

Body Composition, Nutrient Intake and *MTHFR* Genotype
in Patients with Peripheral Arterial Disease

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Dedication

This dissertation is dedicated to my family, for their tireless love and support.

And also to all the Whithams and Fraynes, and to Urvashi Mulasi,
for your loving encouragement and humor.

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Abstract of the Dissertation

Background: Peripheral artery disease (PAD) is a progressive disease characterized by its impact on physical mobility, and a high rate of vascular comorbidities and events. Despite its inclusion in the same grouping of conditions as cardiovascular (CVD) and cerebrovascular diseases, PAD garners much less attention, both in a clinical setting and in research. There is a significant body of research on the roles of nutrition and lifestyle in CVD and stroke etiology, yet the equivalent data for PAD is sparse. The impact of nutrition and related factors on PAD is poorly understood, and in need of expansive clarification.

Methods: We conducted a literature review of all available research on nutrition and body composition in PAD, and used our assessments to design a cross-sectional study of these variables in a sample of PAD patients. We created a conceptual model of how nutrition-related variables may be associated with various aspects of PAD severity, and examined these associations within recruited participants.

Results: Participants' diets were characterized by relatively high intakes of fat, sugar and sodium, as well as by low or inadequate intakes of crucial micronutrients. Several dietary factors were found to be significantly associated with more severe physical or psychosocial PAD symptoms. The majority of participants were also overweight or obese, and total body weight and abdominal obesity were associated with worse scores on tests of PAD severity. Additionally, we encountered several key obstacles to identifying and recruiting patients for this study.

Conclusions: Although our sample size was relatively small, there is evidence to suggest that there are nutrition-related factors that are associated with the severity of PAD patients' symptoms and overall quality of life. Recruitment methods are in need of revision to obtain larger, more statistically powered samples, and we have outlined potential approaches for doing so. Lastly, this study establishes a precedent for future studies to implement interventions in PAD patients using already-established vascular guidelines for nutrition.

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General Introduction

Definition of Peripheral Artery Disease

Peripheral Artery Disease (PAD), also known as Peripheral Vascular Disease or Peripheral Arterial Occlusive Disease, is not a single, narrowly delineated condition, but rather is a general term for a range of noncoronary arterial syndromes. Broadly, PAD can be used to describe a variety of arteriosclerotic processes that lead to: One, the deposition of fatty plaques on arterial walls that progress to atheroma formation and thrombosis or thrombotic embolism; or two, a degradation of the structural integrity of arterial walls that results in thinning or thickening of the wall (dilation or dysplasia) and aneurysm formation¹. These syndromes affect arteries in limbs and visceral organs, particularly the abdominal aorta, lower extremity arteries, renal arteries and mesenteric arteries. While other peripheral arterial beds may also be affected by similar syndromes (e.g. the arteries in the upper extremities or the vertebral column¹), there is little research in these areas, and clinical guidelines focus primarily on visceral organs and the lower limbs.

The arteriosclerosis that defines PAD can be viewed as a systemic process, one that can promote or aggravate arterial disease in the brain and heart. People with PAD often have a concurrent history of cardiovascular disease (CVD)^{2,3} and have a significantly increased risk of experiencing CVD events, including myocardial infarction, stroke and death. Depending on the location of a stenosis or aneurysm, other outcomes may include ischemia in the mesenteric artery bed, ischemic renal failure, rupture of an aneurysm or tissue necrosis and limb loss.

Risk Factors, Signs and Symptoms

Many risk factors for PAD are also risk factors for CVD, including, but not limited to dyslipidemia, obesity, diabetes mellitus, hypertension, a family history of CVD or PAD and smoking^{4,5}. Smoking is a particularly significant risk factor – the risk of developing

lower extremity PAD has a dose-dependent association with daily frequency and years of smoking⁶⁻⁹. Approximately 80% of PAD patients are current or former smokers^{10,11}, although this percentage may underestimate the total impact of cigarette use by not accounting for nonsmoking patients who cohabit with smoker spouses or relatives.

The location of an aneurysm or stenosis can in large part dictate patients' accompanying clinical signs and symptoms. For example, patients with acute ischemia in a mesenteric artery will likely report abdominal pain, while a patient with PAD in a renal artery (i.e. renal artery disease, or RAS), may be asymptomatic and be diagnosed only when a clinician measures the presence of refractory hypertension^{1,12}.

Nevertheless, PAD can be present in different locations within the body simultaneously. Lower extremity PAD is the more common form, and involves pain, cramping and discomfort in the legs, specifically calves, thighs, or buttocks. For many symptomatic PAD patients, their primary experience is of intermittent claudication (IC), in which physical exertion brings on pain or cramping, and is relieved by rest¹³. Another common, more severe set of symptoms is critical limb ischemia (CLI, also known as acute limb ischemia), in which a rapid-onset lack of blood flow in a leg causes acute pain, tingling or numbness, and is not alleviated by rest¹³.

Lastly, and perhaps more critically, a large proportion of people with PAD are asymptomatic prior to an ischemic event. Estimates vary, but at any given time up to half of people with PAD do not have symptoms¹⁴. Also, it is difficult to assess what proportion of the asymptomatic truly do not experience lower extremity pain or discomfort - PAD is a disease of aging like CVD¹⁵, and many people ascribe leg symptoms to old age or other coexistent conditions, thereby categorizing themselves as "asymptomatic"¹.

PAD Prevalence and Disease Burden

Given the difficulty of assessing PAD prevalence accurately, published estimates vary. Within the U.S., available data suggest that between 4 and 14% of people aged 50

to 80 years old have one or more PAD syndromes¹⁶. Additionally, approximately 70% of patients with lower extremity PAD and/or CVD concurrently suffer from renal artery stenosis (RAS)¹⁷, while up to 85% of patients with aneurysms in their lower extremities simultaneously have abdominal aneurysms^{18,19}. PAD in mesenteric arteries is less common (both acute and chronic intestinal ischemia are relatively rare^{1,12}), but without treatment is almost always fatal¹. PAD tends to affect men and women equally, though women are significantly more likely to develop intestinal ischemia¹. When stratified by race and ethnicity, Latinos have a higher prevalence of PAD than Caucasians, but have lower rates than African Americans, who have the highest PAD rates, along with a significantly greater risk of experiencing poor disease outcomes²⁰⁻²² (see Manuscript 1, “Ethnic Disparities” section). The high prevalence of PAD and the interrelated nature of its various syndromes and comorbidities contribute to a significant disease burden. As of 2008, annual health care costs for PAD exceeded 21 billion dollars, surpassing costs of CVD treatment by approximately 23%²³.

Current PAD Treatment Guidelines

In 2006, the Committee to Develop Guidelines for Peripheral Arterial Disease first published evidence-based clinical recommendations for PAD screening and treatment¹, with a more recent update in 2011¹². These recommendations comprise the first major published effort to assess comprehensively available evidence on PAD treatments and interventions. The guide emphasizes clinicians’ general consensus that PAD has historically received considerably less attention than CVD, and that the substantial health and cost burden merit concern^{4,24}.

Clinicians’ approaches to treating PAD are contingent on a variety of factors, including the severity of patients’ symptoms, the location and size of a stenosis or aneurysm, the relative health of the surrounding vascular anatomy, and an assessment of the likelihood that a given intervention will yield lasting results. Medications frequently

comprise the first line of intervention and are tailored to specific goals; for example, to decrease the risk of a CVD event, clinicians may prescribe singly or in combination lipid-lowering medications, anti-hypertensives, glucose control drugs for diabetics or anti-thrombotic drugs. For patients reporting IC or CLI, additional medications may also include analgesics, platelet inhibitors and drugs that inhibit vascular cell adhesion, while RAS treatment may further incorporate medications targeted towards inhibiting angiotensin receptors and renal calcium channels^{1,12}.

Revascularization is recommended for patients in various stages of disease, including when stenosis has completely occluded blood flow, when an aneurysm is at imminent risk of rupture (or has already ruptured), when tissue is in danger of necrotizing, or when a patient finds that discomfort and hampered physical mobility become intolerable. In these circumstances, guidelines again delineate evidence-based approaches to treatment. Procedures are generally categorized as endovascular or operative. Endovascular treatments may for instance be catheter-based thrombolysis of an occlusion or balloon angioplasty stent placement. More invasive procedures are used when endovascular approaches are not viable – a patient may have other serious concurrent health conditions that limit the effectiveness of percutaneous interventions or perhaps the patient's vasculature is not sufficiently stable or healthy to sustain such a procedure. In such circumstances, surgical interventions may include bypass grafting, resection of necrotic tissue, or limb amputation^{1,12}.

Regardless of treatment approach, a clinician's primary goals center on improving quality of life and preserving the viability of limbs and organs, while attempting to reduce the risk of ischemic events. Nevertheless, even as these approaches can be highly effective, there remains much that is unknown about non-pharmacological and non-operative ways of treating, or perhaps even preventing PAD. Such approaches may be comprised of physical activity interventions, nutrition programs, or other lifestyle-

based measures. There is a body of research on physical activity²⁵⁻²⁷ that has shown the potential for exercise programs to improve patients' physical function. While this area warrants further exploration, there remains a persistent gap in PAD research in regard to nutrition. Given the validated importance of nutrition in CVD treatment²⁸, it may be an important area of research that has the potential to influence clinical practice. This dissertation is an attempt to begin to bridge this knowledge gap, and in doing so, expand the body of research on the role nutrition may play in clinical PAD care.

Manuscript 1:

The role of nutrition and body composition in peripheral arterial disease.

Note: This manuscript first appeared in *Nature Reviews Cardiology*, 2012 November; 9(11):634-43, PMID: 22922595, doi: 10.1038/nrcardio.2012.117.

Abstract

Objectives. To provide an evidence-based review regarding the influences of nutrition and body composition on atherosclerotic lower extremity peripheral arterial disease (PAD).

Design. Critical review.

Methods. Review of the published literature (1990 to present via PubMed) examining associations between nutrition, body composition and PAD. Seventy studies were selected, and methodologic and data quality were critically assessed.

Results. There is strong evidence that decreased intake of folate and vitamin D is associated with greater risk of PAD and worsened walking impairment and that abdominal obesity is associated with greater risk of PAD and greater concentrations of pro-inflammatory biomarkers. There is moderate evidence that increased intakes of niacin and insoluble fiber are associated with improved serum lipids and decreased thrombotic biomarkers and that homozygosity for the *MTHFR* T-allele negatively impacts homocysteine metabolism and thus promotes the progression of PAD severity. There is no evidence that vitamins A, C, E, B₆ or B₁₂ play a significant role in PAD, or that essential fatty acid supplementation improves clinical outcomes.

Conclusions. There is a relative paucity of research on nutrition and body composition in relation to PAD, as compared to other cardiovascular diseases, suggesting that further research is essential to elucidate this relationship.

Introduction

Atherosclerosis remains the number one cause of mortality in the United States¹⁴, and is recognized as a set of distinct clinical syndromes (coronary heart disease, ischemic stroke, and PAD) that affects both individuals and communities. While the coronary and stroke syndromes are better known as traditional components of the cardiovascular disease (CVD) burden, peripheral artery disease (PAD) is equally common and is associated with an equal or higher risk of morbid and mortal events and a higher health economic cost^{23,29}. PAD is defined as atherosclerosis of the abdominal aorta and arteries outside the heart, and the term is most commonly used to describe arterial disease of the infrarenal aorta and the lower extremities³⁰. It is estimated that PAD affects 4 to 14% of the U.S. population from ages 50 to 80 years¹⁶. Cardiovascular ischemic events (e.g., heart attack and stroke) are frequent outcomes, and patients experience a significantly increased risk of leg amputation and premature mortality^{4,16}.

Like other forms of CVD, PAD results from one or more risk factors including smoking, diabetes mellitus, hypertension, obesity and dyslipidemia, as well as a family history of CVD or PAD^{4,5}. **Figure 1** presents a model that depicts how these genetic and modifiable lifestyle factors may interact to contribute to PAD risk. Unlike other CVD, however, the body of research on PAD is less robust. Recent data document that the disease is persistently underdiagnosed and undertreated, both in terms of lifestyle interventions and pharmacological therapies^{4,24,31}.

A series of potential explanations may underlie the past knowledge gaps and lower investigational priority of PAD compared to other CVD. For example, PAD is frequently asymptomatic prior to causing a recognized ischemic event. It is estimated that only 25 to 50% of people with PAD exhibit difficulty walking and/or leg symptoms. Standards of clinical care present another barrier, as the majority of care providers either lack training in measuring ankle-brachial indices (ABI) (the standard PAD diagnostic

tool), or feel that time constraints limit their ability to conduct these simple tests³².

Overall, there is a profound lack of public awareness about PAD^{4,24}, and this low awareness, high prevalence and cost have led to the creation of new evidence-based PAD clinical care guidelines²².

The purpose of this critical review is to summarize the current evidence base regarding the role of nutritional and anthropometric factors on PAD incidence and risk, to assess the strength of the evidence for these associations, and to outline concepts that may help future investigators in the design of nutrition-focused PAD clinical research. We have approached this review using the criteria presented in **Figure 2** to rate the quality of each study.

Results of Critical Review

We identified eleven factors relating to nutrition or body composition that have been investigated in the context of PAD incidence. Current evidence for an association between each factor and PAD is summarized in **Table 1**³³⁻¹⁰³, including key causality hypotheses, the proposed biological mechanism for each association, the number and types of studies published, results, and potential avenues of future investigation.

Micronutrients

B vitamins: folate, B₆, B₁₂ and niacin

Currently, it is thought that inadequate dietary intake and/or absorption of B vitamins may influence PAD etiology via homocysteine (Hcy) regulation. As a crucial intermediary in amino acid synthesis (from dietary methionine), Hcy cycles between conversion to the cysteine precursor cystathionine, and remethylation back to methionine¹⁰⁴. While the conversion to cystathionine is vitamin B₆-dependent, remethylation requires folate and vitamin B₁₂ as cofactors¹⁰⁴. Evidence suggests that a folate deficiency contributes to a decreased ability to maintain the cycle's homeostasis. A build-up of unmetabolized serum Hcy may lead to potentially toxic concentrations, and

has been found to contribute to endothelial dysfunction, vasoconstriction and inflammation^{105,106}. Nevertheless, it is unclear precisely how low folate intake or high Hcy concentrations promotes PAD pathogenesis.

Cross-sectional studies of folate intake and PAD outcomes have used a range of assessment methods, including self-reported food frequency questionnaires (FFQ), 24-hour dietary recall interviews, as well as measurement of serum folate concentrations. These studies provided concordant data that participants with lower folate intake were at significantly greater risk of having PAD than those consuming greater quantities. Reported results ranged from a 33% increase in prevalence risk comparing lowest to highest intakes⁷², to a significantly higher prevalence of folate deficiency in PAD cases versus controls (6.4% versus 2.9%) . Prospective cohort study and clinical trial evidence is more equivocal. The sole epidemiological study⁸³ calculated intake data from nutrition tables published prior to the introduction of folate fortification in 1996³³, thus potentially underestimating dietary intake, and only observed a protective association between PAD risk and folate among subjects taking folate-containing multi-vitamins (relative risk for highest intake versus lowest = 0.67). One clinical trial⁶⁶ evaluated folate supplementation alone, while two^{36,42} used multi-nutrient supplements, limiting the ability to discriminate causal pathways. Nevertheless, Carrero et al³⁶ observed significant improvement in patients' walking distance and ABI scores (in the supplement group, a 3.5-fold increase in walking distance from baseline, and a 13% increase in ABI). All three clinical trials observed significant reductions in serum Hcy concentrations. The same case-control studies that evaluated folate's association with PAD also assessed B₁₂^{35,101} and concluded that there is no association between B₁₂ and either PAD prevalence or incidence.

Two clinical trials performed by de Jong et al⁴² and Carrero et al⁹ included B₆ in the multi-nutrient supplements ingested by PAD patients. Thus, while both studies

concluded that B₆ supplementation decreases serum Hcy concentration and improved vascular function, the B₆-specific effects cannot be determined independently of the other compounds included in the supplements.

Finally, a small number of studies have assessed the association of niacin intake with PAD-related lipid profiles or clinical outcomes. Based on the premise that niacin is known to inhibit hepatic LDL cholesterol synthesis^{107,108} and that it may influence endothelial inflammatory mechanisms in PAD etiology¹⁰⁹⁻¹¹¹, five clinical trials^{37,45,54,61,85} have been performed to assess the effect of niacin supplementation on lipid profiles or walking function in individuals with PAD. Three of the trials^{37,54,85} included niacin with antioxidant supplements, possibly confounding the interpretation of the outcomes. All trials that assessed lipids and inflammatory biomarkers observed significant changes (decreases in fibrinogen and prothrombin³⁷, increases in serum HDL cholesterol^{45,85}) while one trial of walking function⁶¹ observed a 37.8% increase in peak walking times among subjects taking niacin compared to those taking a placebo.

Vitamin D

Only within the last decade have researchers begun to investigate vitamin D and its relationship to PAD. Low vitamin D status is associated with increased incidence of various cardiovascular diseases, including heart failure, myocardial infarction, and hypertension¹¹²⁻¹¹⁴. One mechanistic hypothesis⁴⁶ posits that decreased sun exposure among physically impaired PAD patients leads to a decline in vitamin D synthesis, causing a decrease in intestinal calcium absorption. This would be anticipated to be associated with greater renal calcium loss and a compensatory increase in parathyroid hormone (PTH) secretion to maintain serum calcium concentrations. A self-perpetuating cycle may begin in which hypovitaminosis D promotes hyperparathyroidism, which increases the risk of bone pain (osteomalacia), and further exacerbates a PAD patient's immobility. Recent data also suggest that hypovitaminosis D may promote arterial

calcification^{102,115}. While the precise mechanisms remain unclear, it is believed that vitamin D may simulate its regulatory role in bone mineralization by promoting osteoblast-like function in arterial smooth muscle cells¹⁰². These osteoblast-like cells may promote calcium deposition in arterial walls, contributing to atherosclerosis.

To date there have been no prospective studies or clinical trials of vitamin D supplementation in individuals with PAD. Nevertheless, six cross-sectional and case-control studies^{46,47,67,82,89,102} have concluded that vitamin D deficiency is significantly associated with increased odds of prevalent PAD and/or PAD severity. NHANES data showed an inverse linear association between hypovitaminosis D and PAD prevalence (two analyses, ranging from 7.7% to 8.1% prevalence)^{67,82}. The most recent study by Zagura et al¹⁰² also measured arterial calcification in PAD cases and healthy controls, and observed a significant association between calcium deposits and PAD, with 6% of PAD patients having arterial calcification compared to less than one percent in healthy controls.

Other dietary components

Antioxidants

Building on previous studies of dietary antioxidants (AOX) and oxidative stress in CVD¹¹⁶⁻¹¹⁹, researchers have also begun to assess what influence AOX may have on similar inflammatory pathways in PAD. The majority of available research focuses on vitamins E, C and beta-carotene^{33,36,37,40,41,44,54,70,72-75,85,97,98}. Seven cross-sectional, case-control and epidemiological studies used a range of assessment methods (questionnaires, 24-hour recalls, etc.) and concluded that subjects with higher dietary AOX intakes were at significantly lower risk of having or developing PAD compared to subjects with lower intakes. Odds ratios for prevalence among highest dietary AOX intake ranged from 0.37 to 0.84 when compared to lowest intakes^{33,72,73}, and observational studies reported risk ratios for PAD incidence ranging from 0.64 to 0.89 for

subjects with highest AOX intake compared to lowest intake^{70,97}. Supplementation trials, however, produced more ambiguous results. All eight randomized trials^{36,37,40,41,54,75,85,97}, used multi-nutrient supplements which, like the B-vitamins studies, limits the ability to discern vitamin-specific associations. Six studies observed no changes in PAD symptoms or ABI measures in the patients who received supplementation compared to baseline. Two trials^{36,97} reported highly varied results. Carrero et al's³⁶ trial that observed improvements in walking distance and ABI also included vitamin E in their supplements, while Tornwall et al⁹⁷ noted that subjects taking only beta-carotene experienced a slight *increase* in risk of requiring vascular surgery (OR = 1.6 in supplemented group versus placebo group), an outcome the authors argued may have been the result of carotenoid uptake into arterial plaques¹²⁰.

Finally, there is a very small body of research on other compounds with anti-oxidant activity. A recent analysis of 2003-2004 NHANES data³⁴ assessed the relationship between selenium intake and PAD prevalence, and noted that participants with higher selenium intakes were at a lower risk of prevalent PAD (OR = 0.67 for highest versus lowest intakes). The same year, Hawkes et al⁶⁰ published results of a clinical trial of selenium supplementation in healthy males. Although participants' serum selenium concentrations increased significantly, the authors did not observe any changes in endothelial markers. A case-control study of dietary flavonoids⁷⁴ observed that PAD cases had significantly lower flavonoid intake when compared to controls, and Grassi et al's⁵⁸ trial of flavonoid supplementation (derived from black tea) observed significant, dose-dependent increases in arterial dilation (up to 2.3%) and decreases in arterial stiffness (mean change in Stiffness Index = -0.71 ± 0.29 m/s). Lastly, one randomized trial⁸⁰ attempted to observe the effects of an L-arginine-enriched nutrition bar on leg pain and walking distance. After two weeks, subjects in the supplementation group reported significantly decreased leg pain and an average increase in pain-free

walking distance of 66%. When this arginine intervention was then evaluated in a larger prospective randomized trial, however, no benefit on claudication was observed¹²¹.

Dietary Fiber

Based on previous studies of dietary fiber and cholesterol in CVD¹²²⁻¹²⁴, five cross-sectional and epidemiological PAD studies^{44,55,63,72,83} have examined dietary fiber. All studies but one⁶³ concluded that greater intake of dietary fiber was associated with decreased risk of incident or prevalent PAD. Odds ratios of PAD prevalence in subjects with highest fiber intake compared to lowest ranged from 0.65 to 0.80^{55,72}. Although to date there have been no prospective dietary intervention trials, the evidence specifically suggests that cereal fiber may be associated with a decreased risk of developing PAD.

Donnan et al⁴⁴ investigated participants in the Edinburgh Artery Study and delineated the impact of varying fiber sources. They concluded that higher intake of cereal fiber, predominantly insoluble in nature, was associated with higher ABI values. Among men specifically, increasing cereal fiber intake (defined as rarely, 1-3 times/week or 4-7 times/week) was associated with an ABI increase of 0.04 per frequency category. Two epidemiological studies of Health Professional Follow-up data^{63,83} further support the cereal fiber hypothesis. Hung et al⁶³ analyzed FFQ data for fruit and vegetable intake and PAD incidence, while Merchant et al⁸³ analyzed fiber intake (total, cereal-based, and fruit/vegetable-based). Whereas Merchant et al⁸ did not observe any significant associations between incident PAD and total fiber intake, upon separating fiber types the authors found a significant correlation between increased cereal fiber intake and lower risk of developing PAD, even after adjustment for other risk factors (CI = 0.67). Hung et al⁶³ on the other hand concluded that fruit and vegetable intake taken as a whole had no association with incident PAD, suggesting that soluble fiber may have little impact in this context.

Fats

A growing body of research has also begun to examine the roles of various types of dietary fats in PAD etiology. Relying on associations between saturated fat intake and heart disease as a precedent¹²⁵, cross-sectional and case-control studies have reached similar conclusions that people with PAD are more likely to consume high total fat⁵⁵ and/or high saturated fat^{39,65} diets. Other research has observed that PAD patients are significantly less likely to consume diets rich in polyunsaturated vegetable fats^{33,98}, particularly polyunsaturated n-3 essential fatty acids (EFA)^{72,74}. The n-3 EFA are of especial interest in vascular research, as it is believed they function as anti-inflammatory agents that counteract the reactive oxygen species associated with endothelial damage and atherosclerotic inflammation^{126,127}.

Results of clinical trials of n-3 EFA supplementation in PAD patients have varied. A short, 12-week trial of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation observed significant increases in walking distance and ABI (mean increases of 82 meters and 0.18, respectively)⁷⁸, while a two-year trial⁷⁵ observed no changes. Leng et al⁷⁵ randomized 120 subjects to receive either an EFA supplement or a placebo, and after two years there were no intra- or inter-group differences in physical function, vascular measures or serum lipid concentrations. A 2007 meta-analysis⁹⁵ analyzed data from six n-3 supplementation trials, and although the authors noted a moderate increase in blood viscosity among subjects taking EFA capsules, supplementation had no discernible net impact on PAD outcomes.

Since the publication of this meta-analysis, a small clinical trial by Schiano et al⁹² observed no improvement in PAD symptoms, either from baseline among subjects receiving n-3 supplements, or compared to the placebo group. In this instance, the small sample size (n = 16 per study arm) and short 3-month trial duration both hampered the ability to observe any possible changes that could take place over the long-term in a

larger cohort. At the time of this writing, a year-long n-3 supplementation by Leyva et al⁷⁶ is underway, with results forthcoming.

Gene – diet interactions

A total of eleven studies have been conducted of single nucleotide polymorphisms (SNPs) and their associations with PAD etiology. It is thought that certain SNPs can result in disordered or inefficient metabolic processes, and that these in turn may predispose people to a greater risk of developing PAD and other vascular diseases.

To date, the vast majority of research on SNPs and PAD has focused on *MTHFR*, the gene that encodes methylenetetrahydrofolate reductase (MTHFR). MTHFR functions as the enzymatic catalyst in remethylating homocysteine to methionine, and it is as crucial to regulating serum Hcy as folate and vitamin B₁₂. The C667T mutation in *MTHFR* is believed to cause an estimated 50% reduction in MTHFR activity, possibly decreasing the body's ability to regulate serum Hcy¹²⁸. Approximately 10% of the human population is homozygous for this point mutation (genotype TT)^{129,130}.

The evidence for a significant association between *MTHFR* mutations and PAD risk remains unclear. Three studies did not measure serum Hcy concentrations^{48,87,131}, while the remaining studies were split between contradictory conclusions. Overall, *MTHFR* genotyping studies assessed blood samples in cohorts ranging from 51⁸⁸ to 940⁴⁸ participants. Authors' observations did not vary by sample size.

Six studies^{38,48,84,91,100,131} reported no significant differences in mutation prevalence between cases and controls and four^{38,48,84,100} of those studies reported similar, and nearly identical, prevalence rates between groups. Two studies^{87,132} observed a higher prevalence of a T allele and/or TT genotype among PAD cases, and concluded that subjects with a T allele had a higher 1.18 to 3.54 risk (odds ratio) of prevalent PAD. While most investigations have reported no deviations from the Hardy-Weinberg Equilibrium (HWE), two studies by Fowkes et al⁴⁸ and Khandanpour et

al¹³² observed an under-representation of TT genotypes among controls, even while reaching opposing conclusions about TT homozygosity and PAD risk. Lastly, two meta-analyses also reported contradictory findings. Zintzaras et al¹³³ and Khandanpour et al⁵¹ analyzed data from seven and nine studies, respectively, with four studies overlapping. Zintzaras et al⁵² concluded there was no difference in genotype distribution between cases and controls, whereas Khandanpour et al⁵¹ reported a statistically significant odds ratio of 1.99 for PAD prevalence in TT genotypes versus CC types.

Lastly, a small number of studies have begun to examine other SNPs in a PAD context. Results are still preliminary, but research into prothrombin G20210A and Factor V Leiden genotypes^{69,84,94} (both co-factors involved in clotting cascades⁶⁹) has so far not found significant differences in genotype distributions.

Body composition

Obesity is a well-established risk factor for CVD^{134,135}, and the impact of body mass and body composition on incident CVD and disease progression remains an area of active investigation¹³⁶. It is now understood that total body weight, as estimated by body mass index (BMI), is not an ideal predictor of overall atherosclerotic risk¹³⁷⁻¹³⁹. An obese person may have a normal metabolic profile with no accompanying signs of CVD, while an only slightly overweight person can experience severe CVD with multiple comorbidities¹³⁴. Analyses of body composition¹⁴⁰⁻¹⁴⁴, defined by the quantity and visceral locations of fat tissue, have revealed that abdominal fat is a more precise predictor of atherosclerosis risk than shear weight^{141,144}. It is also increasingly understood that abdominal adipose mass is not inert, but is a metabolically active tissue capable of secreting hormones that influence serum lipid concentrations, glucose and insulin regulation, inflammatory pathways, and endothelial function^{140,142,143}. As a result, CVD research now frequently employs waist circumference (WC) singly, or with hip circumference (HC) as a measure of abdominal obesity¹⁴⁵.

Studies of body composition and PAD have not been consistent in their assessment methods. Of 18 cross-sectional and prospective observational studies, 10 assessed both BMI and abdominal obesity in their cohorts, whereas the remaining 8 measured only BMI. Of the 10 studies that measured abdominal adiposity, the majority measured body circumferences, while one used DEXA body density scans⁵³. These 10 studies had concordant results, for example, Makdisse⁷⁹ et al reported a 59% prevalence of abdominal obesity among PAD patients compared to 35% among controls⁷⁹, Planas et al⁸⁶ observed an odds ratio of 1.68 of PAD prevalence in the abdominally obese versus healthy-weight subjects, and Golledge et al⁵⁷ observed a risk ratio of 1.16 for CVD events or death in PAD patients with greater abdominal fat deposition compared to leaner participants. Of those, all but three^{49,77,86} also reported that greater total obesity (as BMI) was associated with more adverse PAD-related outcomes compared to leaner subjects. Of the 8 studies that only measured BMI, five still concluded that total obesity increased odds of adverse outcomes, including having a lower limb amputation⁵⁰ or experiencing a more rapid decline in peak walking distance⁸¹.

Interestingly, two of the remaining studies^{51,71} reported that increased BMI was significantly *inversely* associated with PAD incidence or prevalence. Galal et al⁵¹ reported that normal-weight subjects experienced a 50% rate of overall mortality compared to 31% among obese subjects, and Kumakura et al⁷¹ observed an 18% decrease in risk of all-cause mortality among people with high BMI versus low. Researchers have termed this phenomenon “the obesity paradox”¹⁴⁶, in which leaner body mass appears to be associated with a greater risk of atherosclerotic events. In the context of PAD, authors ascribe the paradox to confounding variables that are both more common among lean subjects, and are independent predictors of atherosclerosis. Specifically, leaner subjects have a higher frequency of smoking and/or chronic obstructive pulmonary disease (COPD), underweight status and overall malnutrition, all

of which can contribute to PAD risk^{51,71,146}. In fact, Ix et al¹⁰³, one of the 5 groups that reported a higher risk of adverse CVD events among obese PAD patients, only observed this association among subjects who had never smoked (HR = 1.32). For the remaining 3 studies that reported an inverse association between PAD and obesity, two reported a significantly greater proportion of smokers, COPD-diagnosed, and/or underweight subjects in their cohorts^{51,71}. This, in addition to their use of BMI as the sole parameter of obesity may explain their inverse results. Overall, the evidence suggests that when these factors are accounted for, higher BMI remains significantly associated with greater PAD prevalence.

Other nutritional issues – ethnic and racial disparities

Nutrition may also be an important mediating factor in racial and ethnic differences in PAD prevalence. African Americans experience a significantly higher burden of PAD compared to their Caucasian counterparts^{20,21,147}, and this disparity is only partly explained by traditional risk factors^{21,148,149}. A recent study by Khawaja et al¹⁵⁰ explored possible reasons for this excess risk, and concluded that while “novel” factors, such as increased serum concentrations of lipoprotein(a) (associated with inflammatory pathways) may partially explain the disparity, African American ethnicity is still significantly associated with higher PAD risk.

A possible nutritional explanation may be found in vitamin D. Evidence strongly suggests there is a racial disparity in vitamin D deficiencies in the United States: people with darker skin pigmentation, particularly African Americans, have significantly higher rates of deficiency compared to their Caucasian counterparts¹⁵¹. This may be due to differing ability to convert sun-derived precursor 7-dihydrocholesterol to pre-vitamin D₃. Populations with darker skin, and therefore higher cutaneous melanin concentrations are less efficient in this conversion process^{152,153}. When African Americans live in northern, less sunny latitudes, they are at especially high risk of developing vitamin D deficiencies.

Two analyses of 2001-2004 NHANES data examined racial differences in serum vitamin D concentrations and PAD. Kim et al⁶⁷ observed that while all ethnicities with vitamin D deficiency are at greater risk of having a cardiovascular disease, African Americans had a higher prevalence of deficiency (97%) than Caucasians or Hispanics (68% and 88%, respectively). Reis et al's⁸⁹ analysis supported these conclusions, and after adjusting for all known PAD risk factors, concluded that hypovitaminosis D accounted for approximately one-third of excess PAD risk among African Americans.

Key implications for future research

To date there are no established dietary guidelines that synthesize evidence into nutritional treatment recommendations for PAD patients. To our knowledge, this is the first critical review of this topic. In order to delineate more precisely how diet and body composition may influence PAD severity and progression, it would seem prudent for future studies to improve on previously used methods. Evidence strongly indicates that abdominal obesity is significantly associated with PAD risk and, and future studies should confirm this observation. Current data suggests that BMI measures alone may not account for all sources of obesity-related risk, and researchers should expand the use of DXA or other measurements to expand this body of data. A prospective clinical trials of weight loss interventions may be required to evaluate this causal relationship more precisely.

Future supplementation trials should also improve on previous PAD-nutrition knowledge limitations. Wide variations in nutrient dosages and trial durations restrict the ability to draw conclusions and thus establish evidence-based clinical recommendations. Additionally, past trials have not assessed baseline intakes and/or bodily concentrations of the nutrients of interest, thus overlooking key deficiencies, and further limiting interpretations of results. Future investigations of genetic contributions to PAD should include data on pre- and post-trial nutrient concentrations and might compare these to

patient genotypes. For example, as some genetic studies have shown that *MTHFR* genotype distribution can vary by PAD status, incorporating a genetic profile, along with Hcy measurements into a folate supplementation trial would greatly strengthen the validity of such a trial's findings. For vitamin D, more attention should be paid to baseline status and racial and geographic influences on endogenous synthesis.

Finally, observational studies of nutrition could also strengthen their approach by assessing baseline nutrient concentrations (even if only in a subset of a larger cohort), and validating FFQ data with 24-hour dietary recalls. While FFQs in large epidemiological studies yield valuable information on population-wide trends, more refined measures like dietary recalls could provide a more detailed body of data on which to base clinical trials.

Clinical implications

The potential for nutritional research to inform PAD clinical practice should not be underestimated. Over 21 billion dollars are spent annually on PAD treatment in the U.S.²³, with per-patient expenditures surpassing the costs of coronary artery disease by 5 percent. Endovascular therapies, such as stents and angioplasty, are now standard PAD treatments²⁹, yet they frequently require repeated interventions¹⁵⁴, and often result in post-intervention complications, both of which in turn contribute to even greater costs¹⁵⁵.

Lifestyle-oriented interventions may be most effective if individuals with PAD are provided “actionable knowledge” regarding the impact of overweight and obesity on their disease. As for other CVD, individuals may enjoy improved health with increased awareness of the synergistic impact of physical activity, andt also of caloric intake and portion sizes as these together contribute to a healthier energy balance. One-on-one counseling that emphasizes foods rich in micronutrients, and that individualizes the

counseling to a given patient's weight status and activity level may prove to be of more benefit than general counseling on "healthy" eating.

Conclusion

A 2009 conference²⁹ on the state of PAD research concluded that a general dearth of pathogenesis studies, a lack of prospective clinical trial-based evidence, and an over-emphasis on late-stage treatment interventions all combine to create a clinical model in which PAD is underdiagnosed and undertreated. The data from this review further supports the need for an expanded research effort that might use nutritional prevention approaches to directly lower the PAD burden. There is adequate preliminary evidence that nutrition and weight-loss-based therapies may be useful components of PAD treatment. Such nutritional interventions may lower PAD incidence, ameliorate functional impairment, and improve ischemic outcomes in PAD patients. These data suggest that it is imperative that research in these areas expands further.

Box 1 | Risk factors for peripheral arterial disease

Lifestyle factors

- Reduced physical activity
- Tobacco use
- Alcohol use

Body composition

- Abdominal obesity
- High BMI
- High ratio of fat:lean body mass

Nutrition

- Insufficient micronutrient intake
- Excessive calorific intake

Comorbidities

- Hypertension
- Insulin resistance
- Dyslipidemia
- Systemic inflammation
- Heart disease

Review criteria

A search for original articles published between January 1990 and April 2012 was performed in PubMed. The search terms used were "peripheral arterial disease", "peripheral vascular disease", "diet", "dietary fiber", "fat intake", "B vitamins", "folate", "niacin", "B₁₂", "vitamin D", "vitamin C", "vitamin E", "β-carotene", "dietary antioxidants", "genetics", "genotype", "nutrigenetics", "nutrigenomics", "MTHFR", "obesity", "excess weight", "body composition", "abdominal obesity", "ethnic differences", "racial differences", and "racial disparities", both alone and in combination. All articles identified were full-text papers published in English. Only clinical and population studies were included.

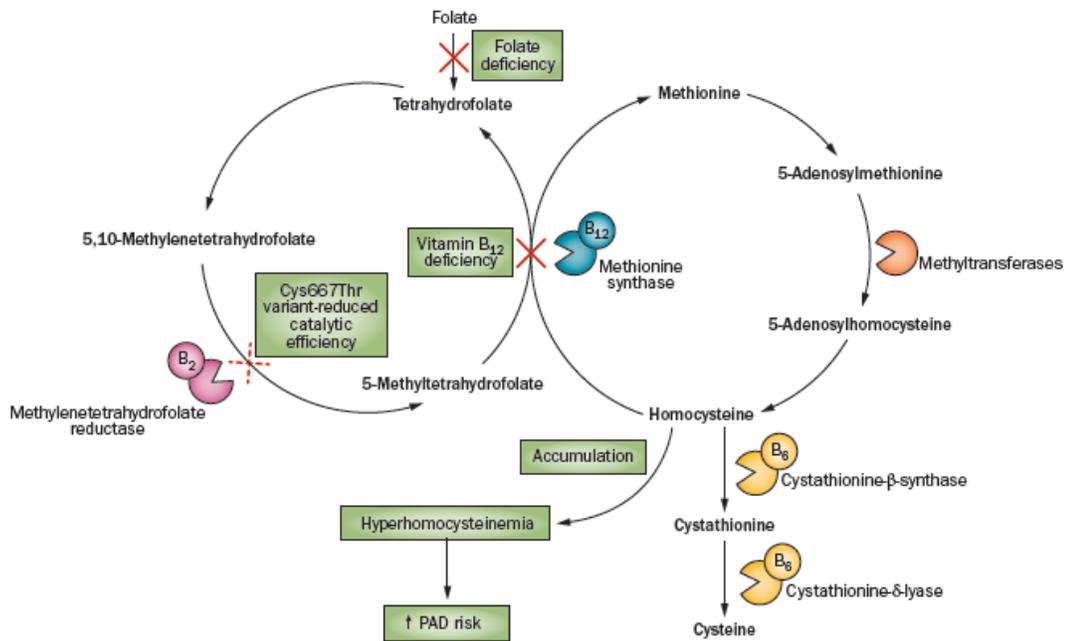


Figure 1 | Potential effects of vitamin B₁₂ and folate deficiencies, and the Cys667Thr variant of the *MTHFR* gene on homocysteine regulation and PAD etiology. Vitamin B₁₂ and the folate substrate 5-methyltetrahydrofolate are cofactors for methionine synthase, which catalyzes the conversion of homocysteine to methionine. Deficiencies in vitamin B₁₂ or folate could, therefore, disrupt homocysteine homeostasis, leading to accumulation of homocysteine, hyperhomocysteinemia, and an increased risk of PAD. Methylenetetrahydrofolate reductase is required for the conversion of folate substrates. The Cys667Thr variant of the enzyme has reduced catalytic efficiency, so the presence of the variant might also lead to disruption of homocysteine homeostasis and possibly increase the risk of PAD. Abbreviations: B₆, vitamin B₆; B₁₂, vitamin B₁₂; PAD, peripheral arterial disease.

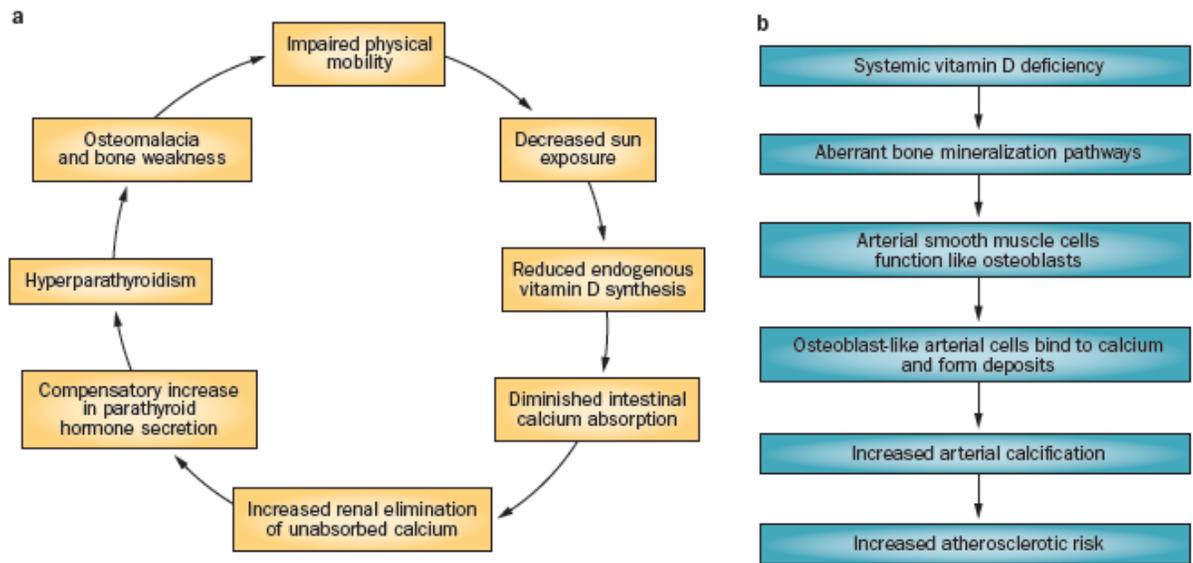


Figure 2 | Potential effects of vitamin D deficiency on the risk and progression of atherosclerosis and PAD. **a** | PAD-induced leg pain and discomfort can lead to decreased walking ability and an increase in sedentary, home-centered habits. This lifestyle, in turn, decreases the frequency and duration of sun exposure, which might lead to a self-reinforcing cycle in which vitamin D deficiency leads to abnormal calcium absorption, hyperparathyroidism, and even greater leg pain and discomfort. **b** | Vitamin D deficiency caused by inadequate sun exposure, dietary intake, or both might result in aberrant calcium metabolism and bone mineralization pathways. These irregularities might lead to alterations in the function of arterial smooth muscle cells and promote calcium deposition on arterial walls, arterial stiffness, and increased risk of atherosclerosis.

Manuscript 2:

Body composition, nutrient intake and *MTHFR* genotype in patients with peripheral arterial disease.

Abstract

Objectives: Nutrition guidelines for prevention and treatment of cerebrovascular and cardiovascular diseases are well-established, yet there are no nutritional guidelines for patients with peripheral arterial disease (PAD). The purpose of this pilot study was to investigate associations between nutritional factors and disease severity in PAD patients. A secondary aim was to assess if current data from existing vascular diet guidelines are adequate to apply to individuals with PAD.

Methods: We conducted a cross-sectional study of current nutritional status amongst 60 free-living PAD patients who were recruited from vascular specialty clinics. Measured nutritional variables included dietary intakes, biomarkers of nutritional status, *MTHFR* genotype, serum adiponectin level and body composition, while PAD severity was measured by assessment of the ankle-brachial indices, walking ability, PAD-associated pain, and psychosocial quality of life.

Results: Increased dietary fat and cholesterol, low serum vitamin D and adiponectin, and increased BMI, total body fat and abdominal adiposity were all associated with more severe clinical and psychosocial PAD symptoms. Increased fiber intake was associated with greater stair-climbing ability.

Conclusions: There is preliminary evidence to support the use of CVD lifestyle guidelines in PAD treatment, but prospective studies with larger sample sizes are needed.

Introduction

Background

Peripheral arterial disease (PAD) is a pressing public health concern. Despite its known high morbidity and mortality amongst cardiovascular diseases (CVD)^{23,29}, and

despite its very high health economic costs^{23,156}, PAD garners substantially less attention. The relationship between established atherosclerosis risk factors for PAD and the other two common manifestations of CVD [coronary heart disease (CHD) and ischemic cerebrovascular disease] and their known clinical outcomes is complex (**Figure 1**). All three of these common atherosclerotic diseases share risk factors, though PAD and the other CVDs are biologically not identical. For example, while diabetes mellitus (DM) and a history of smoking are both established risk factors for incident CHD and stroke, they are particularly strong predictors of PAD risk^{9,157}. PAD is also distinctive in its impact on ambulation and physical function, and is associated with major exertional lower extremity ischemic symptoms (claudication) that are experienced as muscle fatigue, discomfort, pain and diminished walking capacity. PAD is also the single most frequent cause of limb amputation¹, and is often associated with progressive functional impairment and profoundly impacts both physical and psychosocial quality of life¹.

Clinical guidelines have established that pharmacological treatment and revascularization procedures can be effective in alleviating PAD symptoms^{1,12}, however the benefits are not always durable¹⁵⁴. Patients often experience post-procedural clinical limitations, and even successful revascularization is often associated with a need for repeated additional invasive interventions. Thus, this approach is unlikely to be sustainable across such a highly prevalent disease¹⁵⁵. Furthermore, patients with advanced comorbidities or unstable vascular anatomy may not be viable candidates for revascularization, leaving this subpopulation with even fewer treatment options. There has been a relative lack of focus in PAD clinical research on methods that might be applied to prevent PAD or to minimize its anatomic and clinical progression, as compared to efforts to simply provide episodic revascularization. For example, the 2009 TransAtlantic Vascular Medicine “PAD2009” conference²⁹ concluded that current

guidelines over-prioritize end-stage treatment while overlooking the sizeable population of people at risk for developing PAD ^{1,12}.

The efficacy of nutrition-based programs for CVD prevention and early intervention is well-established ¹⁵⁸, but there has been insufficient research to develop equivalent guidelines to prevent or lower PAD risk via nutritional intervention. Since PAD biologically differs from other common CVD, it is possible that the nutritional contributions to each PAD risk factor may differ as well. To date, the majority of PAD research on nutrition has focused on general dietary trends. These studies by necessity have been limited to patients who retain at least moderate walking ability and do not suffer from other incapacitating health conditions. As a result the overall nutritional habits of PAD patients with diminished physical capacity remain largely unexamined. Given the lack of data, there is a critical need to establish whether existing nutrition and body composition recommendations for other CVD can be applied to PAD, or whether PAD is sufficiently biologically distinct so as to warrant its own clinical recommendations. This pilot study aimed to evaluate the nutritional status and body composition of individuals with symptomatic PAD. In the long-term, this study establishes the groundwork for a larger intervention study that would focus more precisely on specific nutritional targets, and aim at modifying patients' PAD symptoms and clinical progression.

Methods

Study design

We conducted a cross-sectional pilot study of the nutritional status and body composition of 60 PAD patients.

Recruitment and study population

Participants were identified through a search of a university-affiliated hospital database and through direct referral from three Twin Cities metropolitan vascular specialty clinics. Inclusion criteria included: an established clinical PAD diagnosis,

English proficiency, consistent access to a telephone, and the ability to attend research clinic visits. Patients were excluded from participation if they were bilateral amputees, had undergone a revascularization procedure within the previous 6 months or if informed consent could not be provided due to dementia or other conditions that might impair cognition. Patients were initially contacted by telephone and then met at an initial screening visit to provide written informed consent. All study documents were approved by the University of Minnesota Institutional Review Board.

Measures

We assessed the severity of each individual's PAD burden as a function of clinical signs, symptoms and quality of life. A conceptual model of factors that determine PAD severity is presented in **Figure 2**. Participants were asked to come to a university-affiliated research clinic for a single three-hour visit that included completion of a survey of demographics, lifestyle and medical history. A trained investigator measured height, weight and waist and hip circumferences. Each participant underwent a total-body dual X-ray absorptiometry (DXA) scan¹⁵⁹ to assess adiposity. Participants were asked to complete three non-consecutive 24-hour dietary recall interviews within a two-month period, the first conducted in-person, and two additional recalls over the telephone. For participants who completed all three recalls, one recall assessed food intake on a weekend day, and two recorded weekday intakes. This multi-pass approach to assessing nutritional status on varying days of the week is considered to be the optimal method for collecting reliable and accurate dietary data¹⁶⁰. A certified interviewer assessed eating habits using the Nutrition Data System for Research (NDSR), a software application developed at the University of Minnesota Nutrition Coordinating Center (NCC) that facilitates standardized recall collection¹⁶¹. Participants used scale models of foods and serving sizes to report their food intake, and were given the NCC Food Amounts Booklets for use during telephone surveys. Dietary intakes were

calculated using the NCC Food and Nutrient Database ¹⁶² and evaluated for portion size, ingredients, nutrient composition and preparation methods. Averages were computed for analysis.

Measures of PAD severity included a research visit-based measurement of the ankle-brachial index (ABI), completion of the Walking Impairment Questionnaire (WIQ) ¹⁶³, and the King's College Hospital's Vascular Quality of Life Questionnaire (VascuQOL) ¹⁶⁴. The ABI was measured using a 5-mHz Doppler device (Elite-100R, Nicolet Vascular Inc., Golden, CO). Systolic blood pressures were recorded at the brachial arteries of both upper extremities, and at the dorsalis pedis and posterior tibial arteries using a standard technique ¹⁶⁵. The same technique was employed on each patient, regardless of the revascularization status of either leg. Each participant underwent two ABI measurements while in a supine position and the average of each pair of readings was recorded. For analysis of PAD severity for this study, we utilized the lower ABI value for each participant. Scores were recorded as continuous measures, as well as categorized by standard clinical guideline-based diagnostic cut points ¹⁶⁶: abnormal (≤ 0.90), borderline (0.91 – 0.99), normal (1.00 – 1.40) or noncompressible (> 1.40).

The WIQ is a validated ¹⁶⁷ 20-item survey designed to measure the severity of PAD limits physical function. It is the predominant instrument used for capturing self-reported walking ability in PAD patients with symptomatic claudication and evaluates four components: Distance, Speed, Stair Climbing and Pain ^{1,168}. Participants answered items on a Likert scale from 0 for "unable to do" to 4 for "no difficulty", and responses were weighted based on the difficulty of each task. When respondents avoided certain activities altogether due to PAD symptoms, they were asked to choose "Didn't do for other reasons".

The VascuQOL is a validated 25-item survey ¹⁶⁴ that assesses patients' perceived quality of life in five domains – Pain, Activity, Symptoms, Emotional and

Social. All questions are equally weighted and scored from 1 to 7; for example, in response to “In the last two weeks I have had pain in the foot or leg when I am at rest”, choosing “All of the time” is coded as a 1, and choosing “None of the time” is coded as a 7. Domain subscores were calculated as averages of the questions assigned to each domain.

Biomarkers

A fasting blood sample was collected from each participant, aliquoted and separated, and frozen at -20°C until analysis. Folic acid concentrations were assessed from whole erythrocytes (chemiluminescent immunoassay, Quest Diagnostics, Wood Dale, IL), as this measure is a more valid indicator of long-term dietary folate intake than serum folic acid¹⁶⁹. Serum 25(OH)D (ng/mL) was measured using the Diasorin LIAISON® chemiluminescence method (Heartland Assays, Ames, IA). Commercially available Quantikine human ELISA kits (R&D Systems, Minneapolis, MN) were used to measure serum adiponectin concentrations (Kurzer lab, Saint Paul, MN). For genotyping, whole blood was extracted using a Qiamp Mini Blood Kit (Qiagen) and manufacturer’s protocol. The methylenetetrahydrofolate reductase (*MTHFR*) gene encodes and determines the efficiency of MTHFR, an enzyme that is crucial for folate metabolism and homocysteine (Hcy) homeostasis. Amplified 15 ng DNA was assessed for *MTHFR* C667T SNPs using a TaqMan SNP Genotyping Assay (Life Technologies Co, Grand Island, NY). The Lightcycler 480 Probes Master (Roche) master mix was run under cycling conditions established by the manufacturer and collected on a real time PCR machine (Institute of Human Genetics, University of Minnesota Masonic Cancer Center, Minneapolis, MN).

Data analysis

Outcome variables were selected for biological plausibility and classified into two categories: PAD severity as a function of clinical signs or symptoms and PAD severity as

a function of psychosocial factors. As this was an exploratory study, in which multiple post-hoc analyses were evaluated in a limited patient sample, we purposefully did not set any Bonferroni corrections to limit the significance of these multiple comparisons. Continuous exposure variables were analyzed against outcomes using linear regression modeling; once using the full data set, and again after removing outliers and heavily influential data points. Only associations that were significant in both models were considered statistically significant. Spearman's correlation coefficients were generated to evaluate monotonic relationships. Ordinal variables were analyzed using Cochran-Mantel-Haenszel tests and proportional-odds cumulative logit modeling. Statistical analyses were conducted using SAS software, version 9.3 (SAS Institute Inc., Cary, NC). All *p*-values are two sided, $\alpha = 0.05$.

Results

Sixty-four PAD patients consented to participation; four withdrew and 60 underwent data collection. The demographic and body composition characteristics of the study cohort are presented in **Table 1**. There were no significant differences in any characteristics by revascularization status, and all revascularized patients had undergone their procedures a minimum of 6 months prior to data collection, thereby mitigating possibly confounding effects of the revascularization intervention on their nutritional habits and status. Half ($n=30$) of the cohort reported having a previous clinical diagnosis of CHD, 9 participants reported a history of stroke, and 4 had a history of both conditions. Over a third were diagnosed with DM type 2, and approximately 75% were classified as overweight or obese. The characteristics of this cohort are similar to those of PAD cohorts in other studies, and to estimates of the general U.S. population (approximately 69% overweight or obese)^{1,170,171}. Further analyses stratifying patients by CVD status, diabetes and other associated comorbidities revealed no significant

differences in any measures of nutritional status, *MTHFR* genotype, physical PAD severity, or quality of life.

Nutritional characteristics of these PAD patients are presented in **Table 2**. Twenty-five participants completed all three dietary recalls, two completed two recalls and 33 completed only the initial in-person recall. Revascularized patients were significantly more likely than patients with native vasculature (NV) to consume a daily diet of 2,000 calories or less ($p = 0.03$). NV patients, however were significantly more likely than revascularized patients to consume the minimum recommended daily calcium intake (1,000 mg)¹⁷², ($p = 0.04$). Participants did not differ by revascularization status for any other dietary variable, WIQ component or VascuQOL domain. Over 58% of the participants had insufficient or clinically deficient serum 25(OH)D concentrations. For *MTHFR*, five participants were homozygous for the CC wild type, and the remaining 55 were split between CT and TT genotypes.

Figure 3 presents results of regression tests for red blood cell (RBC) folate and body-mass index (BMI) in relation to various parameters of clinical PAD severity. One-unit increases in both RBC folate and BMI were significantly associated with lower (more severe) WIQ scores; BMI was also inversely associated with WIQ-Stairs scores (data not shown, -1.73 ± 0.62 , $p = 0.08$; $r = -0.32$, $p = 0.01$).

Figure 4 presents Cochran-Mantel-Haenszel tests between BMI and VascuQOL parameters of clinical and psychosocial severity. Waist circumference was significantly associated with VascuQOL-Symptoms ($p = 0.02$) and inversely associated with WIQ-Stairs (-0.62 ± 0.29 , $p = 0.04$; $r = -0.27$, $p = 0.04$). Similarly, serum adiponectin was inversely associated with WIQ-Distance scores (-19.05 ± 5.87 , $p = 0.002$). Two dietary variables were associated with WIQ components: cholesterol was inversely correlated with WIQ-Pain scores ($r = -0.26$, $p = 0.05$), and fiber was positively related to WIQ-Stairs ($+26.53 \pm 8.71$, $p = 0.004$; $r = +0.26$, $p = 0.04$). The percent of caloric intake as fat was

inversely associated with ABI ($p = 0.04$; $r = -0.31$, $p = 0.02$), serum 25(OH)D was significantly associated with VascuQOL-Pain scores ($p = 0.01$), and total energy intake was positively associated with WIQ-Distance scores ($+3.45 \pm 1.42$, $p = 0.02$).

Figure 5 presents significant associations between physical activity and psychosocial parameters, namely Total VascuQOL and VascuQOL-Social scores ($p = 0.02$, $p = 0.005$, respectively). Lastly, proportional-odds cumulative logit modeling yielded one significant association for dietary fat and ABI: At the 21-35% level of caloric intake as fat, the estimated odds of an ABI score below any diagnostic threshold (abnormal, borderline, normal, noncompressible) are 0.23 times the estimate of odds at the >35% level of fat intake ($p = 0.04$, CI 0.06 – 0.91).

Discussion

Few prior studies have reported the dietary patterns and nutritional status of patients with PAD. In our study, participants reported consuming diets high in protein and processed meat products, refined carbohydrates (e.g. white bread and pasta), saturated fat, cholesterol and added sodium. Participants consumed ≤ 2 servings/day of fruits, vegetables and whole grains (versus recommended intakes¹⁷³), and despite multivitamin supplement use in over half of participants, the majority of participants did not meet Daily Recommended Intakes (DRI)¹⁷³ for zinc, or vitamins A, C, E or K. These observations mirror dietary trends observed in both CVD-specific populations and the total U.S. population¹⁷⁴⁻¹⁷⁶.

This study observed significant associations between markers of increased adiposity and greater clinical PAD severity. Higher BMI and waist circumference was significantly associated with more severe clinical PAD symptoms. Of 60 participants, 20 were clinically overweight (BMI 25 to 29.99) and 24 were clinically obese (BMI ≥ 30). Both total and abdominal obesity's association with a range of related conditions, including DM and other common CVD are well-established^{177,178}. Previous research has

also established that adiponectin, which is secreted by abdominal lipocytes, is inversely correlated with abdominal adiposity and vascular risk ¹⁴⁰. We observed that a 1 ng/mL increase in adiponectin concentration was associated with a 19-point decrease in WIQ-Distance scores. To date there have been no other studies of adiponectin and quality of life, however our results are supported by Golledge et al's ⁵⁷ finding of a positive association between serum adiponectin concentrations and mean walking distance in PAD patients. Lastly, the association between increased total body fat and greater WIQ-measured clinical severity, particularly the Stair Climbing component, suggests that of all of the above factors; total and abdominal obesity, serum adiponectin and total body fat; singly or in combination may be significant predictors of a worsened clinical disease burden compared to leaner participants. In support of this, all but three participants had waist-to-hip ratios categorized as high-risk ¹⁷⁹, and DXA scans of android-gynoid adiposity showed that over two-thirds of the participants had ratios over 1.0.

Nutritional endpoints showed variable associations. The percent of energy intake as fat was negatively correlated with ABI score and participants consuming fat calories in excess of the recommended 35% ¹⁷³ experienced significantly greater odds of having lower, more severe scores. Greater total energy intake was unexpectedly associated with higher WIQ-Distance scores. However, since over half the participants consumed fewer than 2,000 calories per day, it is possible that participants with greater energy intake were more likely to consume foods rich in nutrients associated with decreased clinical severity.

Dietary cholesterol was correlated with more severe pain symptoms, while increased dietary fiber consumption was associated with improved ability to climb stairs. As with other CVD, it is thought that dietary fiber influences PAD symptoms by improving blood glucose control and by mediating cholesterol synthesis ¹⁸⁰. This may explain the

especially large increase of an estimated 26 points in WIQ-Stairs scores for every 1 gram increase in fiber intake.

Serum vitamin 25(OH)D was significantly correlated with VascuQOL-Pain scores, and participants with increased serum concentrations were more likely to report less severe limbic pain. Thirty-five participants had serum 25(OH)D concentrations at suboptimal levels (< 30 ng/mL). Hypovitaminosis D is a significant risk factor for both PAD^{47,67,82} and CHD and stroke^{181,182}, although the mechanisms for vitamin D's action in vascular etiology remain unclear. Hypothesized pathways include calcification of endothelial tissue and increased calcium loss leading to bone pain^{102,114,115}. Calcium metabolism is dependent on vitamin D intake and synthesis, and while the majority of participants met daily calcium intake requirements (revascularized participants were significantly more likely both to meet intake requirements, and to use calcium supplements), our findings suggest inadequate vitamin D might contribute to unabsorbed dietary calcium and more severe PAD-related pain.

Greater RBC folate concentrations were significantly associated with lower WIQ-Speed scores, but since the score decrease was very minimal (~one-tenth of a point per 1 ng/mL), this association is likely spurious. Over 30% of participants were clinically deficient in folate, likely resulting from the participants' generally low intake of folate-rich foods (i.e. fruits and vegetables, whole grains). Inadequate folate intake is associated with increased CVD and PAD risk, as well as with increased serum Hcy concentrations, which is itself an independent risk factor for PAD^{1,91}. *MTHFR* genotype might mediate these associations, and in this study heterozygous type frequency was nearly identical to the general population (45% compared to 43%)^{129,130}. Unexpectedly, and after re-confirming genotyping data, we observed that only 8.3% of the participants had the CC wild type, compared to 47% in the general population^{129,130}, and 46.7% was homozygous for the TT variant, compared to 5 to 10% of the general population

^{129,130,183}. The TT variant encodes a less efficient MTHFR protein (~50% reduced activity ¹⁰⁰), which in turn results in decreased folate metabolism, and may drive increases in Hcy concentrations. It remains unclear, however if the TT genotype is an important mediator of folate and Hcy-related PAD risk ^{132,133}, and we are unable to substantiate why the majority of our participants were homozygous for the variant. Previous CHD and PAD genotyping studies have yielded discordant results, with some reporting moderately higher frequency of the TT type, and others observing equal or lower frequencies ^{100,129,177,183}.

Lastly, we observed an overall tendency towards decreased VascuQOL-Social and Emotional PAD severity in participants with lower BMI and/or more frequent physical activity. Although further proportional-odds analysis did not reveal significant associations, these trends indicate that more overweight and sedentary patients may experience more social isolation and depressed moods than their leaner, more active counterparts. Future lifestyle studies of PAD patients should develop more comprehensive assessments of psychosocial factors to complement the VascuQOL survey. There is evidence that depressive mood disorders are highly prevalent among patients with various CVD conditions, and that a depressed state contributes to worse vascular outcomes ¹⁸⁴⁻¹⁸⁶. Depressed patients may also be less likely to participate in research studies, which underscores the need to assure inclusive recruitment methodologies.

To the best of our knowledge, this is the first attempt to comprehensively assess markers of PAD severity in relation to patients' nutrition and body composition. This is also the first study to use precise quantitative and qualitative tools to measure PAD patients' dietary habits, without relying on generalized surveys. As participants tended to have consistent daily eating habits with minimal intra-personal variation, this strengthens the reliability of our findings.

There were a number of limitations inherent in this study. First, we note the relatively small sample size of this study. Despite this, such a sample size is not unusual when PAD treatments or outcomes are measured in other cohorts. As well, the WIQ and VascuQOL surveys are validated for PAD patients in studies of this size, are correlated¹⁸⁷, and we were able to detect significant associations. When a limited PAD sample is recruited from a vascular specialty clinic (convenience) population, likely reflecting a larger PAD disease burden than might be derived from a primary care population, we note that our results should thus not be generalized. Patients with severely limited physical mobility or age-related cognitive difficulties might differ in eating habits or body composition from a broader population-based sample. This study also excluded participation of individuals with PAD that were asymptomatic, and excluded individuals “at risk” for incident PAD. As a cross-sectional pilot study, the associations we have reported between nutritional variables and PAD severity cannot establish causality. Finally, self-reported dietary and lifestyle habits may have been affected by recall bias.

Conclusions

These data demonstrate, for the first time, potential associations between clinical and psychosocial PAD symptoms and self-reported nutritional status and body composition. We observed that individuals with PAD report eating habits that are similar to the broader US population and to other cohorts with other CVDs. These data can provide the basis for a much broader cross-sectional evaluation of the effect of nutrition on incident PAD, on PAD progression, and on recovery from revascularization interventions. The current data suggest that individuals with PAD might benefit from increased consumption of dietary fiber, vitamin D-rich foods, folate-rich fruits, vegetables and whole grains, as well as efforts to promote weight loss for overweight and obese PAD patients. These actionable nutritional measures could be incorporated into future intervention trials to assess their benefit or harm. Future research can be designed that

would confirm the likely impact of nutrition on PAD outcomes and that could inform PAD care guidelines, with a potential benefit on both patient outcomes and healthcare costs.

Figure 1. Risk factors and outcomes of peripheral arterial disease (PAD), coronary heart disease (CHD) and ischemic stroke.

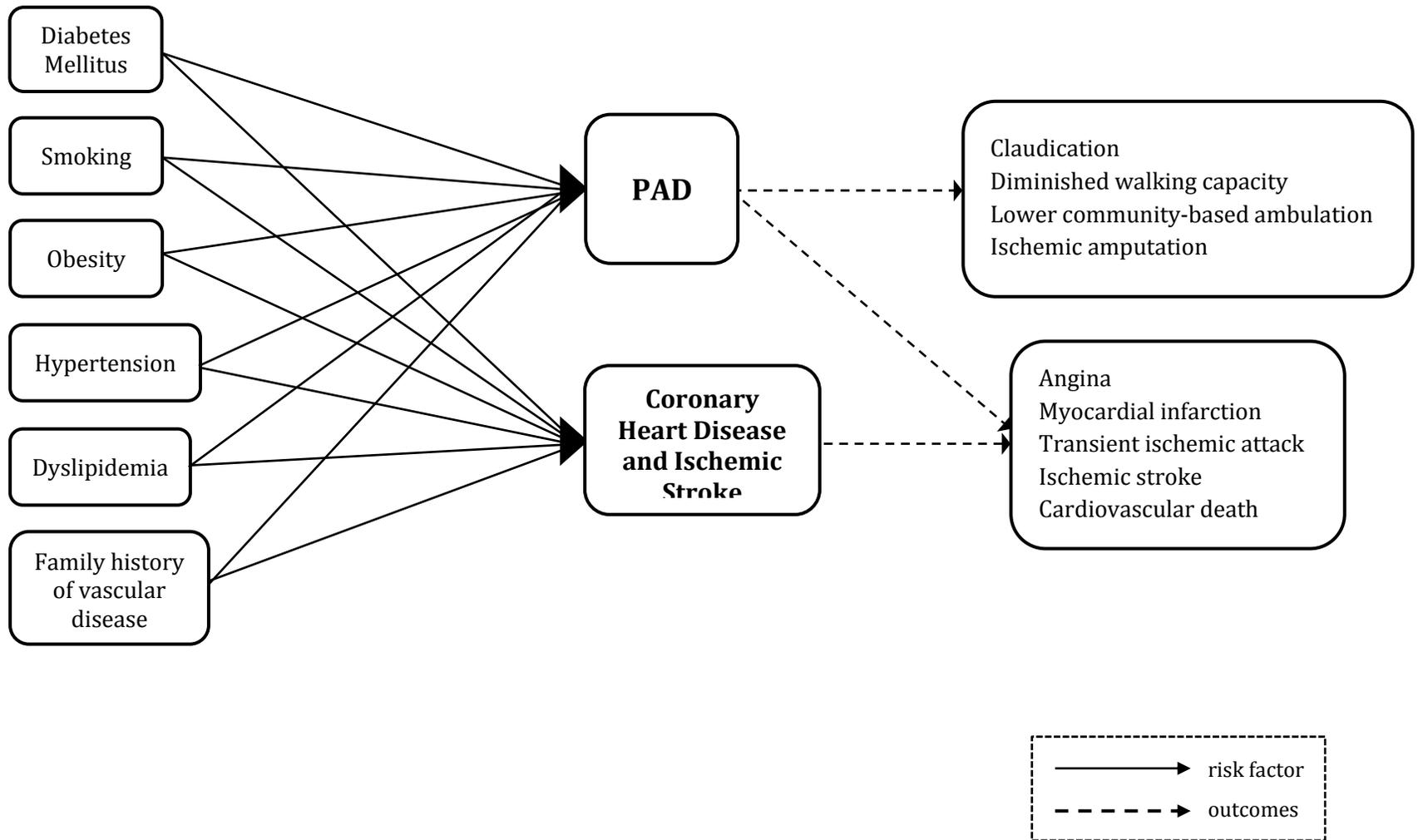


Figure 2. Conceptual model of PAD severity.

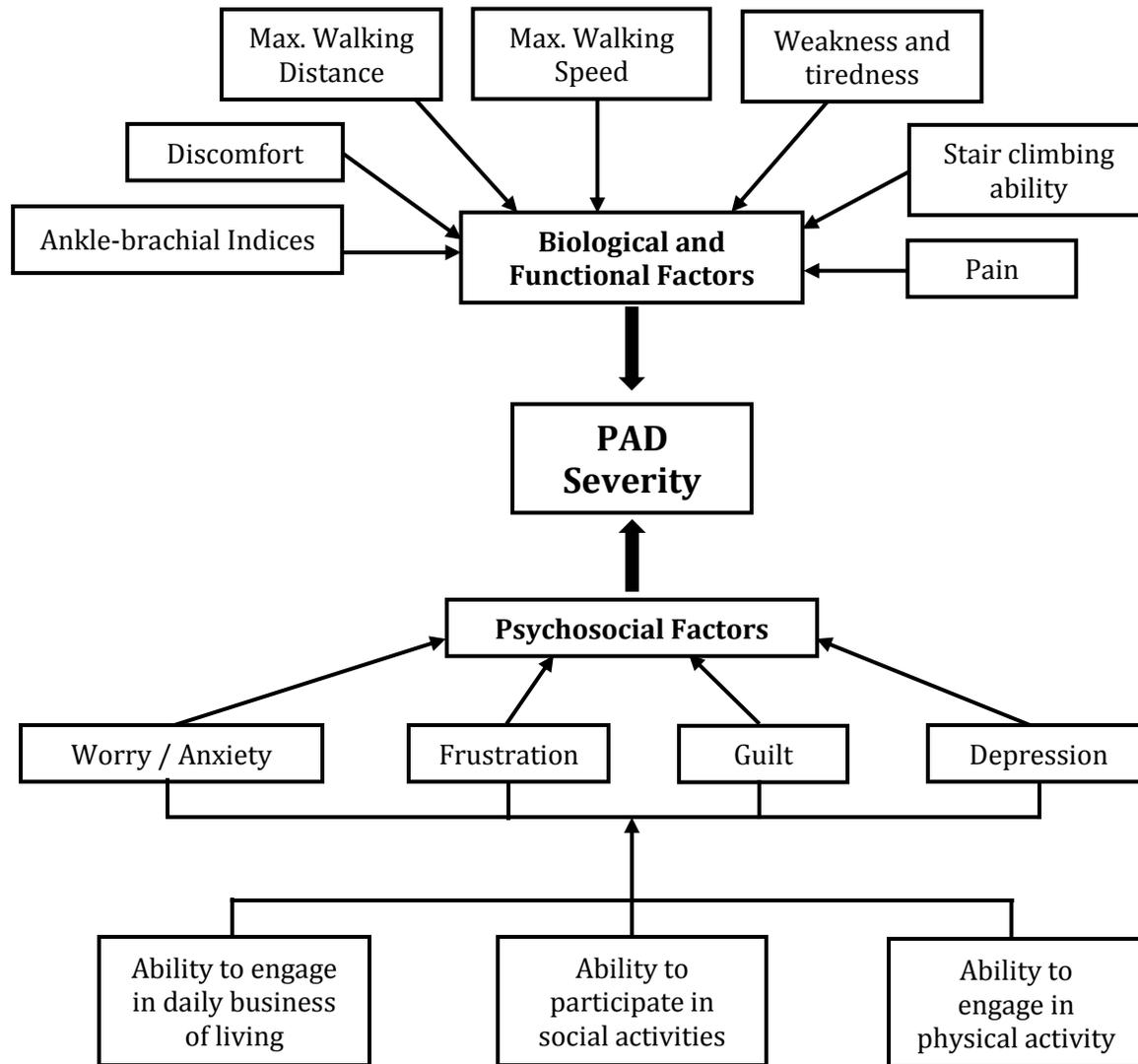


Table 1. Demographics and body composition characteristics of the study cohort by revascularization status¹.

	Total n=60 (%)	Native PAD Cohort n=25 (%)	Revascularization Cohort, n=35 (%)
Sex			
Male	40 (66.7)	18 (45.0)	22 (55.0)
Female	20 (33.3)	7 (35.0)	13 (65.0)
Age (mean years ± SD)	68.7 ±10.7	67.8 ±10.4	69.3 ±11.1
Race/Ethnicity			
White, non-Hispanic	53 (88.3)	22 (41.5)	31 (58.5)
African-American / African	6 (10.0)	2 (33.3)	4 (66.7)
Native American	1 (1.7)	1 (100)	0 (0.0)
Annual Household Income			
< \$20,000	10 (17.0)	4 (40.0)	6 (60.0)
\$20,000 - \$39,999	14 (23.7)	5 (35.7)	9 (64.3)
\$40,000 - \$59,999	12 (20.3)	5 (41.7)	7 (58.3)
> \$60,000	23 (39.0)	11 (47.8)	12 (52.2)
Education			
Some high school	3 (5.0)	0 (0.0)	3 (100.0)
High school graduate	6 (10.0)	1 (16.7)	5 (83.3)
Some college	13 (21.7)	8 (61.5)	5 (38.5)
Technical/vocational training	11 (18.3)	5 (45.5)	6 (54.5)
College graduate	17 (28.3)	7 (41.2)	10 (58.8)
Postgraduate degree	10 (16.7)	4 (40.0)	6 (60.0)
Alcohol (drinks⁸ per week)			
0	35 (58.3)	16 (45.7)	19 (54.3)
1 – 3	12 (20.0)	2 (16.7)	10 (83.3)
4 – 6	4 (6.7)	2 (50.0)	2 (50.0)
7 +	9 (15.0)	5 (55.6)	4 (44.4)
Ankle-brachial index² (index leg)			
Total (mean ± SD)	0.69 ±0.32	0.72 ±0.29	0.67 ±0.35
> 1.40 (noncompressible)	1 (1.7)	0 (0.0)	1 (100.0)
1.00 - 1.40 (normal)	5 (8.5)	3 (60.0)	2 (40.0)
0.91 - 0.99 (borderline)	12 (20.3)	4 (33.3)	8 (66.7)
≤ 0.90 (abnormal)	41 (69.5)	18 (43.9)	23 (56.1)
BMI³			
Total (mean ± SD)	28.8 ±5.6	28.7 ±5.2	28.9 ±6.0
Underweight (< 18.5)	0 (0.0)	0 (0.0)	0 (0.0)
Normal (18.5 - 24.99)	16 (26.7)	6 (37.5)	10 (62.5)
Overweight (25.0 – 29.99)	20 (33.3)	8 (40.0)	12 (60.0)
Obese class I (30.0 – 34.99)	19 (31.7)	10 (52.6)	9 (47.4)
Obese class II (35.0 – 39.99)	2 (3.3)	0 (0.0)	2 (100.0)
Obese class III (≤ 40.0)	3 (5.0)	1 (33.3)	2 (66.7)

Medical history			
History of CVD	30 (50.0)	11 (36.7)	19 (63.3)
History of stroke	10 (16.7)	5 (50.0)	5 (50.0)
Emphysema and/or COPD	12 (20.0)	5 (41.7)	7 (58.3)
Cancer	14 (23.3)	4 (28.6)	10 (71.4)
Atherosclerosis Risk Factors⁴			
Current smoker	12 (20.0)	6 (50.0)	6 (50.0)
Former smoker	38 (63.3)	17 (44.7)	21 (55.3)
Never smoker	10 (16.7)	2 (20.0)	8 (80.0)
Type 2 diabetic	21 (35.0)	8 (38.1)	13 (61.9)
Hypertensive	45 (75.0)	22 (48.9)	23 (51.1)
Obesity diagnosis	10 (16.7)	4 (40.0)	6 (60.0)
Physical Activity⁵ (times per week)			
None	4 (6.7)	2 (50.0)	2 (50.0)
1x	10 (16.7)	6 (60.0)	4 (40.0)
2 – 3x	12 (20.0)	4 (33.3)	8 (66.7)
> 3	34 (56.7)	13 (38.2)	21 (61.8)
Waist circumference⁶ (cm)			
Total (mean ± SD)	106 ±13.9	107 ±13.8	105 ±14.1
Low risk (women ≤ 88, men ≤ 102)	36 (60.0)	17 (47.2)	19 (52.8)
High risk (women > 88, men > 102)	24 (40.0)	8 (33.3)	16 (66.7)
Waist-to-hip ratio⁷			
Total (mean ± SD)	0.99 ±0.09	1.01 ±0.09	0.98 ±0.08
Low risk (Fem ≤ 0.85, Male ≤ 0.90)	3 (5.0)	1 (33.3)	2 (66.7)
High risk (Fem > 0.85, Male > 0.90)	57 (95.0)	24 (42.1)	33 (57.9)
Android-gynoid ratio (mean ± SD)	1.07 ±0.19	1.11 ±0.22	1.05 ±0.16
% Total body fat (± SD)	34.7 ±7.3	34.5 ±5.8	34.8 ±8.2
% Trunk fat (± SD)	35.3 ±7.2	35.4 ±5.9	35.2 ±8.0
Serum adiponectin (ng/mL) (mean ± SD)	103 ±76.4	102 ±85.4	103 ±70.6

1 *p*-values not shown – participants did not differ significantly by revascularization status for any characteristic; participants with a history of 1+ endovascular or operative procedure(s) to treat PAD were categorized as revascularized

2 Ankle-brachial index values include measures taken from revascularized patients as well as patients with native arteries; PAD status was objectively assessed in other ways besides ABI

3 BMI category definitions from clinical guidelines of the National Institutes of Health (NIH) ¹⁸⁸

4 Smoking, diabetes mellitus, hypertension and obesity are established risk factors for PAD ¹

5 Physical activity defined as an aggregate of frequency of 15+ minute intervals of mild (minimal effort) exercise, moderate (not exhausting) exercise and strenuous (rapid heartbeat) exercise

6 Waist circumference cut off thresholds from clinical guidelines of the National Institutes of Health (NIH) ¹⁸⁸

7 Waist-to-hip ratio cut off thresholds from 2008 World Health Organization Expert Consultation ¹⁷⁹

8 An alcoholic beverage contains 0.6 ounces pure alcohol: 12 oz beer, 8 oz malt liquor, 5 oz wine, 1.5 oz spirits ¹⁸⁹

Table 2. Nutritional characteristics¹ of study cohort by revascularization status².

	Total n=60 (%)	Native Vasculature n=25 (%)	Revascularized PAD n=35 (%)	p value ³
Total kcal³				
Total (mean ± SD)	1976 ±788.6	1899 ±720.0	2031 ±840.1	NS
< 1,500	16 (26.7)	10 (16.7)	6 (37.5)	
1,500 – 1,999	19 (31.7)	3 (15.8)	16 (84.2)	
2,000 – 2,499	15 (25.0)	7 (46.7)	8 (53.3)	
≥ 2,500	10 (16.7)	5 (50.0)	5 (50.0)	0.030
Total fat (% of total kcal⁴)				
Total (mean grams ± SD)	76.8 ±44.5	69.9 ±34.2	81.8 ±50.4	NS
≤ 20%	3 (5.0)	2 (66.7)	1 (33.3)	
21 – 35%	34 (56.7)	14 (41.2)	20 (58.8)	
> 35%	23 (38.3)	9 (39.1)	14 (60.9)	NS
Dietary n-3 fatty acids (g) (mean ± SD)	10.7 ±1.2	1.5 ±0.8	1.8 ±1.4	NS
Dietary cholesterol				
Total (mean mg ± SD)	276.9 ±165.2	270.6 ±141.9	281.4 ±181.9	NS
DRI guideline ⁵ ≤ 300 mg	37 (61.7)	15 (40.5)	22 (59.5)	
Exceeds DRI > 300 mg	23 (38.3)	10 (43.5)	13 (56.5)	NS
Protein				
Total (mean grams ± SD)	79.3 ±31.7	77.4 ±24.3	80.6 ±36.3	NS
DRI guideline (≤ 46 g Fem, 56 g Male)	9 (15.0)	4 (44.4)	5 (55.6)	
Exceeds DRI (> 46 g Fem, 56 g Male)	51 (85.0)	21 (41.2)	30 (58.8)	NS
Carbohydrates				
Total (mean grams ± SD)	238.3 ±112.5	233.6 ±108.6	241.6 ±201.5	NS
DRI guideline (≤ 130 g)	8 (13.3)	4 (50.0)	4 (50.0)	
Exceeds DRI (> 130 g)	52 (86.7)	21 (40.4)	31 (59.6)	NS
Dietary fiber				
Total (mean grams ± SD)	22.0 ±16.6	22.3 ±17.5	21.7 ±16.2	NS
DRI guideline (≥ 25 g Fem, 38 g Male)	5 (8.3)	1 (20.0)	4 (80.0)	
Below DRI (< 25 g Fem, 38 g Male)	55 (90.0)	24 (43.6)	31 (56.4)	NS
Added dietary sugars				
Total (kcal) (± SD)	264.1 ±236.0	286.1 ±268.0	248.3 ±212.9	NS
DRI guideline ≤ 25% total kcal	56 (93.3)	23 (41.1)	33 (58.9)	
Exceeds DRI > 25% total kcal	4 (6.7)	2 (50.0)	2 (50.0)	NS
Caffeinated beverages⁶ (no. 8-oz servings)				
≤ 1.5	14 (23.3)	6 (42.9)	8 (57.1)	
1.6 – 2.5	15 (25.0)	5 (33.3)	10 (66.7)	
2.6 – 4	11 (18.3)	4 (36.4)	7 (63.6)	
> 4	20 (33.3)	10 (50.0)	10 (50.0)	NS

Dietary sodium				
Total (mean mg ± SD)	152.1 ±91.3	141.9 ±92.8	159.4 ±90.9	NS
DRI guideline < 1500 mg	22 (36.7)	11 (50.0)	11 (50.0)	
Exceeds DRI ≥ 1500 mg	38 (63.3)	14 (36.8)	24 (63.2)	NS
Dietary Calcium⁷				
Total (mean mg ± SD)	143.9 ±82.1	168.5 ±106.4	126.4 ±54.4	NS
DRI guideline (≥ 1000 mg)	44 (73.3)	22 (50.0)	22 (50.0)	
Below DRI (< 1000 mg)	16 (26.7)	3 (18.2)	13 (81.3)	0.040
Dietary Niacin				
Total (mean mg ± SD)	194.5 ±101.6	207.3 ±121.3	185.3 ±84.5	NS
DRI guideline (≥ 16 mg)	50 (83.3)	22 (44.0)	28 (56.0)	
Below DRI (< 16 mg)	10 (16.7)	3 (30.0)	7 (70.0)	NS
Serum 25(OH)D⁸				
Total (mean ng/mL ± SD)	30.0 ±15.8	32.1 ±19.0	28.4 ±13.2	NS
Optimal (≥ 30 ng/mL)	25 (41.7)	13 (52.0)	12 (48.0)	
Insufficient (20-30 ng/mL)	20 (33.3)	5 (25.0)	15 (75.0)	
Deficient (< 20 ng/mL)	15 (25.0)	7 (46.7)	8 (53.3)	NS
Red Blood Cell Folate⁹				
Total (mean ng/mL ± SD)	665.1 ±182.1	689.3 ±186.6	648.4 ±179.7	NS
Sufficient (≥ 280 ng/mL)	37 (62.7)	17 (47.2)	19 (52.8)	
Deficient (< 280 ng/mL)	22 (37.3)	7 (31.8)	15 (68.2)	NS
MTHFR C667T Genotype [count (%)]				
Total	60 (100.0)	25 (41.7)	35 (58.3)	
CC genotype (wild type)	5 (8.3)	2 (40.0)	3 (60.0)	
CT genotype	27 (45.0)	13 (48.1)	14 (51.9)	
TT genotype (variant)	28 (46.7)	10 (35.7)	18 (64.3)	NS

1 Nutrient intakes calculated via Nutrition Data System for Research (NDSR)^{161,162,190}

2 Participants who have a history of 1+ endovascular or operative procedure(s) to treat PAD were categorized as revascularized

3 Participants total caloric intake differed significantly by revascularization status, $p = 0.03$

4 Percent of total kcal as dietary fat cut off guidelines from the Department of Health and Human Services 2010 guidelines¹⁷³

5 DRI: Dietary Reference Intakes based on Recommended Dietary Allowances (RDA) and Adequate Intake (AI) guidelines^{172,191-196}

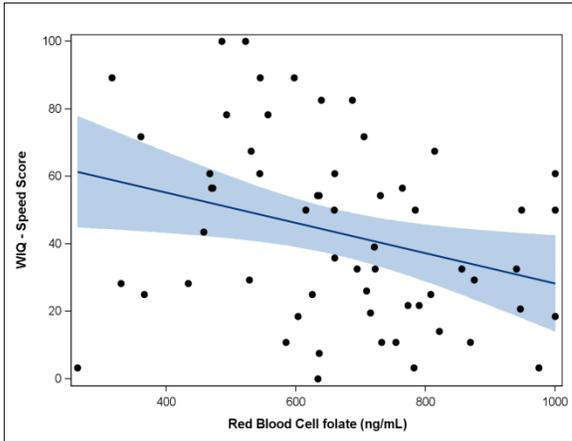
6 Caffeinated beverages include regular and half-caffeine coffees, caffeinated teas (black, green and white), caffeinated soft drinks

7 Participants dietary calcium intake differed significantly by revascularization status, $p = 0.04$

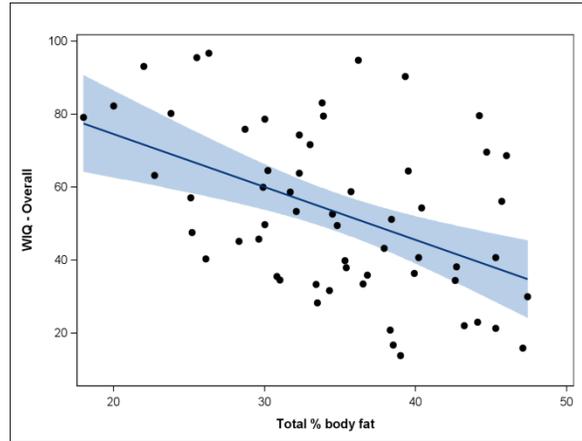
8 Serum vitamin 25(OH)D cut offs derived from current clinical guidelines¹⁸²

9 Red blood cell (RBC) folate (5-methyltetrahydrofolate) cut offs based on diagnostic lab reference ranges¹⁹⁷

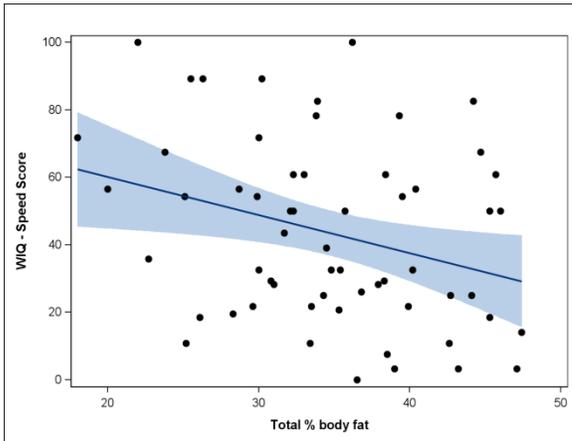
Figure 3. Regression estimates for red blood cell folate and total body fat (%) in relation to each measurement of clinical PAD severity.



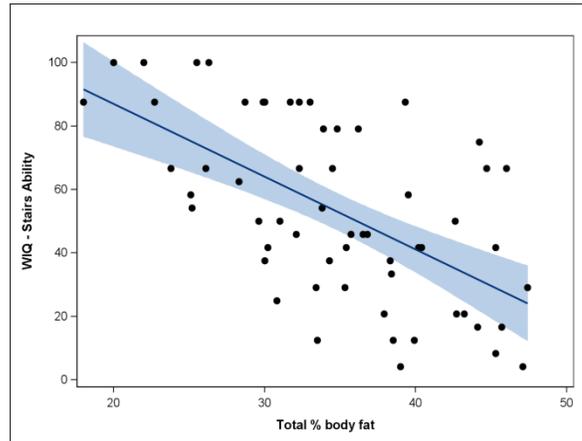
a) Estimate for the effect of 1 ng/mL increase in RBC folate on WIQ-Speed scores, -0.11 ± 0.02 , $p < 0.001$.



b) Estimate for the effect of 1 % increase in total body fat on WIQ-Overall scores, -1.45 ± 0.36 , $p = 0.0002$.

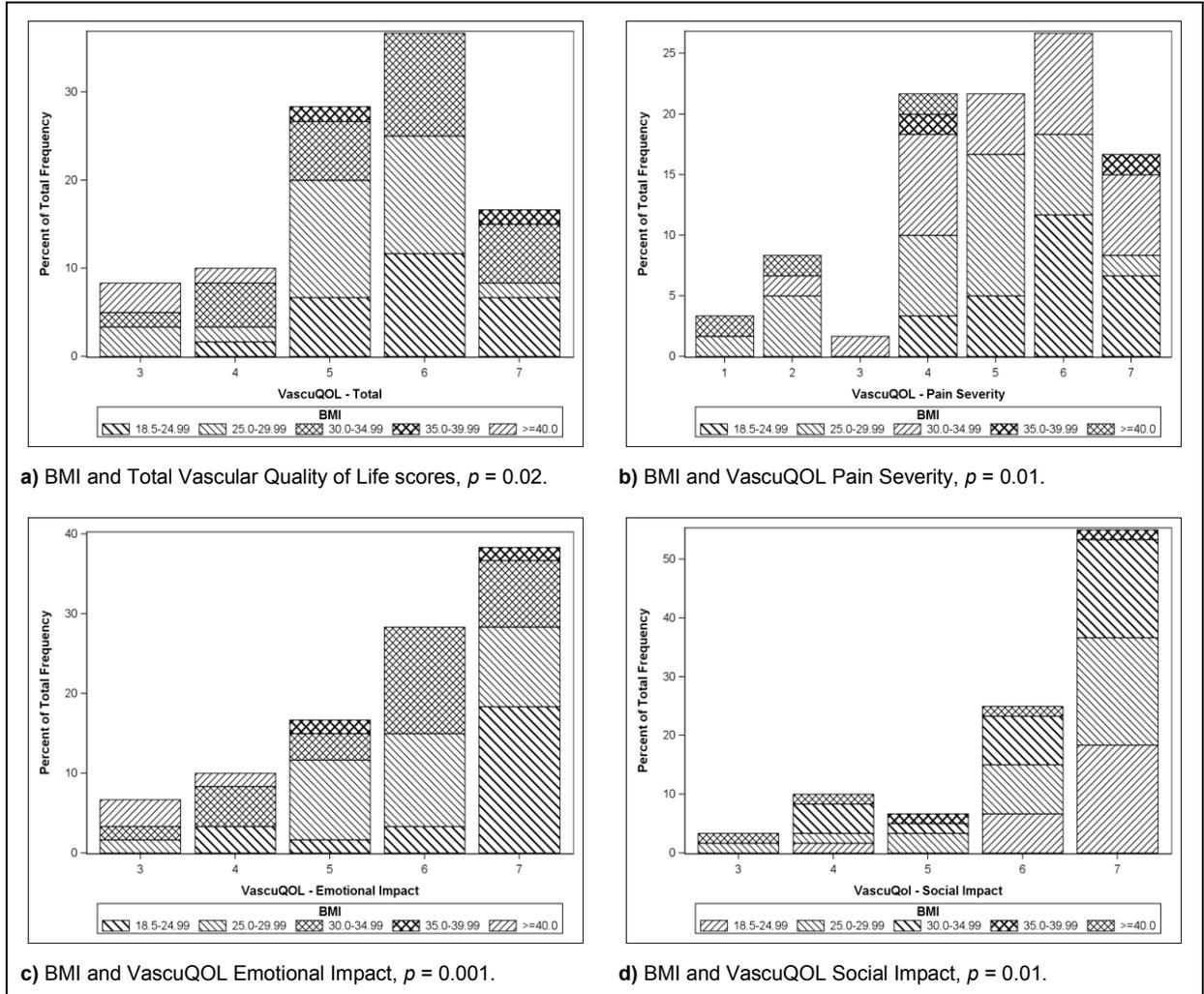


c) Estimate for the effect of 1 % increase in total body fat on WIQ-Speed scores, -1.58 ± 0.44 , $p = 0.0008$.



d) Estimate for the effect of 1 % increase in total body fat on WIQ-Stair Climbing scores, -2.86 ± 0.38 , $p < 0.0001$.

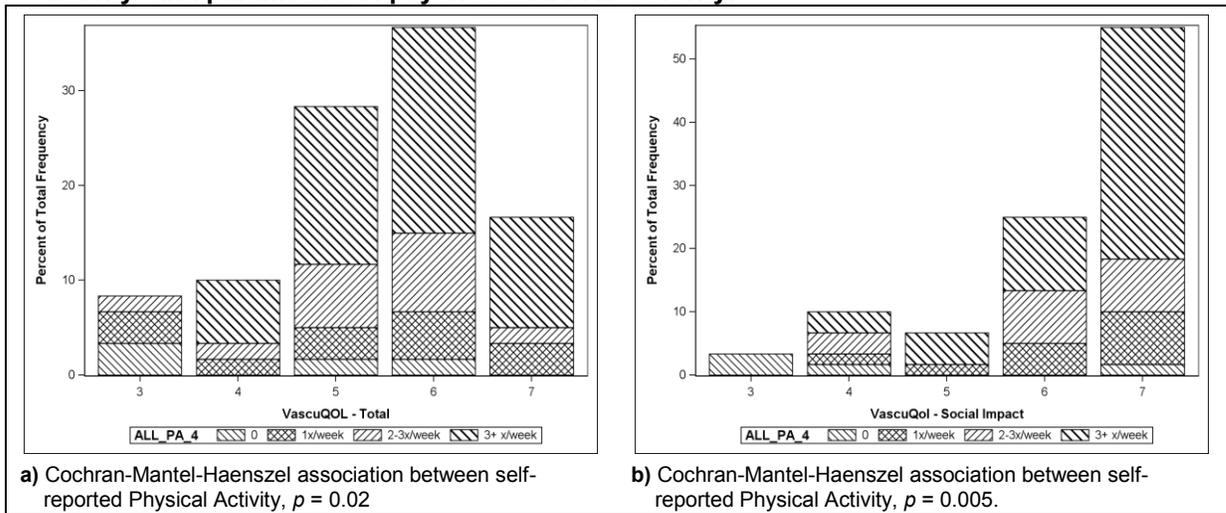
Figure 4. Selected Cochran-Mantel-Haenszel associations between BMI¹ and parameters of clinical and psychosocial PAD severity².



¹ Cut off points for BMI based on current World Health Organization definitions of normal weight, overweight, obesity and extreme obesity¹⁹⁸

² VascuQOL-Pain categorized as a parameter of clinical PAD severity; VascuQOL-Total, -Emotional Impact and -Social Impact categorized as parameters of psychosocial PAD severity.

Figure 5. Selected Cochran-Mantel-Haenszel associations between frequency of physical activity¹ and parameters of psychosocial PAD severity.



1 Physical Activity defined as aggregate of frequency of 15+ minute increments of Mild (e.g. easy walking, bowling, golf), Moderate (e.g. fast walking, baseball, tennis, easy bicyling, easy swimming, yoga) or Strenuous (e.g. running, vigorous swimming, long distance bicycling, soccer, football) physical activity.

Manuscript 3:

Recruitment challenges: obstacles to enrolling patients with peripheral arterial disease (PAD) for a feasibility study of nutrition and body composition.

Key words: recruitment, vascular disease, peripheral arterial disease, elderly, nutrition, diet, obstacles, challenges

A. Abstract

Objectives: To describe recruitment to a cross-sectional pilot study of nutritional status and body composition in patients with peripheral arterial disease (PAD). PAD is a group of syndromes characterized by both chronic and progressive atherosclerosis with a high burden of cardiovascular morbidity and mortality.

Methods: Potential subjects were identified either from hospital billing records or referred directly by vascular clinics. Patients were invited to participate, and recruited subjects were asked to come to a research clinic for data collection.

Results: Between May 2012 and April 2013, 1,446 patients were identified. One hundred sixty-five patients (11.4%) responded to recruitment requests. Of the responders, 34 (20.6%) were deemed ineligible, 20 (12.1%) declined to participate, and we were unable to sustain sufficient contact with 47 (28.5%) patients to complete the consent process. The final enrollment was 64 participants (64/1,446; 4.4%), and 4 subjects (6.3%) subsequently withdrew from the study prior to data collection.

Conclusions: Recruiting PAD patients for a nutrition study presents a variety of challenges, due largely to the burdens of living with PAD and coexistent illnesses, and patients' reluctance or inability to travel for research.

B. Introduction

Definitions and Prevalence

Peripheral Arterial Disease (PAD), also known as Peripheral Vascular Disease (PVD) comprises a range of vascular syndromes. Characterized by arteriosclerosis in

vasculature other than the heart or brain, the term most commonly refers to atherosclerotic disease in the infrarenal aorta and the lower extremities¹. PAD is essentially a disease of systemic inflammation. Fatty plaque deposits on arterial walls diminish or block blood flow, which can lead to thrombosis or thrombotic embolism¹. Similarly, thinning and dilation of the lumen or dysplastic thickening may also impair blood flow, eventually forming aneurysms¹. PAD is the most common cause of limb amputation, and regardless of localization or type of stenosis, all PAD syndromes are associated with a significant risk of cardiovascular events, including infarction and stroke^{1,12}. PAD patients are also at a significantly increased risk of cardiovascular disease (CVD)-related mortality, with rates exceeding those for CVD alone^{23,29}.

At any point, up to 14% of Americans have PAD, and it is estimated that there are over 10 million sufferers in all of North America^{16,199,200}. Accurate data are difficult to collect, however, as half to two-thirds of affected people are asymptomatic prior to an ischemic event¹⁴, and prevalence estimates do not account for people whose clinical measures fall on the cusp of official diagnosis¹. Established risk factors for PAD include smoking, diabetes mellitus, dyslipidemia, hypertension, obesity and a family history of CVD or PAD^{4,5}. As a result, patients experience a high frequency of comorbid conditions that can exacerbate atherosclerotic progression^{1,201}. Finally, the health cost burden of PAD is also significant. Over 21 billion dollars are spent annually on treatment in the U.S., which surpasses CVD treatment expenditures by up to 23%^{23,29}.

Treatment Approaches

Clinical guidelines for PAD treatment center around two concerns: improving patients' quality of life, and decreasing the risk of incident CVD events^{1,12}. Clinicians often initiate treatment with pharmacological interventions, and these focus on mitigating the pain and impaired walking ability that are hallmarks of PAD, and on modifying risk factors that contribute to ischemic risk. The former may be treated with analgesics, while

the latter are treated with medications such as lipid-lowering statins, anti-thrombotics and glycemic control drugs for diabetics. Additionally, depending on a patient's age, vascular anatomy and comorbid conditions, clinicians will employ revascularization procedures to restore blood flow in affected arteries. Revascularization may take the form of less-invasive endovascular procedures, for instance balloon angioplasty or thrombolysis, or more invasive open surgery such as bypass grafting or necrotic tissue resection^{1,12,201}.

Nevertheless, PAD is a progressive illness, and the benefits of revascularization are generally not durable, regardless of treatment type¹⁵⁴. Additionally, revascularization procedures frequently result in complications that require repeated intervention, and further contribute to disease and health cost burdens¹⁵⁵. A recent clinicians' conference²⁹ reported that PAD is generally underdiagnosed and undertreated, and that an emphasis on late-stage treatment has created gaps in research relative to other vascular diseases. Consequently, there is an emerging body of research in lifestyle-based therapies for preventing or treating PAD, particularly exercise²⁵⁻²⁷, and to a smaller degree nutrition¹⁷⁷.

Obstacles to Research

In the nascent area of nutrition research, studies of PAD patients have largely relied on subpopulations of epidemiological cohorts¹⁷⁷. These studies reported generalized observations of eating habits and dietary patterns, and broadly delineated some nutrients of interest. More specific investigations of patients' nutritional status, however, have come across significant challenges. Sample sizes are small, ranging from 16⁹² to 232⁴² in cross-sectional, prospective and randomized trial studies alike¹⁷⁷. PAD patients tend to be older, have multiple comorbidities and have diminished physical mobility^{1,24}, all of which create a population that is difficult to recruit and retain.

Small sample sizes limit the validity of nutrition studies in PAD research. Internal biases, such as convenience sampling and self-selection bias by healthier patients

hinder the generalizability of study results to a wider population. The inability to recruit sufficiently large cohorts also has implications for clinical practice. Small sample sizes limit the ability to detect potentially significant associations and outcomes, calling into question whether taking advantage of patients' time and investigators' resources is useful or ethical. Describing obstacles to recruitment and retention is an important step in expanding the body of research on nutrition and PAD, and is crucial to developing evidence-based recommendations for clinical practice. In this paper, we describe the experiences and obstacles encountered while recruiting patients for a cross-sectional study of nutritional status, body composition and PAD severity.

C. Methods and Analysis

Recruitment and data collection took place simultaneously and lasted 12 months, from May 2012 to April 2013. Inclusion criteria were: a clinical diagnosis of PAD, at least one intact leg, English proficiency, consistent access to a telephone and the ability to procure one's own transportation to and from the research clinic. Initially, all identified patients were considered eligible for recruitment regardless of revascularization status, however patients were excluded if they had undergone revascularization procedures within the previous 6 months. In an effort to delineate more precisely the associations between nutrition, body composition and PAD severity, recruitment criteria were modified at the 6-month mark to prioritize patients with native vasculature. Exclusion criteria included: unconfirmable PAD diagnosis, lack of English literacy, lack of transportation, as well as any patients who were double leg amputees, were wheelchair-bound, or had diminished ability to provide informed consent due to dementia, Alzheimer's disease or other cognitive limitations.

Patients who met all inclusion criteria were invited to participate via telephone or letter, and completed the consent process in person at the research clinic. Each participant was asked to complete one clinic visit, and two telephone dietary interviews.

Table 1 presents data collection variables. Participants were asked to fast for a minimum of 8 hours prior to their clinic visits, and were offered a full breakfast immediately after completing a blood draw. As compensation for their time, participants were given modest financial reimbursement after the clinic visit, and again after completing both telephone dietary interviews. Consent forms stipulated that if blood tests indicated any abnormality (e.g. vitamin D deficiency), patients would be notified and given information to convey to their physicians.

All data were examined for completeness and accuracy. Outcome variables were selected for biological plausibility and classified into two categories; PAD severity as a function of clinical signs/symptoms and PAD severity as a function of psychosocial factors. Continuous exposure variables were analyzed against outcomes using linear regression modeling. Spearman's correlation coefficients were generated to evaluate monotonic relationships. Ordinal variables were analyzed using Cochran-Mantel-Haenszel tests and proportional-odds cumulative logit modeling.

D. Results

A university-affiliated health care organization conducted a database search of all patients with PAD-related billing entries in the greater Minneapolis/St. Paul area. A total of 1,374 people was identified, and each patient was mailed a recruitment packet consisting of contact information, a summary of the study, transportation directions and a consent form. Additionally, vascular clinicians from three area hospitals screened and referred PAD patients to the study. Clinicians personally provided information to patients they deemed eligible candidates for participation, and with a patient's consent, his/her contact information was provided to the study coordinator. Seventy-two patients were identified using this method, yielding a total of 1,446 potential participants.

Of the 1,374 PAD patients mailed recruitment packets, 93 (6.8%) contacted the study coordinator. Including the 72 patients referred by clinicians, recruitment was

attempted with a total of 165 patients, or 11.4% of the initial pool. Of these, 39 (23.6%) declined to participate. All reasons for non-participation are presented in **Table 2**. Most frequent reasons were either an inability to maintain contact with patients to proceed with obtaining consent and scheduling, or difficulty with transportation to the research site. An additional 28 (17%) patients could not be contacted at all, and 34 (20.6%) patients were considered ineligible. A total of 64 patients consented to participation, and four participants did not attend their scheduled visits. Follow up telephone calls were made to establish reasons for non-attendance; 2 participants had difficulty with driving to or finding the clinic, and 2 declined to give a reason. Of the 60 remaining participants, 25 (41.7%) completed the clinic visit and both telephone interviews. Two patients completed only one additional telephone interview, and 33 (55%) solely attended the clinic portion of the study. Upon finishing their clinic visits, 3 of the 35 non-completes declined to take part in telephone interviews, but gave no reason for doing so. The remaining 32 participants were considered non-completes as they could not be reached after repeated follow up attempts. There were no significant differences (clinical or otherwise) between patients who completed the study, partially completed or dropped out.

E. Discussion

This study underscores the difficulties inherent in recruiting PAD patients for a study of nutrition and body composition. Patients were excluded primarily for having a history of revascularization (if recruited after the 6-month midpoint of the study) or for being unable to confirm their PAD diagnoses. Among the unconfirmable cases, 2 categorically denied having a PAD diagnosis, whereas the remaining patients stated that they “likely” or “probably” had been diagnosed with PAD, but were struggling at the time with other chronic illnesses. These patients could not recall if their clinicians had mentioned PAD to them when discussing their various treatments, but when asked to elaborate further, reported having PAD-like symptoms (intermittent claudication or critical

limb ischemia¹³). This observation has been reported in other papers, where a high prevalence of comorbid conditions coupled with patients' lack of familiarity with PAD result in a more limited pool of eligible subjects^{24,201}. More recently, an evaluation of strategies for recruiting Latino PAD patients for an exercise intervention encountered similar difficulties²⁰². Throughout our recruitment efforts, potential participants reported a variety of comorbidities that influenced their decision whether or not to participate. Patients expressed that diminished physical function due to CVD, diabetic neuropathy, emphysema, COPD and after-effects of strokes and related surgeries contributed to a disease burden that made the daily business of living difficult, including those patients that consented to participation.

The second key reason for non-participation related to transportation. Patients who declined to participate for this reason stated that they either: One, physically felt unable to drive and did not have a friend or relative to drive them; Two, considered themselves able drivers but felt anxious about navigating an urban area; or Three, said they were able drivers, but deemed making the trip to participate in a study to be too burdensome. While there is a wide-ranging network of public transportation in the Minneapolis/Saint Paul metro area, very few potential subjects were willing to consider utilizing this option.

It is difficult to discern to what extent transportation was an obstacle due to patients' disease burden or because of other factors. An important alternate factor may be age. PAD and other vascular diseases tend to affect people aged 50 and older, but many patients are over the age of 65¹⁶. As a result, the declining physical mobility and cognitive function that are characteristic of aging may have a substantial impact on patients' receptiveness to recruitment. Several reviews have concluded that older adults comprise a uniquely challenging population in study recruitment, and are therefore significantly under-represented in clinical research²⁰³⁻²⁰⁷. A whole range of factors

shapes this dynamic, but most common among them are the aforementioned issue of transportation, as well as the difficulty of identifying and contacting potential subjects who are functionally limited and/or homebound. Moreover, a general mistrust of interacting with strangers or medical professionals is a recurring observation. Reviewers reported that older patients tend to approach people unknown to them with suspicion, and frequently feel wary of sharing personal information with researchers^{203-205,207}. Lastly, older patients regularly express a misperception regarding the purpose of research studies²⁰⁶, and many confusedly view participation as an optional or unnecessary component of clinical treatment²⁰⁷.

It is possible that a combination of these reasons prevented the majority of identified PAD patients (93.2%) from considering participation in this study. There is an overall consensus that the existing body of literature has provided scant or incomplete descriptions of techniques used for recruiting older participants²⁰³⁻²⁰⁸, further limiting the ability to identify the methods that may be most efficacious. Nevertheless, there are approaches that future researchers may use to improve recruitment. Identifying and contacting older subjects in statistically significant numbers requires an especially large input of resources, manpower and time²⁰⁴. Enlisting the assistance of physicians that directly interact with patients can be an important source of participants^{206,207}. Physicians contend with a heavy workload and may not be willing or able to use time with patients to relay information about a research study, particularly when they feel they have no personal incentive to do so^{206,209}. Consideration should be given to establishing a mutually beneficial partnership with health care providers and organizations – one that encourages clinicians to incorporate study recruitment into daily interaction with patients, while researchers share findings in an immediately translatable and clinically relevant manner. Such an approach may be more effective than mail-outs to patients identified

via hospital billing records – a method that ultimately did not produce a high yield of recruits in this study.

During the planning stage, recruitment of older subjects should also incorporate input from the target population itself. Focus groups and clinic-based interviews of older patients can delineate what methods and inducements may be most likely to attract potential subjects, and create a greater sense of researcher-subject cooperation in the process^{203,205}. Piloting the recruitment strategy with a small representative sample can then help refine the study plan further²⁰⁵, and clarify issues with written materials, consent forms, etc. that may be burdensome or convoluted for older patients²⁰⁷. Finally, consideration should also be given to structuring studies so that recruitment and data collection take place in the same location, preferably the same locations where patients receive regular care, thereby mitigating difficulties with transportation.

The racial/ethnic makeup of the target population may be a less apparent, but possibly significant reason for our difficulty recruiting PAD patients. Minorities in general and African Americans in particular carry a disproportionately higher burden of both PAD and CVD, with greater rates of limb amputation and vascular-related mortality than their Caucasian counterparts^{20,21,147}. This study recruited subjects from a racially diverse region – over 17% of the metro-area population belong to a minority race or ethnicity, primarily Latinos and African Americans²¹⁰. Nonetheless, out of 60 participants none were Latino, and 6 identified as black/African American. While this constitutes 10% of the cohort, oversampling African Americans was an important study goal that remained unmet.

There is a variety of explanations for this outcome. Both public health and clinical care institutions are generally unaware of the importance of screening for PAD and its highly prevalent risk factors⁴. For minority groups like African Americans, however, this phenomenon is exacerbated further. Evaluations of racial disparities in research have

observed that African Americans with PAD make use of medical care less frequently than Caucasians, and if diagnosed, tend to receive less comprehensive treatment^{21,211,212}. Parsing the causes, socioeconomics and otherwise, of systemic racial/ethnic disparities in medical treatment is beyond the scope of this paper. Nonetheless, in regard to recruiting minorities for a PAD study, the recurring issue of mistrust may be especially pertinent. Various studies have concluded that past abuses of research subjects and culturally-imbedded mistrust of physicians disinclines African Americans and other minorities from participating in clinical research²¹³⁻²¹⁵. Older minorities are also markedly wary of interacting with researchers²¹⁶, and mistrust arising from both advanced age and race/ethnicity may have discouraged the PAD patients contacted for this study from being receptive to recruitment.

Even so, there is evidence to suggest that this population's distrust of clinicians and researchers may be effectively mitigated in future studies. Communication is a primary concern; studies have reported that minorities feel physicians are frequently vague in their communication to patients and provide insufficient information about their illnesses and treatments^{213,217}. When asked to participate in studies, minorities have stated that they are inclined to think researchers will intentionally withhold important details of study involvement^{214,216}, and even subject participants to unnecessary risks²¹⁴. The process of informed consent may be perceived as confusing and misleading as well²¹⁸. In order to address these concerns, more recent studies have attempted other methods with measurable success. Clinical studies of African Americans and Latinos reported that community-based participatory research (CBPR)²¹⁹, in which community representatives are involved in study design, recruitment and implementation, have yielded significant rates of recruitment and participation²²⁰⁻²²³. As a non-traditional approach, CBPR bypasses institution-based research and brings data collection efforts to subjects' home areas. In doing so, transportation difficulties are also largely avoided.

Furthermore, participants have reported feeling personally invested in the success of such studies²²⁰, and have helped to modify study materials so that they are culturally tailored to the target population²²². A CBPR-like approach may be effective in PAD research, regardless of patients' racial/ethnic background, and should be considered as a viable option in future studies.

Finally, mental health may also be a significant mediator of study recruitment. There is considerable evidence that mood disorders, particularly depression, are significantly associated with CVD. Prevalence studies have observed that having depressive symptoms is an independent risk factor for CVD and CVD events^{185,224-227}. The Nurses' Health Study reported that depression was a significant risk factor for CVD-related mortality in women²²⁸, and an assessment of Australian patients found that self-reported mental health was a greater predictor of CVD outcomes than self-reported physical health²²⁹.

PAD studies have reached comparable conclusions^{184,230}. A large epidemiological study of 1,024 CVD patients observed a significant positive association between depressive symptoms and both prevalent PAD and ensuing PAD events¹⁸⁶. Remes et al's²³¹ case-control assessment of 131 PAD patients similarly reported significantly worse scores on depressive symptom surveys compared to matched healthy controls. Studies of PAD patients' physical function also mirror these findings; depressive symptoms are associated with greater pain and decreased maximum walking ability²³², as well as a faster rate of functional decline in depressive versus non-depressed patients²³³. Lastly, investigators reported that depressive patients who have undergone revascularization treatment consistently report worse post-procedural symptoms²³⁴, and experience a higher incidence of CVD events and mortality than non-depressives²³⁵.

Beyond the worsened mood states that can accompany physical decline in people with PAD, there is much that is still unknown about the role of mental health in treating the disease, and to date there have been no studies of mental health in PAD recruitment. Whereas the American Heart Association recommends screening for depression in all vascular patients, this guideline is consistently disregarded and mood disorders largely remain untreated²²⁵. Such a dynamic need not continue, however, and consideration should be given in future PAD studies to screen potential subjects for depression, regardless of whether they have a previously-established clinical diagnosis. If as a result it becomes evident that depressive patients largely decline study participation, such an observation should form the basis for revising recruitment approaches, possibly by collaborating with mental health clinicians and social workers who specialize in elderly populations, and who are trained to provide mental health care.

Recently, a small intervention trial found that after 2 months of home-based, self-administered mood therapy education, 13 PAD patients experienced a significant improvement in depressive symptoms and overall mental health²³⁶. Such a finding is preliminary and must be replicated with larger cohorts. Nevertheless, it offers the possibility that addressing mental health issues in a non-clinical setting may be beneficial both to patients and for recruitment efforts. Assessments of the homebound elderly additionally concluded that this population (which is more likely to suffer from depression than able-bodied counterparts), experiences very limited improvement in mood using pharmacological treatment²³⁷, which further supports the use of alternative methodologies.

F. Summary and Conclusion

This paper evaluated the feasibility of recruiting vascular patients for a study of nutritional status, body composition and PAD severity. Primary obstacles to recruiting a statistically significant number of participants included patients feeling burdened by their

symptoms and coexistent chronic illnesses, and difficulties with using or obtaining transportation. As a cross-sectional viability investigation, this study had relatively limited resources and was unable to employ multi-site recruitment. Nevertheless, data collection was completed and results of patients assessments are presented elsewhere.

Challenges in recruiting and retaining subjects should be addressed in future studies, and there is a body of evidence that offers potentially useful strategies. Consideration should be given to community- and home-based recruitment, particularly in cooperation with community representatives. Special emphasis should be placed on collecting stakeholders' feedback on how best to approach potential participants and how to tailor study materials to maximize clarity and ease of use. Whenever possible, data collection must take place either in subjects' home environments or in the clinical settings where they already receive regular care. Such an approach can alleviate difficulties with transportation, and help build a more fruitful partnership with clinical organizations .

Moreover, if recruitment takes place in health care settings that predominantly serve racial and ethnic minorities, this will allow for data collection from a more diverse cohort.

Finally, in order to address the significant issue of depressive mood disorders in patients, PAD researchers should give strong consideration to partnering with mental health care providers and specialized social workers. While these strategies will still limit recruitment to the symptomatic, diagnosed sub-population PAD patients, they will contribute to a more efficient and effective methodology.

Table 1. Variables of interest in a cross-sectional study of nutritional status, body composition and disease severity in patients with peripheral arterial disease (PAD).

Category	Variable	Measurement
Biomarkers ^a	Vitamin D	Serum 25(OH)D
	Folic acid	Red blood cell folate
	Adiponectin	Serum adiponectin
	MTHFR activity	<i>MTHFR</i> genotype
Body composition	Body mass	BMI (kg/m ²)
	Abdominal adiposity	Waist, hip circumferences
	Total adiposity	Whole-body DXA scan
PAD severity ^b	Peripheral blood flow	Ankle-Brachial Index (ABI)
	Walking ability	Walking Impairment Questionnaire (WIQ)
	Pain, related symptoms	WIQ + Vascular Quality of Life Survey (VascuQOL)
	Psychosocial stressors	VascuQOL
Diet and nutrition	Nutrient intakes	Nutrition Data System for Research (NDSR) ^c
	Eating habits	
Other	Demographics ^d	Surveys
	Medical history ^e	

a Serum 25(OH)D and red blood cell folate are validated markers of long-term dietary status^{169,182}; MTHFR, Methylene tetrahydrofolate reductase - an enzyme necessary to folate metabolism

b ABI is the standard diagnostic tool for PAD¹; WIQ and VascuQOL questionnaires are validated surveys of symptoms, walking ability and quality of life in people with vascular disease^{163,164}

c NDSR is a standardized, validated program for collecting 24-hour dietary recall data^{161,162}

d Demographic variables included questions about age, race/ethnicity, income, education and marital status

e Medical history variables included smoking history, frequency and type of physical activity and history of diagnoses of CVD, obesity, pulmonary conditions, cancer, hypertension and diabetes mellitus

Table 2. Reasons for non-participation.

Classification (<i>n</i> = 77)	Reason Detail	<i>n</i>
Expressed interest (<i>n</i> = 23)	Could not be reached after initial contact	19
	Consented, but dropped out prior to data collection	4
Not interested (<i>n</i> = 20)	Unable or unwilling to procure transportation	11
	Too busy, work responsibilities	2
	No reason given	7
Ineligible (<i>n</i> = 34)	History of revascularization(s)	14
	Unconfirmable PAD diagnosis	11
	Double leg amputee	3
	Dementia / Alzheimer's disease	2
	Wheelchair-bound	1
	Recent bodily injury	1
	Lack of English proficiency	1
	Deceased between initial contact and follow up	1

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Appendix A. Demographic Survey

Demographics & Medical History

General Questions About You:

1.

What is your **gender**?

Male

Female

<input type="checkbox"/>	1
<input type="checkbox"/>	2

2.

What is your **age**? _____ years

3.

What is the highest level of **education** you have completed?

Some high school

High school graduate

Some college

Technical/vocational training

College graduate

Postgraduate degree

<input type="checkbox"/>	1
<input type="checkbox"/>	2
<input type="checkbox"/>	3
<input type="checkbox"/>	4
<input type="checkbox"/>	5
<input type="checkbox"/>	6

4.

What is your total yearly household **income**?

Less than \$10,000

\$10,000 to \$19,999

\$20,000 to \$39,999

\$40,000 to \$59,999

\$60,000 to \$79,999

\$80,000 to \$99,999

\$100,000 or more

<input type="checkbox"/>	1
<input type="checkbox"/>	2
<input type="checkbox"/>	3
<input type="checkbox"/>	4
<input type="checkbox"/>	5
<input type="checkbox"/>	6
<input type="checkbox"/>	7

5.

What is your current **marital status**?

Single, never married

Married

Separated

Divorced

Widowed

<input type="checkbox"/>	1
<input type="checkbox"/>	2
<input type="checkbox"/>	3
<input type="checkbox"/>	4
<input type="checkbox"/>	5

6.

What is your **race/ethnicity**?

- | | | |
|-----------------------------------|--------------------------|---|
| Caucasian | <input type="checkbox"/> | 1 |
| Hispanic / Latino | <input type="checkbox"/> | 2 |
| African-American | <input type="checkbox"/> | 3 |
| Native American | <input type="checkbox"/> | 4 |
| African (Somalia, Ethiopia, etc.) | <input type="checkbox"/> | 5 |
| Asian | <input type="checkbox"/> | 6 |
| East Indian | <input type="checkbox"/> | 7 |
| Middle Eastern | <input type="checkbox"/> | 8 |
| Other _____ | <input type="checkbox"/> | 9 |

Regarding Your Medical History and Personal Habits:

1.

On average, how many **alcoholic beverages** do consume in one week?

(**Note:** One drink = 1 can or bottle of beer, one shot of liquor, or 4 ounces of wine)

- | | | |
|--------------|--------------------------|---|
| 0 | <input type="checkbox"/> | 1 |
| 1 - 3 | <input type="checkbox"/> | 2 |
| 4 - 6 | <input type="checkbox"/> | 3 |
| 7 - 10 | <input type="checkbox"/> | 4 |
| More than 10 | <input type="checkbox"/> | 5 |

2.

Which statement best describes your **cigarette smoking** habits?

- | | | |
|---|--------------------------|---|
| I have never smoked. | <input type="checkbox"/> | 1 |
| I used to smoke, but don't anymore. | <input type="checkbox"/> | 2 |
| I currently smoke, but only occasionally. | <input type="checkbox"/> | 3 |
| I smoke on a daily basis. | <input type="checkbox"/> | 4 |

3.

Considering an average week, how often do you do **strenuous exercise** (heart beats rapidly) for **more than 15 minutes**?

(examples: running, vigorous swimming, long distance bicycling, soccer, football)

- | | | |
|----------------------------|--------------------------|---|
| Never | <input type="checkbox"/> | 1 |
| Once per week | <input type="checkbox"/> | 2 |
| 2 to 3 times per week | <input type="checkbox"/> | 3 |
| More than 3 times per week | <input type="checkbox"/> | 4 |

4.

Considering an average week, how often do you do **moderate exercise** (not exhausting) for **more than 15 minutes**?

(examples: fast walking, baseball, tennis, easy bicycling, easy swimming, yoga)

- | | | |
|----------------------------|--------------------------|---|
| Never | <input type="checkbox"/> | 1 |
| Once per week | <input type="checkbox"/> | 2 |
| 2 to 3 times per week | <input type="checkbox"/> | 3 |
| More than 3 times per week | <input type="checkbox"/> | 4 |

5.

Considering an average week, how often do you do **mild exercise** (minimal effort) for **more than 15 minutes**? (examples: easy walking, bowling, golf)

- | | | |
|----------------------------|--------------------------|---|
| Never | <input type="checkbox"/> | 1 |
| Once per week | <input type="checkbox"/> | 2 |
| 2 to 3 times per week | <input type="checkbox"/> | 3 |
| More than 3 times per week | <input type="checkbox"/> | 4 |

6.

What **medications for PAD** have you taken (in the past or currently)?

- | | | |
|---|--------------------------|---|
| Prescription blood thinners | <input type="checkbox"/> | 1 |
| Cholesterol-lowering drugs | <input type="checkbox"/> | 2 |
| Over-the-counter blood thinners (aspirin) | <input type="checkbox"/> | 3 |

7.

What **surgeries** have you had to treat your PAD?

- | | | |
|--------------------------------|--------------------------|---|
| None | <input type="checkbox"/> | 1 |
| Stent (one) | <input type="checkbox"/> | 2 |
| Stents (more than one) | <input type="checkbox"/> | 3 |
| Amputation | <input type="checkbox"/> | 4 |
| Stents & amputation | <input type="checkbox"/> | 5 |

8.

Have you ever been diagnosed with any of the following? (mark all that apply)

- | | | |
|--|--------------------------|---|
| Heart disease | <input type="checkbox"/> | 1 |
| Stroke | <input type="checkbox"/> | 2 |
| High blood pressure | <input type="checkbox"/> | 3 |
| Diabetes (type 2) | <input type="checkbox"/> | 4 |
| Insulin resistance / Pre-diabetes | <input type="checkbox"/> | 5 |
| Emphysema | <input type="checkbox"/> | 6 |
| COPD (Chronic Obstructive Pulmonary Disease) | <input type="checkbox"/> | 7 |
| Cancer | <input type="checkbox"/> | 8 |
| Obesity | <input type="checkbox"/> | 9 |

PLEASE STOP HERE

Appendix B. BMI, Waist/Hip Circumferences and ABI Data Sheet

BMI

HT _____ cm	WT _____ kg	BMI _____
WC _____ cm HC _____ cm		

ABI

Arms	1st			2nd			Average				
Right										Highest Average Arm: R L	<u>Box A</u>
Left											
Ankles	1st			2nd			Average				
Right DP										Highest RIGHT Average Ankle: DP PT	<u>Box B</u>
Right PT											
Left DP										Highest LEFT Average Ankle: DP PT	<u>Box C</u>
Left PT											

Box B _____ ÷ **Box A** _____ = **Right**
ABI

Box C _____ ÷ **Box A** _____ = **Left**
ABI

Notes: _____

Appendix C. Walking Impairment Questionnaire

Walking Impairment Questionnaire (WIQ)

Walking Impairment: These questions ask about the reasons **why you are having difficulty walking**. We would like to know how much difficulty you had walking during the past week. By difficulty, we mean how hard it was or how much physical effort it took to walk because of each of these problems.

	Pain Severity	No Difficulty	Slight Difficulty	Some Difficulty	Much Difficulty	Unable To Do	Didn't Do For Other Reasons
1	Pain, stiffness or aching in your joints (ankles, knees or hips)?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
2	Weakness in one or both of your legs?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
3	Pain or discomfort in your chest?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
4	Shortness of breath?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
5	Heart palpitations?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
6	Other problems? (Please list).	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>

Walking Distance: Report the degree of physical difficulty that best describes how hard it was for you to walk on **level ground without stopping to rest** for each of the following distances during the last week.

	Distance	No Difficulty	Slight Difficulty	Some Difficulty	Much Difficulty	Unable To Do	Didn't Do For Other Reasons
1	Walking indoors, such as around your home?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
2	Walking 50 feet?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
3	Walking 150 feet (½ block)?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
4	Walking 300 feet (1 block)?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
5	Walking 600 feet (2 blocks)?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>

6	Walking 900 feet (3 blocks)?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
7	Walking 1500 feet (5 blocks)?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>

Walking Speed: Report the degree of difficulty that best describes how hard it was for you to walk one city block on level ground at each of these speeds without stopping to rest during the last week.

	Speed	No Difficulty	Slight Difficulty	Some Difficulty	Much Difficulty	Unable To Do	Didn't Do For Other Reasons
1	Walking one block slowly?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
2	Walking one block at an average speed?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
3	Walking one block quickly?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
4	Running or jogging one block?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>

Stair Climbing: For each of these questions, report the degree of physical difficulty that best describes how hard it was for you to climb stairs without stopping to rest during the past week.

	Stairs	No Difficulty	Slight Difficulty	Some Difficulty	Much Difficulty	Unable To Do	Didn't Do For Other Reasons
1	Climbing one flight of stairs?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
2	Climbing two flights of stairs?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>
3	Climbing three flights of stairs?	<input type="checkbox"/> 4	<input type="checkbox"/> 3	<input type="checkbox"/> 2	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/>

Appendix D. Walking Impairment Questionnaire (WIQ) Scoring

Pain	Weight	Denominator
Question 1	2	
Question 2	2	
Question 3	3	88
Question 4	4	
Question 5	5	
Question 6	6	
Distance		
Question 1	20	
Question 2	50	
Question 3	150	
Question 4	300	14,080
Question 5	600	
Question 6	900	
Question 7	1,500	
Speed		
Question 1	2	
Question 2	2	46
Question 3	3	
Question 4	5	
Stairs		
Question 1	1	
Question 2	2	24
Question 3	3	

Appendix E. Vascular Quality of Life Questionnaire (VascuQOL)

Vascular Quality of Life Questionnaire (VascuQOL)

Instructions: These questions ask you how you have been affected by poor circulation to your legs over the last two weeks. You will be asked about the symptoms you have had, the way that your activities have been affected and how you have been feeling. Please read each bit of the answer and then tick the one that applies best to you. If you are unsure about how to answer a question, please give the best answer you can.

There is no right or wrong answer.

Please answer every question. Thank you.

1. In the last two weeks I have had pain in the leg (or foot) when walking

(tick one)

- | | |
|---------------------------|----------------------------|
| 1. All of the time | <input type="checkbox"/> 1 |
| 2. Most of the time | <input type="checkbox"/> 2 |
| 3. A good bit of the time | <input type="checkbox"/> 3 |
| 4. Some of the time | <input type="checkbox"/> 4 |
| 5. A little of the time | <input type="checkbox"/> 5 |
| 6. Hardly any of the time | <input type="checkbox"/> 6 |
| 7. None of the time | <input type="checkbox"/> 7 |

2. In the last two weeks I **have been worried that I might injure my leg**

(tick one)

- | | |
|---------------------------|----------------------------|
| 1. All of the time | <input type="checkbox"/> 1 |
| 2. Most of the time | <input type="checkbox"/> 2 |
| 3. A good bit of the time | <input type="checkbox"/> 3 |
| 4. Some of the time | <input type="checkbox"/> 4 |
| 5. A little of the time | <input type="checkbox"/> 5 |
| 6. Hardly any of the time | <input type="checkbox"/> 6 |
| 7. None of the time | <input type="checkbox"/> 7 |

3. In the last two weeks **cold feet have given me**

(tick one)

- | | |
|--|----------------------------|
| 1. A very great deal of discomfort or distress | <input type="checkbox"/> 1 |
| 2. A great deal of discomfort or distress | <input type="checkbox"/> 2 |
| 3. A good deal of discomfort or distress | <input type="checkbox"/> 3 |
| 4. A moderate amount of discomfort or distress | <input type="checkbox"/> 4 |
| 5. Some discomfort or distress | <input type="checkbox"/> 5 |
| 6. Very little discomfort or distress | <input type="checkbox"/> 6 |
| 7. No discomfort or distress | <input type="checkbox"/> 7 |

4. In the last two weeks, because of the poor circulation to my legs, **my ability to take exercise or to play any sports has been**

(tick one)

- | | | |
|--|--------------------------|---|
| 1. Totally limited, couldn't exercise at all | <input type="checkbox"/> | 1 |
| 2. Extremely limited | <input type="checkbox"/> | 2 |
| 3. Very limited | <input type="checkbox"/> | 3 |
| 4. Moderately limited | <input type="checkbox"/> | 4 |
| 5. A little limited | <input type="checkbox"/> | 5 |
| 6. Only very slightly limited | <input type="checkbox"/> | 6 |
| 7. Not at all limited | <input type="checkbox"/> | 7 |

5. In the last two weeks **my legs have felt tired or weak**

(tick one)

- | | | |
|---------------------------|--------------------------|---|
| 1. All of the time | <input type="checkbox"/> | 1 |
| 2. Most of the time | <input type="checkbox"/> | 2 |
| 3. A good bit of the time | <input type="checkbox"/> | 3 |
| 4. Some of the time | <input type="checkbox"/> | 4 |
| 5. A little of the time | <input type="checkbox"/> | 5 |
| 6. Hardly any of the time | <input type="checkbox"/> | 6 |
| 7. None of the time | <input type="checkbox"/> | 7 |

6. In the last two weeks, because of the poor circulation to my legs, **I have been restricted in spending time with my friends or relatives**

(tick one)

- | | |
|---------------------------|----------------------------|
| 1. All of the time | <input type="checkbox"/> 1 |
| 2. Most of the time | <input type="checkbox"/> 2 |
| 3. A good bit of the time | <input type="checkbox"/> 3 |
| 4. Some of the time | <input type="checkbox"/> 4 |
| 5. A little of the time | <input type="checkbox"/> 5 |
| 6. Hardly any of the time | <input type="checkbox"/> 6 |
| 7. None of the time | <input type="checkbox"/> 7 |

7. In the last two weeks **I have had pain in the foot (or leg) after going to bed at night**

(tick one)

- | | |
|---------------------------|----------------------------|
| 1. All of the time | <input type="checkbox"/> 1 |
| 2. Most of the time | <input type="checkbox"/> 2 |
| 3. A good bit of the time | <input type="checkbox"/> 3 |
| 4. Some of the time | <input type="checkbox"/> 4 |
| 5. A little of the time | <input type="checkbox"/> 5 |
| 6. Hardly any of the time | <input type="checkbox"/> 6 |
| 7. None of the time | <input type="checkbox"/> 7 |

8. In the last two weeks **pins and needles or numbness in my leg (or foot)** have caused me

(tick one)

- | | | |
|--|--------------------------|---|
| 1. A very great deal of discomfort or distress | <input type="checkbox"/> | 1 |
| 2. A great deal of discomfort or distress | <input type="checkbox"/> | 2 |
| 3. A good deal of discomfort or distress | <input type="checkbox"/> | 3 |
| 4. A moderate amount of discomfort or distress | <input type="checkbox"/> | 4 |
| 5. Some discomfort or distress | <input type="checkbox"/> | 5 |
| 6. Very little discomfort or distress | <input type="checkbox"/> | 6 |
| 7. No discomfort or distress | <input type="checkbox"/> | 7 |

9. In the last two weeks **the distance I can walk has improved**

(tick one)

- | | | |
|---|--------------------------|---|
| 1. Not at all (tick this if distance is unchanged or has decreased) | <input type="checkbox"/> | 1 |
| 2. A little | <input type="checkbox"/> | 2 |
| 3. Somewhat | <input type="checkbox"/> | 3 |
| 4. Moderately | <input type="checkbox"/> | 4 |
| 5. A good deal | <input type="checkbox"/> | 5 |
| 6. A great deal | <input type="checkbox"/> | 6 |
| 7. A very great deal | <input type="checkbox"/> | 7 |

10. In the last two weeks, because of the poor circulation to my legs, **my ability to walk has been**

(tick one)

- | | | |
|--|--------------------------|---|
| 1. Totally limited, couldn't walk at all | <input type="checkbox"/> | 1 |
| 2. Extremely limited | <input type="checkbox"/> | 2 |
| 3. Very limited | <input type="checkbox"/> | 3 |
| 4. Moderately limited | <input type="checkbox"/> | 4 |
| 5. A little limited | <input type="checkbox"/> | 5 |
| 6. Only very slightly limited | <input type="checkbox"/> | 6 |
| 7. Not at all limited | <input type="checkbox"/> | 7 |

11. In the last two weeks **being (or becoming) housebound has been a concern of mine**

(tick one)

- | | | |
|----------------------|--------------------------|---|
| 1. A very great deal | <input type="checkbox"/> | 1 |
| 2. A great deal | <input type="checkbox"/> | 2 |
| 3. A good deal | <input type="checkbox"/> | 3 |
| 4. Moderately | <input type="checkbox"/> | 4 |
| 5. Somewhat | <input type="checkbox"/> | 5 |
| 6. A little | <input type="checkbox"/> | 6 |
| 7. Not at all | <input type="checkbox"/> | 7 |

12. In the last two weeks **I have been concerned about having poor circulation to my legs**

(tick one)

- | | |
|---------------------------|----------------------------|
| 1. All of the time | <input type="checkbox"/> 1 |
| 2. Most of the time | <input type="checkbox"/> 2 |
| 3. A good bit of the time | <input type="checkbox"/> 3 |
| 4. Some of the time | <input type="checkbox"/> 4 |
| 5. A little of the time | <input type="checkbox"/> 5 |
| 6. Hardly any of the time | <input type="checkbox"/> 6 |
| 7. None of the time | <input type="checkbox"/> 7 |

13. In the last two weeks **I have had pain in the foot (or leg) when I am at rest**

(tick one)

- | | |
|---------------------------|----------------------------|
| 1. All of the time | <input type="checkbox"/> 1 |
| 2. Most of the time | <input type="checkbox"/> 2 |
| 3. A good bit of the time | <input type="checkbox"/> 3 |
| 4. Some of the time | <input type="checkbox"/> 4 |
| 5. A little of the time | <input type="checkbox"/> 5 |
| 6. Hardly any of the time | <input type="checkbox"/> 6 |
| 7. None of the time | <input type="checkbox"/> 7 |

14. In the last two weeks, because of the poor circulation to my legs, **my ability to climb stairs has been**

(tick one)

- | | | |
|--|--------------------------|---|
| 1. Totally limited, couldn't climb stairs at all | <input type="checkbox"/> | 1 |
| 2. Extremely limited | <input type="checkbox"/> | 2 |
| 3. Very limited | <input type="checkbox"/> | 3 |
| 4. Moderately limited | <input type="checkbox"/> | 4 |
| 5. A little limited | <input type="checkbox"/> | 5 |
| 6. Only very slightly limited | <input type="checkbox"/> | 6 |
| 7. Not at all limited | <input type="checkbox"/> | 7 |

15. In the last two weeks, because of the poor circulation to my legs, **my ability to take part in social activities has been**

(tick one)

- | | | |
|---|--------------------------|---|
| 1. Totally limited, couldn't socialise at all | <input type="checkbox"/> | 1 |
| 2. Extremely limited | <input type="checkbox"/> | 2 |
| 3. Very limited | <input type="checkbox"/> | 3 |
| 4. Moderately limited | <input type="checkbox"/> | 4 |
| 5. A little limited | <input type="checkbox"/> | 5 |
| 6. Only very slightly limited | <input type="checkbox"/> | 6 |
| 7. Not at all limited | <input type="checkbox"/> | 7 |

16. In the last two weeks, because of the poor circulation to my legs, **my ability to perform routine household work has been**

(tick one)

- | | | |
|---|--------------------------|---|
| 1. Totally limited, couldn't perform housework at all | <input type="checkbox"/> | 1 |
| 2. Extremely limited | <input type="checkbox"/> | 2 |
| 3. Very limited | <input type="checkbox"/> | 3 |
| 4. Moderately limited | <input type="checkbox"/> | 4 |
| 5. A little limited | <input type="checkbox"/> | 5 |
| 6. Only very slightly limited | <input type="checkbox"/> | 6 |
| 7. Not at all limited | <input type="checkbox"/> | 7 |

17. In the last two weeks **ulcers in the leg (or foot) have given me pain or distress**

(tick one)

- | | | |
|---|--------------------------|---|
| 1. All of the time | <input type="checkbox"/> | 1 |
| 2. Most of the time | <input type="checkbox"/> | 2 |
| 3. A good bit of the time | <input type="checkbox"/> | 3 |
| 4. Some of the time | <input type="checkbox"/> | 4 |
| 5. A little of the time | <input type="checkbox"/> | 5 |
| 6. Hardly any of the time | <input type="checkbox"/> | 6 |
| 7. None of the time (tick this if you do not have leg ulcers) | <input type="checkbox"/> | 7 |

18. Because of poor circulation to my legs, **the overall range of activities that I would have liked to do in the last two weeks has been**

(tick one)

- | | | |
|---|--------------------------|---|
| 1. Severely limited – most activities not done | <input type="checkbox"/> | 1 |
| 2. Very limited | <input type="checkbox"/> | 2 |
| 3. Moderately limited – several activities not done | <input type="checkbox"/> | 3 |
| 4. Slightly limited | <input type="checkbox"/> | 4 |
| 5. Very slightly limited – very few activities not done | <input type="checkbox"/> | 5 |
| 6. Hardly limited at all | <input type="checkbox"/> | 6 |
| 7. Not limited at all – have done all the activities that I wanted to | <input type="checkbox"/> | 7 |

19. In the last two weeks **the poor circulation to the legs have made me feel frustrated**

(tick one)

- | | | |
|---------------------------|--------------------------|---|
| 1. All of the time | <input type="checkbox"/> | 1 |
| 2. Most of the time | <input type="checkbox"/> | 2 |
| 3. A good bit of the time | <input type="checkbox"/> | 3 |
| 4. Some of the time | <input type="checkbox"/> | 4 |
| 5. A little of the time | <input type="checkbox"/> | 5 |
| 6. Hardly any of the time | <input type="checkbox"/> | 6 |
| 7. None of the time | <input type="checkbox"/> | 7 |

20. In the last two weeks **when I do get pain in my leg (or foot) it has given me**

(tick one)

- | | | |
|--|--------------------------|---|
| 1. A very great deal of discomfort or distress | <input type="checkbox"/> | 1 |
| 2. A great deal of discomfort or distress | <input type="checkbox"/> | 2 |
| 3. A good deal of discomfort or distress | <input type="checkbox"/> | 3 |
| 4. A moderate amount of discomfort or distress | <input type="checkbox"/> | 4 |
| 5. Some discomfort or distress | <input type="checkbox"/> | 5 |
| 6. Very little discomfort or distress | <input type="checkbox"/> | 6 |
| 7. No discomfort or distress | <input type="checkbox"/> | 7 |

21. In the last two weeks **I have felt guilty about relying on friends or relatives**

(tick one)

- | | | |
|---------------------------|--------------------------|---|
| 1. All of the time | <input type="checkbox"/> | 1 |
| 2. Most of the time | <input type="checkbox"/> | 2 |
| 3. A good bit of the time | <input type="checkbox"/> | 3 |
| 4. Some of the time | <input type="checkbox"/> | 4 |
| 5. A little of the time | <input type="checkbox"/> | 5 |
| 6. Hardly any of the time | <input type="checkbox"/> | 6 |
| 7. None of the time | <input type="checkbox"/> | 7 |

22. In the last two weeks, because of the poor circulation to my legs, **my ability to go shopping or carry bags has been**

(tick one)

- | | |
|---|----------------------------|
| 1. Totally limited, couldn't go shopping at all | <input type="checkbox"/> 1 |
| 2. Extremely limited | <input type="checkbox"/> 2 |
| 3. Very limited | <input type="checkbox"/> 3 |
| 4. Moderately limited | <input type="checkbox"/> 4 |
| 5. A little limited | <input type="checkbox"/> 5 |
| 6. Only very slightly limited | <input type="checkbox"/> 6 |
| 7. Not at all limited | <input type="checkbox"/> 7 |

23. In the last two weeks **I have worried I might be in danger of losing a part of my leg or foot**

(tick one)

- | | |
|---------------------------|----------------------------|
| 1. All of the time | <input type="checkbox"/> 1 |
| 2. Most of the time | <input type="checkbox"/> 2 |
| 3. A good bit of the time | <input type="checkbox"/> 3 |
| 4. Some of the time | <input type="checkbox"/> 4 |
| 5. A little of the time | <input type="checkbox"/> 5 |
| 6. Hardly any of the time | <input type="checkbox"/> 6 |
| 7. None of the time | <input type="checkbox"/> 7 |

24. In the last two weeks **the distance I can walk has become less**

- | | | |
|--|--------------------------|---|
| 1. A very great deal | <input type="checkbox"/> | 1 |
| 2. A great deal | <input type="checkbox"/> | 2 |
| 3. A good deal | <input type="checkbox"/> | 3 |
| 4. Moderately | <input type="checkbox"/> | 4 |
| 5. Somewhat | <input type="checkbox"/> | 5 |
| 6. A little | <input type="checkbox"/> | 6 |
| 7. Not at all – tick if distance is unchanged or has increased | <input type="checkbox"/> | 7 |

25. In the last two weeks **I have been depressed about the poor circulation to my legs**

(tick one)

- | | | |
|---------------------------|--------------------------|---|
| 1. All of the time | <input type="checkbox"/> | 1 |
| 2. Most of the time | <input type="checkbox"/> | 2 |
| 3. A good bit of the time | <input type="checkbox"/> | 3 |
| 4. Some of the time | <input type="checkbox"/> | 4 |
| 5. A little of the time | <input type="checkbox"/> | 5 |
| 6. Hardly any of the time | <input type="checkbox"/> | 6 |
| 7. None of the time | <input type="checkbox"/> | 7 |

THANK YOU - this completes the Walking Impairment and Vascular Quality of Life Questionnaires.