PERIPHERAL ARTERIAL DISEASE: INCIDENCE, RISK FACTORS, AND DIAGNOSIS

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ABSTRACT

This dissertation includes three manuscripts related to peripheral arterial disease. The first examines the effects of assigned glycemic control strategy on the incidence of peripheral arterial disease in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial. The BARI 2D results show that patients assigned to control their type 2 diabetes with a strategy that primarily used insulin sensitizing agents (metformin and/or thiazolidinediones) experienced fewer incident cases of peripheral arterial disease than patients assigned to a glycemic control strategy that primarily used insulin providing agents.

The second manuscript extends this work by examining risk factors for peripheral arterial disease in the BARI 2D trial. The analyses included traditional cardiovascular risk factors as well as biomarkers indicative of inflammation, coagulation, and fibrinolysis. In patients treated with insulin sensitizing medications, biomarkers of inflammation and related processes were associated with lower extremity outcomes while this was not the case for patients treated with insulin providing medications, a useful mechanistic insight into how the different types of diabetes drugs may affect the progression of atherosclerosis.

The third manuscript reports the results of a data collection project evaluating the reproducibility and reliability of two methods for measuring the ankle-brachial index. Reproducibility was

excellent for Doppler-measured ABI, while the Colin oscillometric device showed moderate reproducibility. Agreement between Colin and Doppler was somewhat poor; therefore, we would not recommend the Colin device for measuring ABI in clinical settings.

Each manuscript contributes uniquely to public health significance. The first suggests that a glycemic treatment strategy based on insulin sensitizers may reduce the progression of atherosclerosis in patients with type 2 diabetes. The second demonstrated that biomarkers of inflammation and fibrinolysis offer additional predictive value over traditional cardiovascular risk factors for incidence of PAD in type 2 diabetes patients treated with insulin sensitizing medications, implying that different types of glycemic control medications may have different mechanistic effects on the progression of atherosclerosis. The third reinforces current guidelines that Doppler ABI should remain the primary diagnostic for PAD in clinical and research settings.

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This work is dedicated to the late Kim Sutton Tyrrell, who sadly passed away in December 2012. Dr. Sutton Tyrrell was the director of the NHLBI-funded Cardiovascular Epidemiology Training Program at the University of Pittsburgh during the author's time as a T-32 trainee and served as an active member of the author's dissertation committee until her health no longer permitted. This research would not have been possible without Dr. Sutton Tyrrell's influence and vision, and her contributions should be recognized.

1.0 INTRODUCTION

Peripheral arterial disease (PAD), also known as peripheral vascular disease (PVD) or lower extremity arterial disease (LEAD), is one manifestation of atherosclerosis, a chronic disease that is one of the leading causes of death and disability in the United States.[1] The chapter that follows begins by describing the development of atherosclerosis and how it manifests as PAD. Following that, the author presents epidemiological statistics to illustrate the prevalence of PAD, the associated morbidity and mortality, and selected risk factors for PAD. A special section is then devoted to PAD in patients with type 2 diabetes mellitus (T2DM), since that patient population is a main focus of this research project. The author also presents a special section describing the ankle-brachial index (ABI), which is the principal screening tool for PAD and is utilized in all three manuscripts contained within this dissertation. The section devoted to the ABI describes its historical development, the epidemiology of the ABI, a brief description of two methods by which ABI can be measured (supplemented by two Appendices that describe the respective methods in greater detail), what is known about the comparability of these two methods, and finally a section describing current guidelines for whom should have an ABI measurement as well as the clinical significance of the ABI.

1.1 PERIPHERAL ARTERIAL DISEASE

1.1.1 Pathophysiology

Atherosclerosis is a complex disease that involves lipid disturbances, endothelial dysfunction, inflammation, vascular remodeling, and the development of lesions in the arterial wall.[2,3,4] The symptoms and ultimate consequences of an atherosclerotic lesion are dependent on factors such as the location, size, and stability of the lesion. Atherosclerotic lesions are most prevalent in the coronary arteries but can also be present in the brain, kidneys, and lower extremities.[5]

The atherosclerotic process begins when endothelial cells over-express certain molecules in the setting of an unfavorable serum lipid profile.[2,3] These molecules, known as adhesion molecules, include P-selectins and E-selectins, intercellular adhesion molecules (ICAM), and vascular-cell adhesion molecules (VCAM).[6] Adhesion molecules promote the migration of leukocytes, the release of cytokines, and the recruitment of monocytes into the intimal layer of the artery; the recruitment of monocytes into the intima provokes an inflammatory response.

The inflammatory response stimulates the migration and proliferation of smooth muscle cells in the area of injury. Concurrently, monocytes in the intima develop into macrophages, which are mediators of the inflammatory response and directly contribute to the atherosclerotic process. The uptake of modified lipoproteins by macrophages leads to the accumulation of cholesterol esters and formation of macrophage-derived "foam cells" within the arterial wall. The ensuing accumulation of lipid-laden cells is referred to as the "fatty streak" (**Figure 1-1**), the earliest visible lesion in the atherosclerotic process.

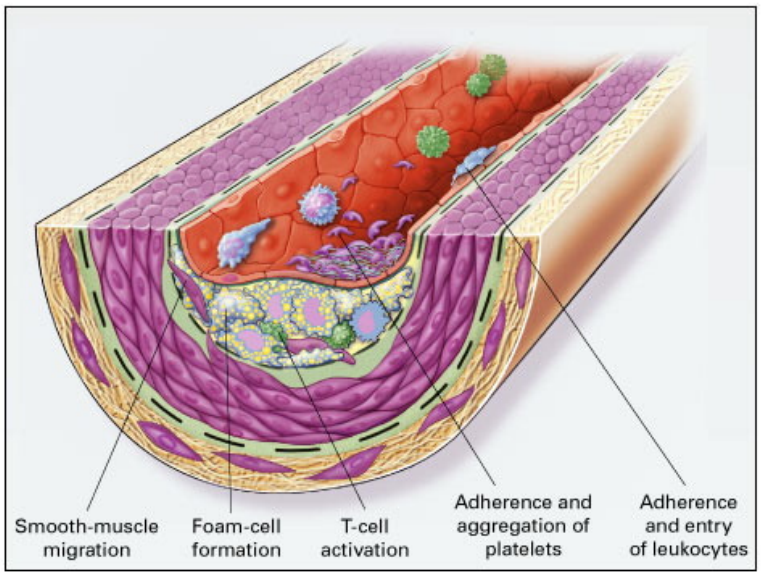


Figure 1-1. Fatty-Streak Formation in Atherosclerosis[a]

Platelet adhesion is another characteristic of the atherosclerotic process. Platelets can adhere to dysfunctional endothelium, exposed collagen, and macrophages. When activated, platelets may release their granules, which contain cytokines and growth factors that may contribute to the migration and proliferation of smooth muscle cells and monocytes.

Initially the only cells thought to proliferate during expansion of atherosclerotic lesions were smooth-muscle cells. However, replication of the monocyte-derived macrophages and T cells may also be involved.[7] The macrophages' ability to produce cytokines, proteolytic enzymes, and growth factors may be critical in the cycle of damage and repair that ensues as lesions progress. With time, the fatty streak evolves into a fibrous plaque.[8] The plaque enlarges gradually over time with continued accumulation of foam cells, which may also accumulate additional lipids.

[a] Reprinted with permission from Ross R, *N Engl J Med* 1999; 340: 115-126. Copyright Massachusetts Medical Society.

Continued proliferation of smooth muscle cells and intracellular matrix produce the definitive plaque, covered by a fibrous cap (pictured in **Figure 1-2**). Acute arterial events occur if the fibrous cap is disrupted; the resulting exposure of the prothrombotic lipid core and subendothelial tissue leads to thrombus formation and flow occlusion.

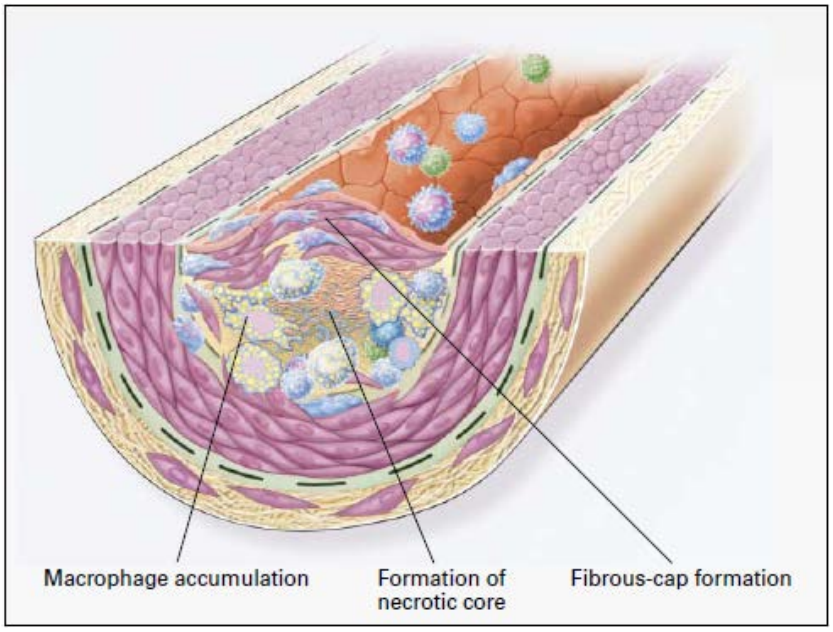


Figure 1-2. Advanced Plaque Formation in Atherosclerosis[b]

During the development of an atherosclerotic plaque, the entire vessel may enlarge in size to maintain normal blood flow through the lumen, a phenomenon known as vascular remodeling. However, once the plaque enlarges to approximately 40% of the vessel diameter, the artery can no longer compensate by vasodilation and the lesion begins to intrude into the lumen. When a hemodynamically significant stenosis is present in the lower extremity, distal pressure and flow are reduced, defining a case of peripheral arterial disease.

[b] Reprinted with permission from Ross R, *N Engl J Med* 1999; 340:115-126. Copyright Massachusetts Medical Society.

The most frequent presenting symptom in patients with PAD is intermittent claudication, which is defined as pain in the leg muscles with ambulation. Claudication is caused by arterial obstruction proximal to affected muscle beds, which limits the normal exercise-induced increase in blood flow and produces transient muscle ischemia during exercise.[9] Many patients with advanced PAD experience some symptoms of intermittent claudication; however, some elderly patients assume that leg pain while walking is part of the normal aging process and do not report their symptoms. Claudication symptoms are typically localized in the calf or thigh, although pain may begin to appear in the foot for patients with severe cases. In advanced stages of PAD, tissue perfusion progresses to ischemic ulceration or gangrene, known as critical limb ischemia (CLI). It is estimated that there is about one annual case of CLI per 100 patients with intermittent claudication. The prognosis is extremely poor for these patients; one-year mortality is approximately 25% and major amputation is eventually required in about 50% of CLI patients.[10] This is discussed further in Section 1.1.3 on morbidity and mortality.

Not all patients undergoing a lower-extremity amputation have experienced a steady progression from claudication to rest pain to CLI to amputation. A 1984 review of 713 patients undergoing below-knee amputations for ischemia found that more than half had experienced no ischemic symptoms as recently as 6 months before the amputation.[11] This seems counterintuitive, because atherosclerosis is a chronic disease that develops over years rather than months, but it demonstrates the complex disease pathology of claudication in PAD patients. One may conclude that many patients with peripheral atherosclerosis will be asymptomatic until the disease is quite advanced, highlighting the importance of screening patients with the ankle-brachial index.

1.1.2 Prevalence

PAD is typically asymptomatic in its early stages, so it is difficult to ascertain the population prevalence in the absence of widespread screening. It is also difficult to compare prevalence estimates because the criterion used to define PAD has evolved over time.[12,13,14] The most reliable prevalence estimate likely comes from a 2007 manuscript written by Allison et al which pooled data from seven community-based studies, applying the studies' age- and ethnic-specific rates to the 2000 U.S. census to estimate population prevalence.[15] This method estimated that at least 6.8 million individuals aged 40 years or older had PAD, approximately 5.8% of the U.S. adult population. According to Allison et al, the prevalence of PAD approximately doubled with each 10-year age increase among adults over 40 years old. This relationship was consistent in all racial/ethnic categories and held true for both men and women (**Figure 1-3**).

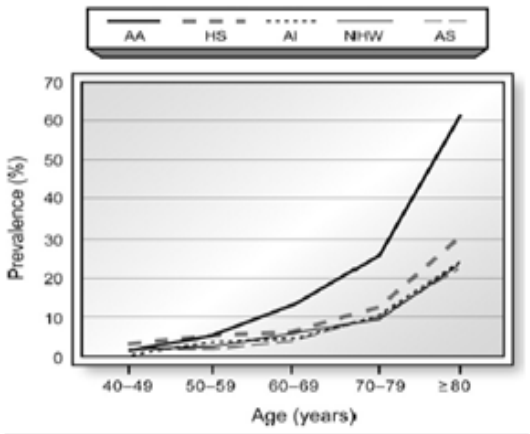


Figure 1. Ethnic-specific prevalence of peripheral arterial disease in men.
AA, African American; AI, American Indian; AS, Asian American; HS, Hispanic; NHW, non-Hispanic white.

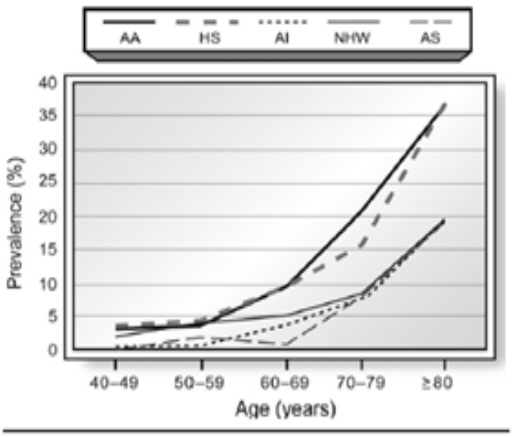


Figure 2. Ethnic-specific prevalence of peripheral arterial disease in women.
AA, African American; AI, American Indian; AS, Asian American; HS, Hispanic; NHW, non-Hispanic white.

Figure 1-3. Ethnic-Specific Prevalence of Peripheral Arterial Disease in Men and Women[c]

[c] Reprinted with permission from Allison et al, *Am J Prev Med* 2007; 32: 328-333

Also visible on **Figure 1-3** is that African-Americans tended to have greater risk of PAD than other racial/ethnic groups in Allison's pooled study. This held true for both men and women, and the increased risk among African-Americans was especially noticeable in the elderly. The higher risk among African-Americans in older age groups is consistent with prior research from the Systolic Hyptertension in the Elderly Program (SHEP),[16] which was a clinical trial evaluating the efficacy of drug therapy for systolic hypertension in the elderly. All participants were at least 60 years old at enrollment and had isolated systolic hypertension, which defines a population with especially high risk for PAD. The ankle-brachial index was measured at 11 of the 16 SHEP field centers as part of an ancillary study to assess the prevalence of PAD and risk factors which might be associated with PAD in the SHEP study population. Participants self-identified as Black race had greater prevalence of PAD than White study participants in all age categories among both men and women. In a multivariable logistic regression which adjusted for potential confounders (age, smoking, body mass index, diabetes, systolic blood pressure, HDL cholesterol, and existing coronary artery disease) there was a significantly greater risk of PAD in Blacks compared to White participants (OR=2.51, 95% CI: 1.84-3.41, $p<0.001$). Therefore, we conclude that the strong relation of PAD with Black race was not explained by differences in baseline characteristics between the racial categories. The SHEP investigators also found that, among Blacks, subgroups that were expected to be at low risk for PAD were not. For example, Blacks who never smoked had nearly twice the prevalence of PAD as Whites who never smoked.

The National Health and Nutrition Examination Survey (NHANES) data have also been used to estimate the prevalence of PAD in the United States. Carried out primarily using surveys, the

NHANES sample is created using a multistage probability sample of the noninstitutionalized civilian United States population designed to oversample the elderly, low-income persons, adolescents, Mexican Americans, and non-Hispanic blacks to provide more reliable estimates for these population subgroups. NHANES 1999–2000 was the first NHANES survey to perform ankle-brachial blood pressure measurements on participants, analyzed by Selvin et al[14] to estimate the prevalence of PAD in the year 2000. According to the NHANES 1999-2000 data, the prevalence of PAD among individuals aged 40 years or older was 4.3% (95% CI 3.1% to 5.5%) in the year 2000, translating to an estimated 5 million adults (95% CI 4 to 7 million) who would be classified as having PAD. Parallel to the SHEP results, the NHANES data suggested that PAD disproportionately affects older individuals and Blacks, and the excess PAD prevalence among Blacks was not explained by other known risk factors. Selvin et al also performed additional analyses to examine the relationships between PAD and other risk factors; NHANES data will be referenced further in Sections 1.1.4 (traditional risk factors), 1.1.5 (novel risk factors) and 1.2.1 (PAD in patients with type 2 diabetes mellitus) where appropriate.

The Atherosclerosis Risk in Communities (ARIC) study[17] also reported the prevalence of PAD in various racial/ethnic subgroups. The ARIC study was a longitudinal investigation designed to assess the natural history and etiology of preclinical and clinical atherosclerotic disease by age, race/ethnicity, and gender. The ARIC cohort was sampled from four U.S. communities (Forsyth County, NC; Jackson, MS; Minneapolis MN; and Washington County, MD) and participants were selected by a probability sample of eligible adults aged 45 to 64 to provide a total of 15,792 men and women. The ARIC data also showed that Black race was associated with increased risk of PAD in all age categories for both men and women (see **Figure 1-4**).

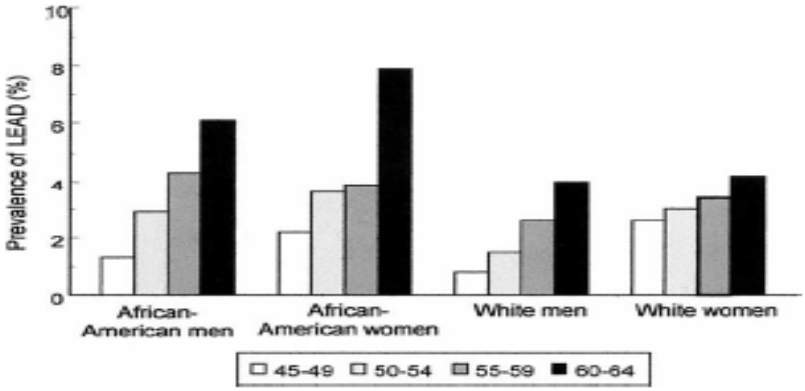


Figure 1. Bar graphs show the prevalence of lower extremity arterial disease (ABI≤0.90) by age group, according to race and gender.
ABI, ankle-brachial index; LEAD, lower extremity arterial disease.

Figure 1-4. Prevalence of Peripheral Arterial Disease in the Atherosclerosis Risk In Communities (ARIC) Study[d]

According to the studies presented here, prevalence of PAD appears to be essentially equivalent in men and women. Neither the SHEP data nor the NHANES data showed any consistent gender differences in PAD prevalence, and the ARIC data generally showed a similar prevalence of PAD when comparing men to women of the same ethnicity and age group (**Figure 1-4**). Allison et al's pooled study (**Figure 1-3**) showed that men had slightly higher rates of PAD than women overall, but also noted that women seemed to have higher prevalence of PAD in the younger age categories, while older men tended to have higher prevalence than women of the same age and ethnic group. Therefore, it appears that overall prevalence is approximately equivalent between men and women.

[d] Reprinted with permission from Zheng et al, *Am J Prev Med* 2005; 29: 42-49.

1.1.3 Morbidity and Mortality

PAD directly increases the risk of functional limitation, physical disability, leg revascularization, and amputation.[18,19,20] While a relatively small proportion (1-3%) of patients with PAD present with critical limb ischemia (CLI), the prognosis for a patient with such advanced disease is extremely poor, with an estimated 25% one-year mortality rate and an additional 30% of patients presenting with CLI likely to undergo amputation within one year (**Figure 1-5**). Following an amputation, quality of life tends to suffer as a result, primarily owing to the restricted mobility.[21]

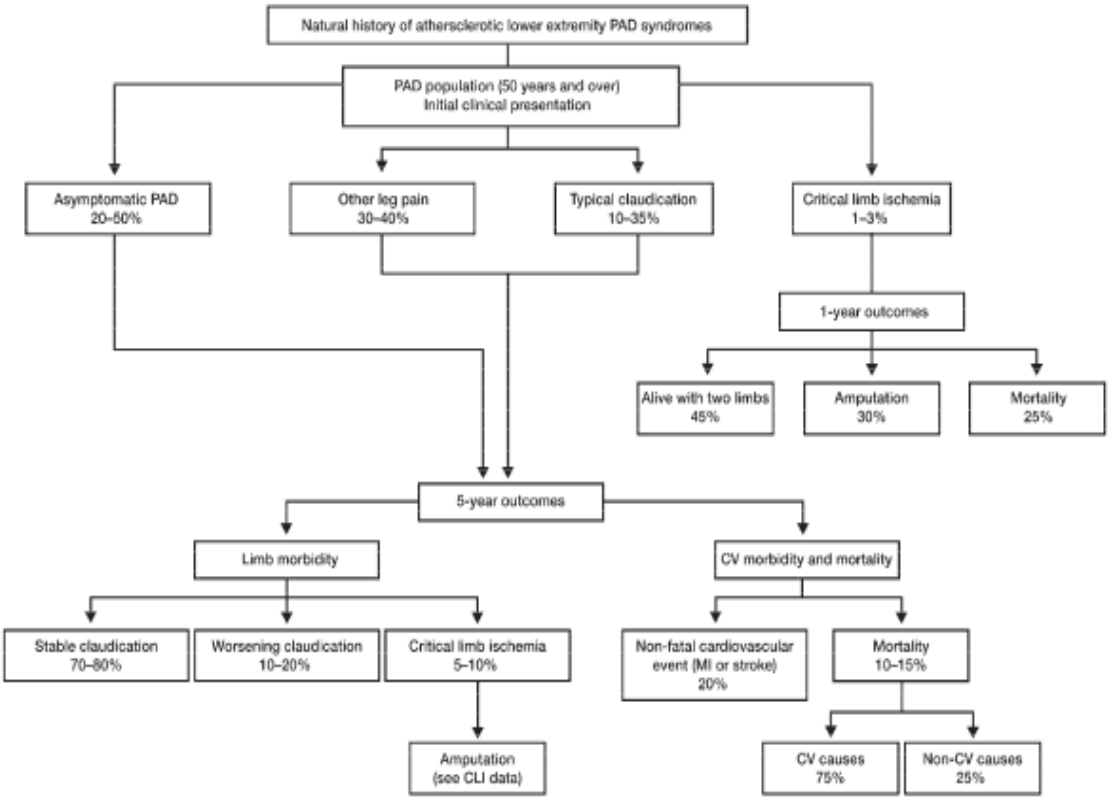


Figure 1-5. Natural History of Peripheral Arterial Disease[e]

[e] Reprinted with permission from Hirsch et al. *Circulation* 2006; 113: 1474-1547.

PAD is not only a condition unto itself, but the presence of PAD is also a marker of generalized systemic atherosclerosis, and therefore PAD is associated with increased risk of cardiovascular and all-cause mortality.[22,23,24,25,26,27] Estimates of the excess mortality risk associated with PAD vary based on the age distribution and disease status of the population in question, but it is clear that patients with PAD consistently have higher mortality risk than those free of PAD in all populations. **Figure 1-6** below shows the precipitous decrease in five- and ten-year survival for patients with intermittent claudication or critical limb ischemia; the mortality risk associated with asymptomatic PAD will be discussed in more detail in Section 1.3 on the ankle-brachial index.

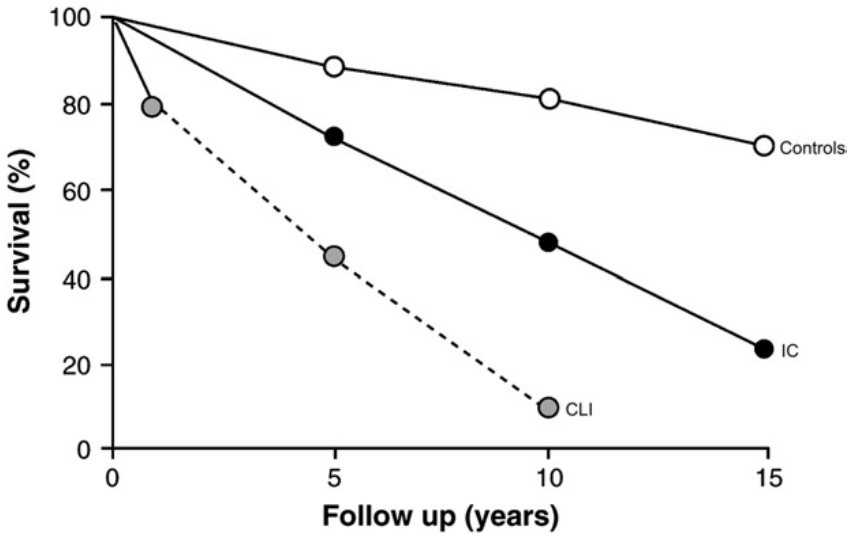


Figure 1-6. Estimated Survival for Patients with Advanced Peripheral Arterial Disease[f]

Admittedly, the relationship between PAD and cardiovascular mortality must be considered in the context of possible coexisting atherosclerosis in other vascular beds. The cause of death in

[f] Reprinted with permission from Norgren et al, *Eur J Vasc Endovasc Surg* 2007; 33: S1-S70.

*IC= Intermittent Claudication; CLI = Critical Limb Ischemia

PAD patients is often something other than the lower extremity disease itself, such as coronary artery disease (CAD). Approximately half of patients with PAD have coexisting CAD.[28] However, the relationship between PAD and other CVD events make it a useful marker of disease as well as a condition that must be monitored in its own right; a recommended treatment algorithm[29] for peripheral arterial disease is shown below (**Figure 1-7**).

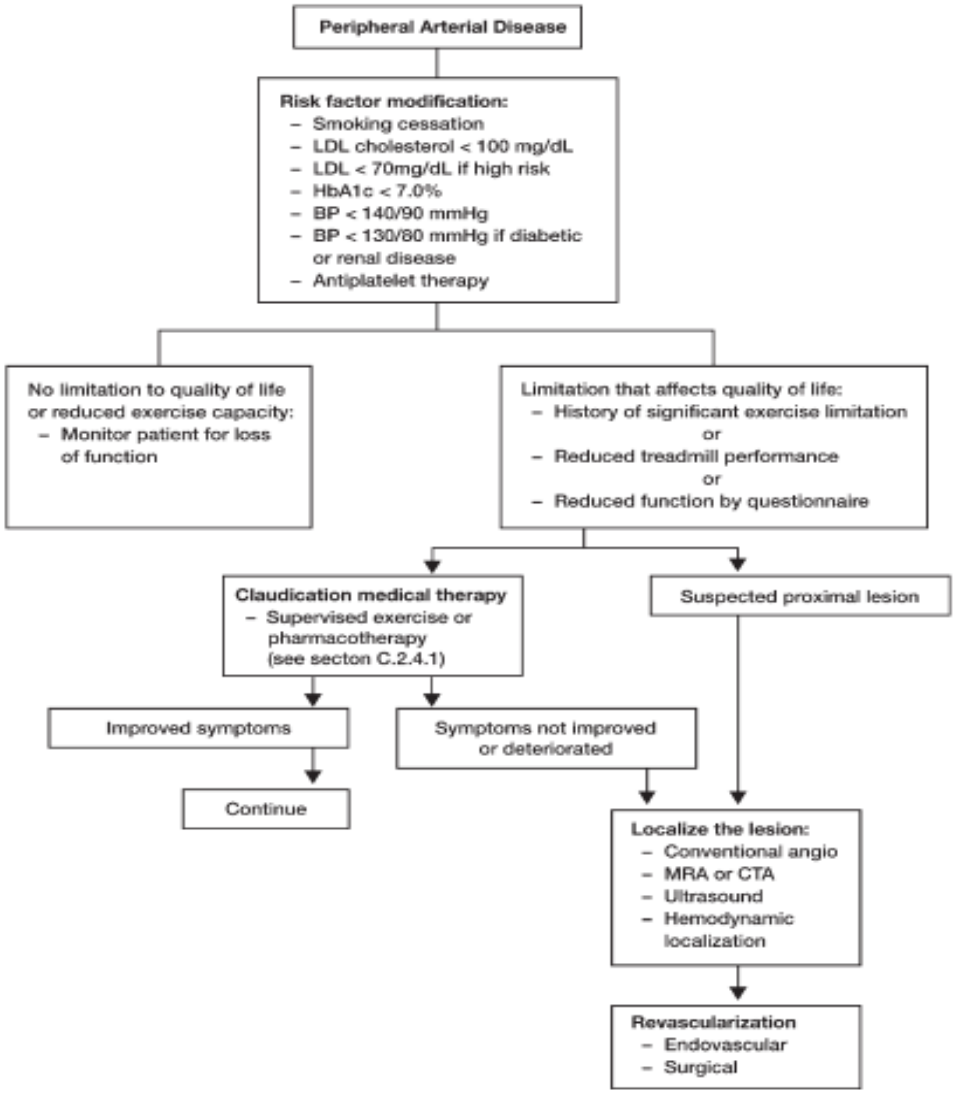


Figure 1-7. Treatment Algorithm for Patients with Known Peripheral Arterial Disease[g]

[g] Reprinted with permission from Hiatt et al *N Engl J Med* 2001; 334: 1608-1621. Copyright Massachusetts Medical Society.

1.1.4 Traditional Cardiovascular Risk Factors

PAD is generally asymptomatic in its early stages, but it is easily detected by screening at-risk patients with an ankle-brachial index (ABI). Therefore, to improve early detection of PAD, knowledge of risk factors is critical to ensure that high-risk patients are screened with an ABI.[28] Many of the known risk factors for PAD are well-established as risk factors for atherosclerosis in all vascular beds, although the magnitude of some risk factors' effects may vary between PAD and other atherosclerotic processes. As demonstrated in **Figure 1-7**, the immediate steps taken to reduce cardiovascular risk in a patient with diagnosed PAD include smoking cessation, treatment of dyslipidemia, treatment of hypertension, and glucose lowering, with additional emphasis on treating these risk factors if a patient is diabetic or has renal disease. Smoking, dyslipidemia, hypertension, elevated glucose (diabetes), and renal disease are all established risk factors for cardiovascular disease at large; each will be discussed briefly here.

Cigarette smoking is the strongest risk factor for both onset and progression of PAD, with estimates ranging from about a twofold greater risk to a fivefold greater risk in current smokers compared to non-smokers. [14,30,31,32] The NHANES 1999-2000 data showed a significantly increased risk of PAD (OR=4.23, 95% CI 1.95-9.17, $p<0.05$) for current smokers compared to nonsmokers. The relative risk associated with smoking varies due to differences in study population; smoking increases risk the most in younger, otherwise healthy populations, while the effect is somewhat smaller in older populations that have a larger overall prevalence of PAD. Physiologically, smoking impairs vasodilation and attenuates production of endothelium-derived nitric oxide (NO), both of which retard the development of atherosclerosis, and therefore

smoking increases risk of atherosclerosis in all vascular beds.[33] Cigarette smoking is more strongly associated with PAD than it is with coronary disease, although the reason is unknown. Therefore, all adult smokers should be considered at high risk for PAD.[28]

Hypertension is also strongly associated with PAD[14,34] showing approximately 1.5- to three-fold increases in risk, again depending on age and composition of the study population. The aforementioned NHANES 1999-2000 data showed a moderately increased risk of PAD (OR=1.75, 95% CI 0.93-3.30), although this was not significant in a fully adjusted model. Hypertension promotes the development of atherosclerotic plaques by its effects on the vasculature: arteries exposed to hypertension have increased permeability, allowing oxidized lipoproteins to migrate into the intima.[35] There is also evidence suggesting that hypertension alters the balance between cellular proliferation and apoptosis.[36] Obviously, since systolic blood pressure is a component of the ankle-brachial index, it would be expected to have some association with PAD risk. This is discussed further in Section 1.3 on the ABI.

Dyslipidemia is associated with increased risk of PAD, but the magnitude of this relationship is somewhat smaller than that observed for smoking and hypertension.[14,37] NHANES 1999-2000 data showed a significantly increased risk of PAD (OR=1.67, 95% CI 1.01-2.74, $p<0.05$) for participants with hypercholesterolemia. Our view of the role of lipids in atherosclerosis has evolved over the past several decades, but it has long been established that higher levels of LDL cholesterol are associated with increased cardiovascular risk, while higher levels of HDL cholesterol are protective.[38] The form of dyslipidemia most common in PAD patients is the

combination of low HDL cholesterol and elevated triglyceride levels commonly seen in patients with diabetes.[30]

Renal insufficiency is also common in patients with PAD and vice versa.[39] However, the causality of this relationship is not entirely clear. The NHANES data[40] showed that the prevalence of low ABI was six times greater in patients with low creatinine clearance, and this relationship remained significant after adjusting for known PAD risk factors. The association between chronic kidney disease (CKD) and PAD is independent of age, ethnicity, diabetes, and hypertension, and although the exact reason is not known, it may relate to the vascular inflammation and elevated homocysteine levels seen in CKD.[37] However, it is uncertain whether i) there is a true causal relationship between CKD and PAD or ii) the frequent coexistence of the two diseases is a reflection of the systemic complications of both atherosclerosis and CKD.

Peripheral arterial disease is also especially common in patients with type 2 diabetes mellitus (T2DM).[14,41] The clinical presentation of PAD also tends to differ in T2DM patients compared to those free of diabetes. It is believed that the abnormnal cluster of hyperglycemia, elevated free fatty acids, and insulin resistance observed in T2DM promotes oxidative stress and endothelial dysfunction, both of which contribute to atherosclerosis. Insulin resistance also intensifies the systemic inflammatory state, promotes thrombosis, and constrains fibrinolysis.[42] The American Diabetes Association recommends a screening ABI every 5 years in patients with diabetes.[43] The relationship between PAD and T2DM will be discussed in much greater depth in Section 1.2 of this document.

Notably, body mass index has generally shown little association with PAD when adjusting for other cardiovascular risk factors.[14,16] Given the strong association between obesity and coronary artery disease, the lack of association between obesity and PAD is somewhat counterintuitive. One hypothesis is that PAD is detected less often among obese patients because the principal diagnostic, the ankle-brachial index, is less sensitive in obese patients because the size of their ankles makes it difficult to take an accurate blood-pressure reading at the ankle (discussed further in Section 1.3 on the ankle-brachial index). Another possibility is that obese patients tend to be less physically active than normal-weight patients and therefore less likely to experience symptoms of claudication that would prompt a physician to examine the patient further.

As reviewed in this section, cigarette smoking, hypertension, dyslipidemia, renal insufficiency, and diabetes are independently associated with peripheral arterial disease. However, treatment targets for these risk factors are generally driven by their respective associations with coronary artery disease; furthermore, since these relationships are already well-documented, further study of these relationships is unlikely to provide a great deal of new information. However, it would be useful to identify additional risk factors beyond the traditional cardiovascular disease risk factors that may enhance our understanding of the pathophysiology of peripheral arterial disease, which may eventually lead to potential therapeutic targets for further study.

1.1.5 Novel Risk Factors

Inflammation, coagulation, and fibrinolysis are interconnected processes[43] and markers related to each process are associated with coronary atherosclerosis[44,45] and peripheral arterial disease.[46,47] Several biomarkers of these three processes are associated with PAD in existing cross-sectional studies, but few of these relationships have been confirmed with data from longitudinal studies. The Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial has longitudinal data on biomarkers related to these processes as well as data on PAD and related outcomes during follow-up. These data could be used to examine which of these biomarkers are associated with incident PAD in a population with existing coronary atherosclerosis, which could help us better understand the systemic atherosclerotic process. The rationale for each biomarker's relationship with atherosclerosis (and, by extension, the risk of developing PAD) will be discussed briefly here.

C-reactive protein (CRP), a marker of inflammation, binds to endothelial cell receptors and also co-localizes with oxidized LDL in atherosclerotic plaques.[48] CRP also promotes leukocyte adhesion and stimulates endothelial production of procoagulant tissue factor,[49] both of which contribute to an atherogenic environment. CRP also increases production of compounds that impair fibrinolysis such as plasminogen activator inhibitor-1 (PAI-1)[50] and inhibits tissue-type plasminogen activator (t-PA),[51] discussed in the section that follows. The associations between CRP and these biomarkers illustrate the complexity of the relationship between inflammation, coagulation, and fibrinolysis. Although discussed with "novel" risk factors for the purposes of this document, C-reactive protein is well established as a risk factor for cardiovascular disease.[52]

According to Ridker's seminal paper, CRP evaluation may have the potential to improve cardiovascular risk prediction models when used as an adjunct to this approach. For each quintile increase in CRP, the adjusted relative risk of suffering a future cardiovascular event is estimated to be 26% for men (95% CI 11% to 44%; $P<0.005$) and 33% for women (95% CI 13% to 56%; $P<0.001$). There is also some evidence linking CRP specifically to PAD: NHANES 1999-2000 data also showed that patients in the highest quartile of CRP had a greater risk than patients in the lowest quartile, although the difference was not statistically significant when adjusting for other cardiovascular risk factors (OR=1.72, 95% CI 0.74-3.99).

CRP is a marker of inflammation, but as mentioned above it might influence the atherosclerotic process by increasing production of compounds that impair fibrinolysis, a process in which a fibrin clot, the product of coagulation, is broken down (see **Figure 1-8**). The acute fibrinolytic response to inflammation and coagulation is the release of plasminogen activators. Tissue-type plasminogen activator (t-PA) is an enzyme found on endothelial cells which catalyzes the conversion of plasminogen to plasmin, which is the major enzyme responsible for breakdown of blood clots.[53] The increase in plasminogen activation and subsequent plasmin generation stimulated by t-PA is counteracted by a delayed but sustained increase in plasminogen activator inhibitor-1 (PAI-1),[54] a protein that functions as the principal inhibitor of t-PA and hence is an inhibitor of fibrinolysis.

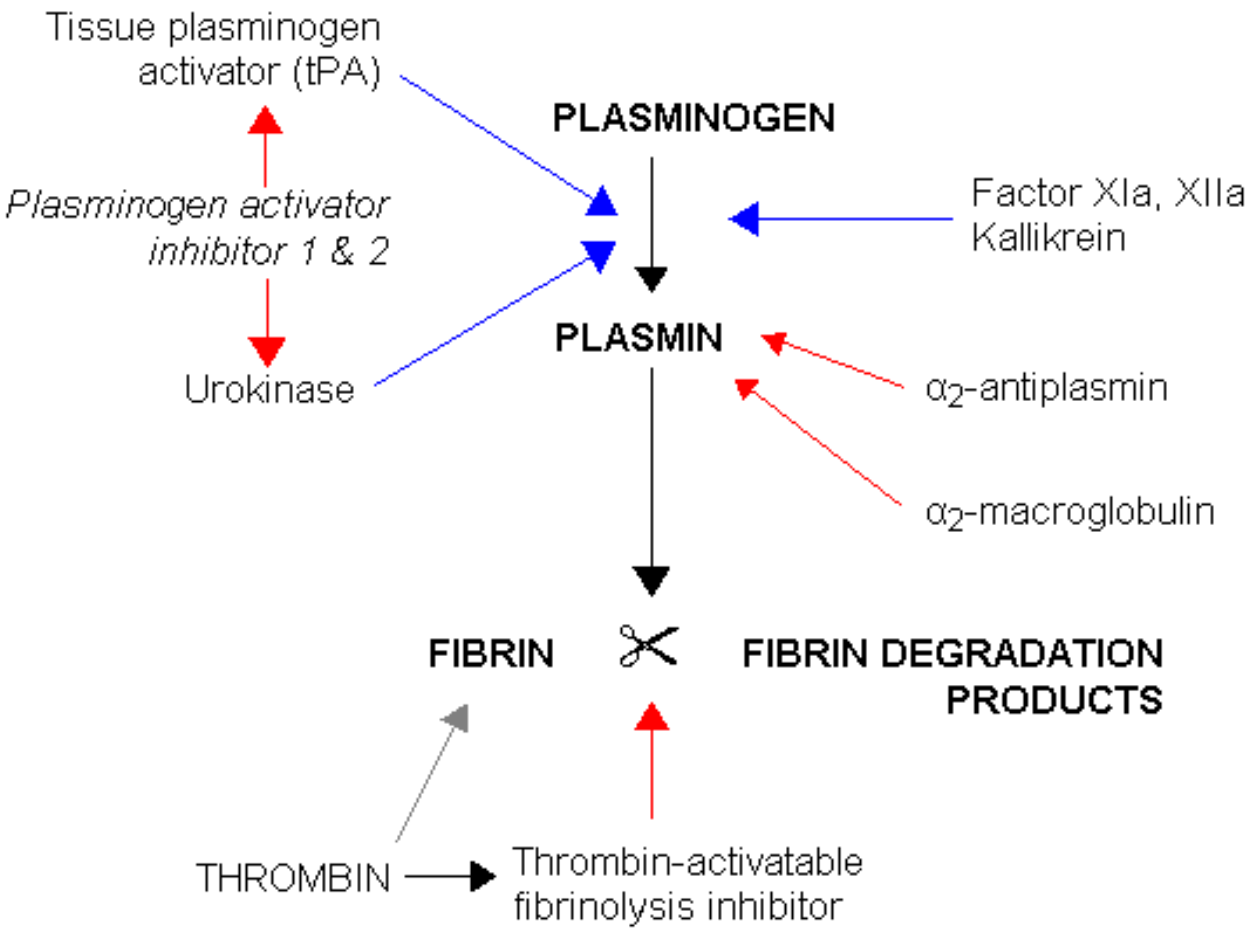


Figure 1-8. Biomarkers' Role In Fibrinolysis

Increased expression of PAI-1 is believed to accelerate the development of atherosclerosis and shifts the balance between thrombosis and fibrinolysis.[55] Increased expression of PAI-1 also is suggestive of insulin resistance and has been associated with vascular inflammation.[56] Lower concentrations of t-PA and greater expression of PAI-1 have each been associated with increased risk of arterial thrombosis.[57,58,59] Since lower levels of t-PA and/or increased expression of PAI-1 are associated with various aspects of the atherosclerotic process as well as thrombotic arterial events, one might expect that they also could be associated with increased risk of PAD.

Another marker of fibrinolysis is fibrinogen, a soluble plasma glycoprotein that is converted into fibrin during coagulation. Fibrinogen is thus a major component of the coagulation cascade and may affect the formation of atherosclerotic plaques through a number of different pathways: fibrinogen promotes the binding and production of high-density lipoproteins,[60] facilitates the migration of adhesion molecules to the endothelial surface,[61] initiates the proliferation and migration of smooth muscle cells,[62] and mediates the binding of monocytes and endothelial cells through ICAM-1 and MAC-1.[63] Fibrinogen can also stimulate expression of proinflammatory cytokines on mononuclear cells and induce production of chemokines by endothelial cells and fibroblasts.[64] While fibrinogen is not a primary therapeutic target or first-line screening test, it is well established as a risk factor for cardiovascular disease.[65] There is also some evidence linking fibrinogen to increased risk of PAD; NHANES 1999-2000 data showed that patients in the highest quartile of fibrinogen had a greater PAD risk than patients in the lowest quartile of fibrinogen, although the difference was not statistically significant when adjusting for other cardiovascular risk factors (OR=1.68, 95% CI 0.67-4.23).

Fibrinogen is converted into fibrin during coagulation; emerging on the other side of the fibrinolytic process is the fibrin degradation product known as D-dimer, a small protein fragment which contains two crosslinked D fragments of the fibrinogen protein.[66] D-dimers are not normally present in human blood plasma except when the coagulation system is activated; its principal use in diagnostic testing is to rule out thrombotic events. However, since the plasma level of D-dimer is a marker of coagulant and subsequent fibrinolytic activity,[67] increased D-dimer might also be associated with increased risk of PAD. Plasma levels of D-dimer are generally correlated with t-PA and PAI-1 activity.[68] However, D-dimer, t-PA and PAI-1 reflect

different aspects of the fibrinolytic system and thus each might provide unique information about the atherosclerotic process.

There is a plausible biological hypothesis linking each of the aforementioned biomarkers with developing atherosclerosis, but only C-reactive protein, fibrinogen, and D-dimer have been identified as risk factors for incident PAD in epidemiological studies. NHANES 1999-2000 data showed that participants in the highest quartile of CRP and fibrinogen had two-fold increased risk of prevalent PAD compared with participants in the lowest quartiles.[69] Both fibrinogen and D-dimer have been associated with increased risk of PAD in cross-sectional studies.[46,70,71] Fibrinogen and D-dimer were also linked to the progression of PAD in the Edinburgh Artery Study;[72] however, neither was significantly associated with PAD progression after adjusting for the level of CRP, suggesting that the processes of inflammation, coagulation, and fibrinolysis may not contribute "independently" to the progression of systemic atherosclerosis but instead that the processes may be reflective of an overall advancing disease state.

The increased risk associated with higher levels of the respective biomarkers is not isolated to "incident" disease but also extends to poorer outcomes among individuals with existing PAD. In patients with existing PAD, the aforementioned biomarkers have been associated with increased risk of functional decline, failure of lower-extremity revascularization procedures, cardiovascular mortality and all-cause mortality.[73,74,75,76,77,78,79] Although these studies combine to suggest that inflammation, coagulation, and fibrinolysis may affect the progression of PAD, most of the existing studies are not ideally suited to describe the association between changes in biomarker levels and PAD outcomes because they are one of the following designs:

i) cross-sectional studies linking current levels of a biomarker to "prevalent" PAD; while this is a useful exploratory analysis, this study is not well-suited to elucidate temporality.

ii) longitudinal studies whereby a "baseline" level of the risk factor is linked to risk of incident PAD. This is highly useful to establish risk factors for future disease, but it would be useful to quantify the relationships between time-varying changes in markers of inflammation, coagulation, and fibrinolysis and the incidence of PAD to determine whether changes in any of these markers provide additional predictive value.

Further study of the association between these biomarkers of inflammatory states and impaired fibrinolysis may improve our understanding of their effects on the development of peripheral atherosclerosis. The next step in understanding these relationships would be a study with these biomarkers examined as time-varying covariates to elucidate how changes in biomarkers profiles may be associated with PAD and/or developing atherosclerosis. If time-varying changes in selected biomarkers show a significant association with disease, we may be able to better target high-risk patients who should be screened and/or treated for PAD, and we may also better understand the mechanism by which atherosclerosis continues to progress throughout the vasculature in patients with type 2 diabetes mellitus, discussed in the following section.

1.2 PERIPHERAL ARTERIAL DISEASE IN TYPE 2 DIABETES

Peripheral arterial disease is highly prevalent in patients with Type 2 Diabetes Mellitus (T2DM). Furthermore, PAD typically presents earlier and progresses more rapidly in T2DM patients than in nondiabetic patients. The epidemiology, clinical presentation, and potential mechanisms of peripheral arterial disease in type 2 diabetes mellitus are discussed below.

1.2.1 Epidemiology

It is well-established that diabetes mellitus is associated with an increased risk of all types of cardiovascular disease, including peripheral arterial disease. Part of this is likely attributable to the multifactorial cluster of additional risk factors that often accompany diabetes, such as hypertension and dyslipidemia. However, elevated glucose levels are generally associated with increased risk of atherosclerotic disease even after adjustment for coexisting risk factors.

The NHANES data suggest that T2DM patients had approximately threefold greater risk of PAD than those free from diabetes.[14] Patients with diabetes often present with more advanced PAD and experience worse PAD outcomes than nondiabetic patients.[80] Peripheral arterial disease in patients with diabetes is associated with substantial functional impairment and adversely affects quality of life.[81] PAD patients with diabetes have a higher risk of leg revascularization and amputation than those without diabetes. [82,83,84] Compared to nondiabetic patients, T2DM patients with coexisting PAD have a significantly increased risk of cardiovascular mortality.[85,86]

1.2.2 Clinical Presentation

The increased risk of PAD in diabetic patients is likely due to the abnormal metabolic state that prevails in T2DM. The abnormnal cluster of hyperglycemia, elevated free fatty acids, and insulin resistance characteristic of patients with diabetes results in oxidative stress and endothelial dysfunction, states that promote the development of atherosclerostic plaques. As a result, the distribution of PAD tends to differ slightly between diabetic and nondiabetic patients. Diabetic patients frequently show involvement of the arteries below the knee, especially the tibial and peroneal arteries, and PAD is more commonly multi-segmental in diabetic patients. [84]

Mobility is especially poor for diabetic patients with PAD. These patients generally have poorer function than nondiabetic patients with PAD, exhibited by shorter mean walking distance and slower walking velocity.[81] This may be due to associated peripheral neuropathy, differences in exertional leg symptoms, and overall greater cardiovascular disease burden in diabetic patients. The decrease in mobility may affect health status and quality of life.[20]

Some diabetic patients with peripheral neuropathy will have decreased pain perception that may delay the recognition of PAD.[87] Peripheral neuropathy and PAD are both known risk factors for foot ulceration and gangrene; patients with both conditions are extremely likely to experience a poor outcome. An estimated 40-60% of diabetic patients with foot ulcers have PAD, which affects wound healing and leads to the need for revascularization, amputation and possibly mortality.[88] Development of dry gangrene is the end-stage presentation of PAD; once disease has advanced to this point, a revascularization and/or a major amputation will be required.

1.2.3 Potential Mechanisms

Several studies have shown that high levels of glycosylated hemoglobin (HbA1c) are independently associated with increased risk of PAD in T2DM. Data from the National Health and Nutrition Examination Survey (NHANES) 1999-2002 showed a positive dose-response relationship between HbA1c and risk of PAD, with a 2.3-fold relative risk for diabetic patients with HbA1c<7% and a 2.7-fold relative risk for diabetic patients with HbA1c≥7% compared to nondiabetic patients.[89] The United Kingdom Prospective Diabetes Study (UKPDS) showed that each 1% increase in HbA1c was associated with a 28% increased risk of PAD.[90,91] The increased risk of PAD incidence associated with poor glycemic control carries over to an increased risk of severe PAD events; data from the Atherosclerosis Risk in Communities (ARIC) study showed that the risk of PAD-related hospitalization was 4.5 times greater among patients in the highest HbA1c tertile than the risk among patients in the lowest HbA1c tertile.[92]

Despite the association between HbA1c and PAD risk, trials of intensive glucose control have thus far failed to demonstrate a significant benefit on most cardiovascular outcomes, including PAD.[93] The Veterans Affairs Diabetes Trial (VADT),[94] the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial[95] and the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial[96] all failed to demonstrate a benefit of intensive glucose-lowering therapy on macrovascular outcomes, despite achievement of significantly lower HbA1c levels in those assigned to intensive therapy. Although HbA1c is a marker of PAD risk, there is no conclusive evidence that lowering HbA1c reduces the risk of PAD.

If glucose control is not the driving factor in PAD risk for T2DM patients, then some other factor may increase the risk of PAD in this population. Some of the unexplained variation in PAD risk among T2DM patients might be due to the insulin resistance which prevails in T2DM. Insulin resistance intensifies the systemic inflammatory state, is prothrombotic, and constrains fibrinolysis;[42] as mentioned in the preceding section, these respective processes are thought to be involved in the atherosclerotic process. Elevated fibrinogen has been associated with increased risk of PAD in patients with diabetes,[97] but no other studies have specifically examined these relationships in T2DM patients. Results from the aforementioned BARI 2D trial have shown that glycemic control strategy focused on improving insulin sensitivity led to changes in biomarker profiles indicative of a profibrinolytic, antithrombotic, and anti-inflammatory state.[98] If longitudinal changes in biomarkers of inflammation, coagulation, and fibrinolysis account for some of the variation in PAD risk, that may i) partially explain the mechanism of increased risk of PAD in T2DM patients and ii) suggest a pathway by which insulin sensitizers reduce the risk of incident PAD, thereby slowing the progression of atherosclerosis in T2DM.

Prior reviews have speculated that insulin sensitizing medications may reduce the risk of incident PAD in T2DM because of their anti-inflammatory properties and metabolic effects.[99,100,101] However, no published data from randomized controlled trials has confirmed this belief. Data from a randomized controlled trial showing that insulin sensitizing medications reduce the risk of PAD independent of changes in glycemic control would suggest that improvements in macrovascular outcomes may be achieved by changing the mechanistic approach to focus on insulin sensitivity.

1.3 ANKLE BRACHIAL INDEX

The ankle-brachial index (ABI) is the principal non-invasive screening technique for peripheral arterial disease. Also known as ankle-arm index (AAI) or ankle-brachial pressure index (ABPI), the ABI is simply a ratio of the brachial systolic blood pressure divided by the ankle systolic blood pressure. Normal ABI values range from 0.91-1.30; an abnormally low ABI is suggestive of peripheral arterial disease. The origin, epidemiology, measurement, and clinical significance of the ABI are summarized in the sections to follow.

1.3.1 Origin

Ankle blood pressure first appeared in medical literature in 1950. University of Southern California physician Travis Winsor reported taking a series of systolic pressure measurements in patients with peripheral arterial disease, including measurements at the ankle, and showed that the gradient of pressure reduction along the arterial tree was greater for patients with peripheral arterial disease than patients with normal arteries.[102] In 1956, Gaskell used ankle systolic pressure to evaluate blood flow before and after an arterial graft in the lower extremity.[103] Strandness and colleagues published a series of articles from 1961-66 using ankle systolic pressures to study various aspects of the occlusive process in the extremities.[104,105,106]

The first study to report the ratio of ankle pressures to brachial pressures was published in 1968. Canadian physician Stefan Carter reported that patients with normal arteries generally presented with an ankle systolic pressure ranging from 100 to 120% of their brachial systolic pressure (corresponding to an ABI of 1.0-1.2), while all patients with peripheral arterial disease had ankle pressures below 80% of their brachial pressure (corresponding to an ABI of 0.8 or less).[107]

Carter's paper also provided statistical evidence that ABI values were lower in patients with peripheral arterial disease than in patients without PAD. The following year, Carter showed that the degree of decrease in ABI was correlated with severity of the occlusion among patients with peripheral arterial disease.[108] In 1970, Yao et al confirmed that severity of occlusion was correlated with degree of decrease in ABI; this study was also the first to report measuring ankle pressures using the Doppler ultrasound technique still used today.[109,110]

1.3.2 Epidemiology

The ankle-brachial index was established as a useful tool for the evaluation of peripheral arterial disease by the end of the 1960's, but its potential utility as a screening tool for cardiovascular risk was not established for many years. The relationship between ankle-brachial index and cardiovascular outcomes was first reported in a large epidemiological study in the early 1990's.

In 1991, McKenna et al published data from a cohort of 744 patients showing that patients with ABI $\leq$ 0.85 had a relative risk of mortality 2.36 times greater than patients with ABI > 0.85, the first published result to suggest that a low ankle-brachial index was associated with increased mortality risk.[111] Two years later, Newman et al published similar results from 1537 subjects enrolled in the Systolic Hypertension in the Elderly Program (SHEP) showing that ABI $\leq$ 0.9 was strongly associated with total mortality (age-adjusted RR=4.1) and cardiovascular mortality (age-adjusted RR=3.7).[112,113] In 1996, Leng et al published data from the Edinburgh Artery Study showing that low ankle-brachial index was independently associated with coronary heart disease and myocardial infarction.[114] Data from the Framingham Study established that low ABI was also associated with an increased risk of stroke.[115] In addition to the associations with

cardiovascular mortality and all-cause mortality, low ankle-brachial index is also associated with limited physical function (**Figure 1-9**).[18]

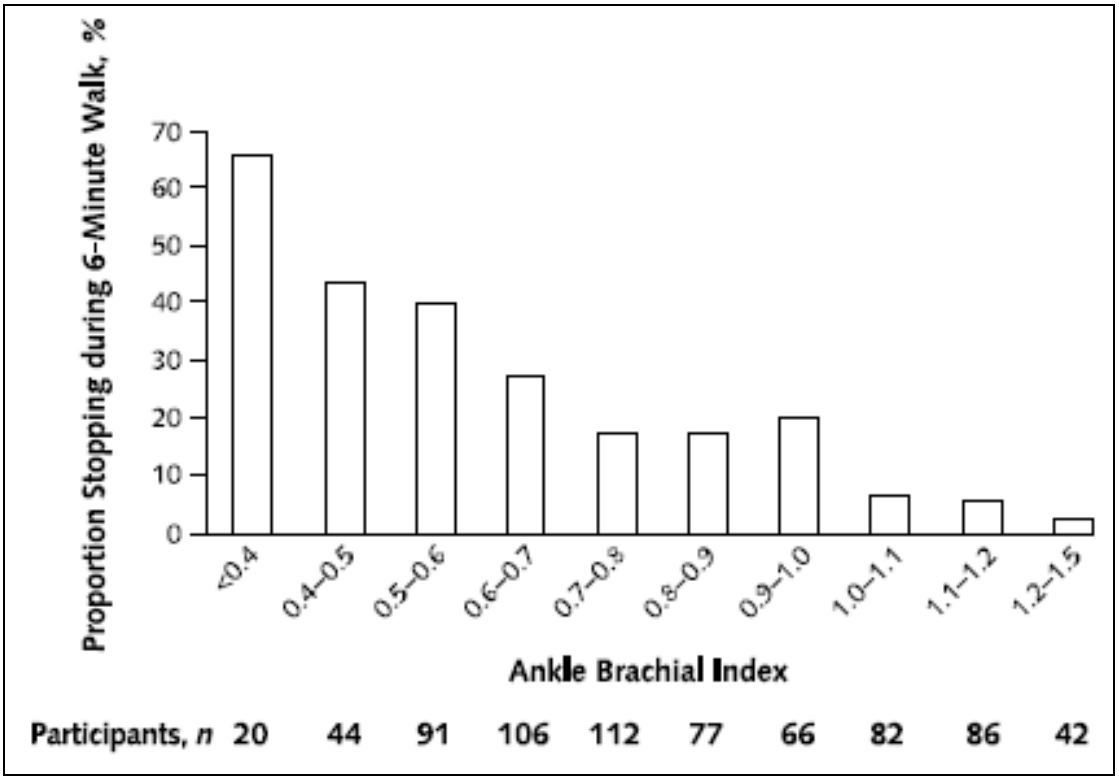


Figure 1-9. Patients Stopping during 6-minute walk test according to ABI Category[h]

Most early studies focused on the risk in patients with a low ankle-brachial index; each of the studies described on the previous page compared patients with low ABI to the rest of the respective study cohort. However, more recent research has examined risk across the entire ABI spectrum, demonstrating that high ABI may also be indicative of increased cardiovascular risk.

[h] Reprinted with permission from McDermott MM et al. *Ann Intern Med* 2002; 136: 873-883

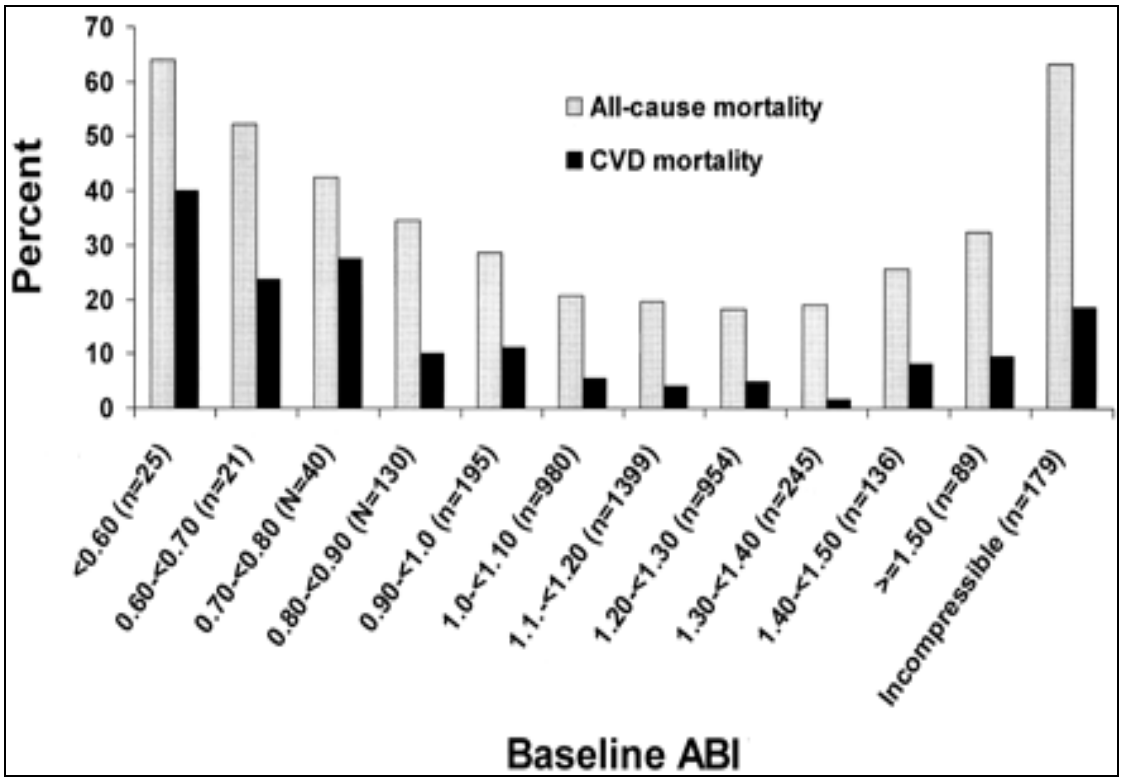


Figure 1-10. All-Cause and Cardiovascular Mortality Across the Spectrum of Ankle-Brachial Index in the Strong Heart Study[i]

In 2004, Resnick et al[116] published data from the Strong Heart Study showing that patients with ABI > 1.4 also had higher risk of cardiovascular events and all-cause mortality compared to patients in the normal range (**Figure 1-10**). These data suggest that the ankle-brachial index does not have a monotonic relationship with cardiovascular risk, but rather a U-shaped relationship where patients with either an abnormally low or an abnormally high ABI have an elevated cardiovascular risk. Data from the Cardiovascular Health Study,[24] the Health ABC Study,[25] and the Multi-Ethnic Study of Atherosclerosis[26] have confirmed the likely U-shaped association between ABI and cardiovascular risk.

[i] Reprinted with permission from Resnick et al *Circulation* 2004; 109: 733-739

The physiological mechanism resulting in a high ABI is usually assumed to be medial arterial calcification (MAC) in the lower extremities. Calcification of a lower extremity artery results in a falsely high reading of the ankle pressure due to stiffness in the arterial wall. In extreme cases, this results in a condition known as a "non-compressible" artery in which the ankle pressure cannot be obliterated. Most studies have shown that patients with non-compressible arteries have similar cardiovascular risk to those with very low ABI (**Figure 1-11**). Notably, the prevalence of high ABI and non-compressible arteries is significantly higher in diabetic patients than the general population.[117]

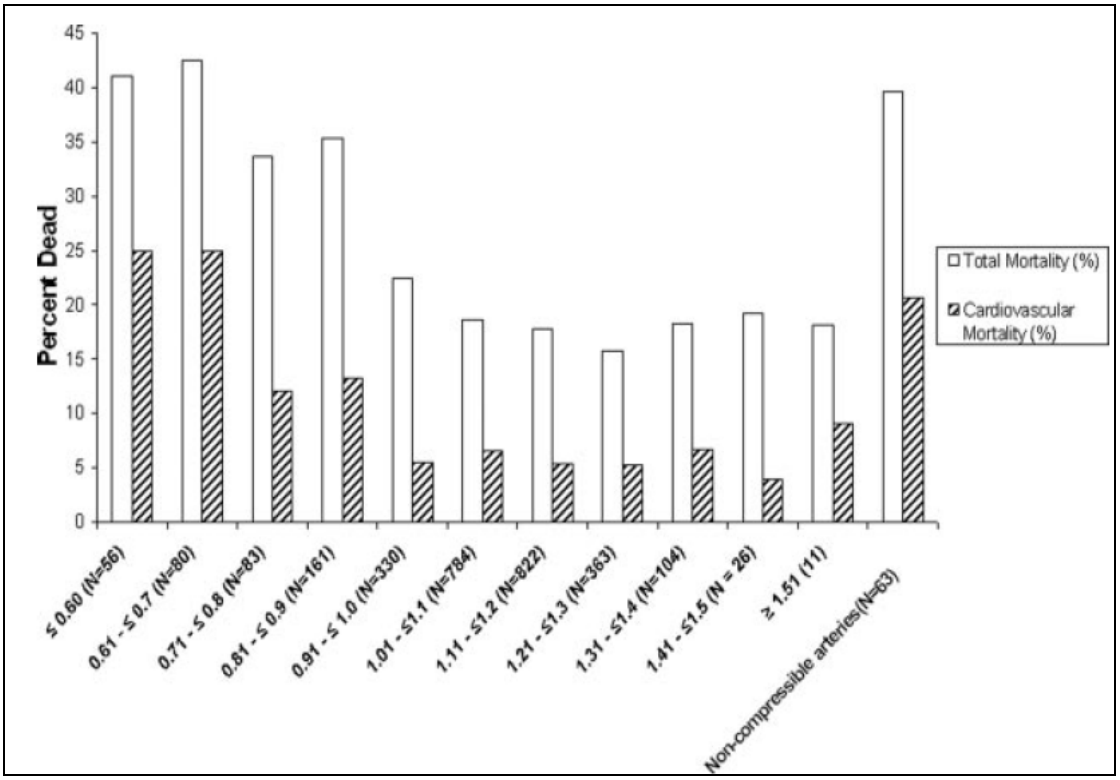


Figure 1-11. All-Cause and Cardiovascular Mortality Across the Spectrum of Ankle-Brachial Index in the Health ABC Study[j]

[j] Reprinted with permission from Sutton-Tyrrell et al. *Stroke* 2008; 39: 863-869

The role of peripheral arterial disease in this relationship is unclear. Some patients with medial arterial calcification may have coexisting PAD, but such calcification also can occur in the absence of any atherosclerotic lesion in the arterial lumen.[118] While several studies have shown elevated risk in patients with high ABI and/or non-compressible arteries, it is uncertain whether this excess risk is isolated to patients with underlying PAD or driven by some other disease process. Notably, in a healthy community-based population, persons with high ABI had similar cardiovascular risk profiles and event rates as persons with normal ABI,[119] and the aforementioned Health ABC data showed that the increased risk at the "high" end of ABI spectrum was far more pronounced in patients with non-compressible arteries than those with high ABI.[25] Therefore, the use of high ABI as a marker of cardiovascular risk is still somewhat controversial.

1.3.3 Measurement of ABI

The most common technique for assessment of the ankle and arm blood pressure involves a continuous wave Doppler system, first reported by Yao et al in 1969.[109] The protocol for measuring ankle-brachial index using Doppler ultrasound has been refined over several decades, but remains generally consistent across the cohort studies referenced above (Strong Heart Study, ARIC, CHS, MESA, and Health ABC). The full Ultrasound Research Laboratory protocol for measuring Doppler ABI in the independent data collection project (see **Appendix A**) was developed based on the techniques established in these cohort studies.

The ankle-brachial index can also be recorded with an oscillometric device (such as the Colin VP-1000 pulse waveform analyzer) that allows simultaneous blood pressure measurements at the right and left brachial and posterior tibial arteries. Testing the reliablity and reproducibility of ABI measures from this device is a principal aim of the independent data collection project in this dissertation; the reliability of similar devices has been documented in a few previous studies and has displayed reasonable agreement with Doppler measurements (a detailed description of these studies can be found in Section 1.3.4). The full Ultrasound Research Laboratory protocol used for the VP-1000 in the independent data collection project is included in **Appendix B**.

1.3.4 Existing Studies of Oscillometric ABI vs. Doppler ABI

Verberk et al published a 2012 meta-analysis[120] which pooled the results of eighteen studies (N=3290) comparing oscillometric ABI measurements to the Doppler method and found an average difference of 0.020 ± 0.018, indicating that oscillometric ABI tended to be higher than Doppler, although this difference was not statistically significant (p=0.28). The meta-analysis also reported a pooled linear correlation coefficient of 0.71 between Doppler ABI and oscillometric ABI. It should be noted that there was substantial heterogeneity between studies included in the meta-analysis; possible explanations include the use of different oscillometric devices, variation between study protocols, and differences in study populations.

Among the eighteen studies included in the meta-analysis mentioned above, eleven different oscillometric devices were used, and only two studies included in the pooled results used the Colin VP-1000 device available in the Ultrasound Research Laboratory. Two other studies have compared ankle blood pressures measured with the Colin to those measured with a Doppler probe, but these studies were not included in the meta-analysis due to lack of ABI data. The four studies comparing Colin and Doppler are summarized briefly here.

Cortez-Cooper et al (2003)[121] compared ankle blood pressure measurements taken with the Colin to those taken with Doppler in 52 healthy participants. This study showed strong linear correlation between ankle pressures (r=0.95) taken with Colin versus Doppler. Ankle pressures measured with Doppler were slightly higher (mean difference = 2.2 mm Hg) than Colin ankle pressures, but this difference was not statistically significant. Following this study, Nukumizu et

al (2007)[122] compared Doppler ankle pressures to Colin ankle pressures in 168 participants from a vascular clinic, all of whom had angiographically confirmed PAD (n=146) or abdominal aortic aneurysm (n=22). The Colin ankle pressures were slightly higher than Doppler ankle pressures, on average, but with no significant evidence of a difference. Nukumizu et al did not report a mean difference, but instead reported the ratios of Colin pressure divided by Doppler pressure for each participant, noting that 135 of 168 (80%) participants had a Colin ankle pressure within 10 percent of their Doppler ankle pressure.

In the largest study comparing Colin ABI measurements to Doppler ABI measurements, Pan et al (2007)[123] performed a population-based study analyzing data from 946 participants in several rural villages south of Shanghai. The Doppler ABI measurements were slightly higher than the Colin ABI measurements (mean difference=0.03). Richart et al (2009)[124] also found that Doppler ABI tended to be slightly greater than Colin ABI in a study of 105 participants but the difference was not statistically significant. The results from the four prior studies comparing Colin and Doppler ankle pressures and/or ABI can be synthesized into the following statements:

1) The Colin has displayed reasonable agreement with Doppler in participants with normal ABI.
2) The Colin may be less accurate for patients with extreme values of ABI (both low and high); in particular, it may provide falsely normal values for patients with very low values of ABI.
3) It is difficult to establish a directional relationship between Colin and Doppler measurements; three of the four studies suggest that the Doppler tends to report slightly higher ankle pressures and/or ABI than the Colin, but this could be accounted for by differences in study populations and protocol.

1.3.5 Clinical Significance and Guidelines

As detailed above, the ankle-brachial index provides valuable information about cardiovascular risk at both ends of the spectrum. Patients with either a low ABI or a high ABI are at increased cardiovascular risk and should be treated accordingly; as such, the ABI should be considered an effective, noninvasive screening tool that can be implemented at low cost: the PARTNERS program demonstrated that ABI technique can be easily performed by both nurses and physicians in the primary care setting.[28] Guidelines established by the American Heart Association[125] call for a screening ABI in high-risk patients meeting one or more of the following criteria:

- Individuals with exertional leg symptoms
- Individuals with nonhealing wounds
- Individuals who are 70 years and older
- Individuals who are 50 years and older with a history of smoking or diabetes.

Congruent with the last recommendation in the list above, the American Diabetes Association recommends that a screening ABI be performed in all diabetic individuals over 50 years of age, regardless of symptoms. If the patient has a normal ABI (0.91-1.30), it is recommended that the test be repeated within five years. However, if a patient presents with claudication symptoms, a screening ABI should be performed immediately regardless of time since the last test.

1.4 SUMMARY

The proposed research will produce three unique manuscripts with the principal focus of improving our knowledge of peripheral arterial disease. This introduction gives an overview of the prevalence of PAD, the effects of established cardiovascular risk factors on PAD risk, pertinent background information on selected novel risk factors that may explain part of the additional risk for PAD in type 2 diabetes, and a history of the principal diagnostic test for PAD and its utility. The specific aims of the three manuscripts are outlined on the following page.

2.0 SPECIFIC AIMS

This dissertation is composed of three manuscripts, each pertaining to peripheral arterial disease.

Specific Aim for Manuscript 1:

Test for difference in PAD incidence between two randomly assigned glycemic control strategies in a large cohort of T2DM patients free of PAD at study entry. This paper will inform physicians of the best treatment practice for diabetes patients at high risk for developing PAD.

Specific Aim for Manuscript 2:

Perform longitudinal analysis of the relationship(s) between established CVD risk factors, novel CVD risk factors, and the incidence of PAD. This paper could improve our knowledge of the pathophysiology of PAD in diabetes as well as provide insights into the effects of different glycemic control medications on the progression of atherosclerosis in this population.

Specific Aim for Manuscript 3:

Evaluate the reliability of ankle-brachial index measurements obtained using the Colin VP-1000 pulse waveform analyzer versus the traditional Doppler ultrasound method of measuring ABI. This project will provide useful quality-control data for the Ultrasound Research Laboratory, which uses the VP-1000 extensively in research studies, and its findings also may be generalized to other research settings.

3.0 MANUSCRIPT 1: FAVORABLE EFFECTS OF INSULIN SENSITIZERS PERTINENT TO PERIPHERAL ARTERIAL DISEASE IN TYPE 2 DIABETES: RESULTS FROM THE BYPASS ANGIOPLASTY REVASCULARIZATION INVESTIGATION 2 DIABETES (BARI 2D) TRIAL

3.1 ABSTRACT

(Oral Presentation at the American Diabetes Association 2012 Scientific Sessions)

Objective: The aim of this manuscript was to report the risk of incident peripheral arterial disease in a large randomized clinical trial that enrolled participants with stable coronary artery disease (CAD) and type 2 diabetes and compare the risk between assigned treatment arms.

Research Design and Methods: The Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial randomly assigned participants to insulin-sensitization (IS) therapy vs. insulin-providing (IP) therapy for glycemic control. Results showed similar five-year mortality in the two glycemic treatment arms. In secondary analyses reported here, we examine the effects of treatment assignment on the incidence of peripheral arterial disease (PAD). 1479 BARI 2D participants with normal ABI (0.91-1.30) were eligible for analysis. The following PAD-related outcomes are evaluated in this manuscript: new low ABI $\leq$0.9, a lower extremity revascularization, lower extremity amputation, and a composite of the three outcomes.

Results: During an average 4.6 years of follow-up, 303 participants experienced one or more of the outcomes listed above. Incidence of the composite outcome was significantly lower among participants assigned to IS therapy than those assigned to IP therapy (16.9% vs. 24.1%; P<0.001). The difference was significant in time-to-event analysis (HR=0.66, 95% CI [0.51, 0.83], P<0.001) and remained significant after adjustment for in-trial HbA1c (HR=0.76, 95% CI [0.59, 0.96], P=0.02).

Conclusions: In participants with type 2 diabetes mellitus that are free from PAD, a glycemic control strategy of insulin sensitization may be the preferred therapeutic strategy to reduce the incidence of PAD and subsequent outcomes.

3.2 INTRODUCTION

Peripheral arterial disease (PAD) is an atherosclerotic condition characterized by chronic occlusion of the arteries in the lower extremities. Prevalence estimates suggest that at least 5 million Americans have PAD.[14,15] The presence of PAD is a marker of generalized systemic atherosclerosis and is associated with cardiovascular morbidity and mortality.[22,23,24,25,26,27]

PAD is especially common in patients with type 2 diabetes mellitus.[41] PAD progresses more rapidly[80] and leads to worse outcomes[84] in type 2 diabetes patients than nondiabetic patients. Type 2 diabetes patients with PAD have a high risk of functional impairment,[81] mobility loss,[19] amputation,[82] and cardiovascular mortality.[85] High levels of glycosylated hemoglobin (HbA1c) is independently associated with increased risk of PAD in type 2 diabetes, suggesting that poor glycemic control may be a risk factor for PAD.[90,91,92,126] Prior reviews have speculated that treatment with insulin sensitizers may reduce the risk of PAD in type 2 diabetes patients.[99,100,101] However, this has never been demonstrated in a randomized controlled trial.

The Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial provides an opportunity to compare the effects of an insulin-sensitizing glycemic control strategy to an insulin-providing strategy on the incidence of PAD in a cohort of participants with type 2 diabetes and documented stable coronary artery disease. We have previously demonstrated that mortality and incidence of major cardiovascular events was comparable in the glycemic control arms.[127] In this report, we present the results of secondary analyses undertaken to examine the association between glycemic treatment assignment and the incidence of PAD.

3.3 METHODS

BARI 2D Trial

A detailed explanation of the BARI 2D trial has been published elsewhere.[128,129] The primary aim of the BARI 2D trial was to determine the optimal treatment for participants with type 2 diabetes mellitus and documented stable coronary artery disease (CAD). The BARI 2D trial utilized a 2x2 factorial design in which participants were assigned at random to initial elective revascularization with intensive medical therapy (REV) versus intensive medical therapy alone (MED) while simultaneously assigned at random to an insulin sensitizing (IS) strategy versus an insulin providing (IP) strategy of glycemic control. All participants were treated medically to achieve targets of HbA1c < 7.0%, LDL cholesterol < 100 mg/dL, and blood pressure ≤ 130/80 mm Hg. All participants received counseling regarding smoking cessation, weight loss, and regular exercise.

BARI 2D included 49 clinical sites throughout North America, South America, and Europe and was coordinated at the University of Pittsburgh. The local institutional review boards approved trial protocol and all participants provided informed consent. Recruitment began in 2001 and continued until 2005; treatment continued until the 6-year visit or the last annual visit before December 1, 2008. The overall study cohort for BARI 2D consisted of 2368 participants. The primary endpoint for BARI 2D was death from any cause, and the principal secondary endpoint was a composite of death, myocardial infarction, or stroke. Results for each of these have been published elsewhere.[127] This manuscript reports the results of a post hoc analysis to examine peripheral arterial disease and related outcomes.

Glycemic Control Strategies

All BARI 2D participants were treated with a target HbA1c < 7.0%. Participants assigned to IP therapy could be treated with sulfonylureas, repaglinide, nateglinide, or insulin itself. Participants assigned to IS therapy could be treated with thiazolidinedones (glitazones) and/or metformin. Alpha-glucosidase inhibitors could be used with either treatment assignment. The trial was not designed to compare specific drugs, but rather to compare the 2 mechanistically different treatment strategies. A detailed description of the BARI 2D glycemic control protocol can be found in Magee et al.[130] Participants with HbA1c>8.0% while taking the assigned treatment were permitted to receive the glucose-lowering drugs from the opposite treatment arm to bring HbA1c within the range 7.0% to 8.0%. Approximately 30% of participants assigned to IS treatment also required medications from the IP arm, while 10% of participants assigned to IP treatment required medications from the IS arm. Nearly 90% of participants in both the insulin-sensitization group and the insulin-provision group were taking their assigned medications at three years.[127]

Diagnosis of Peripheral Arterial Disease

The following lower-extremity outcomes were analyzed: recorded decrease in ankle-brachial index (ABI) to abnormal level (ABI $\leq$ 0.9), lower extremity revascularization, and lower extremity amputation. This manuscript reports incidence of each individual outcome as well incidence of a composite PAD outcome including participants with any one of the individual outcomes. Only participants with a normal ABI (0.91-1.30) at study entry were eligible for the primary analysis in this manuscript; the cutoff values for low, normal, and high ABI are chosen in accordance with values published in an ADA consensus statement on peripheral arterial

disease in diabetes. In secondary analysis, we report the incidence of lower extremity revascularization and amputation among participants with low ABI (<0.9) at study entry.

We have previously reported the baseline prevalence and predictors of abnormal ABI in the BARI 2D trial.[131] Participants with abnormal ABI at study entry were excluded from the primary analysis in this manuscript because i) participants with low ABI at study entry were deemed as already having PAD and ii) participants with high ABI and/or non-compressible arteries are likely to have arterial calcifications that would make it difficult to diagnose PAD using the ankle-brachial index. Participants with a history of lower extremity revascularization or lower extremity amputation were also excluded from the primary analysis because of the likelihood that these participants already suffered from PAD. The algorithm for determining PAD status in this manuscript is as follows:

Each participant's ABI was measured at study entry and annually thereafter. All ABI measurements were taken by certified technicians using a Doppler probe. Participants were asked to rest in a supine position for 5 minutes, after which the technician recorded the systolic blood pressure of the brachial artery of both arms and the posterior tibial artery of both ankles. The higher of the two brachial pressures was used to calculate ABI for each leg, and participants were classified according to their lowest ABI. Participants with normal ABI (0.91-1.30) at study entry were defined as incident cases of PAD if they had an ABI≤0.9 during follow-up with a decrease of at least 0.1 from their baseline measurement.

Participants who had a lower extremity revascularization or amputation during follow-up were included in the composite PAD outcome, even in the absence of a recorded low ABI. We acknowledge that lower extremity revascularization and amputation may be performed for reasons other than atherosclerotic PAD, so the incidence of each outcome is reported in this manuscript as well as the composite PAD outcome to allow reader assessment of practical implications. Intermittent claudication was not considered as an outcome because BARI 2D did not employ a validated claudication questionnaire.

Statistical Methods

The primary comparative analyses were performed according to the intention-to-treat principle. In addition, we performed "per-protocol analyses" that include only participants who remained on assigned treatment without any use of medications from the opposing treatment arm after the initial 6 months of the trial when study treatments were to be implemented and adjusted. Descriptive statistics include means ± SDs and proportions; medians and interquartile ranges are presented for highly skewed data. Variables were compared between the two randomized glycemic control strategies (IS vs. IP) using t-tests, Wilcoxon tests, and chi-squared tests for continuous, skewed continuous, and categorical data, respectively.

Kaplan-Meier estimates of the 5-year event rates were calculated for outcomes of interest and log-rank tests were used to compare the incidence of each PAD outcome by assigned glycemic control strategy for patients with normal ABI at baseline, as described above. Time-to-event for each lower extremity outcome was defined as time from date of randomization to the event (new low ankle-brachial index, lower extremity revascularization, or lower extremity amputation); for

the composite outcome, time-to-event was defined as time from date of randomization to the first event. In the absence of an event, participants were censored at their last full-protocol follow-up visit. We performed a second analysis to compare incidence of lower extremity revascularization and amputation in patients with low ABI at baseline to assess the incidence of severe outcomes in these patients. We also calculated Kaplan-Meier event rates for the composite PAD outcome stratified by insulin use at study entry to see if the effects of assigned glycemic control strategy were consistent regardless of previous use of insulin therapy.

Cox proportional-hazards models were used to estimate hazard ratios and associated 95% confidence intervals for IS strategy with IP as reference group. Time-to-event was defined in the same fashion described above. A second model was constructed with in-trial HbA1c included as a time-varying covariate, updated at the time of each new ABI measurement, to determine whether potential differences between IS and IP strategy were attenuated by adjustment for glycemic control. The effects of assigned cardiovascular treatment strategy and the interaction between the assigned glycemic control strategy and cardiovascular treatment strategy were also tested. P-values less than 0.05 were considered statistically significant; no adjustment was made for multiple comparisons.

3.4 RESULTS

The BARI 2D study population consisted of 2368 participants with type 2 diabetes mellitus and stable CAD. Only participants with normal ABI at study entry and no history of PAD were selected for this paper's primary analysis. 889 participants were excluded due to history of PAD, missing baseline ABI, or abnormal baseline ABI, leaving 1479 BARI 2D participants eligible for this analysis (**Figure 3-1**). The participants excluded from this analysis were generally older, heavier, more likely to be smokers, had higher systolic blood pressure, higher pulse pressure, a longer duration of diabetes, and were more likely to have renal dysfunction at study entry than those included in this analysis (data not shown). Since many of the participants excluded from this analysis had a history of PAD, this is consistent with expectations.

Participants included in the intention-to-treat analysis were age 61.9 ± 8.0 years old, 72% male, and 15% African-American. The distribution of baseline lipid values, blood pressure, glycemic control, and markers of renal function were similar between the assigned glycemic treatment groups (**Table 3-1**). The mean ABI at study entry was 1.10 in the IS group and 1.09 in the IP group ($p=0.23$). There were no significant differences in major baseline demographic or clinical characteristics between the assigned glycemic treatment groups.

Incidence of the composite PAD outcome was significantly lower in the IS arm than in the IP arm (16.9% vs. 24.1%, $p<0.001$, event rates for all intention-to-treat comparisons in **Table 3-2**). On the time-to-event curve, the difference becomes noticeable after three years and increases during longer follow-up (**Figure 3-2**). Examining each clinical outcome separately, participants

assigned to IS therapy had a significantly lower rate of low ABI (16.5% vs. 22.7%, p<0.001) and amputation (0.1% vs. 1.6%, p=0.002) and a moderately lower rate of lower extremity revascularization (1.1% vs. 2.6%, p=0.07) than those assigned to IP therapy. Among participants with low ABI at baseline, we observed no significant difference between the IS arm and IP arm in risk of lower extremity revascularization (7.7% vs. 6.7%, p=0.68) or lower extremity amputation (3.4% vs. 7.2%, p=0.08).

The lower risk of PAD and related outcomes in participants assigned to IS therapy was also significant in time-to-event analyses using Cox models (HR for IS vs. IP therapy for composite outcome=0.66, 95% CI [0.51, 0.83], p<0.001, **Table 3-3**). The effects of glycemic control strategy were partially attenuated by adjustment for in-trial HbA1c; however, even with adjustment for in-trial HbA1c, there was a significantly lower risk of PAD in participants assigned to IS therapy (adjusted HR for IS vs. IP therapy for composite outcome=0.76, 95% CI [0.59, 0.96], p=0.02). None of the PAD outcomes were associated with assigned cardiovascular treatment strategy, and no interactions between glycemic control and cardiovascular treatment strategies were statistically significant (p>0.10 for all).

Among participants in the per protocol analysis, there were significant baseline differences between the glycemic control arms in average duration of diabetes, proportion using insulin at study entry, and HbA1c (**Table 3-4**) but not in other risk factors such as age, smoking, race or baseline ABI. As in intention-to-treat analyses, the results generally favor IS therapy for each outcome (incidence of composite outcome 12.4% vs. 26.0%, p<0.001; event rates for all per-protocol comparisons shown in **Table 3-5**). The effects of IS therapy are also significant in per-

protocol analyses when using time-to-event analyses (HR for IS vs. IP therapy for composite outcome= 0.44, 95% CI [0.31, 0.62], p<0.001, **Table 3-6**). As in the intention-to-treat analyses, the effect of glycemic control strategy is partially attenuated by adjustment for in-trial HbA1c in the per-protocol analysis, but still significant (adjusted HR for IS vs. IP therapy for composite outcome= 0.54, 95% CI [0.37, 0.79], p=0.002).

Insulin treatment at study entry was associated with greater risk of the composite PAD outcome (patients on insulin: 26.3% vs. patients not on insulin: 18.5%, p=0.01). There was a significant difference in risk for the composite PAD outcome between the glycemic control arms regardless of whether participants were receiving insulin at study entry (**Figure 3-3 and Figure 3-4**). The incidence of PAD was lower in the IS arm among participants receiving insulin at study entry (IS: 20.7% vs. IP: 31.9%, p=0.01) and also those not receiving insulin at study entry (IS: 15.6% vs. IP: 21.3%, p=0.01). The difference in PAD incidence between the IS arm and the IP arm tended to emerge earlier for participants on insulin at study entry.

3.5 DISCUSSION

In a large cohort of participants with stable CAD and type 2 diabetes mellitus, participants assigned to insulin sensitizing therapy experienced significantly fewer cases of incident PAD than participants assigned to insulin providing therapy over an average of 4.6 years of clinical follow-up. The difference in PAD risk between the glycemic control arms was significant regardless of assigned cardiovascular treatment strategy and consistent in both intention-to-treat analyses and per-protocol analyses. The results also favored insulin sensitizing therapy for patients receiving insulin at study entry as well as those not receiving insulin at study entry. This is the first randomized controlled trial to demonstrate that treatment with insulin sensitizing agents reduces the risk of incident peripheral arterial disease in participants with type 2 diabetes mellitus.

The reduction in risk of amputation and/or revascularization with IS therapy was not as pronounced for patients with low ABI at baseline, although the incidence of each outcome was still lower for patients assigned to IS therapy than for patients assigned to IP therapy. This could be explained in two ways. First, perhaps IS therapy does have a benefit for these patients, but there was insufficient sample size to detect an effect in the subgroup with low ABI at study entry. Alternatively, it's possible that the benefit of IS therapy on peripheral outcomes is lessened in patients with existing PAD because the disease in the lower extremities has advanced beyond "prevention" of the atherosclerotic process in this vascular bed.

The only previous study to demonstrate a similar result is the PROactive trial, which found that treatment with pioglitazone versus placebo resulted in a moderate decrease in the rate of leg

revascularizations and amputations among participants free from PAD at study entry.[83] Notably, the BARI 2D results demonstrate an even stronger benefit of insulin sensitizing therapy in this population. This may be explained by the shorter follow-up time in PROactive (three years) than BARI 2D (five years), since the BARI 2D results showed that the treatment benefit of IS therapy began to emerge around three years and progressively increased thereafter. It could also be a result of moderate differences in treatment protocol; PROactive randomly assigned patients to pioglitazone or placebo in the setting of continued additional diabetes therapy, whereas BARI 2D assigned patients to insulin sensitizing strategy or insulin providing strategy.

One potential mechanism through which IS treatment may have reduced the risk of PAD incidence in BARI 2D is better glycemic control; we have previously reported that participants assigned to IS had lower HbA1c than participants assigned to IP during BARI 2D follow-up.[98] However, the treatment difference reported here was significant after adjustment for in-trial HbA1c, suggesting that IS therapy conferred a benefit beyond better glycemic control.

Furthermore, previous trials of intensive glucose-lowering therapy have not demonstrated a consistent reduction in macrovascular outcomes. The Veterans Affairs Diabetes Trial (VADT), the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial and the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial all failed to demonstrate that intensive glucose-lowering therapy reduced the risk of macrovascular outcomes.[94,95,96] With that in mind, the BARI 2D results are particularly encouraging because the decreased risk of PAD in participants assigned to insulin sensitizing therapy was significant even while adjusting for in-trial glycemic control, suggesting that

improvements in macrovascular outcomes may be achieved by changing the mechanistic approach rather than targeting a lower HbA1c.

A second plausible mechanism of the reduced PAD risk with IS therapy may be the anti-inflammatory effects of thiazolinediones used in the BARI 2D trial, which may retard atherosclerosis development and progression.[132,133] We have previously reported that the IS strategy led to changes in biomarker profiles indicative of a profibrinolytic, antithrombotic, and anti-inflammatory state. [98] This could contribute to the lower incidence of PAD in the IS group. While thiazolinediones have been shown to induce and maintain the regression of carotid intima-media thickness in participants with type 2 diabetes,[134] to our knowledge no previous study has reported an effect on peripheral arterial disease. However, because the BARI 2D trial was designed to examine mechanistically different treatment strategies rather than individual drugs, we cannot say for certain whether thiazolinediones alone were responsible for the reduction in PAD risk.

It is also plausible that the observed results reflect a harmful effect of IP therapy rather than a protective benefit of IS therapy. Hyperinsulinemia has long been a known risk factor for atherosclerosis, although the causality of the relationship is controversial, and the mechanism is unclear. One study suggests that hyperinsulinemia may promote atherosclerosis by promoting macrophage foam cell accumulation.[135] However, this is not clearly established; further research is needed to determine the potential atherogenic effects of insulin.

To date, no pharmacologic therapies have proven to reduce the risk of incident PAD in type 2 diabetes patients. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study has reported lower amputation rates in patients assigned to treatment with fenofibrate versus placebo; however, PAD status at study entry was not reported for the FIELD results, so we are uncertain whether these findings extend to incident cases of PAD.[136] For patients with PAD, current ACC/AHA guidelines recommend aggressive management of atherosclerotic risk factors to reduce future cardiovascular events.[137] Exercise conditioning[138] and smoking cessation[139] have proven beneficial effects for those with PAD, but these are generally recommended for all type 2 diabetes patients regardless of their effects on PAD risk. Notably, while all participants in BARI 2D received intensive medical therapy, counseling regarding smoking cessation, and regular exercise, treatment with insulin sensitizing agents still resulted in fewer cases of incident PAD than treatment with insulin providing agents.

One potential limitation of this research is the composition of the BARI 2D population, which was restricted to patients with documented coronary artery disease suitable for elective revascularization and type 2 diabetes mellitus. Given that both of these conditions are independently associated with peripheral arterial disease, the BARI 2D population is at very high risk for PAD, and therefore, the findings from this research may not extend to those at lower risk. Further study will be needed to determine if insulin sensitizing medications offer the same benefit to lower-risk patients. Additional limitations include the fact that individual drugs cannot be evaluated because of the trial design, which assigned patients to a mechanistic treatment strategy rather than a specific drug, and the lack of a standardized claudication questionnaire, which might have resulted in the diagnosis of a few more PAD cases during follow-up.

3.6 CONCLUSION

In summary, we have reported that an insulin sensitizing strategy for glycemic control resulted in fewer incident PAD cases, lower extremity revascularizations, and lower extremity amputations than treatment with insulin providing agents in type 2 diabetes patients. The difference between glycemic treatment arms remained significant with adjustment for in-trial HbA1c, suggesting that insulin sensitizers confer a benefit independent of glycemic control. Our results suggest that treatment of type 2 diabetes patients with insulin sensitizers might reduce the morbidity and treatment cost of PAD in this population.

3.7 TABLES AND FIGURES

Table 3-1. Baseline Characteristics of BARI 2D Patients with Normal ABI at Study Entry (N=1479), by Assigned Glycemic Control Strategy

	Assigned Glycemic Control Strategy		
	IS (N=735)	IP (N=744)	P-Value
Age at Study Entry, Years	61.8, 8.9	62.0, 8.7	0.68
Sex (Male), %	71.7	71.9	0.92
Race (Black), %	16.2	14.5	0.37
BMI, kg/m^2	31.6, 5.9	31.4, 5.6	0.48
Current Smoker, %	11.7	11.4	0.87
Cholesterol, mg/dl	166.7, 41.0	170.2, 39.6	0.11
LDL, mg/dl	94.4, 33.0	96.7, 31.8	0.18
HDL, mg/dl	37.6, 9.6	38.2, 10.1	0.25
Triglycerides*, mg/dl	146 (99-217)	152 (108-220)	0.37
Systolic BP, mm Hg	130.9, 18.7	130.0, 19.1	0.39
Diastolic BP, mm Hg	75.0, 11.0	74.3, 10.6	0.26
Pulse Pressure, mm Hg	55.9, 15.1	55.7, 14.7	0.78
CRP*, (ug/mL)	2.1 (1.0-5.7)	2.2 (1.0-5.2)	0.72
Number of Vessels >50% Stenosis			
0/1	37.6	32.4	0.07
2	33.2	38.7	
3	29.1	28.8	
Proximal LAD > 50% Stenosis	12.1	15.2	0.08
Left Ventricular Ejection Fraction	57.6, 10.7	58.1, 10.7	0.42
Baseline ABI	1.10, 0.10	1.09, 0.11	0.23
Duration of DM, Years	9.4, 8.0	9.9, 8.2	0.25
HbA1c, % (mmol/mol)	7.6 (60), 1.6	7.7 (61), 1.6	0.10
Diabetic Peripheral Neuropathy*	50.8%	49.2%	0.75
eGFR**	77.6 (63.8-91.5)	76.6 (64.2-91.5)	0.93
eGFR<60, %	19.8	18.8	0.62
ACR*, mg/g	10.7 (4.8-42.4)	10.8 (5.2-34.6)	0.80
Albuminuria, %			
No Albuminuria (ACR<30)	70.0	72.8	0.49

Table 3-1 Continued

Microalbuminuria (30<ACR<300)	22.6	20.1	
Macroalbuminuria (ACR>300)	7.4	7.1	
Diabetes Medications At Study Entry			
Insulin, %	25.6	25.7	0.97
Sulfonylurea, %	53.4	53.4	0.99
Metformin, %	55.9	55.8	0.98
Thiazolidinedones, %	20.6	17.2	0.10

IS =Insulin Sensitizing Assignment; IP = Insulin Providing Assignment; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein; BP = blood pressure; CRP = C-reactive protein; LAD = left anterior descending; ABI = ankle-brachial index; DM = diabetes mellitus; HbA1c= glycosylated hemoglobin; eGFR = estimated glomerular filtration rate; ACR = albumin/creatinine ratio

*Diabetic peripheral neuropathy assessed using Michigan Neuropathy Screening Instrument (clinical MNSI score ≥ 2)

**Triglycerides, eGFR, ACR, and CRP presented as median (Q1-Q3) because of their skewed distributions

Table 3-2. Cumulative Incidence of Lower Extremity Outcomes, by Assigned Glycemic Control Strategy, Intention-To-Treat Analysis

	Patients with Normal ABI at Study Entry (N=1479)		
	IS (N=735)	IP (N=744)	P-Value
Peripheral Arterial Disease*	124 (16.9%)	179 (24.1%)	<.001
Incident Low ABI	121 (16.5%)	169 (22.7%)	0.001
Lower Extremity Revascularization	8 (1.1%)	17 (2.6%)	0.07
Lower Extremity Amputation	1 (0.1%)	12 (1.6%)	0.002
	Patients with Low ABI at Study Entry (N=430)		
	IS (N=207)	IP (N=223)	P-Value
Lower Extremity Revascularization	14 (6.7%)	17 (7.7%)	0.69
Lower Extremity Amputation	7 (3.4%)	16 (7.2%)	0.08
Revascularization and/or Amputation	19 (9.2%)	25 (11.2%)	0.48

IS =Insulin Sensitizing Assignment; IP = Insulin Providing Assignment

*Patients are classified as an incident case of peripheral arterial disease if any of the following occur: i) ABI < 0.9 with a decrease of 0.1 from baseline, ii) lower extremity revascularization, iii) lower extremity amputation

Table 3-3. Effects of Assigned Glycemic Control Strategy on Lower Extremity Outcomes (N=1479)

		Unadjusted			Adjusted for in-trial HbA1c		
Outcome	**# Events**	HR	95% CI	P-Value	HR	95% CI	P-Value
Peripheral Arterial Disease	303	0.66	0.51, 0.83	<.001	0.76	0.59, 0.96	0.02
Incident Low ABI	290	0.68	0.54, 0.85	0.001	0.73	0.57, 0.93	0.002
Lower Extremity Revascularization	25	0.47	0.20, 1.09	0.08	0.58	0.24, 1.37	0.22
Lower Extremity Amputation	13	0.08	0.01, 0.63	0.02	0.12	0.02, 0.91	0.04

HR = Hazard Ratio; 95% CI = 95% confidence interval

*Hazard Ratios are for IS arm vs. IP arm (reference group)

Table 3-4. Baseline Characteristics of Participants in Per Protocol Analysis (N=942)

	IP (N=643)	IS (N=299)	P-Value
Age at study entry, mean, sd	61.8, 8.7	62.3, 9.2	0.4413
Male, %	71.7	75.9	0.1738
Black, %	14.5	12.4	0.3869
BMI, mean, sd	31.4, 5.6	30.6, 5.2	0.0385
Current Smoker, %	10.7	10	0.7395
Total cholesterol mg/dl, mean, sd	171.1, 39.4	164.6, 39.1	0.0211
LDL mg/dl, mean, sd	97.3, 31.3	93.7, 31.7	0.1144
HDL mg/dl, mean, sd	38.4, 10.2	37.2, 9.1	0.0648
Triglyceride mg/dl, mean, sd	184.8, 154.6	171.1, 113.3	0.1283
Systolic BP average, mean, sd	130.2, 19.1	130.3, 19.4	0.9599
Diastolic BP average, mean, sd	74.7, 10.7	76.2, 10.4	0.0436
Duration of DM, mean, sd	10.3, 8.2	6.6, 6.7	<.0001
On insulin at study entry, %	28.1	5.0	<.0001
HbA1c %, mean, sd	7.8, 1.6	7.0, 1.3	<.0001
ACR mg/g, mean, sd	125.9, 468.5	38.4, 122.9	<.0001
Albuminuria categories, %			
No albuminuria	72	79.5	0.0022
Micro albuminuria	20.7	18.7	
Macro albuminuria	7.3	1.8	
ABI, mean, sd	1.09, 0.1	1.10, 0.1	0.2684

Table 3-5. Cumulative Incidence of Lower Extremity Outcomes, by Glycemic Control Strategy, Per Protocol Analysis (N=942)

	IS (N=299)	IP (N=643)	P-Value
Peripheral Arterial Disease*	37 (12.4%)	167 (26.0%)	<.001
Incident Low ABI	36 (12.0%)	158 (24.6%)	<.001
Lower Extremity Revascularization	3 (1.0%)	15 (2.3%)	0.16
Lower Extremity Amputation	0 (0.0%)	12 (1.9%)	0.002

*Patients are classified as an incident case of peripheral arterial disease if any of the following occur: i) ABI < 0.9 with a decrease of 0.1 from baseline, ii) lower extremity revascularization, iii) lower extremity amputation

Table 3-6. Effects of Glycemic Control Strategy on Lower Extremity Outcomes, Per Protocol Analysis (N=942)

		Unadjusted			Adjusted for in-trial HbA1c		
Outcome	**# Events**	HR	95% CI	P-Value	HR	95% CI	P-Value
Peripheral Arterial Disease	204	0.44	0.31, 0.62	<.001	0.54	0.37, 0.79	0.002
Incident Low ABI	194	0.44	0.31, 0.63	<.001	0.54	0.37, 0.80	0.002
Lower Extremity Revascularization	18	0.43	0.12, 1.48	0.18	0.70	0.18, 2.63	0.59
Lower Extremity Amputation	12	**	**	**	**	**	**

HR = Hazard Ratio; 95% CI = 95% confidence interval

*Hazard Ratios are for IS arm vs. IP arm (reference group)

**Hazard Ratio cannot be estimated for amputation because, in per-protocol analysis, all amputation events occurred in IP arm and there are no amputation events in IS arm

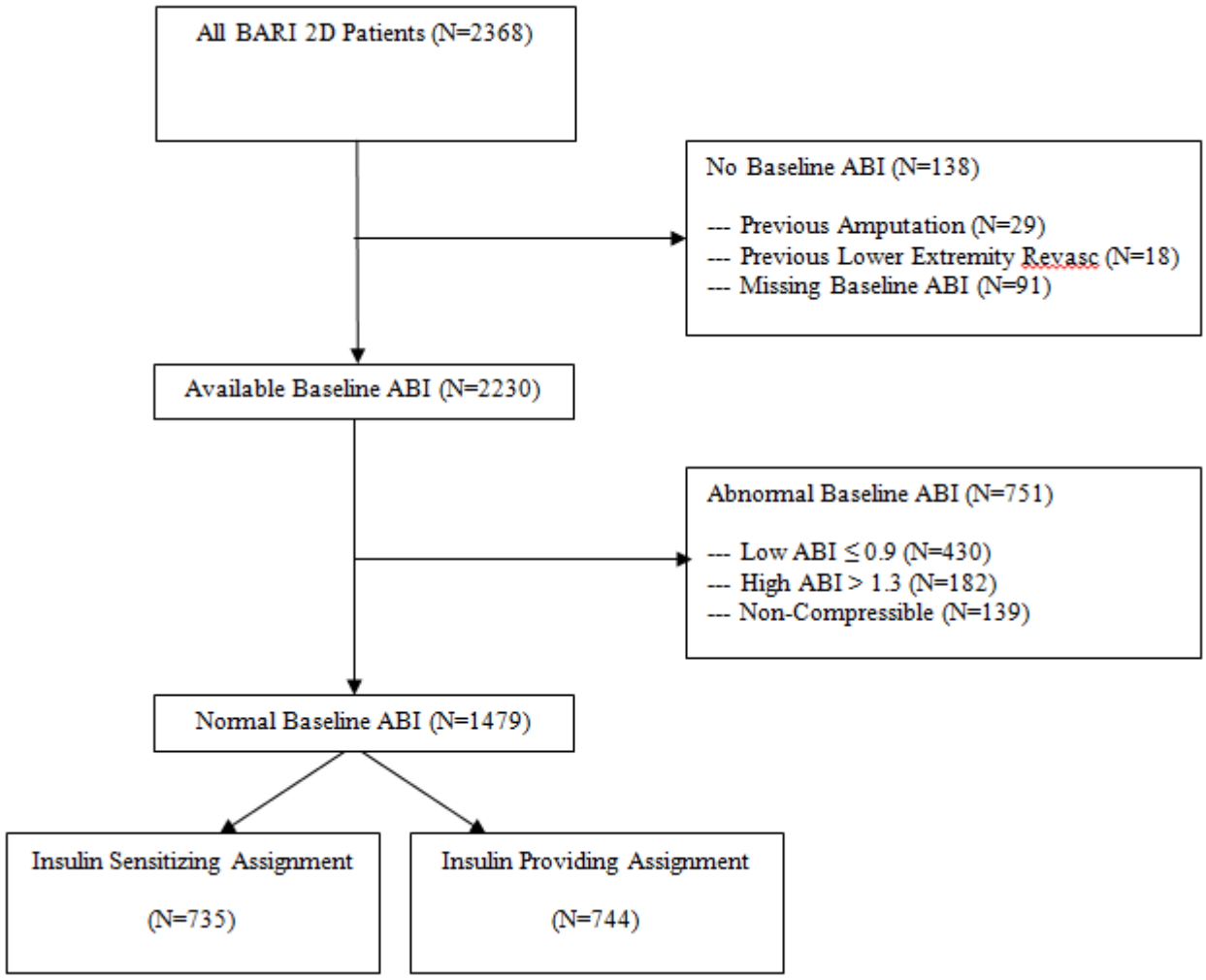


Figure 3-1. Flowchart of Ankle-Brachial Index Measurements Available in All BARI 2D Patients (N=2368)

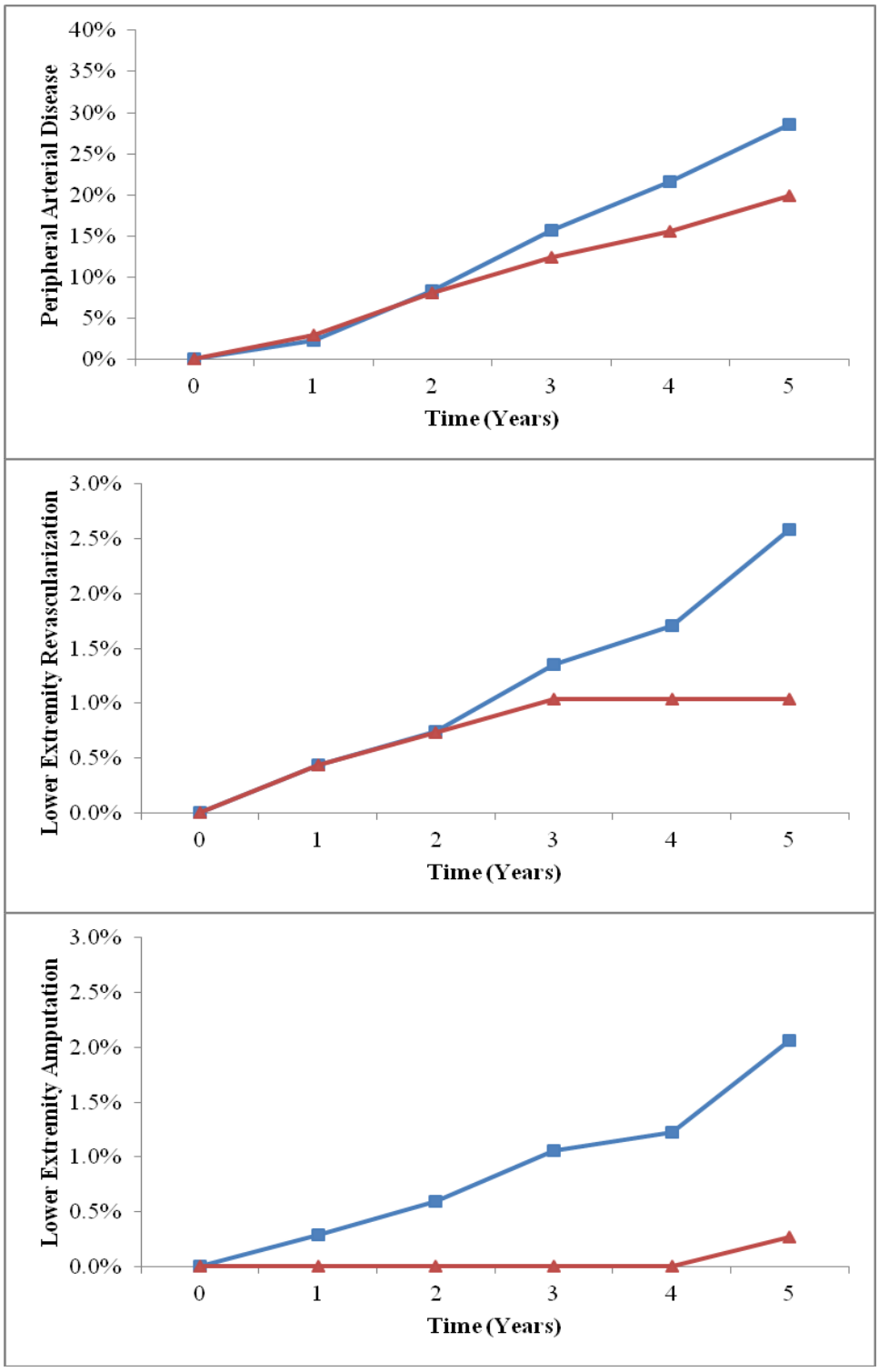


Figure 3-2. Cumulative Incidence of Peripheral Arterial Disease and Related Outcomes, by Assigned Glycemic Control Strategy

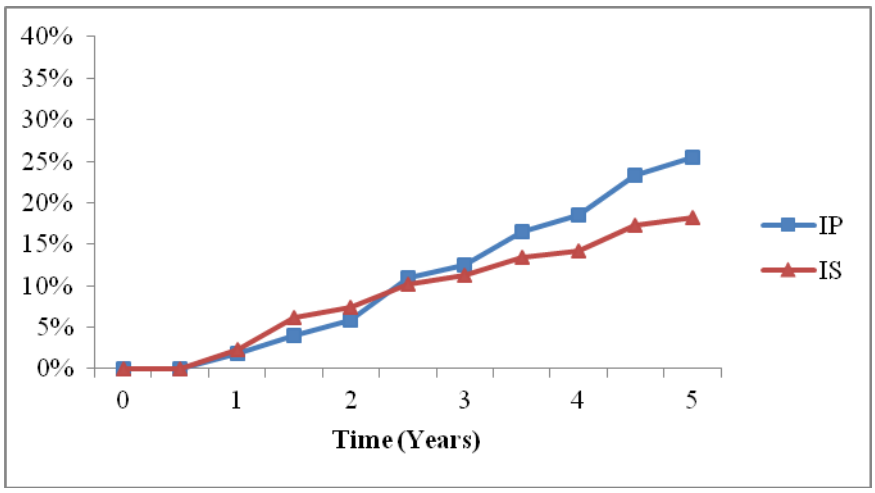


Figure 3-3. Cumulative Incidence of Peripheral Arterial Disease Among Patients Not On Insulin at Baseline, by Assigned Glycemic Control Strategy

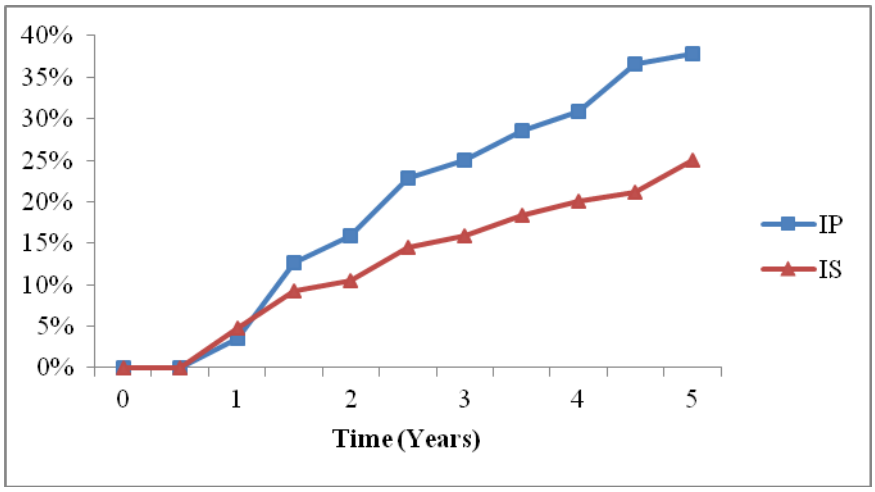


Figure 3-4. Cumulative Incidence of Peripheral Arterial Disease Among Patients On Insulin at Baseline, by Assigned Glycemic Control Strategy

4.0 MANUSCRIPT 2: RISK FACTORS FOR PERIPHERAL ARTERIAL DISEASE IN TYPE 2 DIABETES: RESULTS FROM THE BYPASS ANGIOPLASTY REVASCULARIZATION INVESTIGATION 2 DIABETES (BARI 2D) TRIAL

4.1 ABSTRACT

(Oral Presentation at the American Heart Association 2012 Scientific Sessions)

Objective: Define risk factors for the incidence of peripheral arterial disease (PAD) in a large cohort of type 2 diabetes mellitus (T2DM) patients, overall and within the context of two different assigned glycemic control strategies.

Research Design and Methods: The Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial randomly assigned participants to insulin-sensitization (IS) therapy vs. insulin-providing (IP) therapy. In these secondary analyses, we examine baseline risk factors for the incidence of lower extremity outcomes within the overall cohort and time-varying risk factors within each assigned treatment arm. 1479 BARI 2D participants with normal ABI (0.91-1.30) were eligible for analysis. Cox proportional-hazards models were used to define the association between each risk factor and incidence of lower extremity outcome.

Results: Age, sex, race, and smoking were all significantly associated with lower extremity outcomes in the BARI 2D cohort. Additional baseline risk factors included systolic blood pressure, pulse pressure, HbA1c, albumin-creatinine ratio, and C-reactive protein. In stratified analyses of time-varying covariates, changes in lipids, systolic blood pressure, pulse pressure, albumin-creatinine ratio, and D-dimer were most predictive among IS patients, while HbA1c was most predictive among IP patients.

Conclusions: In participants with type 2 diabetes mellitus that are free from PAD, traditional cardiovascular risk factors accounted for most predictive value of incident lower extremity outcomes. Inflammatory biomarkers such as C-reactive protein, fibrinogen, and D-dimer also were associated with increased risk of lower extremity outcomes, but may have more predictive value in patients treated with insulin sensitizing medications.

4.2 INTRODUCTION

Peripheral arterial disease (PAD) is a critical manifestation of atherosclerosis, a chronic disease that is one of the leading causes of death and disability in the United States.[1] Prevalence estimates of PAD have steadily risen over the past several decades, and recent epidemiological data suggests that approximately 8 million Americans likely have PAD.[12,13,15] Patients with PAD have increased risk of functional limitation, physical disability, leg revascularization, and amputation.[18,19,20] The presence of PAD is indicative of generalized systemic atherosclerosis and therefore associated with increased risk of all-cause mortality and cardiovascular mortality.[22,23,24,25,26,27]

PAD is especially common among patients with type 2 diabetes mellitus (T2DM). Patients with T2DM have an abnormal cluster of hyperglycemia, elevated free fatty acids, and insulin resistance that results in oxidative stress and endothelial dysfunction, key steps in the atherosclerotic process that could lead to PAD.[140,141] NHANES data suggest that patients with T2DM have a threefold increased risk of PAD compared to the general population.[14] PAD also tends to progress faster and lead to worse outcomes in T2DM patients than in nondiabetic patients.[84] The American Diabetes Association has previously issued a consensus statement with guidelines for the diagnosis and management of PAD in patients with diabetes, in recognition of the common nature and severity of this problem.[41]

In addition to the excess risk for patients with T2DM, previous studies have identified a number of risk factors for PAD including age, race, smoking, hypertension, and lipids.[16,30,31,32,34] The direction of these relationships generally matches those between the same risk factors and

coronary artery disease (CAD), although the magnitude of some relationships varies; for example, smoking carries a stronger relationship with PAD than it does with CAD. There is also growing evidence that several biomarkers indicative of inflammation and/or coagulation such as C-reactive protein (CRP), D-dimer, and fibrinogen may be associated with increased PAD risk and/or worse outcomes in peripheral arterial disease.[73,74,75,76,142,143,144,145]

In the context of developing atherosclerosis, CRP, fibrinogen, and D-dimer are indicative of distinct but related processes pertaining to inflammation, coagulation, and fibrinolysis.[146] An uncontrolled coagulatory response to inflammation may lead to a situation in which coagulation and/or thrombosis contribute to disease; for example, thrombus formation on a ruptured atherosclerotic plaque is the pathologic basis of an acute thrombotic event. Even before an acute event, elevated levels of these biomarkers are indicative of inflammatory and coagulatory cascades that may correspond with the development of atherosclerosis. The acute fibrinolytic response to inflammation results in the release of tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1),[54] which may also provide predictive value.

Results from the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial suggest that T2DM patients treated with an insulin sensitizing (IS) regimen experienced improvements in biomarker profiles not observed in those assigned to an insulin providing (IP) regimen.[98] While this did not result in a reduction in all-cause mortality, patients assigned to the IS strategy experienced significantly lower incidence of PAD than those assigned to the IP strategy.[147] Therefore, we sought to determine whether the relationship between these changing biomarker profiles and incidence of PAD is influenced by the assigned glycemic control strategy.

All BARI 2D participants had clinically significant atherosclerosis in the coronary arteries at study entry; however, incidence of new PAD is still informative because it represents the advancement of generalized atherosclerosis. Identifying risk factors for incident PAD in this population improve our understanding of how insulin sensitizing and insulin providing medications affect the progression of atherosclerosis. There is little longitudinal data exploring how time-varying changes in these biomarkers are associated with new atherosclerosis, and few existing studies have explored these biomarkers as risk factors for incident PAD in a population of T2DM patients. Therefore, our principal aim is to establish the associations between inflammatory biomarkers and incidence of PAD in T2DM patients; as a secondary aim, we aim to define risk factors for PAD incidence in the context of each of the BARI 2D glycemic control strategies.

4.3 METHODS

BARI 2D Trial

The Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial was designed to determine optimal treatment strategy for patients with stable coronary artery disease and type 2 diabetes mellitus.[128] BARI 2D participants were randomly assigned via 2x2 factorial design to prompt coronary revascularization with intensive medical therapy (REV) versus intensive medical therapy alone (MED) and simultaneously randomly assigned to either an insulin sensitizing (IS) glycemic control strategy or an insulin providing (IP) strategy. All participants were treated medically to achieve targets of HbA1c < 7.0%, LDL cholesterol < 100 mg/dL, and blood pressure ≤ 130/80 mm Hg as well as given counseling for smoking cessation, weight loss, and exercise. BARI 2D was coordinated at the University of Pittsburgh and included 49 clinical sites throughout North America, South America, and Europe. Recruitment began in 2001 and continued until 2005; treatment continued until the 6-year visit or the last annual visit before December 1, 2008. The overall study cohort for BARI 2D consisted of 2368 participants. The primary endpoint for BARI 2D was death from any cause, and the principal secondary endpoint was a composite of death, myocardial infarction, or stroke. This manuscript reports the results of post hoc analyses that examine associations between baseline and time-varying cardiovascular risk factors and PAD outcomes.

Definition of Lower Extremity Outcomes

The primary outcome reported in this manuscript is a composite lower extremity outcome used in previous BARI 2D analyses. Participants were considered an "event" if they experienced any of the following lower extremity outcomes: decrease in ankle-brachial index to abnormal level

(ABI ≤ 0.9), lower extremity revascularization, or lower extremity amputation. 1479 participants with normal ABI (0.91-1.30) at study entry were eligible for the primary analysis in this manuscript; those with abnormal ABI at baseline were excluded for reasons enumerated previously. The range for normal ABI is chosen based on guidelines published in a 2003 ADA consensus statement regarding PAD in diabetes.

Assays of Biomarkers

The biomarker assays used in BARI 2D were previously reported by Sobel et al (34). Plasminogen activator inhibitor-1 (PAI-1) activity, PAI-1 antigen, tissue plasminogen activator (tPA), and insulin were measured in the fibrinolysis core laboratory at the University of Vermont in samples obtained at baseline, 1 month, 3 month, 6 month, and every 6 months thereafter over 5 years of follow-up. PAI-1 activity was assessed using a modified chromogenic substrate enzymatic assay developed by Chmielewska and Wiman. PAI-1 antigen and tPA levels were determined with commercially available enzyme-linked immunoassay kits (Trinity Biochcech PLC, Bray, Wicklow, Ireland). CRP, D-dimer, and fibrinogen were assayed at the same core laboratory as part of an ancillary study, with data through the first 24 months of follow-up. Fibronogen was measured by the Claus method, and D-dimer was measured immunoturbidimetrically with STA-Liatest D-Dimer reagents (Diagnostica Stago, Parsippany, New Jersey) on a STA Compact (Roche Professional Diagnostics, Basel, Switzerland). HbA1c was assayed in whole blood samples in the BARI 2D chemistry laboratory in Minneapolis or core laboratories in Brazil and Europe.

Statistical Methods

Baseline descriptive statistics are reported as means ± SDs for continuous variables; medians and interquartile ranges are presented for skewed data, and proportions are reported for categorical variables. The baseline distributions of all risk factors and biomarkers were compared across the assigned glycemic treatment arms using t-tests, Wilcoxon tests, and chi-squared tests for continuous, skewed continuous and categorical data, respectively. Baseline characteristics are presented separately for the assigned glycemic treatment arms in order to accompany the stratified analyses described later in this section.

Cox proportional-hazards models were used to estimate hazard ratios and associated 95% confidence intervals for the associations between each potential risk factor and composite lower extremity outcome. Time-to-event was calculated from the date of randomization to first recorded lower extremity event; participants with no event were censored at their last full-protocol follow-up visit. Most predictor variables were examined as continuous variables; a log-transformation was applied to those with skewed distributions and/or nonlinear associations with outcome. The first series of Cox models were constructed to assess the effects of each risk factor while adjusting for known PAD risk factors that demonstrated significant univariate associations with PAD (age, sex, race, and baseline smoking status).

To determine which of the baseline risk factors showed the strongest independent association with lower extremity outcomes when adjusting for other candidate variables as well as the aforementioned four "known" risk factors (age, sex, race, and smoking), we constructed a multivariate model using forward selection with age, sex, race, and baseline smoking status

forced to enter the model plus all candidate variables that met a significance level of $p \leq 0.10$ also included in the final multivariate model.

We then performed a similar process using the same risk factors assessed as time-varying covariates, updating each value annually to be consistent with the availability of updated ABI measurements (also performed annually). Since previous BARI 2D analyses have shown differential trends in several candidate variables as well as differences in the incidence of lower extremity outcomes between glycemic control arms during the trial, we tested for interactions between assigned glycemic control strategy and each of the time-varying risk factors; there were several significant interactions between candidate variables and assigned treatment, suggesting that stratified analyses are appropriate. Within each glycemic control arm, we constructed separate models for each potential risk factor (adjusting for age, sex, race, and smoking) and then created multivariate models determined by forward selection, using the same process described above for the baseline models.

SAS version 9.2 (SAS Institute, Cary, NC) was used for all statistical analyses. Nominal p-values are reported; p-values less than 0.10 were considered statistically significant in this exploratory analysis, and no adjustment was made for multiple comparisons.

4.4 RESULTS

The BARI 2D trial enrolled 2368 participants with T2DM and coronary artery disease; baseline characteristics of the study population have been reported previously.[129] Of the 2368 BARI 2D participants, 1479 were eligible for this paper's primary analyses; the participants excluded were generally older, heavier, more likely to be current smokers, had higher systolic blood pressure, higher pulse pressure, and longer duration of diabetes than those included in this analysis. Since most of those excluded were removed from the analysis due to pre-existing PAD, it is not surprising that excluded participants generally had more cardiovascular risk factors.

Table 4-1 describes the baseline characteristics of the 1479 participants included in this paper's primary analysis. Participants included in our analytic sample were 61.9 ± 8.0 years, 72% male, 15% identified as Black race, and 12% were current smokers. Baseline distributions of BMI, lipids, blood pressure, HbA1c, albumin-creatinine ratio, and biomarkers of interest were similar between the assigned glycemic treatment groups; there were no significant differences in major demographic or clinical characteristics.

Three hundred and three participants (20.5%) experienced one or more of the lower extremity outcomes included in the composite lower extremity outcome. 290 of these participants had at least one recorded low ABI during follow up, 25 had a lower extremity revascularization, and 13 required a lower extremity amputation. **Table 4-2** displays the associations between baseline risk factors and incidence of the composite lower extremity outcome when adjusting for age, sex, race, and baseline smoking status (each of which was significantly associated with the composite outcome in a multivariate model; see **Table 4-3**). Baseline HbA1c was significantly associated

with the incidence of lower extremity outcomes (HR= 1.19, p<0.01). Systolic blood pressure, pulse pressure, albumin-creatinine ratio, and C-reactive protein were also significantly associated with lower extremity outcomes when adjusting for age, sex, race, and smoking.

Table 4-4 displays the results of a forward selection algorithm with age, sex, race, and smoking forced to enter the model and all other baseline variables shown in Table 2 eligible as candidates. HbA1c again shows the strongest association (HR=1.19, p<0.01), followed by log-transformed albumin-creatinine ratio (HR=1.08, p<0.05) and then log-transformed D-dimer (HR=1.15, p<0.10); the selection algorithm terminates after this step since no other variable is associated with outcome at p<0.10 significance level with the aforementioned variables included in the model. Notably, D-dimer did not show a significant relationship in the first set of models, but was only significantly associated with outcome in models that also adjusted for HbA1c. If D-dimer is not considered a candidate variable, then CRP would enter the model instead, and if neither D-dimer nor CRP were candidate variables, then fibrinogen would enter the model; this is possibly due to correlation between these variables (**Table 4-5**).

The observed associations are noticeably different when the risk factors are modeled as time-varying covariates (**Table 4-6**); recall that these analyses are stratified due to the differential effects of assigned treatment on risk factors of interest. Among those assigned to IP strategy, HbA1c is the strongest predictor (p<0.01) with albumin-creatinine ratio the only other candidate variable showing any significant relationship with the composite lower extremity outcome. Among those assigned to IS strategy, several predictors show significant relationships including BMI, lipids, systolic blood pressure, pulse pressure, albumin-creatinine ratio, and D-dimer.

Notably, time-varying HbA1c is not a significant predictor for those assigned to IS therapy, although it was the strongest for those assigned to IP therapy.

When using the forward selection algorithm, the stratified analyses show that the strength of selected risk factors' association with outcome varies by glycemic control strategy (**Table 4-7**). Age, sex, smoking, and baseline HbA1c are strongly associated with outcome for the IP patients, while only moderately so for the IS patients. Of the other candidate variables, baseline HbA1c and change in HbA1c are predictive of outcome in the IP patients, while baseline D-dimer and change in D-dimer are significantly associated with outcome in the IS patients (only after adjustment for HbA1c).

4.5 DISCUSSION

The BARI 2D trial was a randomized controlled trial designed to determine the optimal treatment strategy for patients with stable CAD suitable for elective revascularization and T2DM. All of these participants have clinically significant atherosclerosis in the coronary arteries; studying the incidence of new PAD and lower extremity outcomes is still informative because it represents the advancement of atherosclerosis into another vascular bed. Using the BARI 2D data to identify risk factors for incident PAD in this population may lead to better mechanistic understanding of how insulin sensitizing and insulin providing medications affect the progression of atherosclerosis.

Approximately 20% of BARI 2D participants with normal ABI at study entry experienced at least one lower extremity event (ABI $\leq$ 0.9, lower extremity revascularization, or lower extremity amputation) within five years of follow-up. As expected, age, sex, race, and baseline smoking were significantly associated with incidence of lower extremity outcomes. When adjusting for the aforementioned risk factors, baseline risk factors predictive of lower extremity events were high systolic blood pressure, pulse pressure, poor glycemic control (higher HbA1c), renal dysfunction (high albumin-creatinine ratio), and a marker of inflammation (CRP). With the exception of pulse pressure, these are all well-established cardiovascular risk factors that have demonstrated a relationship with PAD incidence in prior research, and the relationship between pulse pressure and PAD risk is understandable given that pulse pressure is strongly associated with high systolic blood pressure.

It is fascinating to note the variation between glycemic control strategies wherein different risk factors are predictive of PAD in our time-varying analyses. We know from previous research that participants assigned to IS therapy experienced improvements in biomarker profiles not observed in those assigned the IP strategy; in addition to lower HbA1c, participants assigned to IS therapy had significantly greater in CRP, fibrinogen, tPA, PAI-1 activity, and PAI-1 antigen than participants assigned to IP therapy.[98] However, in the stratified analysis with the biomarkers viewed as time-varying covariates and adjustment for changes in HbA1c, it was change in D-dimer that was the most predictive of PAD risk in participants assigned to IS therapy, ironically the only one of the biomarkers that did not differ over time between the glycemic control arms. If D-dimer were not considered a candidate variable for this model, then CRP would have entered the model as a predictor of PAD incidence, and the same is true for fibrinogen. These relationships were significant only in participants assigned to IS therapy. It is possible that the improved biomarker profiles in the patients assigned IS therapy signified an environment less favorable to further development of atherosclerosis than that present in patients assigned to IP therapy. In contrast, HbA1c showed a much stronger association with the incidence of PAD in participants assigned to IP therapy.

It is difficult to say why the magnitude of time-varying change in HbA1c's relationship with PAD was different in the glycemic treatment arms. Adjusting for age, sex, race, and smoking status, our results showed a statistically significant 17% increased hazard ratio in those assigned to IP therapy for each 1% increase in HbA1c, but a nonsignificant 5% increased hazard in those assigned to IS therapy. One possibility is that the better overall glycemic control in the IS arm dampened the effects of HbA1c on PAD outcomes by pushing the majority of participants into

an HbA1c range where there was relatively little effect of glycemic control on the development of new atherosclerosis, while a greater proportion of participants in the IP arm remained in a higher range of HbA1c and therefore may have been in a range where there was more of an effect on atherosclerosis.

As mentioned briefly above, our baseline models showed relationships generally consistent with prior research regarding traditional cardiovascular risk factors and PAD. Both systolic blood pressure (SBP) and pulse pressure were associated with lower extremity outcomes in our baseline models. Hypertension is not a direct cause of atherosclerosis per se, but it may promote atherosclerosis by its effects on the vasculature. Arteries exposed to hypertension have increased permeability, which results in an increased ability of oxidized lipoproteins to migrate into the intima,[35] and hypertension also may affect vascular wall remodeling by altering the balance between cellular proliferation and apoptosis.[36] SBP is one of the components used to calculate the ankle-brachial index and is also an established predictor of PAD from previous research, so one would expect a significant association between SBP and PAD incidence. Increased pulse pressure is generally indicative of arterial stiffness, thought to be more damaging to the heart than to the peripheral vasculature, so it is interesting to note that pulse pressure also demonstrates a strong relationship with PAD; however, since pulse pressure is strongly related to SBP, it is not surprising to see a strong association between pulse pressure and risk of PAD.

HbA1c has demonstrated a positive association with increased risk of PAD in several studies. The United Kingdom Prospective Diabetes Study (UKPDS) showed that each 1% increase in HbA1c was associated with a 28% increased risk of PAD,[90] later confirmed by a meta-analysis

showing the same estimated 28% increased PAD risk per 1% increase in HbA1c.[91] Several plausible biological mechanisms have been proposed to explain a possible relationship between chronically elevated blood glucose levels and atherosclerosis.[148] Glucose can react with many different proteins, causing structural alterations and subsequently impaired protein and tissue function; such alterations may contribute to endothelial dysfunction, changes in arterial distensibility, plaque formation, and atherosclerosis.[149,150,151,152]

Higher albumin-creatinine ratio, indicative of worsening kidney function, was also predictive of lower extremity outcomes in BARI 2D. Because the kidney is a highly vascular organ, it could be argued that systemic atherosclerotic disease could be associated with declining renal function, but the directionality of this relationship is largely unclear. Cross-sectional data from NHANES 1999-2000[40] and the Cardiovascular Health Study[153] both demonstrated a relationship between chronic kidney disease and abnormal ABI; however, as cross-sectional studies, these studies show nothing about temporality. Longitudinal results from the ARIC study suggested that reduced eGFR was a risk factor for incident PAD,[154] and the BARI 2D data presented here also suggest that higher albumin-creatinine ratio was predictive of incident peripheral arterial disease, suggesting that renal insufficiency may be causally related to progression of atherosclerosis. Potential physiological mechanisms by which renal dysfunction might affect the atherosclerotic process include altered calcium-phosphorus metabolism, homocysteine metabolism, lipoprotein(a) metabolism and alterations in inflammatory and coagulation pathways.[155,156]

C-reactive protein, fibrinogen, and D-dimer also showed significant associations with PAD risk in one or more of the models presented in this paper. Since these biomarkers are representative

of distinct but interrelated processes pertaining to inflammation, coagulation, and thrombosis and their values are somewhat correlated, particularly fibrinogen and CRP, often they would not show predictive value when placed in models together, but when any one of the three was considered a candidate variable, they showed a significant association with PAD risk (although, for fibrinogen, this was only true when viewed as a time-varying covariate; baseline fibrinogen showed no predictive value for PAD incidence). Both CRP and fibrinogen have been associated with PAD in cross-sectional studies,[143] and fibrinogen has been associated with increased risk of developing PAD in diabetes.[97] NHANES data has also showed that participants in the highest quartile of CRP and fibrinogen had a 2.0-fold increased odds of prevalent PAD compared with participants in the lowest quartiles.[69] Among individuals with existing PAD, higher levels of inflammatory biomarkers are associated with increased mortality, increased cardiovascular mortality, and increased risk of failure of lower-extremity revascularization procedures.[77,78,79] To our knowledge, no previous research has shown changes in D-dimer to be linked to increased risk of PAD incidence; two studies have shown D-dimer associated with risk of functional decline in patients with existing PAD.[75,76]

Our study findings must be considered carefully in context of the trial's strengths and limitations. This is a post-hoc secondary analysis of a randomized controlled trial in which all participants had CAD and T2DM; the effects of these risk factors may be different in the general population. Several of the biomarkers included in this analysis, including CRP and fibrinogen, were only collected through 24 months of follow-up, and thus their relationships with PAD risk in our analyses using time-varying covariates may not be fully accurate. It also should be noted that our primary outcome was a composite of low ABI, lower extremity revascularization, and lower

extremity amputation used in a prior BARI 2D publication on peripheral arterial disease. We felt this outcome was appropriate because ABI was only measured annually, and wished to capture lower extremity events of clinical significance that may have occurred without a low ABI being measured.

We have previously reported the baseline prevalence of PAD in the BARI 2D trial and the baseline risk factors associated with prevalent PAD at study entry[131] as well as the incidence of PAD in each glycemic control arm. [147] This manuscript extends our previous work by reporting the associations between traditional and nontraditional risk factors for PAD and lower extremity outcomes during follow-up. After adjusting for age, sex, race, and smoking, our data showed that hypertension, HbA1c, albumin-creatinine ratio, and several biomarkers indicative of inflammation and antithrombotic activity were positively associated with PAD risk. It is uncertain whether these are causal relationships between the risk factors and PAD, or whether these are merely reflective of advancing systemic disease. Nonetheless, it appears that in addition to high HbA1c, several nontraditional risk factors such as CRP, fibrinogen, and D-dimer may have potential to identify T2DM patients at highest risk for developing further atherosclerosis.

4.6 CONCLUSION

This manuscript reports the associations between traditional and nontraditional risk factors for PAD and lower extremity outcomes during follow-up. After adjusting for known PAD risk factors of age, sex, race, and smoking, our data showed that hypertension, HbA1c, renal dysfunction, and biomarkers indicative of inflammation, coagulation, and fibrinolysis were positively associated with PAD risk. It is uncertain whether these are causal relationships or merely reflective of advancing systemic disease. Nonetheless, it appears that in addition to high HbA1c, several nontraditional risk factors such as CRP, fibrinogen, and D-dimer may have potential to identify T2DM patients at highest risk for developing further atherosclerosis. Notably, the markers of inflammation, coagulation, and fibrinolysis were associated with lower extremity outcomes among patients treated with insulin sensitizing medications, but this was not the case for patients treated with insulin providing medications, suggesting that these medications may have a differential effect on these processes and subsequently on the development of atherosclerosis in patients with type 2 diabetes.

4.7 TABLES AND FIGURES

Table 4-1. Baseline Characteristics of Participants Available for PAD Analysis

Characteristic	IP (N=744)	IS (N=735)	p-value
Age (years)	62.0 ± 8.7	61.8 ± 8.9	0.633
Gender (male), %	71.9	71.4	0.858
Black Race, %	14.7	16.2	0.402
Current Smoker, %	11.5	11.6	0.925
BMI (kg/m^2)	31.4 ± 5.6	31.6 ± 5.9	0.453
LDL cholesterol (mg/dl)	96.8 ± 31.8	94.5 ± 33.1	0.177
HDL cholesterol (mg/dl)	38.3 ± 10.2	37.6 ± 9.5	0.213
Triglycerides (mg/dl)	183.3 ± 149.5	177.7 ± 123.6	0.437
Systolic Blood Pressure (mm Hg)	130.2 ± 19.2	130.9 ± 18.7	0.474
Diastolic Blood Pressure (mm Hg)	74.4 ± 10.7	75.0 ± 11.0	0.302
Pulse Pressure (mm Hg)	55.9 ± 15.1	55.7 ± 14.7	0.780
HbA1c (%)	7.7 ± 1.6	7.6 ± 1.6	0.107
ACR (mg/g)^	10.9 (5.2-34.6)	10.8 (4.8-42.4)	0.826
CRP (ug/mL)^	2.1 (1.0-5.7)	2.2 (1.0-5.2)	0.720
Fibrinogen (mg/dl)^	356 (295-422)	350 (291-409)	0.232
D-Dimer (µg/ml FEU)^	0.32 (0.19-0.57)	0.30 (0.18-0.55)	0.781
PAI-1 activity (au/ml)^	16.0 (10.0-27.0)	16.0 (10.0-26.0)	0.960
PAI-1 antigen (ng/ml)^	23.0 (15.0-35.0)	23.0 (15.0-34.0)	0.406
TPA (ng/ml)^	9.6 (7.3-12.0)	9.7 (7.2-12.0)	0.638

^Presented as median (Q1-Q3)

IP = Insulin Providing Assignment; IS = Insulin Sensitizing Assignment; BMI= Body Mass Index; LDL=Low-Density Lipoprotein; HDL=High-Density Lipoprotein; HbA1c=Hemoglobin A1c; ACR=Albumin/Creatinine Ratio; CRP= C-Reactive Protein; PAI-1 = Plasminogen Activator Inhibitor-1; TPA= Tissue Plasminogen Activator

Table 4-2. Associations^ Between Baseline Risk Factors and Incidence of Lower Extremity Outcomes

Risk Factor	Hazard Ratio	95% CI
BMI	1.01	0.99-1.03
LDL cholesterol (per 10 mg/dl)	1.01	0.97-1.05
HDL cholesterol (per 10 mg/dl)	0.99	0.81-1.05
Triglycerides (per 10 mg/dl)	1.00	0.99-1.01
Systolic Blood Pressure (per 10 mm Hg)	1.05*	0.99-1.11
Diastolic Blood Pressure (per 10 mm Hg)	0.97	0.86-1.08
Pulse Pressure (per 10 mm Hg)	1.11**	1.03-1.19
HbA1c (per 1.0%)	1.18***	1.10-1.26
Log(ACR)	1.13**	1.06-1.21
Log(CRP)	1.10*	1.00-1.21
Log(D-Dimer)	1.09	0.96-1.24
Log(Fibrinogen)	1.06	0.68-1.66
Log(PAI-1 Activity)	0.97	0.82-1.16
Log(PAI-1 Antigen)	1.01	0.84-1.22
Log(TPA)	0.80	0.61-1.06

^ separate models for each candidate variable; each model adjusted for age, sex, race, and smoking status

BMI= Body Mass Index; LDL=Low-Density Lipoprotein; HDL=High-Density Lipoprotein; HbA1c=Hemoglobin A1c; ACR=Albumin/Creatinine Ratio; CRP= C-Reactive Protein;
PAI-1 = Plasminogen Activator Inhibitor-1; TPA= Tissue Plasminogen Activator

*P<0.10; **P<0.05; ***P<0.01

Table 4-3. Multivariate Associations^ Between Selected Variables and Incidence of Lower Extremity Outcomes

Risk Factor	Hazard Ratio	95% CI
Age (Per 10 years)	1.32	1.19-1.46
Sex (Female vs. Male)	1.55	1.22-1.98
Race (Black vs. non-Black)	1.47	1.11-1.94
Smoking (Current vs. Former/Never)	2.07	1.52-2.83

^Variables simultaneously included in multivariate Cox proportional-hazards model

Table 4-4. Multivariate Associations^ Between Baseline Risk Factors and Incidence of Lower Extremity Outcomes

Forced Into Model	Hazard Ratio	95% CI
Age (per 10 years)	1.34***	1.17-1.52
Sex (Female vs. Male)	1.45***	1.10-1.92
Race (Black vs. non-Black)	1.40**	1.01-1.93
Smoking (Current vs. Former/Never)	2.38***	1.67-3.41
Additional Candidate Variables	Hazard Ratio	95% CI
HbA1c (per 1.0%)	1.19***	1.10-1.29
Log(ACR)	1.08**	1.00-1.17
Log(D-Dimer)	1.15*	0.99-1.34

^Model created using forward selection algorithm with age, sex, race, and smoking forced to enter model and all risk factors listed in Table 2 eligible as candidate variables

HbA1c=Hemoglobin A1c; ACR=Albumin/Creatinine Ratio

*P<0.10; **P<0.05; ***P<0.01

Table 4-5. Correlations Between Baseline Values of Candidate Variables

	BMI	LDL	HDL	Trig	SBP	DBP	PP	A1c	ACR	CRP	D-Dm	Fibr	PAI-1 Act	PAI-1 Ant	TPA
BMI		-0.007	-0.439	0.045	0.034	0.016	0.027	-0.013	0.032	0.150	0.019	0.136	0.205	0.168	0.039
LDL			0.144	0.004	0.125	0.172	0.036	0.155	0.069	0.058	-0.027	0.045	-0.016	-0.011	0.074
HDL				-0.329	0.105	0.031	0.111	0.008	0.086	-0.051	-0.018	0.007	-0.180	-0.169	-0.169
Triglycerides					-0.046	0.022	-0.075	0.152	0.084	-0.003	-0.030	0.079	0.304	0.272	0.284
SBP						0.619	0.820	0.031	0.179	-0.011	0.007	0.041	-0.022	-0.015	-0.047
DBP							0.060	0.102	0.090	-0.023	-0.075	-0.002	0.089	0.089	0.076
PP								-0.036	0.164	0.004	0.063	0.054	-0.094	-0.086	-0.116
HbA1c									0.129	0.046	-0.004	0.129	0.027	0.041	0.063
ACR										0.057	0.088	0.203	-0.046	-0.048	-0.027
CRP											0.163	0.442	0.027	0.036	0.077
D-Dimer												0.114	-0.069	-0.019	-0.001
Fibrinogen													0.009	0.016	0.194
PAI-1 Activity														0.812	0.369
PAI-1 Antigen															0.422
TPA															

BMI= Body Mass Index; LDL= Low-Density Lipoprotein Cholesterol; HDL= High-Density Lipoprotein Cholesterol; Trig= Triglycerides; SBP= Systolic Blood Pressure; DBP= Diastolic Blood Pressure; PP= Pulse Pressure; A1c=Hemoglobin A1c; ACR= Albumin/Creatinine Ratio; CRP= C-Reactive Protein; D-Dm = D-Dimer; Fibr= Fibrinogen; PAI-1 Act = PAI-1 Activity; PAI-1 Ant= PAI-1 Antigen; TPA= Tissue Plasminogen Activator

Table 4-6. Associations^ Between Time-Varying Risk Factors and Incidence of Lower Extremity Outcomes, Stratified By Assigned Glycemic Treatment

	IP Patients (N=744)		IS Patients (N=735)	
Risk Factor	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Body Mass Index	1.00	0.98-1.03	1.04***	1.01-1.06
LDL (per 10 mg/dl)	1.03	0.97-1.08	1.06**	1.00-1.11
HDL (per 10 mg/dl)	0.99	0.85-1.13	0.79**	0.62-0.96
Triglycerides (per 10 mg/dl)	1.00	0.99-1.01	1.01	1.00-1.02
Systolic BP (per 10 mm Hg)	1.00	0.91-1.10	1.13**	1.03-1.23
Diastolic BP (per 10 mm Hg)	0.96	0.80-1.12	0.96	0.78-1.14
Pulse Pressure (per 10 mm Hg)	1.02	0.91-1.13	1.19**	1.07-1.32
HbA1c (per 1.0%)	1.17***	1.06-1.29	1.05	0.93-1.19
Log(ACR)	1.08*	0.97-1.18	1.11*	1.00-1.23
Log(CRP)	1.10	0.97-1.25	1.09	0.94-1.26
Log(D-Dimer)	1.02	0.84-1.22	1.29*	1.05-1.58
Log(Fibrinogen)	1.45	0.84-2.52	1.29	0.65-2.57
Log(PAI-1 Activity)	1.00	0.84-1.21	1.07	0.85-1.35
Log(PAI-1 Antigen)	0.99	0.78-1.26	1.14	0.85-1.51
Log(TPA)	0.98	0.65-1.48	0.95	0.65-1.40

^ separate models for each candidate variable; each model adjusted for age, sex, race, and smoking status

IP = Insulin Providing Assignment; IS = Insulin Sensitizing Assignment; BMI= Body Mass Index; LDL=Low-Density Lipoprotein; HDL=High-Density Lipoprotein; HbA1c=Hemoglobin A1c; ACR=Albumin/Creatinine Ratio; CRP= C-Reactive Protein; PAI-1 = Plasminogen Activator Inhibitor-1; TPA= Tissue Plasminogen Activator

*P<0.10; **P<0.05; ***P<0.01

Table 4-7. Multivariate Associations^ Between Time-Varying Risk Factors and Incidence of Lower Extremity Outcomes, Stratified By Assigned Glycemic Treatment

	IP Patients (N=744)		IS Patients (N=735)	
	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Age (per 10)	1.45***	1.23-1.67	1.22*	0.97-1.48
Sex (Female)	1.53**	1.08-2.17	1.45*	0.94-2.24
Race (Black)	1.46	0.97-2.18	1.37	0.83-2.26
Smoking (Current)	2.95***	1.91-4.56	1.80*	0.99-3.26
Baseline HbA1c	1.24**	1.10-1.40	1.14	0.97-1.35
Change in HbA1c	1.13**	1.01-1.26	0.89	0.76-1.04
Baseline Log(D-Dimer)	Not Included		1.38**	1.04-1.81
Change in Log(D-Dimer)	Not Included		1.30**	1.00-1.68

^Stratified models created using forward selection algorithm with age, sex, race, and smoking forced to enter model and all risk factors listed in Table 2 eligible as candidate variables
*P<0.10; **P<0.05; ***P<0.01

5.0 MANUSCRIPT 3: MEASUREMENT VARIATION OF THE ANKLE-BRACHIAL INDEX WITH AUTOMATED OSCILLOMETRIC DEVICE VERSUS DOPPLER ULTRASOUND

5.1 ABSTRACT

Objective: This data collection project was designed to evaluate the reproducibility and reliability of ankle-brachial index (ABI) measurements obtained using 1) the Colin VP-1000 oscillometric device and 2) the Doppler ultrasound method.

Research Design and Methods: This project was carried out at the University of Pittsburgh's Epidemiology Ultrasound Research Laboratory. 40 participants were enrolled, each of whom had their ABI measured in the same sequence (Colin, Doppler, Colin, Doppler) so that each participant had two ABI measurements taken with Colin and two ABI measurements taken with Doppler, allowing for within-method reproducibility as well as between-method comparisons.

Results: Within-subject agreement was reasonable for the Colin (intra-class correlation = 0.77), and 36 of 40 participants (90.0%) had an absolute difference ≤ 0.10 between the two Colin measurements. Within-subject agreement was even stronger for the two Doppler measurements (intra-class correlation = 0.91); 38 of 40 participants (95.0%) had an absolute difference ≤ 0.10 between the two Doppler measurements. The between-method agreement was less than ideal (intra-class correlation coefficient = 0.44); Doppler ABI measurements tended to be higher than Colin ABI measurements on the same participant, and only 26 of 40 participants (65.0%) had an absolute difference ≤ 0.10 between the two methods.

Conclusions: The Colin VP-1000 is capable of measuring ABI with an acceptable level of reproducibility; however, agreement between the Colin and the gold-standard Doppler method is less than ideal. Our study results do not support the replacement of Doppler ABI measurements with Colin ABI measurements in a clinical setting.

5.2 INTRODUCTION

The ankle-brachial index (ABI) is the principal screening technique for peripheral arterial disease (PAD)[157] and an established predictor of cardiovascular morbidity and mortality.[23,24,25,26] Also known as the ankle-arm index (AAI) or ankle-brachial pressure index (ABPI), the ABI is a simple ratio of the systolic blood pressure measured at the ankle divided by the brachial systolic blood pressure. ABI measurements ranging from 0.91-1.30 are considered normal; an ABI≤0.9 is suggestive of atherosclerotic PAD, while an ABI>1.3 suggests the presence of medial arterial calcification, making it difficult to obtain an accurate reading in the lower extremity (1). Epidemiologic studies have shown that patients with abnormal ABI at either end of the spectrum have an increased risk of cardiovascular events and mortality.[24,25]

The most common method of measuring ABI uses a continuous-wave Doppler probe for detection of arterial flow and a pneumatic cuff to determine systolic blood pressure in each limb. The pneumatic cuff is placed over the appropriate limb and inflated until arterial flow ceases noted by the lack of audible sound heard on the Doppler; the cuff is then deflated slowly until the flow signal reappears, and the cuff pressure at reappearance of a signal is the systolic pressure for that limb. The systolic pressure at each ankle is divided by the higher of the two brachial pressures to calculate the ABI for each leg. In 1969, Yao et al published the first study to report measurement of ankle blood pressures using the Doppler method.[109,110] Despite some controversy regarding appropriate measurement protocol[158] and calculation[159,160] of the ankle-brachial index using Doppler, a 2012 AHA Scientific Statement concludes that the Doppler method is to be the most reliable way to determine ABI and provides guidelines for standardization.[157]

The principal alternative to the Doppler method relies on oscillometric measurement of blood pressure at each limb. The Colin VP-1000 oscillometric device allows simultaneous pressure measurement at each of the brachial arteries as well as the posterior tibial arteries in each ankle. Oscillometers measure the magnitude of pressure vacillation at each arterial site as the cuffs are simultaneously deflated from suprasystolic pressures; as pressure in the cuff decreases and approaches systolic blood pressure, oscillation rapidly increases and eventually reaches a peak, after which decrease of the cuff pressure causes oscillation to decrease. Systolic blood pressure is calculated when the oscillation increases rapidly; diastolic blood pressure is calculated when the oscillation decreases rapidly. The ABI is calculated by dividing the systolic pressure at each ankle by the higher of the two brachial systolic pressures to establish ABI for each leg.

Automated devices such as the Colin have several advantages over the Doppler method for measuring ABI. The Doppler method requires a trained sonographer or physician and can still be prone to measurement biases and errors; in contrast, measuring ABI with the Colin requires little specialized training and is free of bias. The Doppler method is also time-consuming, while the Colin reduces the time required to obtain an ABI by measuring all four limbs simultaneously. The time required for measurement is one of the barriers to the inclusion of ABI in regular office visits; the ability to measure ABI in less time and without highly trained personnel could make it more practical to include as part of routine office visits. However, before the Colin can be recommended for mainstream use, it must be proven accurate and reliable when compared against the gold-standard Doppler method.

Most studies to date have shown reasonable agreement between oscillometric ABI measurements and Doppler ABI measurements; the aforementioned 2012 AHA Scientific Statement (1) on the ankle-brachial index concluded that correlation between the two methods is acceptable, although the studies upon which this statement was based used varying criteria to define an acceptable level of agreement. It should be noted that some discrepancies may be partially explained by differences in measurement protocol; for example, most oscillometric devices (including the Colin described in this manuscript) measure the ankle pressure at the posterior tibial (PT) artery, while Doppler measurements are sometimes taken at the dorsalis pedis (DP) artery since it can be easier to locate the DP pulse in the ankle than the PT pulse.

In addition to evaluating agreement between Colin measurements and Doppler measurements, we must also establish within-method reproducibility for each technique. To date, Doppler measurements have generally shown slightly better reproducibility than oscillometric ABI measurements[161,162] although oscillometric devices have demonstrated acceptable reproducibility for use in research studies.[163,164] In the present study, we report within-method reproducibility for ABI measurements taken with the Colin VP-1000 oscillometric device at the Epidemiology Ultrasound Research Laboratory (URL) at the University of Pittsburgh, as well as within-method reproducibility for ABI measurements using the traditional Doppler method. We also report between-method agreement for Colin ABI measurements against Doppler ABI measurements.

5.3 METHODS

The URL at the University of Pittsburgh conducts noninvasive vascular testing for epidemiological research studies, and therefore must evaluate validity and reproducibility of its research measurements for quality control. The present study has three aims: i) evaluate the reproducibility of ABI measured with the Colin VP-1000 oscillometric device, ii) evaluate the reproducibility of ABI measured with the Doppler method, and iii) evaluate agreement between the two methods.

Participant Recruitment and Enrollment

The study was approved by the Institutional Review Board of the University of Pittsburgh. Volunteers were recruited through advertisements posted in the URL and the Graduate School of Public Health at the University of Pittsburgh, as well as verbal invitations issued by the Principal Investigator and/or laboratory staff members. Fifty-four potential participants agreed to schedule a study visit; forty (74.1%) attended the scheduled visit and completed all study procedures.

Study Visit Procedure

Participants were greeted at the URL by the principal investigator and gave informed consent. Next, they completed a pretest questionnaire with information about basic demographics, known comorbidities of peripheral arterial disease (diabetes, high blood pressure, and high cholesterol) and three behaviors that might acutely affect ABI: smoking, caffeine consumption, and exercise. Specifically, participants were asked whether they had smoked, consumed caffeine, or exercised since midnight; since most visits occurred in the morning or early afternoon, this determines whether they had performed the behavior within approximately 12 hours of their visit.

Participants were also asked to report the time and provide appropriate details (for example, a participant that reported consumption of caffeine was asked to report the beverage consumed - i.e. tea, coffee, soft drink, energy drink - as well as the number of cups consumed). Participants were not asked to refrain from these behaviors, but the data were available to perform sensitivity analyses. After completing the questionnaire, participants were escorted into an exam room, asked to remove their socks and shoes, and instructed to rest in the supine position for 5 minutes. After the rest period, the attending observer entered the room and began the examination.

Clinical Examination

The observer first obtained simultaneous blood pressure readings at the right and left brachial and posterior tibial arteries using the VP-1000 according to the protocol outlined in **Appendix A**. After the first VP-1000 measurement, the observer allowed 5 minutes for the vessels to return to resting state, then began the sequence of pressure measurements required to calculate ABI using the Doppler: first the right brachial pressure, then the left brachial pressure, then the right ankle pressure, and finally the left ankle pressure according to the protocol outlined in **Appendix B**. The observer again allowed a 5-minute rest period, and the process was repeated so each participant had two ABI measurements taken with each method (four ABI measurements total) in the following sequence: Colin #1, Doppler #1, Colin #2, and Doppler #2. Once all four ABI measurements were finished, the observer handed the data collection forms to the principal investigator to verify that form was completed appropriately. The principal investigator asked the participant if they had any questions or concerns, awarded the participant compensation and the visit was concluded.

Baseline Characteristics of Study Participants

We describe the study population using baseline characteristics from the pretest questionnaire, reporting means ± standard deviations for continuous variables and proportions for categorical variables. Much of this data is self-reported on the pretest questionnaire, so accuracy cannot be guaranteed; however, these are not primary analytical variables in our study

Statistical Methods: Notation

Please refer to the following definitions while reviewing the statistical methods:

- $Colin_{1i}$= first Colin ABI measurement on the i^{th} participant
- $Colin_{2i}$= second Colin ABI measurement on the i^{th} participant
- $|Colin_{2i} - Colin_{1i}|$ = absolute value of the difference between the i^{th} participant's second Colin ABI and the i^{th} participant's first Colin ABI
- $Colin_{AVGi}$ = average of the two Colin measurements on the i^{th} participant
- $\overline{Colin_{AVG}}$ = overall mean of all participants' Colin ABI measurements

- $Doppler_{1i}$= first Doppler ABI measurement on the i^{th} participant
- $Doppler_{2i}$= second Doppler ABI measurement on the i^{th} participant
- $|Doppler_{2i} - Doppler_{1i}|$ = absolute value of the difference between the i^{th} participant's second Doppler ABI and the i^{th} participant's first Doppler ABI
- $Doppler_{AVGi}$ = average of the two Doppler measurements on the i^{th} participant
- $\overline{Doppler_{AVG}}$ = overall mean of all participants' Doppler ABI measurements

Statistical Analysis: Within-Method Comparisons

We report the linear correlation coefficient and intra-class correlation coefficient between paired measurements for each method (i.e. $Colin_{1i}$ vs. $Colin_{2i}$), and we also report the mean difference between measurements for each method (i.e. $Colin_{2i}$ - $Colin_{1i}$) with a 95% confidence interval for the difference. We produced scatterplots and Bland-Altman plots for a graphical representation. The Bland-Altman plots show the difference between measurements (i.e. $Colin_{2i}$ - $Colin_{1i}$) on the y-axis versus the average of the two measurements ($Colin_{AVGi}$) on the x-axis, with reference lines drawn at the limits of agreement (mean difference ± 1.96*SD). We also report the mean absolute difference ($|Colin_{2i} - Colin_{1i}|$) and the proportion of participants with absolute difference < 0.10 between measurements. Each of the analyses described here were performed for both the Colin measurements and the Doppler measurements.

Statistical Analysis: Between-Method Comparisons

We performed similar statistical analyses to evaluate between-method agreement of the Colin ABI measurements against the Doppler method. We calculated the linear correlation coefficient and intra-class correlation coefficient between the averaged measurements of each method ($Colin_{AVGi}$ vs. $Doppler_{AVGi}$), and again produced a scatterplot and Bland-Altman plot for graphical representation. For the between-method comparison, the Bland-Altman plot shows the difference between the averaged Colin measurements and averaged Doppler measurements ($Colin_{AVGi}$ - $Doppler_{AVGi}$) on the y-axis versus the overall average of the paired measurements on the x-axis. We also report the mean absolute difference ($|Colin_{AVGi} - Doppler_{AVGi}|$) between methods and the proportion of subjects with absolute difference < 0.10 between methods.

Power and Sample Size

We performed sample size calculations based on an equivalence test for paired means[165] with significance level $\alpha = 0.05$ and the following hypotheses:

$$\text{Ho: } \overline{\text{Colin}_{AVG}} - \overline{\text{Doppler}_{AVG}} \leq -0.10 \textbf{ OR } \overline{\text{Colin}_{AVG}} - \overline{\text{Doppler}_{AVG}} \geq 0.10$$

$$\text{Ha: } -0.10 < \overline{\text{Colin}_{AVG}} - \overline{\text{Doppler}_{AVG}} < 0.10$$

We chose 0.10 as the limit of equivalence since ABI is often categorized into intervals of that width in epidemiologic studies. We assumed standard deviation of 0.15 for both Colin ABI and Doppler ABI measurements based on previously published studies and expert opinion.[164,166] This sample size calculation also requires estimating the between-method correlation; for the equivalence test specified here, there is a negative association between the between-method correlation and the required sample size to guarantee a fixed level of power, meaning that smaller between-method correlation requires a larger sample size to declare equivalence. Thus, underestimating the between-method correlation is the conservative approach since it yields a larger sample-size estimate than overestimating the between-method correlation. A systematic review comparing oscillometric methods and Doppler reported a pooled correlation of 0.71 between methods;[120] however, this review included many studies that used oscillometric devices other than the Colin, so we cannot be certain that the Colin will have the same level of correlation with Doppler. Therefore, we performed sample size calculations using lower estimates of the correlation (0.2, 0.4, 0.6), noting that if the actual correlation was greater than our chosen estimates, we would retain sufficient power to declare equivalence between methods.

Under the conditions outlined above, a sample size of 33 pairs with correlation 0.4 (**Table 5-1**) has 90% power to detect equivalence when the margin of equivalence is from -0.10 to 0.10 and the actual mean difference is 0. We set an enrollment target of 40 participants to account for unusable data. The additional sample size also allowed us to perform useful sensitivity analyses.

5.4 RESULTS

Characteristics of Study Participants

Table 5-2 displays baseline characteristics of our study participants. Participants were generally young (mean age 35.4 ± 11.9 years, range 22-63 years) and the majority self-reported white race (82.5%), although the sample also included black (7.5%) and Asian (10.0%) participants. The sample included both males (30.0%) and females (70.0%). Most participants were normal weight (65.0%) or overweight (22.5%), with five (12.5%) meeting criteria for "obese" according to BMI (calculated from self-report height and weight). Few participants reported common PAD comorbidities such as diabetes (5.0%), high blood pressure (10.0%), or high cholesterol (12.5%). No participants reported smoking within a 12-hour window preceding the visit, 25 participants (62.5%) reported consuming caffeine prior to their study visit, and 16 participants (40.0%) reported exercising prior to their study visit.

Distribution of Individual Pressure Measurements

The mean and standard deviation of each pressure component involved in the calculation of ABI (left brachial, right brachial, left ankle, right ankle) are shown in **Table 5-3** for each of the four ABI measurements performed in this study (Colin #1, Doppler #1, Colin #2, Doppler #2). Notably, while the brachial pressures were distributed similarly across all four measurements, the ankle pressures recorded with the gold-standard Doppler method were an average of 7.7 mm Hg higher than those recorded with the Colin. This difference was present in the first and second measurement run. Discussed in detail later in this manuscript, the difference in ankle pressures should manifest itself as slightly higher ABI measurements with the Doppler than with the Colin.

Colin Reproducibility

The first Colin measurement and second Colin measurement each have approximately normal distributions with no significant difference in the mean ABI from the first run to the second run (first: 1.05 ± 0.11, second: 1.08 ± 0.09, **Table 5-4**). Within-subject agreement was reasonably good across the two Colin measurements, with an intra-class correlation coefficient = 0.77 and a linear correlation coefficient = 0.81 (**Figure 5-1**). The within-subject differences between the first and second Colin measurements followed an approximately normal distribution with mean 0.03 (95% CI: -0.03, 0.09). The mean absolute difference was 0.06 (SD=0.04), and 36 of 40 participants (90.0%) had an absolute difference ≤ 0.10 between the two Colin measurements. Only one of the 40 participants had a difference outside Bland-Altman limits of agreement (**Figure 5-2**) calculated based on the distribution of Colin measurements in our study.

Doppler Reproducibility

As reported for the Colin, the distributions of each of the Doppler ABI measurements were approximately normal with no significant difference in the mean ABI from the first to the second (first: 1.14 ± 0.13, second: 1.15 ± 0.12, **Table 5-4**). Within-subject agreement was strong for the Doppler, with intra-class correlation = 0.91 and linear correlation = 0.92 (**Figure 5-3**). The within-subject differences between the first and second Doppler measurements followed an approximately normal distribution with mean 0.01 (95% CI: -0.05, 0.07). The mean absolute difference was 0.04 (SD=0.04), and 38 of 40 participants (95.0%) had absolute difference ≤ 0.10 between the two Doppler measurements. Two of 40 participants fell outside Bland-Altman limits of agreement (**Figure 5-4**) based on the study distribution of Doppler measurements.

Colin vs. Doppler Agreement

The between-method comparison resulted in an intra-class correlation coefficient = 0.44 and linear correlation coefficient = 0.64 (**Figure 5-5**). The differences between Colin ABI and Doppler ABI measurements followed a roughly normal distribution, although there was one significant outlier (difference of 0.38). Doppler ABI tended to be higher than Colin ABI; according to the equivalence test described in the power and sample size section, we do not have sufficient evidence to conclude that the methods are equivalent (mean difference = 0.08, 95% CI: 0.05-0.11, p=0.105). The mean absolute difference between methods was 0.09 (SD=0.09), and 26 of 40 participants (65.0%) had an absolute difference ≤ 0.10 between the respective means for the two methods. Only one of the 40 participants fell outside the Bland-Altman limits of agreement (**Figure 5-6**) calculated based on the distribution of our study data; however, it must be noted that the limits of agreement are wider for between-method comparison than within-method comparisons.

We can also evaluate agreement using categories of low, normal, and high ABI defined by AHA guidelines described in the introduction (**Table 5-6**). 32 of 40 participants are classified "normal" by each method, and one participant is deemed "high" by both methods, yielding categorical agreement for 33 of 40 subjects. However, the kappa statistic is only 0.17, indicative of poor agreement between the methods. Most of the discordance stemmed from 6 participants classified as "high" by Doppler and "normal" by Colin, while only one was classified "low" by Colin and "normal" by Doppler. The lack of any participants with low ABI by both methods makes it impractical to calculate a kappa statistic for detection of low ABI vs. non-low ABI.

Sensitivity Analyses

We also performed analyses stratified by caffeine intake and exercise prior to study visit to see if results were consistent (**Table 5-7**). The correlation between Colin measurements was slightly higher for those who abstained from caffeine compared to those who consumed (r=0.90 for abstainers vs. r=0.73 for consumers, p=0.13), while the opposite was true for Doppler measurements (r=0.86 for abstainers vs. r=0.95 for consumers, p=0.13), although neither of these differences were statistically significant. In the analyses stratified by exercise (**Table 5-8**), the correlation coefficient between Colin measurements was lower for those who abstained compared to those who exercised (r=0.64 vs. r=0.91, p=0.03), while there was little difference between correlations for Doppler measurements (r=0.90 vs. r=0.95, p=0.30). Mean absolute differences were distributed similarly for each comparison, suggesting that caffeine intake and exercise did not significantly alter reproducibility of ABI measurements in this study; it should be noted that these are based on small groups, so power to detect such differences is limited.

5.5 DISCUSSION

We evaluated the reproducibility of ABI measurements taken using both the Colin VP-1000 oscillometric device and the Doppler method at the University of Pittsburgh's Epidemiology URL. The Doppler ABI measurements had excellent reproducibility, with an intra-class correlation > 0.9 and 95% of participants with an absolute difference less than 0.10 between the two Doppler measurements. Reproducibility of Colin ABI was not quite as strong, but still showed reasonable agreement with a intra-class correlation > 0.8 and 90% of participants having an absolute difference less than 0.10 between repeated Colin measurements.

There was significant evidence of nonequivalence between methods; ABI measurements taken with Doppler were generally higher than Colin measurements on the same participant (mean difference=0.08), and 14 of 40 (35%) participants had mean Doppler ABI at least 0.10 greater than their mean Colin ABI, large enough to be clinically significant. Since brachial pressures were distributed similarly between methods, it appears that the differences in ABI were primarily driven by the differences in ankle pressures.

One possible explanation is the sequence in which the measurements were taken. Recall that the first ABI measurement was taken using the Colin, followed by the first sequence of Doppler measurements, then the Colin again, and finally the Doppler again. If systemic pressure rose with each measurement, one would expect higher ankle pressures with the Doppler. However, this seems unlikely because ankle pressures from the second Colin measurement were significantly lower than both the Doppler measurement that came before it and after it.

A second possible explanation is that the time required to take four separate Doppler pressures may lead to an inaccurate evaluation of inter-site blood pressure ratios; changes in systemic pressure may occur during the time required to take four separate measurements needed to calculate ABI.[167] In this study, recall that Doppler measurements were taken in the following sequence: left brachial, right brachial, left ankle, right ankle. This process can take several minutes, during which the patient may experience changes in systemic pressure. It is also possible that Doppler ABI is prone to observer error if there are consistent differences in the time taken to hear the flow signal and record the pressure for the arm compared to the ankle.

A third possible explanation is the difference between the Colin and Doppler measurement sites used to obtain the ankle pressures. The pressure cuff is placed slightly more distally for ankle pressure measurement with a Doppler probe than it is for measurement with the Colin; therefore, it is possible that ankle pressures tend to be higher using the Doppler because of greater distal amplification of systolic BP generated by pressure-wave reflections within the arterial tree.[168] Furthermore, recall that the ankle pressure can be taken at either the dorsalis pedis (DP) or the posterior tibial (PT) arteries; since the PT pulse is missing or impossible to locate in approximately 10-20% of the population, the DP is commonly used for Doppler ankle pressure measurements, and was the measurement site used in this study. However, the Colin always measures ankle pressure using the PT pulse. There is no published evidence that the DP ankle pressure differs systematically from the PT ankle pressure, but it is possible that the difference in measurement site could explain some of the systematic difference, noting that our results showed Doppler ankle pressures that tended to be higher than Colin ankle pressures.

While the Colin ABI tended to be lower than the Doppler in our study, the Colin retains possible utility as a PAD screening tool if it classifies participants in the same category of low (ABI≤0.9), normal (0.9<ABI≤1.3), and high (ABI>1.3) as the Doppler method. 33 participants had an ABI in the "normal" range according to the Doppler, and 32 of these were also identified as "normal" by their Colin ABI; however, since there were no participants with low ABI according to the Doppler, we cannot appropriately evaluate the sensitivity of the Colin as a PAD screening tool. Our study would require a larger sample with more participants outside the "normal" range, especially in the "low" range since that is indicative of peripheral arterial disease.

Comparison to Previous Studies

Within the last three years, several studies have evaluated validity of various oscillometric devices against the Doppler method. A 2012 meta-analysis (18) pooled the results of eighteen studies (N=3290) comparing oscillometric ABI measurements to the Doppler method and found an average difference of 0.020 ± 0.018, indicating that oscillometric ABI tended to be higher than Doppler, although this difference was not statistically significant (p=0.28). This conflicts with our findings that the Doppler measurements were higher than the Colin oscillometric measurements. The meta-analysis also reports a pooled linear correlation coefficient of 0.71 between Doppler ABI and oscillometric ABI, slightly greater than the linear correlation of 0.64 observed in our study. It should be noted that there was substantial heterogeneity between studies included in the meta-analysis; possible explanations include the use of different oscillometric devices, variation between study protocols, and differences in study populations.

Among the eighteen studies included in the meta-analysis mentioned above, eleven different oscillometric devices were used, and only two studies included in the pooled results used the Colin device (two other studies have compared ankle blood pressures measured with the Colin to those measured with Doppler, but are not included in the meta-analysis due to lack of ABI data). The four studies comparing Colin and Doppler measurements are briefly summarized below.

Cortez-Cooper (2003)[121] compared ankle blood pressure measurements taken with the Colin to those taken with Doppler in 52 participants. This study showed strong linear correlation between ankle pressure measurements (r=0.95) taken with Colin versus Doppler, higher than the correlation between Colin and Doppler ankle pressures observed in the present study (r=0.8). Ankle pressures measured with the Doppler were slightly higher than the Colin ankle pressures, although the mean difference between methods (2.2 mm Hg) was smaller than that observed in our study (8.0 mm Hg). Direct comparison with our results is difficult because it is not clear whether the patient or the limb was the unit of analysis, nor is it clear exactly how the methods are compared (i.e. average of the two limbs; lower of the two limbs). Also, since this study reported only ankle pressures, we cannot evaluate how its results compare to our ABI results.

Nukumizu (2007)[122] compared Doppler ankle pressures to Colin ankle pressures in 168 participants from a vascular clinic, all of whom had angiographically confirmed peripheral arterial disease (n=146) or abdominal aortic aneurysm (n=22). The Colin ankle pressures were slightly higher than Doppler ankle pressures, on average; the authors reported the ratios of Colin pressure divided by Doppler pressure for each participant, and noted that 135 of 168 (80%) participants had a Colin ankle pressure within 10 percent of their Doppler ankle pressure.

Notably, the agreement was significantly worse for PAD patients than non-PAD patients, suggesting that the Colin may be less reliable in patients with PAD. One limitation which might influence these results: the study methods do not mention any rest period between Doppler measurement and Colin measurement. In the absence of a rest period between measurements, these results may be influenced by artificially elevated readings for Colin ankle pressure.

Pan (2007)[123] performed a population-based study analyzing data from 946 participants in several rural villages south of Shanghai. The Doppler ABI measurements were slightly higher than the Colin ABI measurements, a similar trend to that observed in our study, although the between-measurement differences were smaller than those observed in our study (mean difference=0.03 for Pan et al vs. 0.08 in our study). Pan et al also noted that the gap between Doppler ABI and Colin ABI increased at higher values of the ABI (greater than 1.2). One limitation worth noting is that this study only performed a Doppler measurement at the right side, so they discarded Colin measurements obtained at the left side.

In the most recent published study comparing Colin ABI vs. Doppler ABI, Richart (2009)[124] measured ABI in 105 participants from a rural area near Flanders, Belgium. In this study, Doppler ABI tended to be slightly greater than Colin ABI, concordant with our study results; however, Richart et al also found that the Colin had better reproducibility than the Doppler, whereas we found slightly better reproducibility using the Doppler method. This study also performed a Doppler measurement only at the right arm and ankle, discarding the Colin data obtained from the left side.

The results from the four prior studies comparing Colin and Doppler ankle pressures and/or ABI combined with the present study can be synthesized into the following statements:

1) The Colin has displayed reasonable agreement with Doppler in participants with normal ABI.
2) The Colin may be less accurate for patients with extreme values of ABI (both low and high); in particular, it may provide falsely normal values for patients with very low values of ABI.
3) No consistent pattern has emerged to establish a directional relationship between Colin and Doppler measurements; there is somewhat weak evidence that the Doppler tends to report slightly higher ankle pressures and/or ABI than the Colin, but this could be accounted for by differences in study populations and protocol.
4) No consistent pattern has shown that either method has definitively better reproducibility. Richart et al reported that the Colin had slightly better reproducibility than Doppler ABI, while our results showed better reproducibility with the Doppler.
5) There is no significant evidence that smoking, exercise, or caffeine intake affects the *reproducibility* of ABI measurements taken in the same visit. However, these behaviors may affect the measurement between visits.

Other Oscillometric Devices

Most studies using other oscillometric devices have found that oscillometric ABI was slightly greater than Doppler ABI or that there was no difference between methods (18). Other than the differences in devices, results may vary due to differences in measurement protocol and study populations (patients referred to vascular laboratories, inclusion of participants with PAD). For example, Beckman (2006)[169] found that ABI measured by the Cas 740 oscillometric device

tended to be higher than Doppler, while our study showed the opposite. However, all participants in this study were referred to the laboratory for evaluation of PAD; since we have noted that the Colin is less reliable than the Doppler for participants with extreme values of ABI, it is possible that this pattern of higher ABI with the Cas 740 oscillometric device is driven by the inclusion of participants with PAD, similar to that described by Nukumizu et al.

Strengths and Limitations

Our findings must be considered in appropriate context of our study's strengths and limitations. The principal investigator trained both of the observers that performed ABI measurements during the study. All four of the ABI measurements on any single participant were performed by the same observer, and all were performed in a single office visit, eliminating potential concerns due to interobserver variability and day-to-day variation in blood pressure.

This study also has a few important limitations. Although taking all four measurements in a single visit eliminates the potential for day-to-day variation in pressures, the Colin and Doppler measurements were always taken in the same sequence, allowing the possibility that performing one measurement first influences the readings in subsequent measurements. A rest period was implemented between each measurement, but the sequence still may have an effect. This study includes only 40 participants, limiting our ability to perform subgroup analyses and sensitivity analyses, and our participants comprised a convenience sample of primarily healthy volunteers. With so few participants demonstrating abnormal ABI values, the generalizability of these findings is likely limited to participants with normal ABI.

It should be noted that we reported our primary results using the patient as the unit of analysis rather than the limb; we followed this convention because most epidemiological studies consider the lower of each participant's left/right ABI as a marker of cardiovascular risk. However, we performed limb-specific analyses (data not shown) that were consistent with the patient-level results, showing similar reproducibility within methods and agreement between methods.

Public Health Implications

The ABI is used primarily as a screening tool for PAD in high-risk patients. However, as one of the least expensive and least invasive markers of systemic atherosclerosis, the ABI could be very useful for CVD risk assessment in primary care. Both low ABI and high ABI are known to be predictive of cardiovascular events, and a 2008 meta-analysis showed that ABI provided additional predictive value when combined with the Framingham Risk Score.[170] Changes in ABI over time also provide predictive value; patients with significant decreases in ABI over time are proven to have increased risk for cardiovascular morbidity and mortality.[171] These results suggest that judicious use of the ABI in primary care settings has potential to improve public cardiovascular health.[172]

Despite this potential, the ABI remains underutilized in primary care.[28] According to practicing physicians, one of the greatest barriers to office-based ABI measurement is the time required for testing.[173] The Colin VP-1000 has potential to mitigate concerns about time by allowing simultaneous pressure measurements of all four limbs; in addition, obtaining an ABI with the Colin also requires little specialized training and could be performed by an office assistant.

5.6 CONCLUSION

In this report, we present evidence that the Colin VP-1000 oscillometric device is capable of measuring ABI with a moderate level of reproducibility; however, agreement between the Colin ABI and the gold-standard Doppler method is less than ideal. Colin ABI measurements were consistently higher than Doppler ABI measurements performed on the same patient, and there is not significant evidence of equivalence between methods. While measuring ABI with the Colin could prove more cost-effective and practical than the Doppler method because it is faster to perform and requires less training, our study results do not support the replacement of Doppler ABI measurements with Colin ABI measurements in a clinical setting.

5.7 TABLES AND FIGURES

Table 5-1. Required Sample Sizes to Achieve 80% Power and 90% Power

		Power	
		80%	90%
Assumed Correlation Between Measurements	0.2	33	127
	0.4	25	31
	0.6	17	22

Table 5-2. Baseline Characteristics of Study Participants

Characteristic	**N=40**
Age, years	35.4 ± 11.9
Gender (Female)	70.0%
Race	
White	82.5%
Black	7.5%
Asian	10.0%
Height, inches	66.8 ± 3.6
Weight, pounds	161.2 ± 47.2
BMI, kg/m^2	25.3 ± 6.6
BMI "Normal" (18.0-24.9)	65.0%
BMI "Overweight" (25.0-29.9)	22.5%
BMI "Obese" (>30.0)	12.5%
Self-Reported Diabetes	5.0%
Self-Reported Hypertension	10.0%
Self-Reported Hypercholesterolemia	12.5%
Smoke within 12 hours of visit	0.0%
Caffeine within 12 hours of visit	62.5%
Exercise within 12 hours of visit	40.0%

*Variables are presented as mean ± SD unless denoted as a percentage

Table 5-3. Descriptive Statistics of Blood Pressure Measurements

		Left	**Right**
Colin #1	Brachial	114.8 ± 12.9	115.9 ± 13.0
	Ankle	127.2 ± 16.5	126.1 ± 17.2
Doppler #1	Brachial	115.6 ± 13.8	114.8 ± 13.9
	Ankle	135.5 ± 19.2	135.0 ± 18.8
Colin #2	Brachial	114.9 ± 12.2	116.7 ± 12.7
	Ankle	129.7 ± 18.1	128.9 ± 18.1
Doppler #2	Brachial	115.8 ± 14.2	114.7 ± 13.3
	Ankle	136.4 ± 18.9	136.2 ± 18.5

All statistics reported as Mean ± SD

Table 5-4. Descriptive Statistics of ABI Measurements

	Mean ± SD	Median (IQR)
Colin #1	1.05 ± 0.11	1.06 (0.98-1.13)
Doppler #1	1.14 ± 0.13	1.12 (1.04-1.21)
Colin #2	1.08 ± 0.09	1.07 (1.03-1.13)
Doppler #2	1.15 ± 0.12	1.12 (1.07-1.21)

Table 5-5. Intra-Class Correlations, Linear Correlations, and Mean Absolute Differences for Primary Comparisons Reported in Text

	Intra-Class Correlation
Colin ABI #1 vs Colin ABI #2	0.77
Doppler ABI #1 vs Doppler ABI #2	0.91
Mean Colin ABI vs Mean Doppler ABI	0.44
	Linear Correlation
Colin ABI #1 vs Colin ABI #2	0.81
Doppler ABI #1 vs Doppler ABI #2	0.92
Mean Colin ABI vs Mean Doppler ABI	0.64
	Mean Absolute Difference
Colin ABI #1 vs Colin ABI #2	0.06 ± 0.04
Doppler ABI #1 vs Doppler ABI #2	0.04 ± 0.04
Mean Colin ABI vs Mean Doppler ABI	0.09 ± 0.09

Table 5-6. Between-Method Agreement by ABI Category

	Colin ABI ≤ 0.9	0.9 < Colin ABI ≤ 1.3	Colin ABI > 1.3
Doppler ABI ≤ 0.9	0	0	0
0.9 < Doppler ABI ≤ 1.3	1	32	0
Doppler ABI > 1.3	0	6	1

Table 5-7. Stratified By Caffeine Intake

	No Caffeine (n=15)	**Caffeine (n=25)**
	Intra-Class Correlation	
Colin ABI #1 vs Colin ABI #2	0.83	0.71
Doppler ABI #1 vs Doppler ABI #2	0.86	0.94
Mean Colin ABI vs Mean Doppler ABI	0.71	0.55
	Linear Correlation	
Colin ABI #1 vs Colin ABI #2	0.90	0.73
Doppler ABI #1 vs Doppler ABI #2	0.86	0.95
Mean Colin ABI vs Mean Doppler ABI	0.74	0.57
	Mean Absolute Difference	
Colin ABI #1 vs Colin ABI #2	0.06 ± 0.04	0.06 ± 0.04
Doppler ABI #1 vs Doppler ABI #2	0.06 ± 0.04	0.03 ± 0.03
Mean Colin ABI vs Mean Doppler ABI	0.09 ± 0.07	0.09 ± 0.09

Table 5-8. Stratified by Exercise Prior to Study Visit

	No Exercise (n=24)	**Exercise (n=16)**
	Intra-Class Correlation	
Colin ABI #1 vs Colin ABI #2	0.61	0.86
Doppler ABI #1 vs Doppler ABI #2	0.90	0.95
Mean Colin ABI vs Mean Doppler ABI	0.45	0.75
	Linear Correlation	
Colin ABI #1 vs Colin ABI #2	0.64	0.91
Doppler ABI #1 vs Doppler ABI #2	0.90	0.95
Mean Colin ABI vs Mean Doppler ABI	0.47	0.78
	Mean Absolute Difference	
Colin ABI #1 vs Colin ABI #2	0.05 ± 0.04	0.06 ± 0.04
Doppler ABI #1 vs Doppler ABI #2	0.04 ± 0.04	0.04 ± 0.03
Mean Colin ABI vs Mean Doppler ABI	0.09 ± 0.09	0.09 ± 0.07

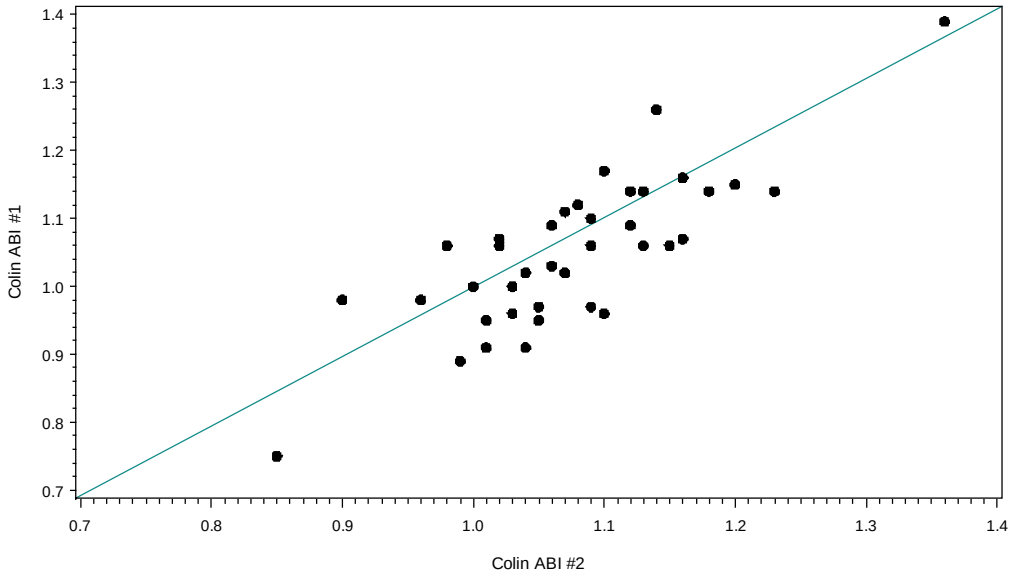


Figure 5-1. Scatterplot of Colin ABI Measurement #1 vs. Colin ABI Measurement #2

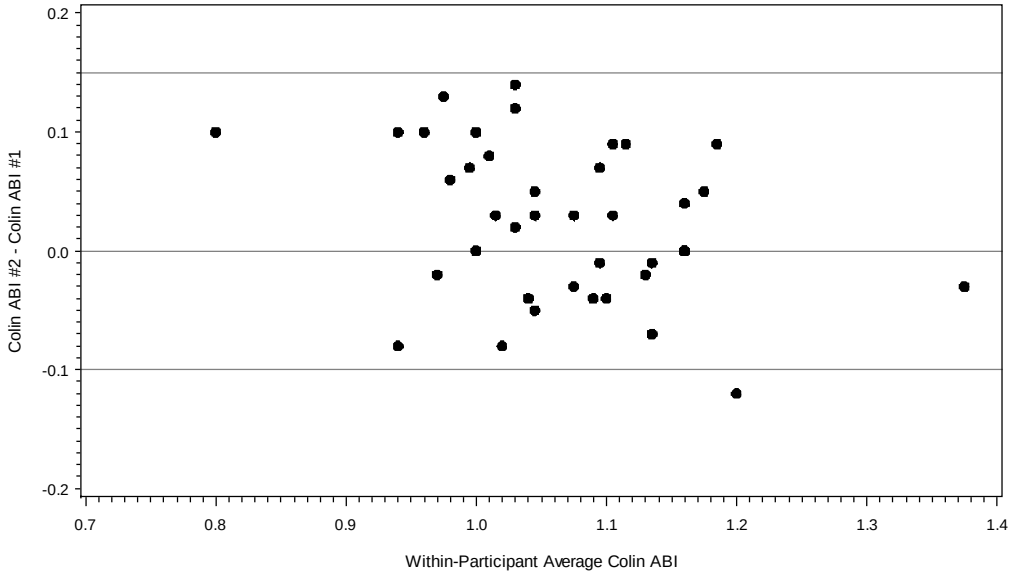


Figure 5-2. Bland-Altman Plot of Difference in Colin ABI Measurements vs. Mean Colin ABI

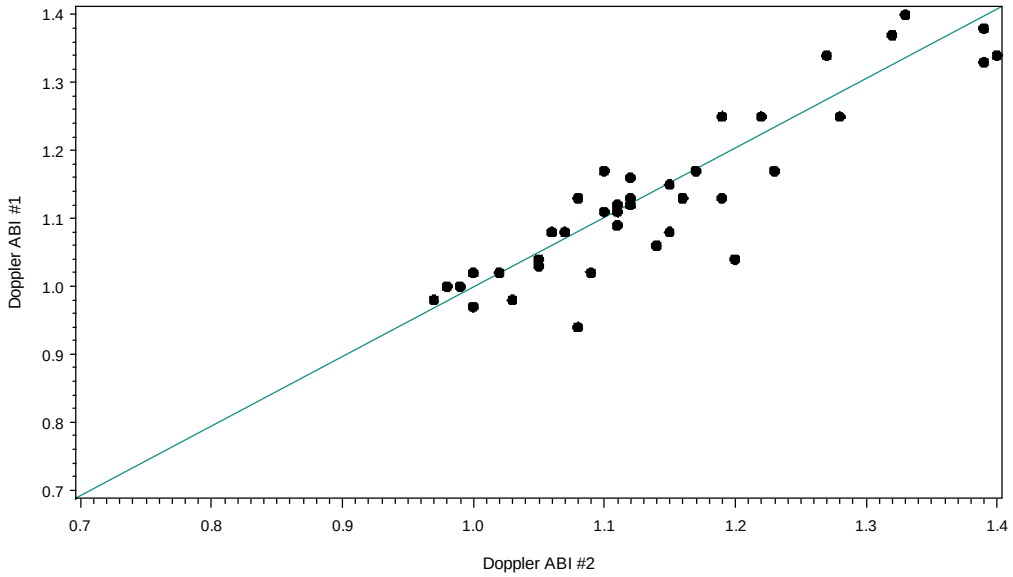


Figure 5-3. Scatterplot of Doppler ABI Measurement #1 vs. Doppler ABI Measurement #2

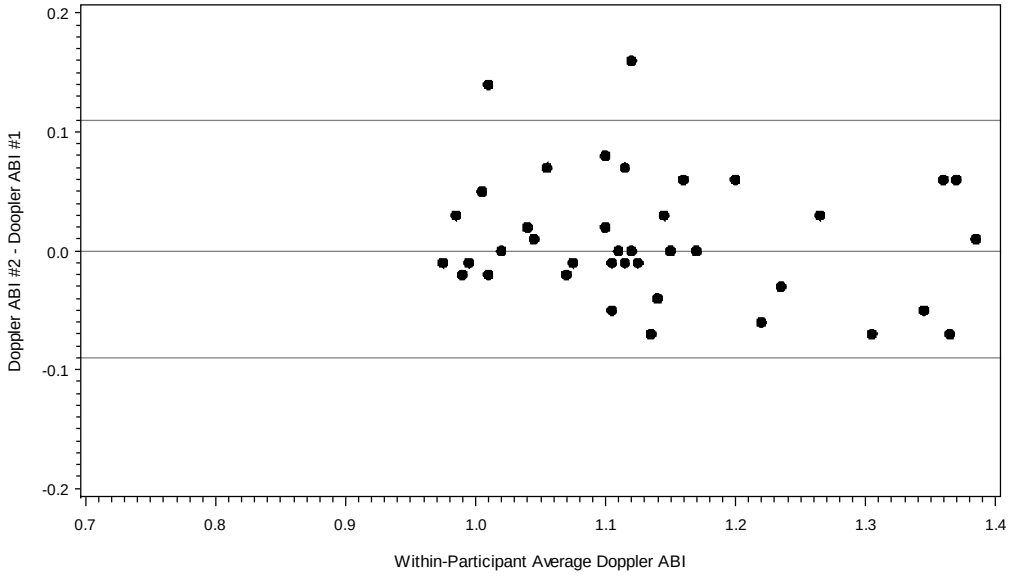


Figure 5-4. Bland-Altman Plot of Difference in Doppler ABI Measurements vs. Mean Doppler ABI

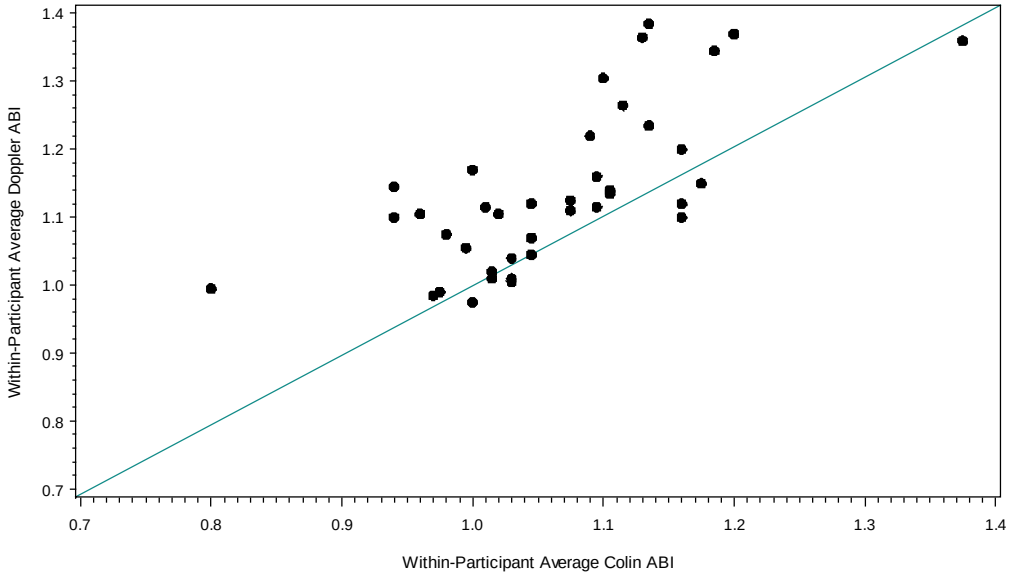


Figure 5-5. Scatterplot of Mean Doppler ABI vs. Mean Colin ABI

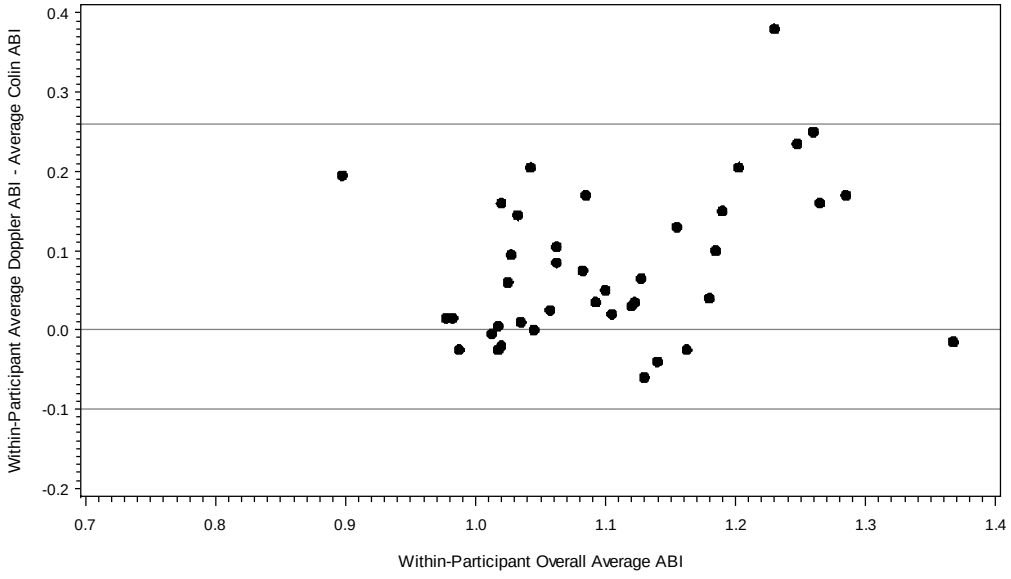


Figure 5-6. Bland-Altman Plot of Between-Method Differences vs. Mean Overall ABI

6.0 SUMMARY OF FINDINGS

The first manuscript reports the incidence of peripheral arterial disease (PAD) in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial. Approximately 20% of the 1,479 BARI 2D patients free from PAD at baseline were diagnosed with new PAD (defined as an ankle-brachial index ≤ 0.9 with a decrease of at least 0.1 from the baseline measurement) or another lower extremity event indicative of advancing PAD (lower extremity revascularization and/or lower extremity amputation) over an average 4.6 years of follow-up. The incidence of PAD and other lower extremity outcomes was significantly lower in patients assigned to an insulin sensitizing strategy than the incidence among those assigned to an insulin providing strategy (16.9% vs. 24.1%, $p<0.001$). Therefore, the BARI 2D results suggest that treatment of type 2 diabetes primarily using insulin sensitizing agents (metformin and/or thiazolidinediones) may result in lower incidence of PAD than treatment with a glycemic control strategy using insulin providing agents. Theoretically, this result implies that treatment with insulin sensitizing medications may slow progression of systemic atherosclerosis in patients with type 2 diabetes and advanced coronary artery disease. The effect of assigned glycemic control strategy remained statistically significant in a Cox proportional-hazards model after adjusting for in-trial HbA1c, suggesting that the insulin sensitizing medications may have conferred a benefit through a pathway besides improved glycemic control. Possible alternative pathways by which insulin sensitizing medications may achieve this include their effects on the interrelated processes of inflammation, coagulation, and fibrinolysis.

The second manuscript builds upon the first by examining a number of potential risk factors for PAD in the BARI 2D trial, first within the entire study cohort and then within the context of the two glycemic control strategies. The analyses included traditional cardiovascular risk factors such as body mass index, lipids, blood pressure, and Hb1Ac as well as novel risk factors indicative of inflammation, coagulation, and fibrinolysis such as C-reactive protein (CRP), fibrinogen, D-dimer, tissue-type plasminogen activator (t-PA), and plasminogen activator inhibitor-1 (PAI-1). Cox proportional-hazards models were used to establish independent associations between the selected baseline risk factors and PAD (while adjusting for age, sex, race, and smoking status) and then multivariable models were used to see which baseline risk factors provided the greatest predictive value. Systolic blood pressure, pulse pressure, HbA1c, albumin-creatinine ratio (ACR), and CRP showed significant associations with PAD in separate models. When assessed using a multivariate model created using a forward selection algorithm, three variables (HbA1c, ACR, and D-dimer) showed significant associations with PAD.

A second set of analyses using each risk factor as a time-varying covariate was performed to assess how longitudinal changes in each risk factor were associated with risk of PAD. These analyses were stratified by assigned glycemic control strategy because of the possibility that the treatment strategy could have influenced the value of the risk factors over time as well as the outcome. Notably, among patients assigned to the insulin providing strategy, only HbA1c and ACR were significantly associated with lower extremity outcomes, while among patients assigned to insulin sensitizing strategy a host of traditional cardiovascular risk factors (LDL, HDL, systolic blood pressure, pulse pressure) as well as D-dimer were significant in models that adjusted for age, sex, race, and smoking status.

In multivariable models, change in HbA1c was the only significant predictor of outcome in the patients assigned to insulin providing strategy, while D-dimer was the most significant predictor of outcome in the patients assigned to insulin sensitizing strategy. If D-dimer were not included as a candidate variable in this analysis, then fibrinogen would have entered the model in our forward selection algorithm (only for the analysis of patients assigned to insulin sensitizing strategy). If neither D-dimer nor fibrinogen were included as candidate variables, then CRP would have entered the model (again, only for patients assigned to insulin sensitizing strategy). This is noteworthy because the markers of inflammation, coagulation, and fibrinolysis were associated with lower extremity outcomes among patients treated with insulin sensitizing medications, but this was not the case for patients treated with insulin providing medications, thereby suggesting that these medications may have a differential effect on these processes and subsequently on the development of atherosclerosis in patients with type 2 diabetes.

The third manuscript reports the results of a data collection project evaluating reproducibility and reliability of two different methods for measuring ankle-brachial index: 1) the Colin VP-1000 oscillometric device and 2) the current gold-standard method involving a Doppler probe. Our results showed excellent reproducibility for ABI measured with the Doppler, while the Colin oscillometric device showed moderately good reproducibility but did not match the excellent reproducibility achieved with Doppler. Agreement between Colin and Doppler was somewhat poor; therefore, based upon these results, we would not likely recommend the use of Colin ABI measurements to diagnose peripheral arterial disease in clinical settings.

7.0 PUBLIC HEALTH SIGNIFICANCE

The public health significance of this research is primarily derived from the first two manuscripts regarding the incidence of peripheral arterial disease in patients with type 2 diabetes. The first manuscript suggests that insulin sensitizing medications (metformin and/or thiazolidinediones) may reduce the incidence of peripheral arterial disease in patients with type 2 diabetes and stable coronary artery disease; this can be interpreted as evidence that an insulin sensitizing strategy reduces the progression of systemic atherosclerosis. The second manuscript builds upon the first by demonstrating that longitudinal changes in biomarkers of inflammation, coagulation, and fibrinolysis are predictive of incident peripheral arterial disease in these patients when treated with insulin sensitizing medications, providing a possible mechanistic insight to how these medications affect the progression of atherosclerosis differently than treatment with insulin providing medications. These manuscripts have implications for both 1) clinicians treating patients with diabetes and 2) bench-science researchers investigating the mechanisms that influence the development of atherosclerosis.

As published previously, all-cause mortality and cardiovascular mortality was comparable between the assigned glycemic control arms in BARI 2D. [127] However, the insulin sensitizing strategy did demonstrate some noteworthy physiological benefits, specifically changes in biomarker profiles indicative of decreased insulin resistance, an altered balance between thrombosis and fibrinolysis favoring fibrinolysis, and diminished intensity of the systemic inflammatory state.[98] It was recognized *a priori* that an association of differences in biomarker profiles with clinical outcomes might not be evident because the ancillary study of CRP,

fibrinogen, and D-dimer was not powered to delineate effects on outcomes. Furthermore, since a systemic inflammatory state and impaired fibrinolysis are thought to affect clinical outcomes over prolonged intervals, and this study was carried out in a population that had quite advanced coronary disease upon study entry, it is unsurprising that beneficial effects of insulin sensitizing therapy on these biomarkers did not translate into significantly better mortality outcomes during BARI 2D follow-up.

The research contained in this dissertation provides further ammunition supporting the benefits of insulin sensitizing therapy and creates at least one fascinating hypothesis. Peripheral arterial disease is one of the foremost markers of "subclinical" atherosclerosis and, as detailed in this document's introduction, is known to be associated with cardiovascular mortality and all-cause mortality. Therefore, first manuscript's finding that an insulin sensitizing strategy may reduce the risk of peripheral arterial disease carries the implication that these drugs may reduce the *progression* of atherosclerosis; therefore, it is possible that if they are used earlier in the disease process and/or in a population with less disease burden, they might reduce the development of coronary atherosclerosis before disease becomes too advanced to observe a clinical benefit. Mechanistically, if the effects of insulin sensitizing medications on developing atherosclerosis occur by their effects on inflammation, coagulation, and fibrinolysis - processes that are not thought to affect clinical outcomes in the short term, but rather over a prolonged interval - then it is possible that these medications would result in cardiovascular risk reduction in a study of patients with less advanced disease at study entry and longer follow-up.

Making this argument even more compelling is the second manuscript's finding that longitudinal changes in biomarkers such as CRP, fibrinogen, and D-dimer were more closely associated with PAD outcomes in the patients assigned to treatment with insulin sensitizing therapy. Therefore, it is possible that patients treated with insulin sensitizing medications that experienced reductions in the systemic inflammatory state and improved fibrinolysis were protected from the continuing development of atherosclerosis, but the patients treated with insulin sensitizers that did not experience these benefits were more prone to the continued development of atherosclerosis (manifesting itself as an incident case of PAD). Further research should be carried out to determine whether insulin sensitizing medications do, in fact, affect the development of atherosclerosis through these specific pathways, particularly if this information can be leveraged to create new therapeutic targets.

All of the arguments thus far point to a benefit of insulin sensitizing medications; it should be noted that the American Diabetes Association (ADA) 2013 Clinical Practice Recommendations recommend an insulin sensitizing drug, metformin, as the first-line therapy for patients with diabetes.[174] Metformin was the most commonly used insulin sensitizing medication in the BARI 2D trial, with over 70% of patients assigned to insulin sensitizing therapy taking metformin at their three-year study visit. While the "insulin providing versus insulin sensitizing" design used in the BARI 2D trial is not well-suited to analyze specific medications because of the complex nature of the glycemic control strategy employed in BARI 2D, it may be reasonably inferred that the benefits of insulin sensitizing medications on peripheral arterial disease outcomes are at least in part due to the use of metformin. Therefore, our research supports the current guidelines by demonstrating an as-yet-unidentified benefit of insulin sensitizing therapy.

The ADA 2013 Clinical Practice Recommendations also offer comprehensive guidelines for cardiovascular risk reduction in patients with type 2 diabetes as follows:

- People with diabetes and hypertension should be treated to a systolic blood pressure goal of < 140 mm Hg; lower systolic blood pressure targets may be appropriate for certain individuals if it can be achieved without undue treatment burden
- LDL-cholesterol targeted statin therapy remains the preferred strategy, targeting an LDL level of less than 100 mg/dL in individuals without overt CVD and an LDL level of less than 70 mg/dL in individuals with overt CVD
- Statin therapy should be added to lifestyle therapy, regardless of baseline lipid levels, for diabetic patients that meet one or more of the following criteria:
    - Family history of CVD
    - Hypertension
    - Smoking
    - Albuminuria
- Aspirin therapy for patients with type 2 diabetes at an increased cardiovascular risk (same risk factors listed above); clopidogrel may also be considered for those with an aspirin allergy or in combination with aspirin for especially high-risk patients
- All patients are advised not to smoke or use tobacco products

Our findings do not have any specific bearing on the aforementioned recommendations, which are similar to those presented in **Figure 1-8** regarding CVD risk reduction for patients with peripheral arterial disease. Thus, we conclude that the first two manuscripts support current practice guidelines by reinforcing the notion that all patients with type 2 diabetes should first be treated using insulin sensitizing medications when practical.

The third manuscript leaves the question of insulin sensitizing medications' effect on atherosclerosis to ask a simpler question regarding the diagnosis of peripheral arterial disease: does the Colin VP-1000 oscillometric device offer potential to measure the ankle-brachial index (the principal diagnostic tool for PAD) with greater reproducibility than the current gold-standard technique using a Doppler probe?

According to our data, the answer is no. The reproducibility of ABI measurements taken using the Colin VP-1000 was inferior to reproducibility of ABI measurements taken using a traditional Doppler probe in all respects. Agreement between the two methods was mediocre at best, certainly less than that necessary to declare the Colin VP-1000 equivalent to the Doppler ABI. Therefore, based on the superior reproducibility displayed by Doppler and the lack of equivalence between Colin-measured ABI and Doppler-measured ABI, we cannot recommend the Colin VP-1000 oscillometric device as a replacement for Doppler-measured ABI in research settings or clinical settings.

In 2012, the American Heart Association issued a Scientific Statement on the measurement and interpretation of the ankle-brachial index in response to a lack of standards for measurement and calculation of ABI.[157] The AHA Scientific Statement notes that the correlation between Doppler-derived and oscillometric-determined ankle pressures and ABIs has been "generally acceptable in most studies with 1 exception" but still recommends that the Doppler method is the most reliable method to determine ABI and should be considered the gold-standard method. Our results support this statement, and therefore are in agreement with current AHA guidelines.

In summary, the research contained in this dissertation:

1. Documents the incidence of peripheral arterial disease in a defined population from a randomized controlled trial of patients with type 2 diabetes and coronary artery disease, illustrating that the atherosclerotic process continues even in those treated aggressively
2. Provides evidence of an as-yet-unidentified benefit of insulin sensitizing medications, the reduction in risk of incident peripheral arterial disease, thereby supporting current guidelines regarding the use of insulin sensitizing medications as the first-line therapy for treatment of type 2 diabetes mellitus
3. Suggests a potential mechanistic pathway through which insulin sensitizing medications may affect the progression of atherosclerosis, which can be studied to see if the pathways involved have any promise as therapeutic targets and/or can be better used to identify those at high risk of developing atherosclerotic plaques
4. Evaluates the reliability and reproducibility of two techniques that can be used to diagnose peripheral arterial disease and provides evidence that the current gold-standard method, using a Doppler probe, should remain the principal technique for evaluation of ABI in research settings and, likely, in clinical settings as well

APPENDIX A

Doppler ABI Testing Instructions

1. Patient Preparation

1) Bring the patient into a quiet room at normal room temperature (21-23 C).
2) Instruct the patient to remove their socks and shoes.
3) Instruct the patient to lie in a supine position with the ankles at heart level and the feet arranged so that the toes are pointed towards the ceiling.
4) Ascertain the appropriate cuff size for each limb
5) Place pressure cuffs approximately 3 cm above the cubital fossa on the arms.
6) Place pressure cuffs approximately 3 cm above the medial malleoulus on the ankle.
7) Allow the patient to remain at rest in the quiet room for 5 minutes.

2. Answer any questions and explain the test using the following sample participant script:

This test will take approximately 5 minutes during which time multiple blood pressures will be taken on each arm and ankle. While the test is being performed we ask you to hold still, remain quiet and not fall asleep.

3. Brachial Pressure Measurements

1) Locate brachial pulse on the right arm
2) Apply small mound of ultrasound transmission gel to the pulse site
3) Place the tip of the Doppler probe on top of the gel at a 45-degree angle
4) Adjust the Doppler probe to obtain the best audible pulse signal
5) Inflate the pressure cuff 20 mm Hg beyond the last audible signal
6) Deflate cuff (2 mm Hg/sec) until first audible signal (**systolic** pressure only)
7) Record the pressure at first audible signal
8) Repeat steps (1-7) using the left arm

4. Ankle Pressure Measurements

1) Locate dorsalis pedis (DP) pulse in the right ankle
2) Apply small mound of ultrasound transmission gel to the pulse site
3) Place the tip of the Doppler probe on top of the gel at a 45-degree angle
4) Adjust the Doppler probe to obtain the best audible pulse signal
5) Inflate the pressure cuff 20 mm Hg beyond the last audible signal*
6) Deflate cuff (2 mm Hg/sec) until first audible signal (**systolic** pressure only)
7) Record the pressure at first audible signal (*note: if the artery cannot be occluded 230 mm Hg, ankle pressure should be recorded as “non-compressible”)
8) Repeat steps (1-7) above using the left ankle

APPENDIX B

Colin VP 1000 Testing Instructions

1. Answer any questions and explain the test using the following sample participant script:

This test will take approximately 5 minutes during which time multiple blood pressures will be taken on each arm and ankle. While the test is being performed we ask you to hold still, remain quiet and not fall asleep.

2. Anthropometric Measurements**:** Five anthropometric measures are required on the **RIGHT** side prior to the Colin test. **All measurements must be taken with a steel measuring tape and recorded in centimeters on the Colin worksheet.** They are described on the following page in sequence:

1) **Carotid to suprasternal notch**:

   Measure the distance from the sampling site of the right carotid artery to the suprasternal notch.

2) **Suprasternal notch to umbilicus:**

   Measure from the suprasternal notch to the inferior (lower) edge of the umbilicus.

3) **Umbilicus to common femoral:**

   Measure from the inferior (lower) edge of the umbilicus to the sampling site of the right common femoral artery.

4) **Femoral to ankle:**

   Measure from the sampling site of the right common femoral artery to the medial malleolus.

5) **Suprasternal notch to antecubital fossa:**

   Measure from the suprasternal notch to the dot placed at the antecubital fossa

6) **Calculate Lcf measurement = (#2) + (#3) - (#1)**

3. To enter subject information on the Colin ID Input Screen rotate the jog dial to the desired digit. When the digit desired is displayed in the blue box, *depress* the jog dial to select. The selected digit will then appear the ID box. Repeat the previous step to choose all digits. If an error is made, select the F1 (correction) button and the last digit entered will be deleted. Pressing **New ID** will delete the entire ID. When ready, press F3 to **confirm**. The ID number will appear in the blue box at the top left-hand corner of the screen.

4. Participant information is entered into the next screen.

1) The sex (gender) field is first and defaults to **male**. If the participant is a female, *depress* the jog dial and the screen will be highlighted in blue. *Rotate* the dial to select female and depress the jog dial again.
2) Rotate the jog dial to move to the next field; use the jog dial to enter the participant's height.
3) Use the jog dial to bypass the Lcf* field (used only with carotid-femoral sensors).
4) Next enter participant's weight and birth date.
5) The fields to the right of the participant information are already entered. They should be completed as follows **and never changed**:

Meas. Part:	Both Arms + Legs
Pressurized Right Ankle:	AUTO
Pressurized Left Ankle:	AUTO
Measurement Times:	1
Wait Time:	10 sec.
Tonometry:	

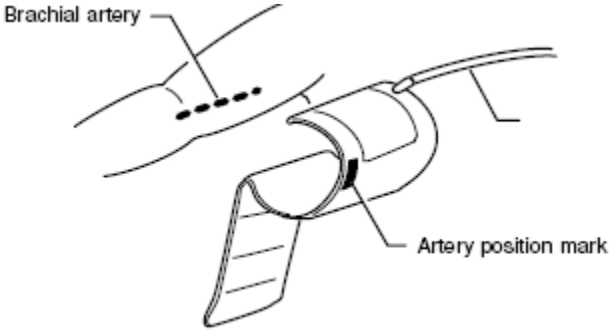
5. Choose a cuff appropriate for the patient by selecting the correct size (determined by the patient's arm circumference).

	Cuff Size (cm)	
Limb Circumference (cm)	Bladder Width	Bladder Length
16-21	8	21
22-26	10	24
27-34	13	30
35-44	16	38
45-52	20	42

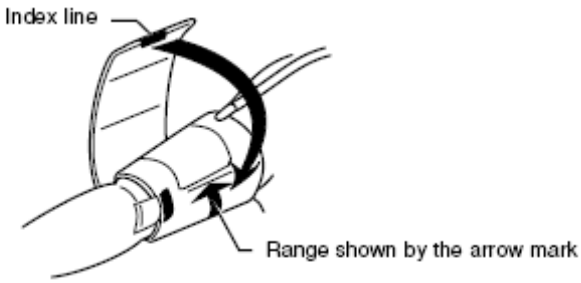
Note**: **Choose the appropriate cuff to avoid any error caused by gap between cuff and ankle in the measurements. If cuff is too large, the blood pressure measurement may be lower than actual value. If cuff is too small, measurement may be higher than actual value.

6. To attach the appropriate cuff to the cuff hose, insert the hose and turn it clockwise to lock.

7. Wrap the brachial cuffs around bare arms or thin clothing. (When applying the cuff above clothing, pull the clothing so that it does not bunch up on the side of the artery. If the cuff is wrapped around the arm with clothing bunched up at the artery site, the blood pressure will measure higher than the actual value.) Place appropriate cuff on each arm (cuffs are marked for left and right). The brachial artery runs down the inside of the arm. Attach the cuffs so that the horizontal artery position mark on the cuff aligns with the artery. See image below:

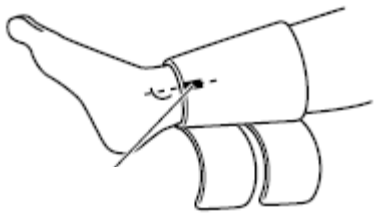


Apply the cuff with the tubing traveling up the arm towards the shoulder. Wrap the cuff snugly so that 2 fingers can be placed between the cuff and the arm. At this time, be sure that the index line is inside the range shown by the arrow mark. See image below:

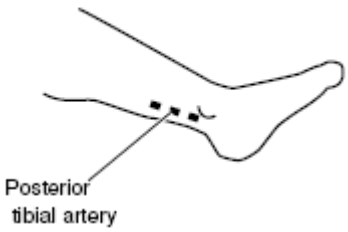


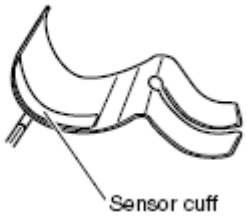
Note: *If the index line does not go inside the range, use a cuff of another size.*

8. Remove socks or stockings for application of ankle cuffs. The cuffs are marked for right and left side. Place the cuffs so that the artery position mark is about two finger widths from the top of the medial malleolus on the inside portion of the leg. See image below:

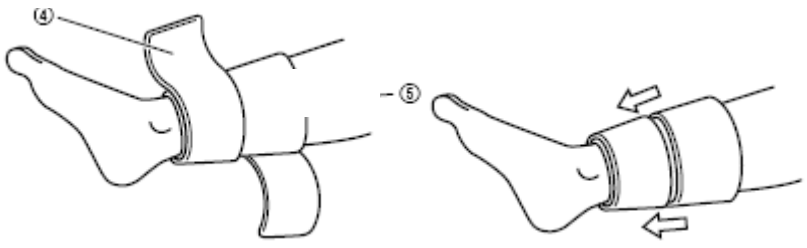


Note: *The sensor cuff segment (see image below) must be in contact with the posterior tibial artery of the ankle for the purpose of detecting the pulse.*

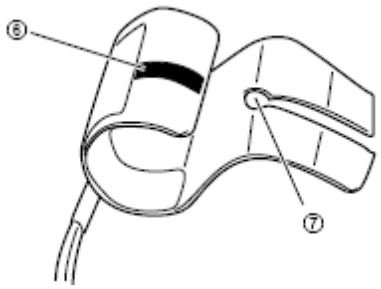




Wrap the lower ankle velcro wrap first then the upper calf velcro wrap. Wrap the cuff so that there is enough space to fit one finger between the cuff and ankle.



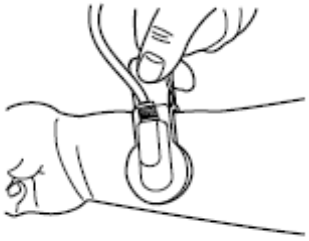
Note: *If a section of the index line can be seen through the cuffs window, measurements can be taken.* See image below:



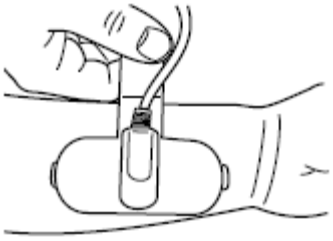
9. Application of the ECG (electrocardiogram) clips:

To attach the electrodes to the ECG clip, press and hold the button on the side of the right ECG clip and insert one electrode to the right ECG clip. Release the button to fix the electrode to the clip. Repeat these steps with the left ECG clip. Remove the protection sheets from all 3 electrodes.

The wrist clips are marked left and right. The left sensor has two electrodes and the right sensor has one electrode. Apply the clips to medial side of the forearm placing sensors just above the area where the radial pulse is palpated.



Right ECG ELECTRODES CLIP



Left ECG ELECTRODES CLIP

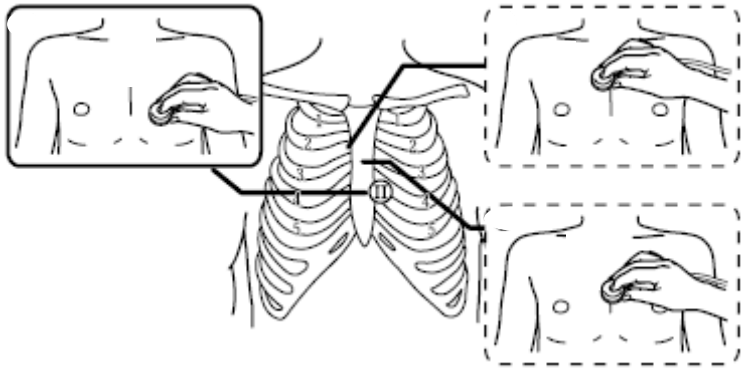
10. Application of the PCG (phonocardiogram) sensor:

Take off the protective sheet (light blue) and put it onto the sensor. Remove the cover sheet that is on the gel pad side. There are three places that the PCG sensor can be placed:

a) The 4th intercostal space to the left of the sternum. *(#4 on diagram*)

b) The 2nd intercostal space directly over the sternum. *(#6 on diagram)*

c) The 3rd intercostal space (centered). *(#5 on the diagram)*



11. Verify all fields on the participant information screen are completed and select F3 to confirm.

Note: *Take care to avoid mistakes in entering information and numerical values because this information will be used in determining analysis results and cannot be changed after the fact.*

12. The measurement screen should now be in view. Inform the participant to hold still and not move while the measurements are in progress.

13. Achieve the following on the screen display before the measure is started:

ECG: OK status

PCG: OK status

Note: It is recommended that at least 3-4 meters be flashing on the PCG level when the measurement starts. While testing can be started even when "OK" is not indicated, the accuracy may be reduced.

14. Depress the **Blue Start Switch** to have the cuffs inflate automatically. The measurements will be recorded for 30-60 seconds and when the measures are complete the cuffs will deflate automatically.

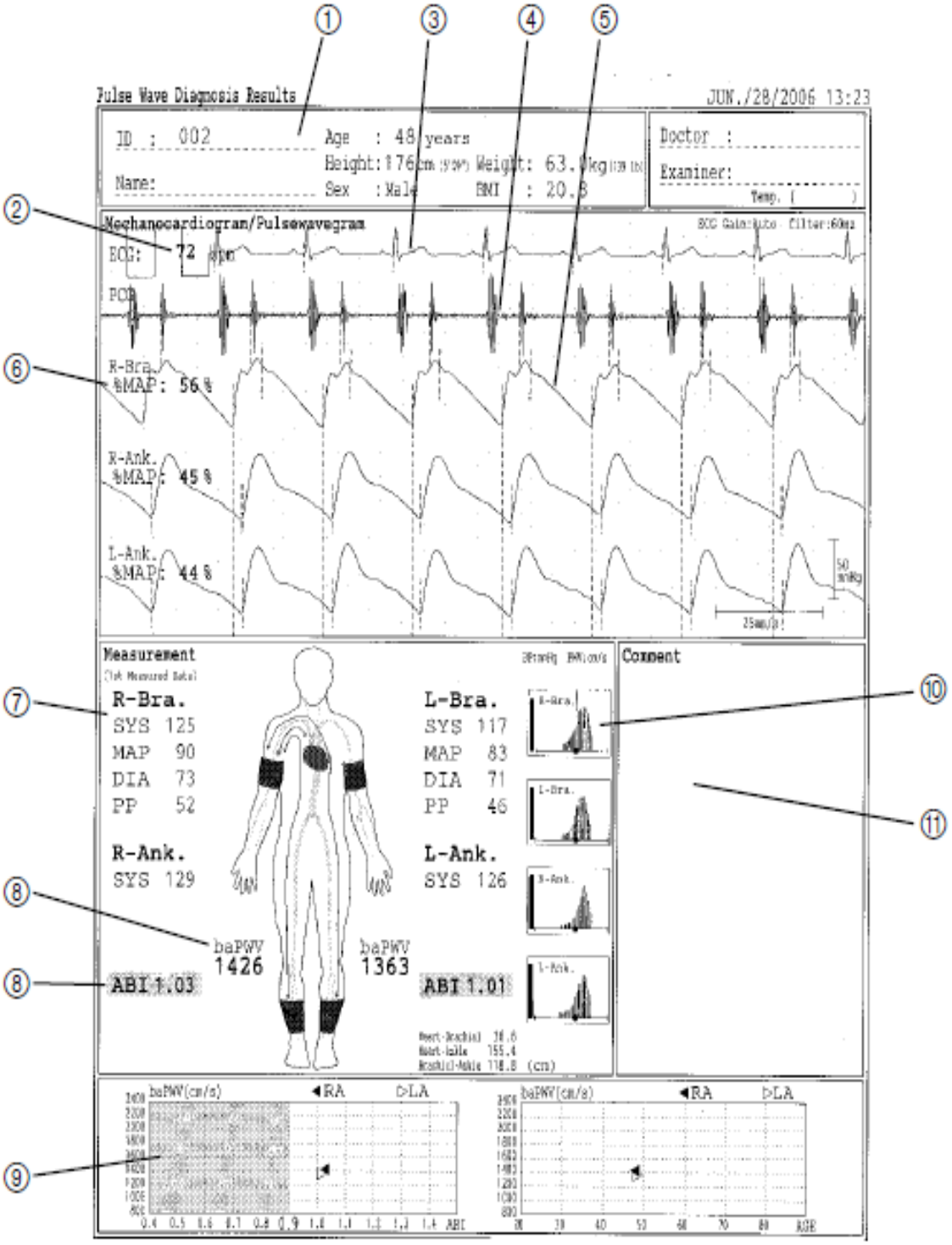
15. Evaluate the results on the display screen for accuracy. Results are automatically printed after each run. The machine will prompt you if waveforms are not adequate and will offer corrective suggestions. Pay attention to screen messages and make changes accordingly (refer to pages 90 thru 97 of the Operation Manual for a *List of Colin Messages/ Troubleshooting* for specific errors and how to make adjustments).

16. Depress the **Orange Stop Switch** to return to the ID Input screen. Re-verify the SWAN IV ID and depress the F3/Confirm button. Repeat steps 12-15 for the start of the 2nd run.

17. Each print out must be assessed for quality and accuracy. Prior to dismissing the participant the numbered areas on the Colin print out (see next page) are to be reviewed for correct data entry.

18. On the *Clinical Measurements* form, complete all the appropriate boxes for each run performed and provide comments (if any) from the machine print out.

The following pages contain a sample printout and numbered descriptions of each element on the Colin printout.


①
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⑥
⑦
⑧
⑧
⑨
⑩
⑪
Pulse Wave Diagnosis Results
JUN./28/2006 13:23
ID : 002
Name:
Age : 48 years
Sex : Male
Doctor :
Examiner:
Mechanocardiogram/Pulsewavegram
ECG: 72
PCG
R-Bra. %MAP: 56 %
R-Ank. %MAP: 45 %
L-Ank. %MAP: 44 %
Measurement
R-Bra.
SYS 125
MAP 90
DIA 73
PP 52
R-Ank.
SYS 129
baPWV
1426
ABI 1.03
L-Bra.
SYS 117
MAP 83
DIA 71
PP 46
L-Ank.
SYS 126
baPWV
1363
ABI 1.01
Comment
baPWV(cm/s)
RA
LA
ABI
AGE

① Patient information

Shows the patient information entered into the patient information input screen.

② Heart rate

Shows the measured heart rate.

③ ECG

Printout of the ECG waveform.

④ PCG

Printout of the PCG waveform.

⑤ PVR waveform

Shows the pulse wave obtained from the measurement. Because the amplitude of these results is calibrated from the measured blood pressure value, the amplitude may be different than that shown on the screen.

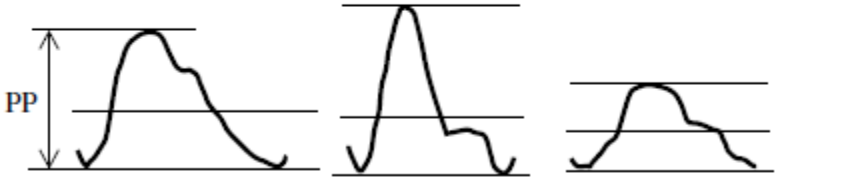
⑥ %MAP

This value is one of the pulse waveform indexes that is calculated from the blood pressure values. It expresses, as a percentage, a value from the area of the wave form (P2) divided by the amplitude of the pulse (P1). This value is calculated with the following formula:

%MAP = P2/P1 ¥ 100 (%)

P1 : Pulse wave amplitude

P2 : Mean value of the area
(the level at which ▨ and ▭ are equal.)



a. Soft (i.e. 45%)　b. Hard (i.e. 38%)　c. Constricted (i.e. 50%)

⑦ Blood pressure values

The shows the blood pressure values for the left and right arm and left and right ankle.

⑧ PWV

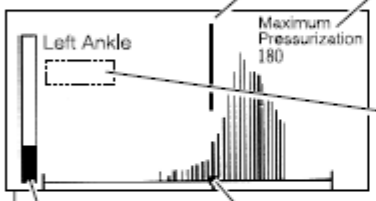
This gives the baPWV value. It measures the start of the brachial pulse wave to the start of the ankle pulse wave.

⑨ ABI value

Shows the right and left ABI values.

⑩ Pulsatile variation graph

This graph shows the pulsatile variation obtained from each cuff.



Synchronization line: This line is not printed if synchronized measurement was not possible.

Inflation upper limit: If the inflation upper limit is set to anything besides "AUTO," that setting is printed here.

When "Estimate" or "First Measurement" is displayed, accuracy may be poor.

The part marked with a black dot is the highest blood pressure value. This will not be printed if measurement was not possible.

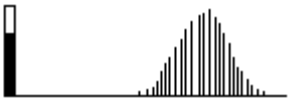
This level meter shows the size of the pulse wave.

Border: when constriction of the upper arm or ankle is suspected, this border will be printed with a thicker line for emphasis.

⑪ Observation

Gives observations based on the test results.

Good Measurement — The display below is a mountain shape and reliability of the measurement is high without noise such as motion artifact.



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