietary intervention.[16] Singh et al. analyzed an Indo-Mediterranean diet in 1000 patients with existing coronary disease or at high risk for coronary disease. When compared with the control diet, the intervention diet group showed significantly reduced sudden death from cardiac causes (6% vs 16%, $p = 0.015$) as well as non-fatal myocardial infarction (21% vs. 43%, $p < 0.001$).[31] Taken altogether, these reports uniformly attributed the plant-based foods with high anti-oxidant phytonutrients as the predominant factor of the cardiovascular health improvement in these patients.

In our study participants, the baseline incidence of hypertension and hypercholesterolemia was 58% and 63%, respectively. Plant-based dietary intervention improved these 2 conditions in 4 months with a resultant incidence of 38% and 42%, respectively. The benefit of dietary intervention in reversing CVD-related risk factors such as diabetes, hypertension, and hyperlipidemia has been previously supported in multiple studies.[35-39] The improvement of vascular endothelial dysfunction in our PAD patients treated with dietary intervention similarly underscores the therapeutic principle that plant-based nutrition can reverse atherosclerotic disease progression.[40,41,42,43] We were unable to analyze the use of concomitant medication, such as hypertensive or diabetic drugs, due to the significant heterogeneity of our patients who use concomitant medications. Given the relatively small sample size in both study groups, it is beyond the scope of our study to analyze the effect of concomitant medication usage. Dr. Dean Ornish and colleagues conducted a trial in 48 patients with CVD randomized to usual care versus lifestyle modification that included a low-fat vegetarian diet and stress management.[44] After 5 years of follow-up, patients in the lifestyle group showed a significant reduction of coronary artery stenosis of 7.9% compared to a 27.7% worsening in the control group based on angiographic assessment. Those treated in the lifestyle group experienced a 60% reduction in cardiac events compared to the control group. The benefit of plant-based lifestyle modification in reversing coronary atherosclerosis has similarly been validated in several studies.[43,45] As a result of these scientific studies, the Centers for Medicare & Medicaid Services (CMS) approved reimbursement for the Ornish lifestyle intervention as part of an intensive cardiac rehabilitation program.[46,47]

One observation based on our practice relates to the challenge of patient adherence to PBD intervention. While we routinely recommend lifestyle modification with dietary advice to PAD patients in our clinical practice, their verbal agreement often

leads to low diet acceptability and lifestyle adherence. In fact, 2 meta-analyses of clinical trials of PBD encompassing 20 studies showed dietary compliance ranged between 55% and 64%,[48,49] as many of these studies relied on a notebook-based food journal. Consequently, we placed a particular emphasis in our study design in an effort to enhance dietary adherence by implementing an online food journal using smartphone-based apps. We believe this empowers patients with a greater sense of behavior accountability, as the ubiquitous smartphone camera allows photographic recording of their meals with ease in contrast to a notebook-based food journal. While there are many smartphone-based dietary apps available for food journaling, we chose specific apps including MyFitnessPal, FatSecret, Yazio, and LoseIt, because they are simple to use and free of charge. This journaling strategy also facilitates patients' efforts to keep track of their own dietary progress as well as a closer online monitoring by the study coordinators. The use of a smartphone-based food journal to document caloric and nutrient intake has been similarly praised by other researchers as a useful strategy to enhance diet adherence and compliance.[50,51,52]

There are several limitations to our study. First, this is an open-label study design with unblinded randomization. All participants and researchers were aware of the treatment strategy, which may have resulted in study bias affecting the treatment outcome. Second, there was a high number of patient dropouts, which affected the sample size and therefore may have influenced the power of the study to detect certain measured parameters. Third, because only 52 out of 157 subjects who were initially screened completed the study, the generalizability of our results is limited. The high dropout rate underscores the challenges patients may encounter with strict dietary compliance. Because significant efforts were made in the study design to ensure patient compliance with the electronic diet journal and frequent counseling by the study coordinator, our results may apply only to individuals capable of adhering to the intervention. Fourth, this is not an intention-to-treat study as our analysis only focused on those who completed the study evaluation. Lastly, although our study showed improved vascular function based on FMD following dietary intervention with PBD, endothelium-independent vasodilation was not investigated in our study. Notwithstanding these limitations, the present findings support the beneficial effect of PBD in reversing endothelial dysfunction and improving many metabolic parameters in patients with PAD.

In conclusion, our study showed that regular consumption of a plant-based diet along with avoidance of processed foods can reverse vascular endothelial dysfunction in patients with peripheral arterial disease. Further studies are needed to determine the mechanistic role of plant-based dietary strategies in reversing vascular endothelial dysfunction in patients with CVD.

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References

1. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014;129:S76–S99.

2. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: executive summary: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2019;74(10):1376–1414.
3. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults. Executive summary: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 2018;71: 1269–1324.
4. Ford ES, Ajani UA, Croft JB, Critchley JA, Labarthe DR, Kottke TE, et al. Explaining the decrease in U.S. deaths from coronary disease, 1980–2000. *N Engl J Med* 2007;356:2388–2398.
5. Muntner P, Carey RM, Gidding S, Jones DW, Taler SJ, Wright JT Jr, et al. Potential U.S. population impact of the 2017 ACC/AHA High Blood Pressure Guideline. *J Am Coll Cardiol* 2018;71:109–118.
6. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* 2003;41: 1769–1775.
7. Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner K, Maurer G, et al. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *Am J Cardiol* 2000;86:207–210.
8. Ghiadoni L, Versari D, Giannarelli C, Faita F, Taddei S. Non-invasive diagnostic tools for investigating endothelial dysfunction. *Curr Pharm Des* 2008;14:3715–3722.
9. Huck CJ, Bronas UG, Williamson EB, Draheim CC, Duprez DA, Dengel DR. Noninvasive measurements of arterial stiffness: repeatability and interrelationships with endothelial function and arterial morphology measures. *Vasc Health Risk Manag* 2007;3:343–349.
10. Tuttolomondo A, Casuccio A, Guercio G, Maida C, Del Cuore A, Di Raimondo D, et al. Arterial stiffness, endothelial and cognitive function in subjects with type 2 diabetes in accordance with absence or presence of diabetic foot syndrome. *Cardiovasc Diabetol* 2017;16:2.
11. Aboyans V, Criqui MH, Abraham P, Allison MA, Creager MA, Diehm C, et al. Measurement and interpretation of the ankle-brachial index: A scientific statement from the American Heart Association. *Circulation* 2012;126:2890–2909.
12. Sidhu PS, Desai SR. A simple and reproducible method for assessing intimal-medial thickness of the common carotid artery. *Br J Radiol* 1997;70:85–89.
13. Franco-de-Moraes AC, de Almeida-Pititto B, da Rocha Fernandes G, Gomes EP, da Costa Pereira A, Ferreira SRG. Worse inflammatory profile in omnivores than in vegetarians associates with the gut microbiota composition. *Diabetol Metab Syndr* 2017;9:62.
14. Kim MS, Hwang SS, Park EJ, Bae JW. Strict vegetarian diet improves the risk factors associated with metabolic diseases by modulating gut microbiota and reducing intestinal inflammation. *Environ Microbiol Rep* 2013;5:765–775.
15. Lithell H, Bruce A, Gustafsson IB, Hoglund NJ, Karlstrom B, Ljunghall K, et al. A fasting and vegetarian diet treatment trial on chronic inflammatory disorders. *Acta Derm Venereol* 1983;63:397–403.
16. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004;292:1440–1446.

17. Alisson-Silva F, Kawanishi K, Varki A. Human risk of diseases associated with red meat intake: analysis of current theories and proposed role for metabolic incorporation of a non-human sialic acid. *Mol Aspects Med* 2016;51:16–30.
18. Cam A, de Mejia EG. Role of dietary proteins and peptides in cardiovascular disease. *Mol Nutr Food Res* 2012;56:53–66.
19. Gomez-Smith M, Karthikeyan S, Jeffers MS, Janik R, Thomason LA, Stefanovic B, et al. A physiological characterization of the Cafeteria diet model of metabolic syndrome in the rat. *Physiol Behav* 2016;167:382–391.
20. Okreglicka K. Health effects of changes in the structure of dietary macronutrients intake in western societies. *Rocz Panstw Zakl Hig* 2015;66:97–105.
21. Manzel A, Muller DN, Hafler DA, Erdman SE, Linker RA, Kleinewietfeld M. Role of "Western diet" in inflammatory autoimmune diseases. *Curr Allergy Asthma Rep* 2014;14:404.
22. Esposito K, Giugliano D. Mediterranean diet and prevention of coronary heart disease. *J Endocrinol Invest* 2002;25:296–299.
23. Esposito K, Maiorino MI, Bellastella G, Panagiotakos DB, Giugliano D. Mediterranean diet for type 2 diabetes: Cardiometabolic benefits. *Endocrine* 2017;56:27–32.
24. Sondergaard E, Moller JE, Egstrup K. Effect of dietary intervention and lipid-lowering treatment on brachial vasoreactivity in patients with ischemic heart disease and hypercholesterolemia. *Am Heart J* 2003;145:E19.
25. Silvestro A, Scopacasa F, Ruocco A, Oliva G, Schiano V, Zincarelli C, et al. Inflammatory status and endothelial function in asymptomatic and symptomatic peripheral arterial disease. *Vasc Med* 2003;8:225–232.
26. Fernandes T, Gomes-Gatto CV, Pereira NP, Alayafi YR, das Neves VJ, Oliveira EM. NO Signaling in the Cardiovascular System and Exercise. *Adv Exp Med Biol* 2017;1000:211–245.
27. Schwartz BG, Economides C, Mayeda GS, Burstein S, Kloner RA. The endothelial cell in health and disease: Its function, dysfunction, measurement and therapy. *Int J Impot Res* 2010;22:77–90.
28. Jamwal S, Sharma S. Vascular endothelium dysfunction: a conservative target in metabolic disorders. *Inflamm Res* 2018;67:391–405.
29. Brevetti G, Silvestro A, Di Giacomo S, Bucur R, Di Donato A, Schiano V, et al. Endothelial dysfunction in peripheral arterial disease is related to increase in plasma markers of inflammation and severity of peripheral circulatory impairment but not to classic risk factors and atherosclerotic burden. *J Vasc Surg* 2003;38:374–379.
30. Tousoulis D, Kampoli AM, Papageorgiou N, Androulakis E, Antoniades C, Toutouzas K, et al. Pathophysiology of atherosclerosis: The role of inflammation. *Curr Pharm Des* 2011;17:4089–4110.
31. Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, et al. Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): A randomised single-blind trial. *Lancet* 2002;360: 1455–1461.
32. Aizawa K, Shoemaker JK, Overend TJ, Petrella RJ. Metabolic syndrome, endothelial function and lifestyle modification. *Diab Vasc Dis Res* 2009;6:181–189.
33. Esposito K, Ciotola M, Giugliano D. Mediterranean diet, endothelial function and vascular inflammatory markers. *Public Health Nutr* 2006;9:1073–1076.
34. Vogel RA, Corretti MC, Plotnick GD. The postprandial effect of components of the Mediterranean diet on endothelial function. *J Am Coll Cardiol* 2000;36:1455–1460.
35. Chiu THT, Pan WH, Lin MN, Lin CL. Vegetarian diet, change in dietary patterns, and diabetes risk: A prospective study. *Nutr Diabetes* 2018;8:12.

36. Mihrshahi S, Ding D, Gale J, Allman-Farinelli M, Banks E, Bauman AE. Vegetarian diet and all-cause mortality: Evidence from a large population-based Australian cohort—the 45 and Up Study. *Prev Med* 2017;97:1–7.
37. Ornish D, Denke M. Dietary treatment of hyperlipidemia. *J Cardiovasc Risk* 1994;1:283–286.
38. Praharaj AB, Goenka RK, Dixit S, Gupta MK, Kar SK, Negi S. Lacto-vegetarian diet and correlation of fasting blood sugar with lipids in population practicing sedentary lifestyle. *Ecol Food Nutr* 2017;56:351–363.
39. Viguiliouk E, Kendall CW, Kahleova H, Rahelic D, Salas-Salvado J, Choo VL, et al. Effect of vegetarian dietary patterns on cardiometabolic risk factors in diabetes: A systematic review and meta-analysis of randomized controlled trials. *Clin Nutr* 2018 Jun;38(3):1133–1145.
40. Diehl HA. Coronary risk reduction through intensive community-based lifestyle intervention: The Coronary Health Improvement Project (CHIP) experience. *Am J Cardiol* 1998; 82:83T-7T.
41. Esselstyn CB Jr. Resolving the coronary artery disease epidemic through plant-based nutrition. *Prev Cardiol* 2001;4:171–177.
42. Ornish D, Brown SE, Scherwitz LW, Billings JH, Armstrong WT, Ports TA, et al. Lifestyle changes and heart disease. *Lancet* 1990;336:741–742.
43. Ornish D, Brown SE, Scherwitz LW, Billings JH, Armstrong WT, Ports TA, et al. Can lifestyle changes reverse coronary heart disease? The Lifestyle Heart Trial. *Lancet* 1990;336: 129–133.
44. Ornish D, Scherwitz LW, Billings JH, Brown SE, Gould KL, Merritt TA, et al. Intensive lifestyle changes for reversal of coronary heart disease. *JAMA* 1998;280:2001–2007.
45. Frattaroli J, Weidner G, Merritt-Worden TA, Frenda S, Ornish D. Angina pectoris and atherosclerotic risk factors in the multisite cardiac lifestyle intervention program. *Am J Cardiol* 2008;101:911–918.
46. Horrigan BJ. Ornish and Pritikin programs approved by CMS. *Explore* (NY) 2010;6:346–348.
47. Zeng W, Stason WB, Fournier S, Razavi M, Ritter G, Strickler GK, et al. Benefits and costs of intensive lifestyle modification programs for symptomatic coronary disease in Medicare beneficiaries. *Am Heart J* 2013;165:785–792.
48. Huang RY, Huang CC, Hu FB, Chavarro JE. Vegetarian diets and weight reduction: A meta-analysis of randomized controlled trials. *J Gen Intern Med* 2016;31:109–116.
49. Barnard ND, Levin SM, Yokoyama Y. A systematic review and meta-analysis of changes in body weight in clinical trials of vegetarian diets. *J Acad Nutr Diet* 2015;115:954–969.
50. Boushey CJ, Spoden M, Zhu FM, Delp EJ, Kerr DA. New mobile methods for dietary assessment: Review of image-assisted and image-based dietary assessment methods. *Proc Nutr Soc* 2017;76:283–294.
51. Bentley CL, Otesile O, Bacigalupo R, Elliott J, Noble H, Hawley MS, et al. Feasibility study of portable technology for weight loss and HbA1c control in type 2 diabetes. *BMC Med Inform Decis Mak* 2016;16:92.
52. Gemming L, Doherty A, Utter J, Shields E, Ni Mhurchu C. The use of a wearable camera to capture and categorise the environmental and social context of self-identified eating episodes. *Appetite* 2015;92:118–125.
53. Esposito K, Giugliano D. Mediterranean dietary patterns and chronic diseases. *Am J Clin Nutr* 2008;88:1179–1180; author reply 80–81.
54. Giugliano D, Esposito K. Mediterranean diet and cardiovascular health. *Ann N Y Acad Sci* 2005;1056:253–260.