PULSE WAVE ANALYSIS IN TYPE 1 DIABETES: RELATIONSHIP WITH HISTORICAL MEASURES AND PREVALENT DISEASE

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Type 1 diabetes (T1D) is associated with numerous complications. These include renal and cardiovascular disease which are the leading causes of morbidity and mortality in T1D. Renal complications also increase the risk for cardiovascular disease. Early detection and treatment of their risk factors may help to prevent or at least delay these complications. This dissertation examines potential risk factors for altered measures of pulse wave analysis (PWA), which have been linked to cardiovascular events and mortality in other populations. It also examines how PWA measures relate to prevalent cardiovascular and renal complications in T1D.

Prospective analyses of potential risk factors for increased arterial stiffness indices, augmentation index (AIx) and augmentation pressure (AP), and decreased estimated myocardial perfusion, i.e. subendocardial viability ratio (SEVR), showed autonomic neuropathy, smoking history, low HDL cholesterol and poorer glycemic control, to be associated with altered PWA measures 18 years later.

Next, cross-sectional analyses between PWA measures and prevalent CVD showed AP and SEVR to be significantly related to coronary artery disease and coronary artery calcification, respectively, although age was the major predictor of both. AP was also higher, although not significantly, and SEVR significantly lower in those with peripheral vascular disease.

Finally, SEVR, but not AIx nor AP, was significantly associated with the presence of microalbuminuria (MA), and preferentially entered multivariate models over brachial blood pressure measures. SEVR was also related to degree of albuminuria in those within the normo- and MA range, and was significantly associated, multivariately, with low renal function.

This dissertation thus yields significant Public Health findings by identifying factors (AN, smoking, glycemic control, lipid levels) that may delay increased arterial stiffness (AIx and AP) and decreased myocardial perfusion (SEVR). As it additionally shows that these same PWA measures are altered in the presence of CVD and renal damage in T1D a potential role for PWA measures, especially SEVR, in risk stratification and early intervention for T1D complications is apparent.

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PREFACE

I would like to thank the members of my committee for their time and understanding with the accelerated timeline for the completion of this dissertation. Thank you for all of your help and input which has greatly increased my knowledge and understanding of the material presented here. I would also like to thank Dr. Trevor J. Orchard, my advisor and my GSR supervisor. I appreciate all of the opportunities you have given me and the time and energy you invested in aiding me in the successful completion of both my MPH and my PhD. I promise to do my best to publish these papers even though my time in Pittsburgh is short.

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1.0 INTRODUCTION

Diabetes is the 6th leading cause of death in the United States and the majority of people with diabetes die from its complications. Diabetes also contributes to the development of heart disease and stroke, the first and third leading causes of death. In fact, heart disease and stroke account for about 65% of deaths in people with diabetes [1]. Complications of diabetes can be prevented or at the very least delayed; therefore early detection is very important.

Increased arterial stiffness (or reduced arterial compliance), is a marker of vascular aging and is also associated with cardiovascular risk [2-6]. Arterial stiffness measures increase with age [7] as well as in the presence of diabetes [8-10] and end-stage renal failure[11, 12]. Microalbuminuria (MA) has been associated with increased arterial stiffness in the general population[13] which may be one of the pathways underlying the associations of MA with advanced atherosclerosis and cardiovascular mortality. MA is also a marker of early diabetes renal disease, itself a risk state for CAD [13, 14]. As changes in arterial stiffness indices can be detected prior to the development of clinical disease, markers of arterial stiffness may therefore be useful in predicting risk for a number of complications of diabetes. This paper will focus on the relatively new technology of radial tonometry to obtain pulse wave analysis (PWA) and resulting arterial stiffness indices, augmentation index (AIx) and augmentation pressure (AP) as well as subendocardial viability ratio (SEVR), an estimate of myocardial perfusion. In particular it will focus on:

1. Examining the relationship between risk factors assessed ~18 years prior earlier and PWA measures in type 1 diabetes (T1D).
2. Determining the relationship between PWA measures and prevalent cardiovascular disease (CVD). Specifically, coronary artery disease (CAD), coronary artery calcification (CAC, a subclinical marker of CAD) and lower extremity arterial disease (LEAD) will be assessed.
3. Examining the relationship PWA measures have with early nephropathy (microalbuminuria) and with renal function, both estimated glomerular filtration rate (eGFR) utilizing the Modification of Diet in Renal Disease (MDRD) equation and Cystatin C measures, in T1D.

2.0 BACKGROUND

2.1 PATHOPHYSIOLOGY OF DIABETES MELLITUS

Diabetes Mellitus (DM) is a group of metabolic diseases which occurs when the body fails to properly metabolize glucose. In the United States, the National Diabetes Data Group (NDDG) criteria for diagnosis and classification of diabetes are used. Diabetes is diagnosed if an individual meets one of three criteria: 1) symptoms of diabetes (polydipsia, polyuria, unexplained weight loss) and a casual (without regard to last time of meal) plasma glucose concentration of ≥200 mg/dl, 2) a fasting plasma glucose (FPG) (at least 8 hours) ≥126 mg/dl, or 3) an oral glucose tolerance test (OGTT) 2-hour value ≥200 mg/dl[15]. Individuals with FPG levels between 100 and 126 mg/dl are considered to have impaired fasting glucose (IFG). A 2-hour post load glucose level between 140 and 199 mg/dl is considered IGT. Both IFG and IGT are referred to as "pre-diabetes". Individuals with pre-diabetes are at increased risk for developing diabetes and cardiovascular disease[16].

To maintain glucose homeostasis there must be balance between glucose uptake and utilization by the cells and production of glucose by the liver. In a normal fasting state, low circulating insulin levels lead to the production of glucose by the liver via gluconeogenesis and glycogenolysis, a reduction in synthesis of glycogen as well as a reduction in the uptake of glucose by cells requiring insulin for uptake. Postprandially, the increased circulating glucose

levels stimulate the secretion of insulin by the beta cells of the islet of Langerhans of the pancreas. The insulin is secreted into the portal venous system, where some is removed by the by the liver. The remainder enters the circulatory system, eventually binding to insulin receptors of skeletal muscle and fat cells where it aides in the translocation of glucose into cells and thus lowers blood glucose levels. The inability to appropriately metabolize glucose in diabetes mellitus, can be due to the failure of the pancreas to produce insulin, as is the case in T1D (T1D), or to the inability of the cells to use insulin (insulin resistance) as occurs in Type 2 diabetes and its early prediabetes state. Failure in either of these areas results in high levels of circulating glucose, hyperglycemia.

T1D occurs in individuals who are genetically predisposed to the disease. Greater than 90% of Type 1 cases occur due to autoimmune destruction of beta cells over time. Though initiation of this autoimmune response may be triggered by an environmental or infectious stimulus, there are many hypotheses as to how and why this occurs, but no solid evidence for specific risk factors have been established therefore T1D is currently considered not preventable[17]. The autoimmune destruction of the beta cells leads to a reduction and eventually, a complete absence of insulin production and secretion. In order for cells to metabolize glucose, exogenous insulin must be administered.

Type 2 diabetes is thus characterized by peripheral insulin resistance, impaired insulin secretion and excessive hepatic glucose production. The peripheral cells become resistant to insulin thereby impairing glucose utilization. The liver also becomes insulin resistant which results in decreased uptake of glucose as well as decreased inhibition of hepatic glucose production. With reduced insulin sensitivity in peripheral tissues and an increased hepatic production of glucose, the result is a state of hyperglycemia. During the initial stage of insulin

resistance however, the pancreas will increase its production and secretion of insulin to compensate. Eventually the beta cells become exhausted and production levels of insulin decrease[18]. Typical treatment of Type 2 diabetes includes lifestyle/behavioral changes such as diet and exercise as well as insulin sensitizing agents. However, some Type 2 patients will eventually need insulin therapy as the beta cells fail to meet the increased demand. It is thought that Type 2 diabetes results from a combination of insulin resistance and a beta cell defect, for many more subjects have insulin resistance than diabetes. Insulin resistance is often related to age and/or obesity. Type 2 diabetes is a very preventable disease. It is usually preceded by impaired glucose tolerance (IGT) and approximately 20-50% of people with IGT will progress to Type 2 diabetes within 10 years. Screening of individuals for IGT followed by lifestyle interventions could prevent, or at the very least delay the onset of the disease[19-21].

2.2 DIABETES EPIDEMIOLOGY

Approximately 20.8 million people in the United States, 7% of the population, have diabetes. An estimated 14.6 million are diagnosed; 6.2 million undiagnosed while a further 54 million have pre-diabetes (impaired glucose tolerance (IGT) or fasting glucose (IFG))[1]. The World Health Organization (WHO) estimated the global prevalence of diabetes to be 171 million (2.8% of the population) in the year 2000 and projects that by 2030, 366 million (4.4% of the population) people worldwide will have diabetes [22]. The rates of diabetes have increased dramatically in the past few decades and will continue to increase due to the aging of the population and to the obesity epidemic. Type 1 accounts for only 5-10% of diabetes cases however, the incidence rates of both Type 1 and Type 2 are increasing worldwide[23]. In 1993,

T1D affected about 1.4 million people in the U.S. and 10-20 million worldwide[24]. Type 1 diabetes is the main chronic disease affecting children. The current estimate of Type 1 worldwide prevalence in children 0-14 years of age is 440,000, or 0.02% of that population with an annual increase in incidence of 3%[23]. One in every 400-600 children/adolescents in the U.S. has T1D. Current estimates of U.S. Type 1 annual incidence rates in youth are 14.3, 22.1, 25.9 and 13.1 per 100,000 for age groups 0-4, 5-9, 10-14 and 15-19, respectively[25].

Development of T1D has both environmental and genetic components. The rates of diabetes vary by race and ethnicity as well as by geographical location. For instance, the highest incidence rates of T1D among children ≤14 years, are in Scandinavia with the annual incidence in Finland at 41.1 per 100,000. In Sweden, the annual incidence rate among the same age group is range is 31.7 per 100,000, while in Lithuania it is 7.8 per 100,000, one of the lowest rates in Europe [23]. The lowest incidence rates known are in China and Japan (1 to 3 per 100,000). Accurate estimates of incidence rates of Type 1 in African countries are currently not available. Known prevalence rates in African countries are quite low however, this is not necessarily due to low incidence, but more likely to high mortality in these areas.

The frequency of high-risk HLA alleles (HLA-DR3 and HLA-DR4), which are found in about 95% of Type 1 patients, is greater in Caucasians [26-28]. However, studies of monozygotic twins show a only a moderate concordance (about 50% or less) and the risk to a first degree relative of someone with type 1 is about 5%[29]. In the U.S. the highest incidence rates are among Non-Hispanic whites, followed by African Americans, Hispanics, then Asian/Pacific Islanders; the lowest incidence rates are in American Indians [25]. The growing incidence of T1D is apparent in Non-Hispanic white children as well as in Hispanic and African

American children [30, 31]. There is also a seasonality effect in T1D as it is diagnosed more frequently in winter and autumn months. This is particularly the case during puberty[32].

Both genetic susceptibility and lifestyle factors contribute to the development of Type 2 diabetes. There is a strong genetic predisposition to Type 2. The concordance in monozygotic twins is approximately 70% [33]. History of Type 2 in a first-degree relative doubles ones risk of diabetes and the lifetime risk of Type 2 for a child of two diabetic parents is about 80% [33, 34]. However, behavioral and lifestyle factors such as obesity, poor diet and sedentary lifestyle, are major contributors to the development of Type 2. Certain racial/ethnic groups are more predisposed to type 2 diabetes than others therefore incidence rates can vary greatly by group within one population. Rates of Type 2 diabetes are highest in the Pacific Islands, intermediate in the U.S. and India and lowest in China and Russia. In the U.S., the racial/ethnic group with the highest prevalence of Type 2 diabetes is American Indians/Alaska Natives. 15.1% of this group, who are 20 years of age or older, has Type 2 and they are 2.2 times more likely than non-Hispanic whites to have diagnosed -diabetes. 13.3% of non-Hispanic blacks, 9.5% of Hispanic/Latino Americans and 8.7% of non-Hispanic whites have Type 2 diabetes[35]. So while the rates of T1D are higher in Caucasians, at least within the U.S. rates of Type 2 diabetes are much lower in the same population.

In the U.S, the total economic cost of diabetes in the year 2002 was estimated to be $132 billion. Direct costs were estimated to be $92 billion a drastic increase from the $44 billion spent in 1997. Approximately 20% of all health care costs are due to diabetes. A large portion of the costs associated with diabetes is due to chronic complications[36]. Beyond the monetary cost there is also the personal cost; persons with diabetes have two times the prevalence of physical

disability compared to those without diabetes[37]. With the increased incidence of diabetes, finding methods to prevent or delay chronic complications is of great importance.

2.3 TYPE 1 DIABETES COMPLICATIONS

Chronic hyperglycemia leads to chronic complications of diabetes. Many of these complications are vascular in nature including microvascular complications such as nephropathy, retinopathy and neuropathy as well as macrovascular complications such as peripheral vascular disease, coronary artery disease (CAD) and cerebrovascular disease.

2.3.1 Neuropathy

Diabetes causes damage to the body's nerves. Diabetic neuropathies are a family of nerve disorders and are classified as 1) peripheral - affecting the limbs, often called Distal Symmetric Polyneuropathy (DSP), 2) autonomic – affecting the autonomic nervous system and associated organs/functions, 3) proximal - sometimes called lumbosacral plexus neuropathy and affects the thighs, buttocks, or hips or 4) focal – muscle pain or weakness caused by sudden weakness of one nerve, or a group of nerves. Approximately 50% of people with diabetes will develop some form of neuropathy. The most common form is DSP [22] affecting approximately 60% of those with T1D over the age of 30 [23]. The risk for diabetic neuropathy increases with increasing diabetes duration [24, 25].

2.3.1.1 Peripheral Neuropathy

Peripheral neuropathy damages nerves in the arms and legs, typically affecting the feet and legs prior to affecting the hands and arms. Symptoms of peripheral neuropathy include numbness/insensitivity, a tingling, burning, or prickling sensation, sharp pains or cramps, extreme sensitivity to touch, loss of balance and coordination. Peripheral neuropathy may also cause muscle weakness and loss of reflexes. Due to numbness, sores may occur on the feet and go unnoticed. Infection of sores may occur and can spread into the bone resulting in a need for amputation. Distal symmetric polyneuropathy (DSP) is the most common form of diabetic neuropathy usually developing in the feet. Signs of DSP during clinical examination can include signs of sensory loss, initially vibratory and later touch and pain, signs of motor loss such as weakness and atrophy, depressed or unelicitable tendon reflexes [26].

In The Pittsburgh Epidemiology of Complications (EDC) Study, by 30 years of diabetes duration, over two thirds were affected by DSP [24]. The incidence density of DSP in this study was 1.2 per 100 patient years among those with diabetes duration less than 20 years, but 3.83 among those with duration 20-30 years. The cumulative incidence of DSP in this population at 20 years ranged from approximately 20-30% while the 25 year cumulative incidence ranged from 35-50% (depending on diagnosis cohort: 1965-1969, 1970-1974 and 1975-1980; earlier cohort has the highest rate)[27]. Risk factors for DSP in the EDC Study were diabetes duration, poor glycemic control (HbA1c level), current smoking, height and hypertension [28]. The EURODIAB study, a Type 1 population sampled from 31 populations throughout Europe, found an overall prevalence of peripheral neuropathy of 28%. In this study, age (mean = 32.7 ± 10.2 years), diabetes duration (mean = 14.7 ± 9.3 years) and HbA1c level (mean = 6.7 ± 1.9%), were significantly associated with peripheral neuropathy. When adjusting for both age and HbA1c,

rates ranged from 11% in those with a diabetes duration of <7 years to 23% in those with diabetes duration of 8-14 years, to 38% with a 15 year duration[29]. Diastolic blood pressure and total cholesterol were also shown to be independent risk factors for DSP [30].

2.3.1.2 Autonomic Neuropathy

Autonomic neuropathy occurs in diabetes due to damage to the nerve fibers of the autonomic nervous system. The autonomic nerve fibers include those that innervate the blood vessels and the heart and therefore a common form of autonomic neuropathy is Cardiovascular Autonomic Neuropathy (CAN). When damage occurs to these nerve fibers , abnormalities in central and peripheral vascular dynamics and heart rate occur[31]. Reduction in heart rate variability is the earliest indicator of heart rate. Patients with CAN cannot adequately regulate cardiac output or redirect blood flow therefore they tend to have exercise intolerance and orthostatic hypotension [32]. Also, since the nerves innervating the heart are affected they can also have asymptomatic ischemia and painless myocardial infarction[33]. Relative risk of mortality associated with CAN (defined as presence of >2 abnormal quantitative autonomic function tests) is was found to be 3.65 (95% C.I.:2.66-4.47) in a pooled estimate of 15 studies [34].

In the Pittsburgh EDC Study, incidence density per 100 person-years of *symptomatic* autonomic neuropathy was 0.83 (0.62-1.1) and 0.36 (0.07-1.1) in those with a diabetes duration of <20 and 20-30 years, respectively. The overall incidence density was 0.78 (0.59-1.0) per 100 person-years. Age of onset adjusted cumulative incidence of symptomatic autonomic neuropathy ranged from 11-21% in those with diabetes duration of 20 years and from 18-25% in those with diabetes duration of 25 years. Incidence of autonomic neuropathy varied by diagnosis cohort, which in the EDC study were 1965-1969, 1970-1974, and 1975-1980. Therefore, given the same

diabetes duration (20 years), those diagnosed with diabetes later (i.e. between 1975 and 1980) were significantly less likely to have incident autonomic neuropathy (cumulative incidence = 11%) than those diagnosed earlier (i.e. between 1965 and 1969; cumulative incidence = 21%) [35]. Autonomic neuropathy is often asymptomatic. However, the presence of asymptomatic CAN can be diagnosed by assessing heart rate variability (HRV) using the expiration-to-inspiration ratio [36]. In the Pittsburgh EDC Study, when CAN was diagnosed by as an abnormal (≤1.1) expiration-to-inspiration ratio during deep breathing, the incidence-density over a mean follow-up time of 4.7-years was 5.9 /100 person-years. Age (relative risk [RR]=2.15, p=0.0001), HbA1 (RR=1.50, p=0.0002) and nephropathy (albumin excretion >200 μg/min) (RR=2.46, p=0.0001) were all significant independent predictors of CAN. When nephropathy was not available to multivariate models, hypertension was significantly predictive as well[37].

2.3.2 Eye Complications

The eye complications associated with diabetes are 1) diabetic retinopathy - damage to the blood vessels in the retina, 2) cataract – the clouding of the eye's lens, cataracts tend to develop at an earlier age in people with diabetes; 3) glaucoma – the increase in fluid pressure inside the eye which can lead to optic nerve damage and loss of vision. Retinopathy is the most prominent eye associated complication in T1D.

2.3.2.1 Diabetic Retinopathy

Retinopathy is the most common eye disease in diabetes and is caused by changes in the blood vessels to the retina such as swelling and leakage of fluids or abnormal development of blood vessels on the surface of the retina. During the initial, asymptomatic stage, there is

decreased retinal blood flow, loss of pericytes and thickening of the retinal basement membrane. As the disease progresses, microaneurysms, increased vascular permeability and retinal ischemia are present. During advanced stages, fibrovascular proliferation and macular edema occur[38].

Although improved management of diabetes and glycemic control in recent decades has led to a decline in microvascular complications, diabetes is still the leading cause of blindness among adults in the U.S. with diabetic retinopathy causing 12,000-24,000 incident cases of blindness annually[39]. Diabetic retinopathy is the most common diabetic eye disease and is caused by changes in the blood vessels of the retina which usually do not begin to occur until after 3-5 years of diabetes duration [40].

There are various stages of progression of retinopathy. The first stages are the non-proliferative stages. In mild non-proliferative diabetic retinopathy (NPDR), microanerysms are present due to weakened capillary walls from pericyte (small cells that can differentiate into fibroblasts or smooth muscle cells and are potential blood flow regulators) loss and endothelial cell damage. During this stage, “dot-blot” hemorrhages (ruptures in the capillary walls of the deep retinal layers), may also be present. “Hard Exudates” may also form, which are made up of lipids leaked from retinal capillaries. In Moderate NPDR, there are microaneurysms as well as dilatation of venules (venous beading) and “cotton wool spots” (slowing or halting of movement of materials in nerve fibers) due to poor perfusion. Macular edema may also occur at this stage due to leaking aneurysms. In severe NPDR, there is noticeable capillary loss, retinal ischemia, and greater than 20 intraretinal hemorrhages in each of the four quadrants, venous beading in at least two quadrants, or prominent intraretinal microvascular abnormalities in at least one quadrant, but no signs of proliferative disease. Finally, in proliferative diabetic retinopathy (PDR), there is neovascularization (forming of new but weak blood vessels) and/or

vitreous/preretinal hemorrhages[41]. During this stage, severe macular edema can occur, and if it is located near the fovea centralis (the center of the macula region of the retina which is responsible for sharp central vision), resulting vision loss. The new vessels formed in PDR are prone to spontaneous bleeding. They may also undergo fibrosis and contraction resulting in detachment of the retina and therefore potential vision loss. Secondary, or Neovascular Glaucoma, may also occur due to neovascularization which is accompanied by fibrous tissue that blocks drainage of fluid causing its accumulation within the eye and an increase in pressure [38].

A recent study of a multi-ethnic cohort in the U.S. shows the prevalence rate of retinopathy among adults 45-85 with diabetes to be 33.2% and that prevalence is higher among blacks (36.7%) and Hispanics (37.4%) than in whites (24.8%) and Chinese (25.7%). Predictors of retinopathy were longer diabetes duration, higher fasting glucose level as well as a greater waist-hip ratio[42]. In Type 1 diabetes specifically, the crude prevalence of diabetic retinopathy was estimated to be between 75-82%. 1 in 300 persons 18 or older have retinopathy due to T1D and 1 in 600 has advanced retinopathy that threatens their vision [43]. In the Pittsburgh EDC study, prevalence of retinopathy (background or worse) in Type 1 participants age 18-29 years ranged from 57% in those with diabetes duration of 5-9 years to 100% in those with duration of 20-29 years. Prevalence rates of retinopathy increased with increasing duration and with age as prevalence rates in those 30 years or older ranged from 93 to 100%[24]. Independent risk factors for retinopathy in the EURODIAB study were age, duration of diabetes, HbA1c, as was previously stated, as well as weight, current smoking, severe ketoacidosis, macroalbuminuria, background and proliferative retinopathy [29].

2.3.3 Nephropathy

In the United States, diabetic nephropathy is the most common cause of kidney failure, accounting for approximately 44% of new cases in 2002 [44, 45]. In the early stages of diabetic nephropathy, thickening of the glomerulus (a cluster of blood vessels in the kidney responsible for filtering blood and forming urine) occurs. When this occurs, the kidney allows more albumin (protein) into the urine resulting in what is called microalbuminuria when the amounts are small and later in macroalbuminuria as nephropathy progresses. Risk factors for nephropathy include poor glycemic control, hypertension and lipid levels [27, 46-50].

Approximately one third of those with Type 1 develop microalbuminuria and about 15-25% will develop proteinuria within the first 20 years of diabetes duration[51, 52]. Diabetic nephropathy is a major predictor of premature death among Type 1 patients[51, 53] . In The Pittsburgh EDC Study, the prevalence rate of microalbuminuria in those ages 18-29 with diabetes duration of 5-9 years was 13%. In this same group, prevalence of macroalbuminuria was about 2%. By 30 year duration, prevalence of overt nephropathy (AER>200μg/min or renal failure) was approximately 40%. Rates of both micro- and macroalbuminuria increased with increasing diabetes duration until macroalbuminuria surpassed micro at diabetes duration of 25-29 years (microalbuminuria ~20%, macroalbuminuria ~18% in females and ~50% in males). In the older age group of ≥ 30 years, prevalence rates of macroalbuminuria were significantly higher than those of the younger age group with similar diabetes duration [24].
Microvascular complications are risk factors for cardiovascular disease in T1D, specifically microalbuminuria and diabetic retinopathy[54-57].

2.3.4 Cardiovascular Complications

Cardiovascular disease (CVD) occurs with greater frequency in those with diabetes mellitus, a finding particularly striking in women [58] whom traditionally are more protected from CVD than men. Atherosclerosis is responsible for the development of many cardiovascular complications. Atherosclerosis is a slow, progressive disease that starts in childhood. It is the development of plaques in the walls of arteries which can result in the overall narrowing of the arteries or, if the plaque breaks away from the vessel wall, a clot, both of which can restrict blood flow to tissues supplied by the specific vessel affected. Plaque is composed of fat, cholesterol, calcium, and other substances found in the blood. Two major types of plaques exist, hard/stable plaques and soft/unstable plaques. Hard plaque causes thickening and hardening of vessels while soft plaque is more likely to cause a blood clot.

Development of plaques is associated with lipid levels and lipoprotein size. Lipid profiles in those with well-controlled T1D without nephropathy or cardiovascular disease, are similar or better compared to levels in those without diabetes [59]. However, poor glycemic control[59], central obesity, and cigarette smoking[60] are associated with important lipid abnormalities. Presence of higher albuminuria levels in T1D have been found significantly associated with higher total and LDL cholesterol, triglycerides, and Apo B levels, and lower HDL cholesterol, HDL/LDL cholesterol ratio and Apo A/Apo B ratio [46]. Elevated levels of low density lipoprotein (LDL) cholesterol, total triglycerides, very low density lipoprotein (VLDL), triglycerides and lower level of high density lipoprotein (HDL) cholesterol are associated with coronary artery disease in both men and women T1D [61]. The cardiovascular complications of T1D include coronary artery disease (CAD), peripheral vascular disease (PVD), cerebrovascular disease, all of which are affected by atherosclerosis.

2.3.4.1 Coronary Artery Disease

Heart disease is the leading cause of diabetes-related deaths [45]. Coronary artery disease (CAD) is increased tenfold or greater in those with T1D [62-64]. CAD occurs when atherosclerosis results blockages of the coronary arteries . As plaques increase in size, coronary arteries narrow and less blood can flow through them. Reduced blood flow means reduced perfusion of the heart with oxygen-rich blood. Lack of oxygen to the muscle can result in angina (chest pain or discomfort), ischemic ECG changes and/or myocardial infarction (when a blood clot forms at the site of plaque in a coronary artery and blood supply is acutely cut off or greatly reduced to part of the heart muscle). If the muscle cells of the heart do not receive oxygen, permanent damage can occur. Over time, CAD can weaken the heart muscle and contribute to heart failure when the heart cannot pump blood effectively to the rest of the body, and arrhythmias (changes in the normal beating rhythm of the heart).

Incidence density of hard CAD (CAD death, history of MI confirmed by ECG Q-waves or hospital records or angiographic stent) in participants of the Pittsburgh EDC study were 0.17 per 100 patient years for those with diabetes duration less than 20 years and 0.98 per 100 patient years with duration 20-30 years. The overall incidence density was 0.36 per 100 patient years after 12 years of follow-up. At 20, 25, 30 years duration cumulative incidence rates were 3.5%, 8%, 15%, respectively[35].

The DCCT found that intensive insulin therapy reduces risk of incident cardiovascular disease compared to conventional therapy. Results in epidemiologic studies examining the relationship between glycemic control (as determined by glycosylated hemoglobin) and CAD in T1D have varied. Lehto et al showed a significant independent association between HbA1 and CAD events in men (not women) in their cohort of older-onset T1D participants without renal

disease after controlling for traditional risk factors[65]. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) showed a relationship between HbA1 level and heart disease mortality but no significant association with myocardial infarction or angina specifically[66]. In the EURODIAB study, HbA1 was not significantly associated with CAD when adjusted for traditional CV risk factors beyond age and diabetes duration[67]. Selvin et al reported in their meta-analysis that HbA1c was not significantly associated with risk of CAD[68]. However, in another meta-analysis improved glycemic control did significantly reduce incident CAD in T1D[69]. In the Pittsburgh EDC Study, there was no significant relationship between baseline HbA1c and incident CAD at the 10-year [70] or at the 16-year follow-up however, in the 16-year follow-up, a positive change in HbA1c from baseline to measurement prior to CAD event was significantly associated with CAD risk even after adjustment for traditional risk factors [71].

Univariate risk factors for CAD at the most recent (16 year) Pittsburgh EDC follow-up were baseline age, diabetes duration, systolic and diastolic blood pressure (and the presence of hypertension), serum creatinine, total and LDL cholesterol levels, triglyceride level, albumin excretion rate, presence of nephropathy and smoking status were all positively, significantly associated with incident CAD while HDL cholesterol was negatively associated[71]. In multivariate analyses, only lower HDL-cholesterol level, higher non-HDL level, longer diabetes duration and positive change in HbA1c and AER, and negative change in BMI between baseline and follow-up were significantly predictive of CAD. A report of the 10-year follow up from the EDC study also showed that insulin resistance (as measured by estimated glucose disposal rate (eGDR)) is associated with incident hard CAD (history of MI, fatal CAD, and coronary revascularization or coronary artery occlusion ≥50% by angiography) events. There is little sex difference in morbidity of CAD in T1D [72], however CAD risk factors do differ by sex[73].

For men in EDC, diabetes duration, HDL cholesterol, fibrinogen, hypertension, and smoking were significantly associated with incidence of CAD. When nephropathy status was available to the model it replaced hypertension, fibrinogen, and smoking. For women, duration, hypertension, waist-hip ratio, physical activity, and depressive symptomatology were all significant predictors of CAD multivariately[73].

A major predictor of CAD in T1D is nephropathy[74-78]. The relative mortality from CVD is increased 40-fold in T1D patients with nephropathy compared with the general population[79]. The association between renal damage and cardiovascular disease may be due to its negative effect on prothrombotic factors. The presence of microalbuminuria (small amounts of the protein albumin in urine) is associated with increased fibrinogen and coagulation factor VIIc in T1D. These factors are more elevated in those with macroalbuminuria compared to those micro- and normoalbuminuria [80]. Levels of apo(a) in T1D patients with either microalbuminuria or macroalbuminuria but no CAD are comparable to CAD patients without diabetes. But in T1D patients without microalbuminuria apo(a) levels are normal[81]. Potentially atherogenic lipoprotein profiles are also associated with the presence of albuminuria in T1D. Jensen et al found that plasma total cholesterol, very low density lipoprotein (VLDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride and fibrinogen levels increase with increasing urinary albumin excretion but that HDL cholesterol levels were unaffected by albuminuria [82]. In the DCCT/EDIC study, medium, and small VLDL were associated with albumin excretion rate (AER) in both men and women. Large VLDL and intermediate density lipoprotein (IDL) was associated with AER in men only. This study also found that LDL particle concentration and ApoB were positively associated with AER, that there was a borderline inverse association between LDL diameter and AER in men and that small HDL was positively

associated with AER[83]. In the Pittsburgh EDC cohort, lipid mass and particle concentrations VLDL subclasses, small LDL, medium LDL, and medium HDL were significantly higher in CAD cases compared to non-cases, and large HDL was decreased[78]. Another factor that may link albuminuria and CAD is its association with left ventricular (LV) dysfunction. Guglielmi et al found that increased LV mass, higher wall thickness to radius ratio and impaired LV relaxation are significantly higher in microalbuminuric T1D patients compared to those with normoalbuminuria and to controls [84].

2.3.4.2 Peripheral Vascular Disease

Peripheral Vascular Disease (PVD) is a disease of blood vessels, often leading to narrowing of blood vessels that are outside the heart and brain. Lower-Extremity Arterial Disease (LEAD) is a specific type of PVD in which the lining of blood vessels in the legs and feet become damaged, leading to buildup of cholesterol and other lipids, causing the arterial wall inner lining to thicken. As the atherosclerosis worsens, the arteries become narrowed or blocked, causing blood flow to decrease. Reduce blood flow to tissues causes can lead to foot ulcers (possibly resulting in amputation) as well as specific symptoms of claudication: discomfort, cramps, or pain in the hips, thighs or calves with walking. The ankle-brachial index (ABI) is used to measure to determine if PAD/LEAD is present. ABI is measured by taking the brachial and ankle systolic pressures. The higher systolic pressure of the anterior tibial or posterior tibial measurement for each foot is then divided by the highest brachial systolic pressure to obtain an ankle brachial pressure ratio. An ABI less than 1.0 is considered abnormal and symptoms of claudication typically occur when ABI is less than 0.9. In persons with diabetes, calcification of the arteries can occur which hardens the arteries and in turn elevate ABI[85]. Determining the

presence of LEAD in T1D often takes into account ABI, history of claudication and lower extremity amputation.

In The Pittsburgh EDC study population traditional cardiovascular risk factors that multivariately predicted incident LEAD were diabetes duration, HbA1, LDL cholesterol and smoking status[86]. Further evaluation showed that increased albumin excretion rate (AER), the presence of hypertension, and increased heart rate were significantly associated with 10-year incidence of LEAD in both males and females. Diabetes duration, HbA1 and AER were predictors in males only, while estimated glucose disposal rate (eGDR) and diabetes duration were predictive in females[87]. PVD is a strong predictor of cardiovascular events [88, 89] and is twice as common among those with diabetes[90, 91].

2.3.4.3 Cerebrovascular Disease

Cerebrovascular disease is a disease of the blood vessels, especially the arteries that supply the brain. It is usually caused by atherosclerosis and can lead to a stroke. Stroke is an interruption of blood supply to part of the brain. This interruption is usually caused by blockage of the arteries or blood vessels that lead to the brain, termed Ischemic Stroke. Ischemic stroke is the most common type of stroke and is a result of atherosclerosis. The build-up of plaque over time causes abnormal blood flow and possibly clots. Clots are referred to as cerebral thrombus if they are stationary in the brain or cerebral embolism if they break loose from elsewhere and move through the circulatory system to the brain. Hemorrhagic stroke is a second type of stroke which is caused by the bursting of weak blood vessels in the brain[92]. The risk of stroke in patients with T1D has been assessed in epidemiological studies. In a multinational study, increased risk of stroke mortality was observed among individuals with type 1 and type 2 diabetes, but there was considerable variation among countries[93, 94]. Risk of total stroke, but

not hemorrhagic stroke mortality, in a U.K. T1D cohort was increased compared to those among the general population [95]. Total stroke mortality was also found to be 4.8-fold higher in women with T1D than in nondiabetic women [96]. In the Nurses' Health Study, T1D was associated with a 6.3-fold higher risk of ischemic stroke and a nearly 4-fold higher risk of hemorrhagic stroke than women without diabetes, even after controlling for age, BMI, and other cardiovascular risk factors[97].

The WHO multinational study of vascular disease in diabetes showed that independent risk factors for stroke in T1D are systolic blood pressure and proteinuria in both men and women. Additionally, probable ECG changes significantly increased risk in T1D men [93].

2.4 VASCULAR AGING AND ARTERIAL STIFFNESS

2.4.1 The Vasculature

The arterial tree of the vascular system functions to deliver oxygenated blood from the left ventricle of the heart through the aorta to large arteries, then to the arterioles and finally the capillaries of tissues and organs. In doing so, it also regulates the flow of blood so that there is continuous flow during systole (the contraction of the heart's ventricles) and diastole (the relaxation period) as well as the reflection of blood back towards the heart for reoxygenation and coronary perfusion. The aorta, large arteries, and the arterioles are made up three layers; 1) a connective tissue outer layer (adventitia)**;** 2) a middle layer of smooth muscle (media); and 3) an inner layer of endothelial cells and some subendothelial connective tissue (intima). The aorta and the large arteries contain a large amount of elastic tissue, so after stretched during systole by the ejection of blood from the left ventricle, the vessels are able to recoil during diastole. This arterial recoiling is important as it helps to propagate the blood forward throughout the arterial tree and also allows for coronary perfusion during diastole. Arterioles are different from the aorta and large arteries because they contain less elastic tissue but more smooth muscle thereby creating resistance and slowing down blood flow[98]. The peripheral resistance created by the arteries and arterioles cause a reflected wave back to the heart. The pulse-wave velocity, the speed at which the forward pressure wave created by the contraction of the left ventricle is transmitted from the aorta to the vascular tree, as well as the distance to peripheral reflecting sites, determine the timing of the wave reflection. In healthy, young individuals the reflection

wave typically reaches the heart during diastole which aides in coronary perfusion. Also, this wave reflection limits the pressure to the periphery where damage to the microcirculatory beds might occur [99]. Increases in arterial stiffness, i.e. increases in the rigidity of arterial walls [100], increases the speed at which forward and reflected waves travel [101]. Therefore, reflected waves occur earlier, eventually occurring during systole rather than diastole, augmenting systolic blood pressure while lowering diastolic blood pressure creating a state of isolated systolic hypertension (ISH), thereby increasing cardiac afterload and decreasing coronary perfusion. Meanwhile, forward waves have increased pulsatile velocity flowing into microcirculation potentially causing damage.

2.4.2 Arterial Stiffness Measures and Indices

Many indices can be used to quantify arterial stiffness; terms and definitions of which are provided in Table 1 (adapted from O'Rourke, et al. [102] and Mackenzie et al. [100]). Multiple factors can influence these indices, such as assumed values of cardiac output that are poorly validated; they may relate proximal diameter change to pressure change at distant sites; or they may be influenced by heart rate or cardiac contractility [100].

Table 1. Definitions, formulae, units for measures of arterial stiffness, compliance or distensibility

TERM	DEFINITION	FORMUAE	UNITS
Arterial Distensibility	Relative diameter change for a pressure increment	$\Delta D/\Delta P x D$	$mmHg^{-1}$
Arterial Compliance	Absolute diameter change for a given pressure increase at fixed vessel length	$\Delta D/\Delta P$	cm/mmHg
Elastic Modulus	Pressure change required for 100% stretch from resting diameter (fixed vessel length)	$\Delta P*D/\Delta D$	mmHg
Young's Modulus	Elastic Modulus per unit area	$\Delta P*D/\Delta D x h$	mmHg/cm
Stiffness Index	Ratio of Ln(Systolic pressure / diastolic pressure) : relative change in diameter	B=Ln(Ps/Pd)/[Ds-Dd/Dd)	No Units
Augmentation Index	Difference between the 2^{nd} and 1^{st} systolic peaks as a percentage of pulse pressure	[(P2-P1)/(PP)] x 100	%

Pulse pressure, the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP), is a well known surrogate marker for systemic arterial stiffness[103]. Although SBP and DBP tend to increase with age[104], after approximately the 5th decade of life ISH tends to occurs as does a decline in diastolic blood pressure resulting in greater pulse pressure (PP). PP is a simple measure of arterial stiffness as it can be measured by the use of a standard sphygmomanometer. However, PP is not the most accurate marker of arterial stiffness as it does not necessarily accurately reflect central pulse pressure[105]. Pulse pressure is a better predictor of coronary heart disease than SBP or DBP alone in hypertensive patients over 50 [106, 107]. Central pulse pressure has been shown to be an independent predictor of all cause mortality in ESRD [108], and of cardiovascular events in those with hypertension[2].

The speed at which the forward pressure wave is transmitted from the aorta to the remainder of the arteries and arterioles is known as pulse-wave velocity. The measurement of PWV is determined by measuring the time it takes for the arterial waveform to pass two reading sites (typically carotid and the femoral arteries) a measured distance apart. This measurement gives information about the distensibility of the vessel being studied rather than information about systemic arterial stiffness [100]. There are invasive and non-invasive techniques for measuring PWV. Non-invasive carotid-femoral PWV is considered the "gold-standard" measurement of arterial stiffness [101]. PWV is considered the gold-standard because it is based on direct measurements of parameters linked to regional arterial stiffness and carotid-femoral PWV specifically, is considered clinically relevant as this measures pressure and stiffness within the aorta and large arteries which are closest to and most influential on the heart[101]. Also, many of the studies that have examined arterial stiffness measures in relation with adverse outcomes have utilized PWV, therefore the risk associated with higher PWV is more understood

than for other measures of arterial stiffness [109]. However, problems that arise with this PWV measurement include use of superficial arteries as central arteries are inaccessible, estimating actual distance between recording sites, reduced accuracy if sites are close in proximity [100]. Also, in the presence of metabolic syndrome, diabetes, peripheral artery disease and obesity, the femoral pressure waveform can be difficult to record accurately[110].

As previously mentioned, numerous longitudinal studies have reported the independent predictive value of arterial stiffness for mortality and CVD. For example, aortic Pulse Wave Velocity (aPWV) has been show to predict all cause mortality in the elderly[111, 112], and in those with impaired glucose tolerance (IGT)[113], or cardiovascular mortality in those with end-stage renal disease (ESRD)[3], hypertension[4], and in the general population[5, 6].

Ultrasound technology is also used to measure arterial stiffness by measuring distensibility and compliance (the inverse of stiffness). This technique is limited to larger and accessible arteries such as the brachial, femoral, carotid arteries and the abdominal aorta and can be operator-dependent[100]. Simultaneous measurement of blood pressure during ultrasound of arteries is necessary. The use of ultrasound to measure arterial stiffness can be expensive and inconvenient due to the size and cost of equipment. Studies that have utilized B-Mode Ultrasonography to measure distensibility (the inverse of stiffness), have shown that reduced distensibility (i.e. increased stiffness) is an independent predictor of cardiovascular outcomes. For instance, Tsivgoulis et al. showed in a case-control study, that reduced common carotid artery (CCA) distensibility was independently associated with ischemic stroke even after adjusting for blood pressure values, diastolic common carotid arterial diameter and height [114]. Stork et al. showed that the calculated Young's Elastic Modulus from B-Mode measures of distensibility are predictive of cardiovascular mortality in elderly men[115]. Liao et al. also used

B-mode ultrasonography using its measurements to calculated Peterson's Elastic Modulus, Young's Elastic Modulus, and the beta stiffness index finding that after adjustment for age, gender, ethnicity, smoking status, HR, obesity and education, a lower modulus or higher beta stiffness index was significantly associated with risk for incident hypertension over a 6-year follow-up. These findings were independent of risk factors for hypertension and baseline blood pressure[116].

There are multiple non-invasive devices available to measure and analyzed the arterial waveform. Applanation tonometry is used to record pressure at either the radial or carotid artery. A transfer function is then used to derive central aortic waveforms which can be used to calculate central blood pressure, the amount of central systolic pressure due to pulse wave reflection (augmentation pressure (AP)) and augmentation index (AIx), the AP as a percentage of the pulse pressure (AIx=(AP/PP) X100) . Carotid AIx has been shown to independently predict all cause cardiovascular mortality in ESRD [117]. Pulse wave analysis is influenced by vasoactive drugs[118], AIx and AP increase with mean arterial pressure (MAP)[119], and are inversely related to body height[120] and heart rate[121]. Arterial stiffness increases with increasing age. A significant relationship between aging and arterial stiffness as measured by a variety of techniques, has been found in numerous cross-sectional studies and a recent study showed that the relationship to be curvilinear in nature[7]. Physiological factors attributed to the aging of the vasculature include progressive thickening of the intima-media[122]. Between 20 and 90 years of age, carotid intima medial thickness (CIMT) increases threefold[123]. This thickening is largely due to intimal hyperplasia[124]. However, the elasticity of the media is also reduced as elastin lamellae thin and become separated[125]. In the larger elastic arteries there is increase in both collagen content and covalent cross-linking of collagen reducing compliance. Arterial

stiffness is also shown to increase due to atherosclerosis development, the slow buildup of plaques composed of fat, cholesterol, and especially calcium, on the inside of walls of the arteries[126]. Lack of physical activity[127], obesity[128], smoking[129], hypertension[130], hypercholesterolemia[131], impaired glucose tolerance[132], Type 2 diabetes[133], and T1D[10, 134, 135] are associated with increased arterial stiffness as well.

Alterations in the function of endothelial cells lining the vasculature, termed "endothelial dysfunction", also occur with increasing age. There is an increase in endothelin, a potent vaso-constrictor and procoagulant[136]. Most important however, is the loss of active/bio-available nitric oxide (NO), which is essential in maintaining vascular tone and reactivity[137] as well as vasodilatation. Oxidative stress, the build-up of oxygen free radicals, occurs with age and in the presence of cardiovascular risk factors such as hyperlipidemia, high blood pressure, smoking and diabetes. These oxygen free radicals directly inactivate NO [138] thereby reducing NO bioavailability causing endothelial dysfunction[139, 140].

Hyperglycemia induces endothelial dysfunction and reduces endothelium-dependent relaxation[141, 142] due to increased production of reactive oxygen species[143-145]. Mechanisms potentially involved in development of endothelial dysfunction due to hyperglycemia include increased formation of glucose-derived advanced glycation end-products[146]; glucose-induced activation of protein kinase C isoforms[147]; and increased glucose into the aldose reductase pathway increasing production of sorbitol[148]. Endothelial dysfunction and arterial stiffness are both associated with diabetes however, studies examining the association between measures of endothelial dysfunction and measures of arterial stiffness show conflicting results [8, 149-152]. Berry et al. measured systemic arterial compliance and endothelium-dependent flow-mediated dilation (FMD) of the right brachial artery in a group of

T1D free of overt micro- and macrovascular complications and a group of control subjects. Although arterial compliance was 29% lower in the T1D group compared to controls, compliance was not significantly related to FMD in the T1D group[8]. In a group of participants, the majority with hypertension, arterial stiffness as measured by pulse wave analysis and FMD were not significantly correlated[152]. Nigam et al. did find a significant association between arterial stiffness and percent flow-mediated dilation in patients with risk factors for CAD and those with established CAD [149]. Nakamura et al. also found a significant association between FMD and both arterial compliance and distensibility in CHF patients[150]. Jadhay et al. showed that PWV was significantly associated with FMD of the brachial artery in a mixed group of participants with hypertension and/or type 2 diabetes.

2.4.3 Arterial Stiffness and Type 1 Diabetes

Isolated increased systolic blood pressure and decreased diastolic blood pressure with the resulting increase in pulse pressure appear to occur earlier in Type 1 diabetes indicating possible accelerated arterial aging [153]. Through measurement of pulse wave velocity, Berry et al showed that arterial compliance is reduced by approximately 29% in those with T1D compared to controls. The Type 1 participants of the study had no evidence of overt micro- or macrovascular disease. This study also measured flow mediated dilation (FMD), a measure of endothelial dysfunction, and examined whether there was a significant relationship between PWV and FMD; there was not[8]. Brooks et al utilized pulse wave analysis via radial applanation tonometry to measure arterial stiffness indices in type 1 diabetes study participants and controls finding that T1D increased aortic AIx as well as reduced estimated myocardial perfusion (as determined using the subendocardial viability ratio (SEVR))[9]. Haller et al used

the same technique demonstrating that arterial stiffness indices were significantly higher in children with T1D compared to controls[10]. However, Ryden et al found arterial stiffness measured non-invasively by sonography of the abdominal aorta and common carotid arteries to be significantly higher in women, but not in men, with T1D compared to controls[154]. These findings held true at a 7-year follow-up[155]. Age is significantly associated with pulse pressure in T1D as it is in the general population; however this association is stronger in the presence of micro- or macroalbuminuria and/or retinopathy[156]. Acute hyperglycemia is also associated with increased arterial stiffness in T1D[157] and insulin resistance in T1D seems to affect typical decreases in large artery stiffness associated with normal insulin action [158].

Some studies have examined the potential link between endothelial dysfunction and arterial stiffness in T1D. A cross-sectional examination within The EURODIAB study found that advanced glycation end products (AGE's) were significantly associated with pulse pressure in T1D [135] suggesting a potential link between oxidative stress and endothelial dysfunction with arterial stiffness. However, Haller et al showed that superoxide dismutase activity and NO are not correlated with arterial stiffness

2.4.4 Pulse Wave Analysis via Applanation Tonometry

The SphymoCor Pulse Wave Analysis device (Atcor, Sydney, Australia) uses applanation tonometry to record peripheral arterial waveforms. These waveforms are used to derive central aortic waveforms by applying a generalized transfer function. Studies have shown that there exists good consistency between measured and derived waveforms as well as the values calculated from these waveforms[159, 160]. Pauca et al. compared SphygmoCor derived waveforms to directly measured aortic waveforms, finding a high correlation between the two

[161]. In the study, ascending aortic and radial artery pressure waves were recorded simultaneously in 62 anesthetized patients prior to the initiation of cardiopulmonary bypass. A generalized transfer function was used to generate ascending aortic pressure waveforms from the radial pulse. Estimated aortic pulse waveforms were compared with simultaneously recorded aortic waves. The two different waveforms were then compared for systolic, diastolic, mean, and pulse pressures. When radial estimated and directly measured aortic pressure waveforms were compared, there was good correspondence for systolic (mean 0.0±4.4 mm Hg), pulse (0.7±4.2 mm Hg), mean (0.5±2.0 mm Hg) and diastolic (0.6±1.7 mm Hg) pressures.

Numerous variables associated with vascular stiffness can be obtained using the SphgymoCor device, which are detailed in Table 2. Studies show a high level of repeatability and reproducibility of SphygmoCor pulse wave analysis measurements. Savage et al. examined intra-observer, inter-observer and long-term reproducibility of pulse-wave analysis utilizing the SphygmoCor device in patients with chronic renal failure and in health controls. This study found that measurements of central aortic mean blood pressure (MBP), indices of arterial stiffness and the subendocardial viability ratio (SEVR) showed excellent reproducibility in all the studies[162]. Wilkinson et al also found good inter-observer reproducibility of AIx measures using the SphygmoCor device in a mixed group of subjects with and without a wide range of cardiovascular risk factors [163]. Papaioannou et al. demonstrated good intra- and inter-observer reproducibility of pulse-wave analysis measures of AIx and reflection time intervals in those with low blood pressures [164]. Crilly et al. completed at repeatability study using inexperienced operators also finding excellent inter- and intra-observer reproducibility over time and across a range of AIx measures[126].

Table 2. Measures obtained by the SphygmoCor Vx Pulse Wave Analysis Device

PARAMETER	DESCRIPTION	FORMULAE	UNITS
Augmentation Pressure (AP)	A measure of contribution the reflected wave makes to the systolic aortic pressure; Pressure difference between the 1st peak and the 2nd Peak	**AP = P2-P1**	mmHg
Augmentation Index (AIx)	AP as a proportion of central pulse pressure (PP).	**AIx = (AP/PP)x100**	%
Subendocardial Viability Ratio (SEVR)	Ratio of diastolic area/min (Tension Time Index (TTI)) and Systolic area/min (Diastolic Time Index (DTI))	**SEVR = (DTI/TTI)x 100**	%
Ejection Duration (ED)	The period of time from the start of the cardiac cycle (T0, aortic valve open) to the end of systole (incisura).	**ED =T(incisura)-T0**	msec
Aortic Systolic Blood Pressure (aSBP)	Maximum pressure of the central waveform		mmHg
Aortic Diastolic Blood Pressure (aDBP)	Minimum pressure of the central waveform		mmHg
Aortic Pulse Pressure (aPP)	Difference between aortic systolic and diastolic pressures	**aPP = aSBP-aDBP**	mmHg
HR	The average heart rate over the captured 10-second data capture period		bpm
End Systolic Pressure (ESP)	Pressure at end of systole		mmHg

3.0 ARTICLE 1: AUTONOMIC NEUROPATHY IS ASSOCIATED WITH INCREASED ARTERIAL STIFFNESS INDICES AND DECREASED ESTIMATED MYOCARDIAL PERFUSION IN TYPE 1 DIABETES

To be submitted for publication

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3.1 ABSTRACT

BACKGROUND: Cardiovascular disease occurs earlier age and with greater frequency in Type 1 diabetes (T1D). Arterial stiffness indices (augmentation index (AIx) and augmentation pressure (AP)), are increased in T1D and are associated with CAD risk. Subendocardial viability ratio (SEVR), an estimate of myocardial perfusion and an indicator of potential for myocardial ischemia, is significantly reduced in T1D. Autonomic neuropathy (AN), a known T1D complication, affects blood pressure and heart rate regulation and is an independent risk factor for cardiovascular morbidity and mortality. We therefore evaluated the predictive value of AN for AIx, AP and SEVR in T1D.

METHODS: Baseline autonomic nerve function was measured in participants in The Pittsburgh EDC Study of childhood onset T1D by heart rate variability during deep breathing. The ratio of R-R interval length during expiration to inspiration (E/I ratio) was calculated. Other cardiovascular and diabetes factors were also assessed. Pulse wave analysis (PWA) was performed using SphgymoCor Px on 144 participants at 18 year examination. Multivariate regression was performed for each PWA measure with baseline factors and adjustment for concurrent potential confounders.

RESULTS: Lower baseline E/I ratio and HDL-cholesterol, and smoking history were associated with higher AIx and AP, and lower SEVR at follow-up (18 years later). Higher baseline HbA1 was also associated with higher AP and lower SEVR.

CONCLUSIONS: AN is associated with increased arterial stiffness indices and decreased estimated myocardial perfusion in T1D some 18 years later. This association persists after adjustment for potential confounders as well as baseline HbA1, HDL-c and smoking history, which were also associated with these PWA measures.

3.2 INTRODUCTION

Cardiovascular disease (CVD) occurs with greater frequency and at an earlier age in those with diabetes mellitus, a finding particularly striking in women [58]. These observations are especially true for those with type 1 diabetes (T1D), in whom coronary artery disease (CAD) is increased tenfold or greater [62, 63]. Much of what is understood regarding risk for CAD in T1D revolves around presence and severity of atherosclerosis and its risk factors (dyslipidemia/hyperlipidemia). Blood flow dynamics and arteriosclerosis, or arterial stiffening, indices of which are measured in a variety of ways [165], are also important risk factors for cardiovascular events and mortality [4, 166-168]. Interestingly, in case-control studies, arterial stiffness indices are shown to be increased in T1D [9, 10, 169]. Indices of arterial stiffness can be measured noninvasively with pulse waveform analysis (PWA)[163, 170, 171],[172] using applanation tonometry (external application of a micromanometer-tipped probe over a peripheral artery) [159, 173]. Via PWA, a variety of indices and hemodynamic parameters can be derived including augmentation pressure (AP) and augmentation index (AIx), both of which provide information about the effects of early wave reflection on central blood pressure, as well as subendocardial viability ratio (SEVR), the ratio of the area under the time-pressure curve during diastole (an estimate of myocardial perfusion) to the area under the curve during systole (an estimate of cardiac workload) and an indicator of potential for myocardial ischemia [174].

Currently, few data are available concerning risk factors for increased arterial stiffness in T1D. Autonomic neuropathy (AN) is a T1D complication that predicts cardiovascular events and mortality [175]. The autonomic nervous system is responsible for regulating heart rate and vascular tone and therefore may contribute to increased arterial stiffness in T1D. Therefore the aim of the present study is to examine the association between autonomic nerve function and

indices of arterial stiffness, AIx and AP, and with SEVR, an estimate of myocardial perfusion, in a T1D population.

3.3 METHODS

Participants in the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study, an 18-year prospective investigation of patients with childhood-onset (age <17 years) T1D were selected for study. Individuals were either diagnosed or seen within 1 year of diagnosis at Children's Hospital of Pittsburgh between 1950 and 1980 and were on insulin therapy at initial discharge [27, 176]. Initial evaluation in the EDC study occurred between 1986 and 1988 then followed by biennially. Full examination at 18 years included PWA. The EDC Study population has been shown to be epidemiologically representative of the T1D population of Allegheny County, Pennsylvania [177]. The study protocol was approved by The University of Pittsburgh Institutional Review Board.

Physical activity was assessed using a survey previously described [178]. A summary estimate of energy expenditure was derived and expressed as kcal/min. Self-reported alcohol consumption (average drinks/week), current and ever smoker status (at least 100 cigarettes during lifetime) and medication (coded according to ATC/DDD codes) use were obtained. Medications of interest in these analyses were those potentially effecting pulse wave reflection indices (i.e. angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARBs), calcium channel blockers (CCBs), beta-blockers (BB) and nitrates [179].

Systolic and diastolic blood pressures (SBP and DBP) were measured using a random zero sphygmomanometer, according to the Hypertension Detection and Follow-Up Program

protocol, after a 5-minute rest [180]. Hypertension (HTN) was defined as blood pressure of ≥130/80 mmHg or the use of antihypertensive medication for the purpose of lowering blood pressure. Height was measured in cm and weight in kg. BMI was calculated from height and weight and expressed as kg/m^2. Waist and hip circumferences were measured 2 times, and a third if the first two were not within 0.5 cm of one another. The means of waist and of hip measurements were used to calculate waist-to-hip ratio (WHR). Total cholesterol (TC) levels were measured enzymatically [181, 182]. High-density lipoprotein cholesterol (HDL-c) levels were determined by means of a precipitation technique (heparin and manganese chloride) with modification [183] of the Lipid Research Clinics method [184]. Non-HDL cholesterol was calculated by subtracting HDL-c cholesterol level from TC level.

Complete blood counts (CBC) were determined using the Coulter S-Plus IV. Urinary creatinine concentrations were measured using an Ekachem 400 analyzer (Eastman Kodak Co, Rochester, NY). For the first 18 months, blood samples were analyzed for hemoglobin A_1 (HbA_1; microcolumn cation-exchange; Isolab, Akron, OH). For the remainder of the baseline clinic visits, automated high-performance liquid chromatography (Diamat; Bio-Rad, Hercules, CA) was performed. Serum creatinine was assayed using an Ectachem 400 Analyzer (Eastman Kodak Co, Rochester, NY)[185] and urinary albumin was measured by immunonephelometry [186, 187]. Albumin excretion rates (AER) were calculated using urinary albumin levels from at least 2 validated timed sample collections. Heart rate response to deep breathing, expiration-inspiration ratio (E/I) was used to test for autonomic neuropathy (AN). An E/I <1.1 was considered abnormal [188].

Certain medications are known to affect measures of arterial stiffness including angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB), beta

blockers, calcium channel blockers and nitrates. In order to account for the potential confounding effect of use of these medications a "Pulse Wave Drug" (PWD) variable was created. PWD use was coded as 0 no use of these medications was reported and 1 if use of one or more of the medications were used.

Of those EDC study participants examined at baseline (n=658), 22.7% (n=150) had died by the 18-year follow-up (November 2004 – November 2006), 78 had moved out of area, 101 declined exam, 19 were lost to follow-up leaving 318 eligible for 18 year exam, 309 for which data was available for this analysis. Pulse waveform analysis (PWA) testing began part way through the 18-year examination period (January 2006), after which 189 subjects were seen and 144 (76%) had PWA. The PWA population thus 144 men and women in attendance at the 18-year follow-up clinic visit after January 1 2006. Aortic augmentation index (AIx), aortic augmentation pressure (AP) and subendocardial viability ratio (SEVR) were derived using waveforms measured at the radial artery using the SphygmoCor Vx version 7.01 (AtCor Medical, Sydney, Australia). In brief, a high-fidelity micromanometer with a frequency response of >2 kHz (Millar Instruments, Houston, TX) was placed on the right radial artery, and gentle pressure was applied until a consistent waveform was produced. After at least 20 sequential waveforms had been acquired, measurement was stopped. Central pressure values were estimated from radial measurements using the software's mathematical transfer function [159, 189]; the accuracy and reliability of which have been validated [163, 172]. The pressure wave created by left ventricular contraction propagates forward until meeting sites of resistance which reflect the wave backward. Stiffer artery walls result in earlier wave reflection [168, 171]. When the reflected wave returns during systole rather than diastole, systolic pressure is increased or "augmented". Augmentation pressure (AP) is a measure of how much the early reflected wave

contributes to central systolic pressure. Augmentation index (AIx) represents the level of augmentation measured and is expressed as a percentage of the pulse pressure (AIx = AP/PP). Heart rate is inversely associated with AIx and AP [121]. Subendocardial viability ratio (SEVR), the ratio of the diastolic area under the curve (AUC) of an arterial pulse wave to the systolic-AUC [190, 191] is a ratio of myocardial perfusion (as coronary artery perfusion takes place primarily during diastole) to myocardial contraction, and is a tonometric, non-invasive measure of myocardial perfusion relative to cardiac workload. The SphygmoCor device provides a quality index (QI) which represents reproducibility of the waveform. If PWA produced results with a QI<80 the measure was repeated. Measures with a QI $\geq$ 80 were included in this study.

Distributional characteristics and normality of all variables were assessed. Student's t-test and One-Way ANOVA were used to compare continuous normally distributed variables between groups while Mann-Whitney U and Kruskal-Wallis tests were used for variables not meeting the parametric assumptions (AP, TG, serum creatinine, AER, WBC). The χ^2 test was used to compare categorical variables between groups.

Pearson's and Spearman's correlations were used for bivariate correlations for normal and non-normal variables, respectively. Linear regression models were created for AIx, AP and SEVR, for which all categorical variables (medication use, smoking history) were coded as either 0 or 1, 1 representing the use/presence. Sex was also categorized as 0 (males) and 1 (female). All continuous variables were standardized to the population prior to multivarate analyses. Linear regression for augmentation pressure used natural logarithmically transformed AP (LnAP) as model residuals were not normally distributed. Forward regression was completed in a stepwise manner, for each PWA measure with baseline variables adjusting for concurrent potential confounders (even if not statistically significant). All statistical analyses were

conducted using SPSS 15.0 for Windows (SPSS, Chicago, IL). Due to the limited sample size factors with p-values <0.10 are reported.

3.4 RESULTS

Characteristics of the PWA study population (n=144, 46.6%) and the remaining EDC population examined at the 18 year follow-up (n=165, 53.4%) are listed in Table 3. The mean±SD age and diabetes duration for the PWA population at baseline were 25.9±7.38 and 17.6±6.70, respectively, which did not significantly differ from the remaining EDC population. The only baseline factor that differed significantly between PWA participants and non-participants was mean heart rate (76.0±12.3 vs. 73.1±11.1; p=.04). At follow-up, those with PWA measures still had significantly higher mean heart rate compared to non-participants as well as lower mean WHR and mean AER (data not shown). PWA participants also had a borderline significantly greater percentage of reported current smokers (15% vs. 8%, p=0.05) but not ever smokers (40.0% vs. 31.7%, p=.13). They also had greater mean±SD alcoholic drinks/week (2.6±6.1 vs. 1.81±5.22, p=0.09) than non-PWA EDC participants.

AIx and AP were highly correlated (r=0.897 p<.001). Both factors were also significantly correlated with height, HR and age (p<0.01). SEVR was not significantly correlated with AIx (r=-.058, p=.49), but was negatively correlated with AP (r=-.214; p=.01). SEVR was also significantly, negatively correlated with age (r=-.292, p<0.001), heart rate (r= -0.665, p<.001), and positively with height (r= 0.229, p<.01) in the PWA population. AIx and AP remained significantly correlated with height after adjustment for sex, but SEVR did not.

Unadjusted correlations between PWA measures and baseline variables are described in Table 4. Baseline E/I ratio, heart rate, energy expenditure in sports activities were all negatively associated with Aix and AP and positively associated with SEVR. Non-HDL-c was positively associated with AP, but not AIx and negatively with SEVR as was AER. BMI was related only to AIx, in a positive direction. AIx and AP were higher in females and SEVR lower compared to males (Table 4). This was also the case between those with a history of smoking compared to those without.

Ninety (62.5%) of the 144 PWA study participants reported PWD use. Most, 88.9% (n=80), were using an ACEI and/or ARB's. Of the 16 participants taking beta blockers, 10 (62.5%) were also taking ACEI/ARBs; 13 of the 18 reporting calcium channel blocker use and 4 of the 5 participants reporting nitrate use were also taking ACEI/ARB.

In multivariate analyses relating baseline PWA measures while adjusting for concurrent potential confounders (age, heart rate, height, sex, PWD use), lower E/I ratio and HDL-c and history of smoking were associated with increased follow-up AIx and Ln(AP) (Table 5). Higher baseline HbA1 was also associated with increased AP. The baseline factors associated with decreased SEVR were lower E/I ratio, higher HbA1 and having a smoking history. When models were adjusted for ACEI/ARB use instead of PWD use, results were similar. However, ACEI/ARB use was more significantly related to PWA measures than was the more inclusive PWD use variable (data not shown).

3.5 DISCUSSION

The main outcome of interest of this study is that baseline autonomic neuropathy, as measured by E/I ratio, is a potent predictor of arterial stiffness, as measured by both augmentation index and augmentation pressure, and of reduced estimated myocardial perfusion, as measured by SEVR, in childhood onset Type 1 diabetes, some 18 years later. Along with autonomic neuropathy, know cardiovascular risk factors such as reduced HDL-c and cigarette smoking were also predictive of increased stiffness indices AIx and AP. Poorer glycemic control (higher baseline HbA1) in this T1D population was also associated with higher AP and with reduced SEVR.

The current results indicate that autonomic neuropathy may exert a pathophysiological role in the development of arterial stiffening. E/I ratio was predictive, multivariately, of AIx and LnAP. Our finding that E/I and arterial stiffness indices are linked is consistent with the finding by Ahlgren et al. that there was a significant correlation between E/I ratio and aortic stiffness, measured as aortic distensibility using ultrasonography, in females with T1D [192]. In our sex adjusted analyses, E/I was related to all three PWA measures. In sex-stratified analyses (data not shown), E/I remained significantly associated with AP and SEVR in females but not in males. However, due to the limited sample size, stratified analyses are not conclusive. In T1D, diabetic autonomic neuropathy is associated with increased all-cause mortality [193] and specifically cardiovascular fatality as well as non-fatal cardiovascular events, especially in those with nephropathy [175]. Cardiovascular autonomic neuropathy (CAN) is shown to be associated with left ventricular hypertrophy and diastolic dysfunction in T1D [194]. As arterial stiffness indices are shown to contribute to left ventricular diastolic dysfunction [195], arterial stiffness may be a link between AN and cardiovascular disease. T1D patients without nephropathy, retinopathy or

neuropathy have preserved vascular function [196, 197] which suggests an intimate relationship between vascular dysfunction and these complications.

Lower HDL-c, a traditional cardiovascular risk factor, but not higher non-HDL-c, was independently predictive of AIx and LnAP. In a study of healthy subjects, Duprez et al. showed that low HDL-c was significantly correlated to AP and AIx in women, but not in men [198]. However, the study examined only univariate correlations. The results of the present study showed that baseline HDL-c was correlated with both AIx and LnAP in partial correlations and in multivariate, sex-adjusted models. In sex-stratified analyses, lower HDL-c remained significantly associated with higher AIx and higher AP in women and with higher AP in men.

Having a history of smoking was significantly associated with all three outcomes. This finding is not surprising as cigarette smoking is an established cardiovascular disease risk factors [199] and is known to be associated with arterial stiffness indices, particularly in those with hypertension [200]. A higher baseline HbA1 was associated with increased AP and decreased SEVR in the present study. This is consistent with the notion that the formation of advanced glycation end products (AGEs) is one of the primary mechanisms thought to be involved arterial stiffening, especially in those with diabetes. Arterial wall exposure to AGEs can cause cross-linking of collagen molecules which in turn reduces arterial elasticity [109]. Advanced glycation end-products (AGEs) are shown to be associated with increased arterial AIx in persons with hypertension [201]. Schram et al showed that AGEs, pentosidine, Nepsilon-(carboxymethyl)lysine and Nepsilon-(carboxyethyl)lysine, were all significantly associated with increased pulse pressure in those with T1D in a cross-sectional analysis, although HbA1c was not [135]. In our study, concurrent HbA1c also was not significantly associated with PWA

measures (data not shown). Baseline HbA1 level may better represent early exposure to hyperglycemia leading to AGE exposure.

Many of the factors associated with augmentation index were also associated with augmentation pressure in this study. This is not surprising as AIx is merely AP/PP x 100. AP is the measure of contribution that the wave reflection makes to the systolic arterial pressure, and it is obtained by measuring the reflected wave coming from the periphery to the centre. As the reflected wave returns earlier in the cardiac cycle, there is a disproportionate rise in SBP and therefore an increase in PP. It has been shown that AIx increases with age in healthy population until approximately 55 years of age, but that AP steadily increases with age without reaching a plateau [202]. The difference is due to the positive association between PP and age. At a certain point it seems that AP is a better representation of vascular aging than AIx because widening pulse pressures result in lower AIx. This may be the case in those with T1D as its presence is associated with accelerated vascular aging [153]. As poorer glycemic control is associated with increased PP [135] this may explain why baseline HbA1 was associated, multivariately, with AP but not with AIx.

This study is not without limitations. For one, the limited sample size; in an attempt to make up for this limitation, p-values ≤ 0.10 were represented. Another limitation is the lack of pulse wave analysis measurement at baseline. Due to this, the factors found significant in this study can only be considered potential predictors of the arterial stiffness indices measured, and should be studied further in prospective study of T1D populations. Blood glucose level has been shown to acutely affect arterial stiffness measures in T1D[157] however, blood glucose measurements at the time of measurement were not taken in this study. Another thing that could be considered a potential limitation is that pulse wave analysis was used and not pulse wave

velocity, the accepted gold standard [101]. PWV is considered the gold-standard because it is based on direct measurements of parameters linked to regional arterial stiffness and carotid-femoral PWA specifically, is considered clinically relevant as this measures pressure and stiffness within the aorta and large arteries which are closest to and most influential on the heart [101]. However, pulse wave analysis is an easy to implement, quick, well-tolerated and reliable technique that can be reasonably implemented in a clinical setting. Central pressure measures derived from PWA are correlated with PWV measures, are already shown to be altered in the presence of T1D [9, 169] are associated with adverse outcomes [112, 117].

Another limitation is that this population is essentially a survivor population and that those who were unable to attend or deceased at the 18 year clinic visit may represent those with greatest risk for complications and possibly those most affected by increased arterial stiffness. Also, compared to those at the 18-year follow-up but without PWA measures, the subpopulation in the present study had significantly lower follow-up AER measures and waist-to-hip ratio and therefore may represent a healthier segment of our T1D population (data not shown). However, these limitations are more likely to hinder finding significant relationships between baseline factors and follow-up PWA measures than demonstrate false relationships.

This is the first study to examine multiple potential risk factors for arterial stiffness in a type 1 population. The findings of this study are significant in that it has found that certain modifiable and/or treatable risk factors, such as cigarette smoking, poor glycemic control, and low HDL-c levels, are associated with increase indices of arterial stiffness and lower estimated myocardial perfusion later in life in T1D. The results of this study also confirm that that the use of anti-hypertensive medications, specifically ACEI/ARB, are associated with lower arterial stiffness indices, but also shows that they do not necessarily improved coronary artery perfusion.

Early testing and treatment for autonomic neuropathy may also be effective in reducing arterial stiffness and in turn cardiovascular morbidity and mortality in those with T1D.

In summary, this study demonstrates that, along with traditional cardiovascular risk factors associated with structural changes in arteries (low HDL, smoking, poor glycemic control), historical measurements of E/I ratio, a measure of autonomic neuropathy, are predictive of increased augmentation index and augmentation pressure (indices of arterial stiffness) and lower subendocardial viability ratio (an estimate of myocardial perfusion) in T1D. These results suggest that autonomic neuropathy plays role in development of arterial stiffness and may be the potential link between these factors and cardiovascular events in type 1 diabetes.

Table 3. Baseline characteristics of The Pittsburgh EDC Study Pulse Wave Analysis population compared to the remaining 18-year follow-up participants

	PWA Study Population N=144	**Remaining 18-year EDC Population[a] N=165**
Female (%)	50.7 (73)	52.1
Age (years)	25.9±7.38	26.7±7.50
Diabetes Duration (years)	17.6±6.70	18.7±7.34
Systolic Blood Pressure (mmHg)	111.4±13.1	110.2±12.5
Diastolic Blood Pressure (mmHg)	71.8±10.1	70.6±10.1
Hypertension (%)	22.2	19.4
Heart Rate (bpm)	76.0±12.3	73.1±11.1**
HbA1 (%)	10.1±1.69	10.1±1.71
Non-HDL-c (mg/dL)	127.2±39.9	128.1±36.7
HDL-c (mg/dL)	54.0±11.0	55.6±14.1
Triglyceride (mg/dL)	94.1±81.9	90.3±53.2
Waist-to-Hip Ratio	0.82±0.07	0.82±0.07
Body Mass Index (kg/m^2)	23.5±3.10	23.4±3.37
Serum Creatinine (mg/dL)	0.90±0.36	0.93±0.78
AER (μg/min)	238.5±653.8	229.0±734.3
Expiration-to-Inspiration Ratio	1.14±0.12	1.13±0.12
WBC ($x10^{-9}/L$)	6.25±1.76	6.20±1.64
Energy Expenditure (kcal/wk)	930.3±1324.9	1112.6±1626.4
Ever Smoker (%)	34.5	28.0
Prevalent CAD (%)	4.2	3.6

Abbreviations: Epidemiology of Diabetes Complications, EDC; high-density lipoprotein, HDL-c, kilocalories, KCAL; albumin excretion rate, AER; white blood cell count, WBC.
Data presented mean±SD or %.
[a]Those included in PWA study compared to the remaining Pittsburgh EDC study population at the 18-year follow-up; continuous variables using t-test or Mann-Whitney U for non-parametric variables (E/I Ratio, WBC, AER, Energy Expenditure, Serum Creatinine, Fibrinogen) and categorical using χ^2.
** $p<0.05$

Table 4. Correlations (or mean±SD[a]) between baseline factors and follow-up pulse wave analysis measures (augmentation index, augmentation pressure and subendocardial viability ratio)

	AIx	AP	SEVR
Expiration:Inspiration Ratio	-.18**	-.32****	.47****
Heart Rate (beats per minute)	-.20**	-.16*	-.44****
Systolic Blood Pressure (mmHg)	-.09	-.03	-.11
Diastolic Blood Pressure (mmHg)	-.00	-.01	-.03
HbA1 (%)	.02	.06	-.12
NonHDL-c (mg/dl)	.11	.153*	-.164*
HDL-c (mg/dl)	.10	.05	.03
Waist-to-Hip Ratio	-.04	.02	.05
Body Mass Index (kg/m^2)	.19**	.11	-.12
Albumin Excretion Rate (µg/min)	.10	.19**	-.22***
Serum Creatinine	.11	.11	-.05
White Blood Cell Count	.11	.12	-.07
Energy Expenditure	-.28****	-.29****	.20**
Males (n=71)[a]	19.4±11.5	7.66±6.36	149.7±29.8
Females (n=73)[a,b]	26.5±9.18	10.4±6.42	135.8±31.2
Never Smoker (n=93)[a]	22.0±11.5	8.42±6.85	145.8±31.1
Ever Smoker (n=49)[a,c]	25.3±9.59*	10.3±5.71	135.1±30.6

AIx, Augmentation Index; AP, Augmentation Pressure; SEVR, subendocardial viability ratio.
*p<.10, **p<.05, ***p<.01, ****p<.001.
[a] mean ± standard deviation of AIx, AP and SEVR for categorical variables.
[b] males compared to females: AIx: p<.001; AP: p=.003; SEVR: p=.01.
[c] never smokers compared to ever smokers: AIx: p=.09; AP: p=.01; SEVR: p=.05.
Pearson's correlations used for all normally distributed variables and comparisons with categorical (sex and ever smoker status) variables. Spearman's correlations for correlations of any non-normal variables (AP, Albumin Excretion Rate, Energy Expenditure, White Blood Cell Count, Alcohol Intake)

Table 5. Baseline predictors of AIx, LnAP and SEVR in multivariate linear regression analysis post potential confounder adjustment

	AIx			Ln(AP)			SEVR		
	β	**Se**	**p**	**β**	**se**	**p**	**β**	**se**	**p**
Concurrent Age	.187	.077	.02	.068	.013	<.001	-.293	.067	<.001
Concurrent HR	-.434	.066	<.001	-.063	.011	<.001	-.674	.055	<.001
Female Sex	.494	.194	.01	.073	.032	.03	-.377	.150	<.001
Concurrent Height	-.322	.094	.001	-.043	.015	.01	--	--	--
PWD Use	-.289	.142	.04	-.039	.026	.05	.077	.118	.52
	Δr^2	**β**	**se**	**p**	**Δr^2**	**β**	**Se**	**p**	**Δr^2**	**β**	**se**	**p**
HDL-c	.054	-.242	.071	.001	.053	-.046	.013	.001	--	--	--	--
E/I Ratio	.023	-.171	.081	.04	.035	-.026	.015	.05	.009	.126	.067	.06
Ever Smoker	.013	.247	.136	.07	.017	.046	.022	.04	.046	-.410	.108	<.001
HbA1	--	--	--	--	.019	.026	.011	.02	.022	-.129	.055	.02
MODEL	.494			<.001	.527			<.001	.679			<.001

Variables are standardized to the population (variable-mean)/standard deviation.
Base models with concurrent age, heart rate (HR), height, sex and use of medications with potential affect on pulse wave analysis measures (PWD use: Angiotensin Converting Enzyme Inhibitors, Angiotensin II Receptor Blocker, Calcium Channel Blocker, Beta Blocker, and/or Nitrate use).
Baseline variables available for forward regression: systolic blood pressure, diastolic blood pressure, NonHDL-c, HDL-c, white blood cell count, albumin excretion rate, serum creatinineHbA1, body mass index, waist-to-hip ratio, Energy expenditure in sports at baseline, Expiration-to-Inspiration Ratio (E/I), serum creatinine.

4.0 ARTICLE 2: PULSE WAVE ANALYSIS INDICES AND PREVALENT CARDIOVASCULAR DISEASE IN TYPE 1 DIABETES

To be submitted for publication

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4.1 ABSTRACT

BACKGROUND: Type 1 Diabetes (T1D) is associated with a high risk for and mortality from premature coronary artery disease (CAD). Coronary artery calcification (CAC), a subclinical marker of CAD, is shown to predict CAD events as is lower extremity arterial disease (LEAD). Pulse Wave Analysis (PWA) arterial stiffness indices are associated with cardiovascular disease (CVD) risk factors and outcomes in various populations. Availability of data regarding the relationship between these measures and CVD in T1D is limited.

METHODS: PWA was performed using the Sphygmocor Px device on 144 participants in The Pittsburgh EDC Study of childhood onset T1D. The cross-sectional association between arterial stiffness indices augmentation index (AIx) and augmentation pressure (AP) and estimated myocardial perfusion via subendocardial viability ratio (SEVR), and prevalent CAD, electron beam computed tomography measured CAC and low (<0.90) ankle-brachial index (ABI).

RESULTS: Higher AP (but not AIx) and lower SEVR were univariately associated with low ABI, prevalent CAD and high CAC score. AP and SEVR's association with CAD and CAC did not remain significant, multivariately, after the addition of age. After exclusion of nitrate use, higher AP was significantly associated with increased risk for hard CAD events over brachial blood pressure measures and age, multivariately and lower SEVR was associated with high CAC. Lower SEVR was also associated with low ABI, multivariately.

CONCLUSIONS: Central pressure augmentation and decreased myocardial perfusion are associated with the presence of low ABI in T1D, consistent with the physiologic process underlying the development of early return of the reflected pulse wave. These factors are also altered in the presence CAD however, age (partially reflecting T1D duration in our early onset population) remains an important determinant of CAD, including subclinical CAD. Medication

use, in particular nitrates, complicates cross-sectional analyses. Exclusion of nitrate use showed that augmentation pressure is in fact associated with hard CAD in T1D and low SEVR with CAC. Prospective studies are needed examine the predictive value of PWA arterial stiffness indices in T1D populations

4.2 INTRODUCTION

Type 1 diabetes (T1D) is associated with high risk (10-fold or greater) of, and increased mortality from, premature coronary artery disease (CAD) [62, 63]. This increased risk is especially apparent in women with T1D, virtually eliminating the traditional female advantage seen in the absence of diabetes [72, 73]. In T1D, the atherogenic process appears to start earlier and occur more rapidly thereby leading to early mortality and morbidity [72, 203] . Traditional factors shown to be associated with increased risk for both CAD and with lower extremity arterial disease (LEAD), (a type of peripheral vascular disease), in T1D include altered lipoprotein metabolism, nephropathy and hypertension [73, 86, 203-205]. Coronary artery calcium (CAC) measured using electron beam tomography can be used as an indicator of atherosclerotic burden [206], and in those with T1D, CAC has been correlated with CAD [207]. Persons with T1D are also at increased risk for LEAD, risk for which is higher in females than in males with T1D [205]. Vascular stiffness can be measured in a variety of ways. One way is via pulse wave analysis to measure the pulse waveform and timing of reflected waves. Increased arterial stiffness increases the velocity of forward blood flow in arteries as well as that of reflected waves. Increased blood flow velocity and earlier reflection of pulse waves results in earlier arrival (within the cardiac cycle) of reflected waves to the aorta causing central pressure

augmentation. This augmentation pressure (AP) can be measured non-invasively using applanation tonomentry and quantified as augmentation index (AIx) which is the AP expressed as a percentage of the aortic pulse pressure (PP) (AIx=AP/PP x100). The subendocardial viability ratio (SEVR), also known as The Buckberg index, can also be determined using radial applanation tonometry. SEVR is an estimate of myocardial perfusion and a measure of the propensity to cardiac ischaemia. It is calculated by dividing the diastolic time-pressure (area under the pressure curve) by systolic time-pressure and is expressed as a percentage. AIx, which has been shown to be elevated in T1D [9, 169], has also been found to correlate with traditional CAD risk factors [131][208, 209], coronary atherosclerosis[210, 211], cardiovascular outcomes [117, 212] and extracoronary atherosclerosis in those with CAD [213].

As arterial stiffness indices such as augmentation pressure, augmentation index and/or pulse wave velocity have been associated with both CAD [117, 210, 214], CAC [215, 216] and lower ankle-brachial index (ABI), (a measure of LEAD) [213, 217]. Since these relationships have yet to be explored in a T1D population, the aim of this study is to examine, cross-sectionally, the relationships between indices of arterial stiffness and myocardial perfusion as measured using applanation tonometry, and prevalent coronary artery disease, coronary artery calcification, and low ABI in population with childhood onset type 1 diabetes.

4.3 METHODS

Participants in the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study, an 18-year prospective investigation of patients with childhood-onset (age < 17 years) T1D were selected for study. Individuals were diagnosed, or seen within 1 year of diagnosis, at

Children's Hospital of Pittsburgh between 1950 and 1980 and were on insulin therapy at initial discharge [27, 176]. Initial evaluation in the EDC Study occurred between 1986 and 1988 and biennial reexamination followed for 10 years, followed by a more limited, survey, follow-up then another full clinical examination at 18 years. The 18 year examination included pulse wave analysis measures via radial applanation tonometry. The EDC Study population has been shown to be epidemiologically representative of the type 1 diabetes population of Allegheny County, Pennsylvania [177]. The study protocol was approved by The University of Pittsburgh Institutional Review Board.

Questionnaires concerning demographic, health care, self-care, and medical history information were sent to participants prior to clinic examinations. Smoking history (at least 100 cigarettes in lifetime), current smoker status and medications use were obtained. All medications were coded according to ATC/DDD codes. Medications with potential effects on pulse wave reflection measures (angiotensin converting enzyme inhibitors (ACEIs), angiotensinogen receptor II blockers (ARBs), calcium channel blockers (CCBs), beta-blockers (BB) and nitrates) were of particular interest and use of 1 or more of these medications was categorized as use of a "pulse wave drug" (PWD). The other type of medication of interest were antilipidemic agents.

During clinic visits, systolic and diastolic blood pressures (SBP and DBP) were measured using a random zero sphygmomanometer, according to the Hypertension Detection and Follow-Up Program protocol, after a 5-minute rest [180]. Hypertension (HTN) was defined as blood pressure of ≥130/80 mmHg or the use of antihypertensive medication for the purpose of lowering blood pressure. Height (in cm) and weight (in kg) were measured and BMI calculated and expressed as kg/m^2. Waist and hip circumferences were twice and a third

time if measurements were not within 0.5 cm. The mean of waist measurements and that of hip measurements were used to calculate waist-to-hip ratio (WHR).

Total cholesterol and triglycerides were measured enzymatically [181, 182]. High-density lipoprotein (HDL) cholesterol level was determined by means of a precipitation technique (heparin and manganese chloride) with modification [183] of the Lipid Research Clinics method [184]. Non-HDL cholesterol was calculated by subtracting HDL cholesterol level from total cholesterol level. Blood samples were analyzed for hemoglobin A_{1C} using the DCA 2000 analyzer (Bayer Diagnostics, Tarrytown, NY).

Soft coronary artery disease was determined as EDC physician-diagnosed angina or ischemic electrocardiogram changes (Minnesota codes 1.3, 4.1 to 4.3, 5.1 to 5.3, and 7.1). Hard CAD events were defined as myocardial infarction confirmed by Q waves on electrocardiogram (Minnesota codes 1.1 or 1.2) or hospital records or angiographic stenosis of 50% or greater, coronary artery bypass surgery, angioplasty, or ischemic electrocardiogram changes (Minnesota codes 1.3, 4.1 to 4.3, 5.1 to 5.3, and 7.1). During the 10, 16 and 18- year follow-up exams, electron beam tomography with a GE-Imatron ultrafast computed tomographic scanner (GE-Imatron, San Francisco, California) was used to assess CAC. Scans were triggered by electrocardiographic (ECG) signals at 80% of the RR interval and obtained in 3-mm contiguous sections of the heart. At year 10, only one CAC score was measured, while at years 16 and 18 two CAC scores were used, the means of which were calculated. The most recent CAC score (or mean of scores) was selected for the present study. CAC scores were also categorized as 1) None/Mild: 0-99, 2) Moderate: 100-399, 3) Severe : ≥400 for univariate and linear trend analyses and as presence of clinically significant CAC (≥100 mm^3) for multivariate analyses.

Resting ankle-brachial systolic blood pressures were taken in the supine position with a Doppler blood-flow detector. The right and left tibialis posterior and dorsalis pedis pressures were compared with the arm pressure, and an ankle-to-brachial index (ABI) was calculated using the arm pressure measurement taken closest in time to the ankle pressure. An ankle-brachial index (ABI) of <0.9 on either side at rest was considered to be evidence of LEAD. An ABI of >1.3 was considered as high ABI. A broader definition of LEAD comprised of low ABI (<0.9), a history of claudication as determined by the ROSE questionnaire[218] or self-reported history of amputation for vascular cause.

Of those EDC study participants examined at baseline (n=658), 22.7% (n=150) had died by the 18-year follow-up (November 2004 – November 2006), 78 had moved out of area, 101 declined exam, 19 were lost to follow-up leaving 318 eligible for 18 year exam, 309 for which data was available for this analysis. Pulse waveform analysis (PWA) testing began part way through the 18-year examination period (January 2006), after which 189 subjects were seen and 144 (76%) had PWA. The PWA population thus 144 men and women in attendance at the 18-year follow-up clinic visit after January 1 2006. Aortic augmentation index (AIx), aortic augmentation pressure (AP) and subendocardial viability ratio (SEVR) were derived using waveforms measured at the radial artery using the SphygmoCor Vx version 7.01 (AtCor Medical, Sydney, Australia). In brief, a high-fidelity micromanometer with a frequency response of >2 kHz (Millar Instruments, Houston, TX) was placed on the right radial artery, and gentle pressure was applied until a consistent waveform was produced. After at least 20 sequential waveforms had been acquired, measurement was stopped. Central pressure values were estimated from radial measurements using the software's mathematical transfer function [159, 189]; the accuracy and reliability of which have been validated [163, 172]. The pressure wave

created by left ventricular contraction propagates forward until meeting sites of resistance which reflect the wave backward. Stiffer artery walls result in earlier wave reflection [168, 171]. When the reflected wave returns during systole rather than diastole, systolic pressure is increased or "augmented". Augmentation pressure (AP) is a measure of how much the early reflected wave contributes to central systolic pressure. Augmentation index (AIx) represents the level of augmentation measured and is expressed as a percentage of the pulse pressure (AIx = AP/PP). Heart rate is inversely associated with AIx and AP [121]. Subendocardial viability ratio (SEVR), the ratio of the diastolic area under the curve (AUC) of an arterial pulse wave to the systolic-AUC [190, 191] is a ratio of myocardial perfusion (as coronary artery perfusion takes place primarily during diastole) to myocardial contraction, and is a tonometric, non-invasive measure of myocardial perfusion relative to cardiac workload. The SphygmoCor device provides a quality index (QI) which represents reproducibility of the waveform. If PWA produced results with a QI<80 the measure was repeated. Measures with a QI≥80 were included in this study.

Distributional characteristics and normality of variables was assessed. Binary categorical variables were coded as 0 or 1, including sex for which females were 1. Student's t-test, for parametric, and the Mann-Whitney U test, for variables not meeting the non-parametric assumptions (augmentation pressure, triglyceride level, albumin excretion rate, serum creatinine) were used to compare continuous variables between cases and non-cases. χ-square test was used to examine the differences in binary categorical variables (i.e.: sex, medication use, smoking status), between cases and non-cases. Pearson's and Spearman's correlations were used, as appropriate, to univariately compare CAC scores with pulse wave reflection measures (AIx, AP, SEVR). CAC scores were also categorized as none/minimal (0-99), mild/moderate (100-399), and severe (≥400) and PWA measures AIx and SEVR were

compared between groups using one-way ANOVA with the Bonferroni correction. AP, as it was non-parametric was compared across CAC categories with the Kruskal-Wallis test. All continuous variables were standardized by subtracting the mean and dividing the by their standard deviation of the study population. Logistic regression was performed, in a stepwise manner, separately for prevalent CAD, low ABI and high CAC score.

4.4 RESULTS

Cross-sectional characteristics of the PWA population from the Pittsburgh EDC Study are detailed in Table 6, along with sex comparisons. Although crude comparisons of AIx, AP and SEVR showed significant differences between the sexes, height adjusted comparisons did not. Males and females were of similar age, diabetes durations, and had similar heart rates, BMI's, HbA1c, WBC, and Non-HDL-c levels and coronary artery calcification scores. Percentage with hypertension or with a smoking history also did not significantly differ between males and females. However, males did have significantly higher SBP and DBP, lower HDLc, and greater waist-to-hip. Overall, 22.9% (n=33) of the population had prevalent CAD (soft or hard events), 12.5% (n=18) with a history of hard and 10.4% (n=15) with a history of soft events. The percentage of males with any type of CAD was higher than in females, but this was not statistically significant (28.2% vs. 17.8%). However, the percentage of hard events was significantly higher in males compared to females (18.3% vs. 6.8%, p=.04). CAC scores were available for 138 (68 males, 70 females) of the 144 PWA participants. Fifty (34.7% of those with CAC scores) of the participants had a CAC score ≥100 mm^3. The percentage of females with CAC score ≥100 mm^3 did not significantly differ from males (32.9% vs. 36.6%, p=.63).

Sixteen participants (11.1%) had an ABI <0.90 and 29 (20.1%) had a high ABI. Presence of low ABI differed by sex as 11 (68.8%) of the 16 cases were female, however this difference was not statistically significant (p=.13). Presence of high ABI also differed by sex with males representing 20 (71.4%) of the 28; this did reach statistical significance at p=.006.

Table 7 shows the rate of medication use by CAD status. Those with prevalent CAD of any type were more likely to be taking a drug with potential effects on PWA measures (78.8% vs 57.7%). ACEI/ARB medication use was fairly prevalent throughout the study population and did not significantly differ between those with and without CAD. A greater percentage of those with hard CAD reported use of every type of PWD medication that did those with soft CAD: ACEI/ARB – 72.2% vs. 40.0%, p=.06; beta-blocker – 33.3% vs. 26.7%, p=.68; calcium channel blocker – 27.8%, p=.94; nitrates – 22.2% vs. 6.7%, p=.22. Eleven of those with hard CAD reported use of 2 or PWD medications compared to only 3 of those with soft CAD.

4.4.1 Pulse Wave Analysis Measures and Coronary Artery Disease

Augmentation index showed no significant univariate associations with any cardiovascular outcome (Figure 1). Univariately, lower SEVR was associated with any and soft CAD and with increasing CAC score category but not significantly with hard CAD (Table 7 and Figure 2). Augmentation pressure, although higher in cases of all outcomes (Figure 3), was borderline significant when comparing any CAD to non-cases and with increasing CAC category but was not significant by CAD type (Table 8).

In multivariate logistic regression for any type of CAD, per standardized unit increase in AP there was an associated 87% increased risk (OR=1.87; 95%CI: 1.17-3.00; p=.009) for CAD in models adjusted for potential confounders (height, heart rate, PWD use). When allowing for

other variables associated with CAD to enter models, age enters and eliminates the significant association between AP and prevalent CAD. Multivariate models for specific CAD type showed a similar occurrence for soft CAD (2.5 times risk for CAD per standard unit increase of AP) but no significant association between AP and hard CAD even in the model adjusted only for potential confounders (data not shown). Multivariate models with SEVR also showed similar results for CAD; per standard unit decrease in SEVR there was a 40% increased risk of any CAD (p=.07), a 45% increased risk for soft CAD (p=.009) but no significant association with hard CAD (p=.77) in confounder adjusted models. The addition of age to multivariate models again diminished the statistical significance of the SEVR-CAD relationship. Due to the potential confounding affect of PWD use, multivariate models were performed after excluding each of the PWD except for ACEI/ARB use as the majority of participants (60%) reported its use and use was not significantly different between CAD cases (57.6%) and non-cases (55.5%). All of the models produced results similar to those already discussed except for those which excluded nitrate use. Higher AP (OR=2.57; 95%CI:1.03-6.42) was significantly related to hard CAD, multivariately, when those taking nitrate medication (n=4) were excluded (Table 8). AP actually entered multivariate the model preferentially over brachial measures of systolic and diastolic blood pressure as well as over age. Results were similar when diabetes duration was available instead of age. Without the availability of AP, age and sex are the major determinants of hard CAD in this population. A univariate comparison shows that in those using nitrate (n=5) medications, AP is lower (8.80 ± 10.2) than in those not (9.05 ± 6.42). SEVR was still not significantly associated, multivariately, with hard CAD when those with nitrate use were excluded, but was associated with having high CAC score (Table 9).

Multivariate models for high (≥100 mm^3) CAC score were similar to those for clinical CAD. AP was borderline significant (OR=1.55; 95%CI: 0.96-2.49; p=.07) in confounder adjusted models with entrance of age having the same effect on the relationship. AP models adjusted for prevalent CAD showed no significant association between AP and high CAD. SEVR was significantly associated with having a high CAC score in confounder adjusted models (OR=0.46; 95%CI: 0.26-0.81) with age reducing the statistical significance (OR=0.61; 95%CI:0.33-1.13; p=.12). CAD adjustment did not significantly alter SEVR models for high CAC.

4.4.2 Pulse Wave Analysis Measures and Ankle-Brachial Index

Cross-sectional characteristics of the PWA population by ABI category are described in Table 10. AIx was not significantly different across ABI categories (Table 10 and Figure 1). Univariately, AP (p=.08) and SEVR (p=.07) were borderline significantly different by ABI category. The most noticeable difference in AP and SEVR was in those with low ABI (Figures 3 and 2, respectively). Other factors that differed across ABI category were age and diabetes duration (those with low ABI being the oldest and having the longest duration) and diastolic blood pressure (again lowest in those with low ABI). Waist-to-hip ratio was also borderline significantly different by ABI category with the normal group having the lowest WHR. Medication use was not statistically different by ABI category, however, a greater percentage (81.3%) of those with low ABI were on at least one of the PWD medications.

Multivariate models for Low ABI, excluding those with high ABI showed that the AP confounder adjusted model, per standardized unit increase in AP, there was a 66% increase risk for low ABI (OR=1.66; 95%CI: 0.95-2.90; p=.08). However, when adjusted for age, the

association between AP and low ABI was not significant (Table 11a). SEVR, however, was significantly associated with low ABI and preferentially entered heart rate adjusted multivariate models over brachially measured systolic and diastolic blood pressure (Table 11b). PWD use was not significantly associated with low ABI and did not significantly alter models when adjusted for. When a broader definition of lower extremity arterial disease was used (including claudication and/or amputation), PWA measures were not significantly associated with presence of LEAD multivariately.

4.5 DISCUSSION

This study found that augmentation pressure, measured non-invasively using radial applanation tonometry, is significantly associated with prevalent coronary artery disease in T1D. It was specifically associated with hard CAD, definition for which comprises of myocardial infarction, a coronary blockage of at least 50% or history of revascularization (coronary artery bypass graph or angioplasty), when use of nitrate medications (n=4 hard CAD cases) was excluded. We also found that lower estimated myocardial perfusion, SEVR, is associated with presence of high CAC (≥100), again when those reporting nitrate use were excluded. This was the case even when adjusting for age and prevalent hard CAD. Another finding was that AP was higher in those with low ABI and remained statistically significant until the model was adjusted for prevalent CAD. However, reduced SEVR remained significantly associated with presence of low ABI, multivariately.

In some of our multivariate models for CAD and high CAC, the addition of age eliminated the significant associations between pulse wave analysis measures and disease.

Age is a major risk factor for CAD as is diabetes duration in T1D[219]. Diabetes duration childhood onset in T1D and age are highly correlated, r=.825 in the current study population. Diabetes duration has been shown to be closely tied to coronary artery calcification progression in T1D in The Pittsburgh EDC Study [220] and age is a known risk factor for degree of CAC [221] Arterial stiffness indices are also very much associated with age [108, 222] and in studies of those with T1D, longer diabetes duration is significantly related to higher values of arterial stiffness measures[192, 223]. Given the relationship between age and T1D duration, and the association both have with CAD, CAC and arterial stiffness indices, it is not entirely surprising that the addition of age to multivariate models for CAD and CAC would impact the relationship between the indices and outcomes. Interestingly, SEVR was similar between those with hard CAD compared to those with soft or no CAD even though we would expect worse CAD to be associated with lower SEVR. However, this may be due to the fact that revascularization is included in the definition of hard CAD in our study. There was pervasive use of medications that are known to influence PWA measures in this T1D population, particularly in those whom had a history of hard CAD events. Approximately 90% of those on PWD's were on an ACEI or ARB which are shown to be very effective, compared to other antihypertensive agents, in reducing values of arterial stiffness indices [224]. However, since a good proportion of non-cases reported ACEI/ARB use. Use of other medications types, such as calcium channel blockers, beta-blockers and nitrates was more common among those with CAD, particularly with hard CAD. Therefore, we chose to examine the relationship between PWA measures and CAD in their absence. Exclusion of calcium channel blockers and beta-blockers each resulted in models similar to those produced when they were included. However, it was by excluding those on nitrates, only 4 participants

with hard CAD, that significant changes to multivariate models occurred. Administration of exogenous nitrate has been shown to increase vasodilatation, arterial compliance and decrease systolic blood pressure [225]. Stokes et al found, in a double-blind randomized cross-over study in elderly hypertensives, that administration of isosorbide mononitrate (ISMN) decreased not only brachial systolic and diastolic ambulatory blood pressures, but also decreased aortic SBP and augmentation pressure [226]. Stokes et al later confirmed the effect ISMN on pulse wave analysis measures in a another study which showed ISMN to decreased SBP by 16 mmHg, pulse pressure by 13 mmHg and AIx by 4% in a group of older hypertensive patients[227].

Presence of a low ABI is associated with increased cardiovascular risk [88, 89] and cardiovascular and all-cause mortality risk in T1D[228]. Previous studies have shown significant association between AIx and ABI in multivariate analyses [213, 217]. While our findings show no significant relationship between low ABI and AIx in our T1D population, we did find a relationship with augmentation pressure and SEVR. AIx is the expression of augmentation pressure as a percentage of pulse pressure and its effectiveness in the representation of increased pulse wave reflection and arterial stiffness in older populations has been questioned [202]. As there is potentially accelerated vascular aging in those with T1D [153], AIx may not be a suitable index of arterial stiffness in this population either. LEAD is due to atherosclerosis in the arteries of leading to or within the legs. It is logical that atherosclerotic changes in lower peripheral vessels contributes to earlier reflection of the pulse wave and to an increase in central systolic pressure augmentation. The earlier the timing of the wave reflection, the greater the AP, the higher the central systolic pressure therefore the greater the pressure the heart must pump against. Overtime, this increased pressure leads to a

reduction in diastolic pressure and duration and it is in diastole that coronary perfusion takes place. The findings of the current study are consistent with this physiologic process in that reduced diastolic pressure leads to impaired coronary perfusion, i.e. lower SEVR.

The cross-sectional design of this study is one of its limitations and a potential reason that PWA measures were not significantly associated with all CAD outcomes. A prospective design using pulse wave analysis measurements to predict CAD events or CAC progression within a T1D population may be more effective in elucidating the relationship between arterial stiffness indices and these outcomes. Most participants who had CAD or high values of CAC reported PWD use, which is to be expected, but is also a limitation of the study. The use of these types of medications may explain the lack of association between PWA measures and some of the CVD outcomes. The limited sample size of our study population is another limitation as a greater sample may have yielded more significant results. A larger sample sized would have also allowed for stratification by medication use, age (younger vs. older) or diabetes duration (shorter vs. longer), as well as sex, which may have contributed to a greater understanding of associations.

In summary, augmentation pressure, an index of arterial stiffness, and subendocardial viability ratio, an estimate of myocardial perfusion, are associated with hard CAD and high CAC, respectively, in our T1D population. These factors are also associated with presence of low ABI, although AP's association is diminished by age adjustment. This is the first study to examine the association pulse wave reflection measures and prevalent cardiovascular disease in a Type 1 diabetes population. Pulse waveform analysis is an easy to implement, non-invasive measure of arterial stiffness indices and further research into the association between these indices and disease in T1D is necessary.

Table 6. Cross-sectional characteristics, and sex comparisons, of The Pittsburgh EDC Pulse Wave Analysis Study Population

	Males	Females	Total
Augmentation Index (%)	21.3 (18.6-24.1)[a]	24.7 (22.0-27.3)	23.0±11.0
Augmentation Pressure (mmHg)	8.55 (6.86-10.2)[a]	9.49 (7.83-11.2)	9.03±6.51
Subendocardial Viability Ratio (%)	143.7 (135.6-151.2)[a]	140.7 (132.6-148.7)	142.2±31.1
Age (years)	45.5±7.70	44.0±7.14	44.7±7.43
Diabetes Duration (years)	37.1±6.37	35.7±7.06	36.4±6.74
HbA1c (%)	7.52±1.39	7.37±1.36	7.44±1.37
Systolic Blood Pressure (mmHg)	119.1±16.9	112.2±15.5**	115.6±16.5
Diastolic Blood Pressure (mmHg)	69.2±8.38	63.9±9.29****	66.5±9.21
Heart Rate (bpm)	77.7±13.2	77.45±12.9	77.6±13.0
Hypertension (% (n))	23.9 (17)	17.8 (13)	20.8 (30)
NonHDL-c (mg/dl)	114.5±15.1	113.3±33.3	113.9±32.7
HDL-c (mg/dl)	52.2±15.1	64.9±16.1****	59.2±16.6
Body Mass Index (kg/m^2)	26.9±4.45	27.7±5.14	27.3±4.81
Waist-to-Hip Ratio	0.91±0.07	0.82±0.09****	0.87±0.9
Serum Creatinine (mg/dl)	1.21±0.70	0.96±0.29**	1.08±.054
White Blood Cell Count (mg/dl)	6.49±1.96	6.38±2.07	6.44±2.01
CAC Score (mm^3)	275.6±554.9	227.9±449.9	251.4±503.1
Ever Smoker (% (n))	44.3 (31)	36.6 (26)	39.6 (57)
Any CAD (% (n))	28.2 (20)	17.8 (13)	22.9 (33)
Hard CAD (% (n))	18.3 (13)	6.8 (5)**	12.5 (18)
Low ABI (% (n))	7.0 (5)	15.1 (11)	11.1 (16)
High ABI (% (n))	31.8 (21)	11.9 (8)	20.1 (29)
PWD use (% (n))	69.0 (49)	56.2 (41)	62.5 (90)
Anti-Lipidemic Med (% (n))	49.3 (35)	41.7 (30)	45.5 (65)

Abbreviations: coronary artery disease, CAD; ankle-brachial index, ABI; pulse wave drug use, PWD.[a]height adjusted mean (95% confidence interval) compared between males and females.
* p<.10, ** p<.05, ** p<.01, **** <.001.

Table 7. Characteristics of CAD cases and non-cases and CAC score category within EDC PWA study population.

	No CAD	Any CAD	Soft CAD	Hard CAD	CAC Score		
					0-99	100-399	≥400
n (%)	111 (77.1)	33 (22.9)	15 (10.4)	18 (12.5)	88 (61.1)	24 (16.7)	26 (18.1)
AIx (%)	22.4±9.82	25.1±14.2	25.0±13.0	25.1±15.5	22.4±10.6	24.0±8.14	24.9±14.4
AP (mmHg)	8.09±4.67	12.2±10.1*	13.8±10.7	10.8±9.61	7.98±5.83	9.29±4.51	12.4±10.5^{*b}
SEVR (%)	144.5±32.4	134.2±25.2*	123.7±23.4**	143.0±18.7	147.3±33.0	136.2±29.6	130.6±24.3^{**b}
Heart Rate (bpm)	77.7±12.5	77.3±14.9	78.4±14.9	76.4±15.3	77.4±13.9	78.2±9.81	76.9±12.2
Age (years)	43.1±6.61	49.8±7.59****	51.0±8.29***	48.9±7.06***	42.3±6.57	46.3±6.60	51.4±6.24^{****b}
T1D Duration (years)	34.8±5.89	41.3±7.08****	43.1±8.42***	39.7±5.54***	34.4±6.10	38.6±5.55	41.6±6.62^{****b}
Systolic BP (mmHg)	113.6±15.1	122.1±19.6***	125.9±16.7***	118.9±21.6	113.4±15.5	117.2±15.4	120.9±14.6^{*b}
Diastolic BP (mmHg)	67.2±9.05	64.1±9.47*	61.9±9.43**	66.0±9.36	67.6±9.18	65.5±8.10	62.9±10.1^{*b}
Non-HDL-c (mg/dL)	115.8±34.2	107.5±26.8	111.8±31.0	103.9±23.1	116.6±33.3	101.9±23.5	111.9±32.7
HDL-c (mg/dL)	59.5±15.9	57.8±18.3	61.3±21.3	54.3±15.0	60.5±16.8	57.0±17.0	57.0±12.9
HbA1c (%)	7.41±1.46	7.55±1.05	7.61±0.95	7.49±1.15	7.51±1.47	7.30±1.26	7.33±1.17
Waist-to-Hip Ratio	0.86±0.09	0.91±0.09***	0.90±0.11*	0.92±0.07***	0.86±0.09	0.88±0.11	0.91±0.08^{**b}
BMI (kg/m^2)	27.0±4.58	28.7±5.90*	28.8±5.28	28.6±6.52	27.2±4.64	27.6±6.05	27.30±4.01
S. Creatinine (mg/dL)	1.03±0.32	1.26±0.94	1.06±0.26	1.42±1.24	1.01±0.28	1.11±0.53	1.30±1.00^{**b}
WBC (x10^{-9}/L)	6.31±2.09	6.89±1.62*	6.30±1.66	7.38±1.47**	6.20±2.07	6.78±1.98	6.88±1.92
PWD Use (% (n))	57.7 (64)	78.8 (26)**	73.3 (11)	83.3 (15)**	48.9	87.5	88.5****
ACEI/ARB (% (n))	55.5 (61)	57.6 (19)	40.0 (6)	72.2 (13)^{a}	0.0	4.2	15.4^{***b}
Beta blocker (% (n))	5.5 (6)	30. 3 (10)****	26.7 (4)**	33.3 (6)***	46.6	83.3	61.5^{***b}

Table 7 continued							
	No CAD	**Any CAD**	**Soft CAD**	**Hard CAD**	**CAC Score**		
					0-99	**100-399**	**≥400**
Calcium Channel Blocker (% (n))	8.2 (9)	27.3 (18)***	26.7 (4)*	27.8 (5)**	3.4	29.2	26.9 ****b
Nitrates (% (n))	0.0 (1)	15.2 (5)***	6.7 (1)	22.2 (4)****	0.0	4.2	15.4***b

Abbreviations: Coronary Artery Disease, CAD; Coronary Artery Calcification, CAC; Augmentation Index, AIx; Augmentation Pressure AP; Subendocardial Viability Ratio, SEVR; Type 1 Diabetes, T1D; Body Mass Index, BMI; White Blood Cell Count, WBC; Angiotensin Converting Enzyme Inhibitor, ACEI; Angiotensin II Receptor Blocker (ARB). CAD cases compared to those without CAD. [a]ACEI/ARB use different (p=.06) by CAD type (hard versus soft). [b]significant linear trend.
*p<.10, ** p<.05, ** p<.01, ****<.001.

Table 8. Multivariate logistic regression model for hard CAD (myocardial infarction, >50% blockage, revascularization) in The Pittsburgh EDC Pulse Wave Analysis study population

Variable	OR	95%CI	p
Female Sex	0.15	0.03-0.74	.02
Augmentation Pressure	2.34	1.04-5.28	.04
Heart Rate	1.16	0.58-2.28	.68
Height	0.85	0.44-1.64	.63

Excludes those reporting nitrate use (n=4). Odds ratios are per standardized unit.
Variables available to the model: augmentation pressure, age, brachial systolic blood pressure, bracial diastolic blood pressure, waist-to-hip ratio, HDL-c, NonHDL-c, sex, HbA1c, anti-lipidemic agent use, PWD use, smoking history, albumin excretion rate.
Model adjusted for potential confounders: heart rate and height

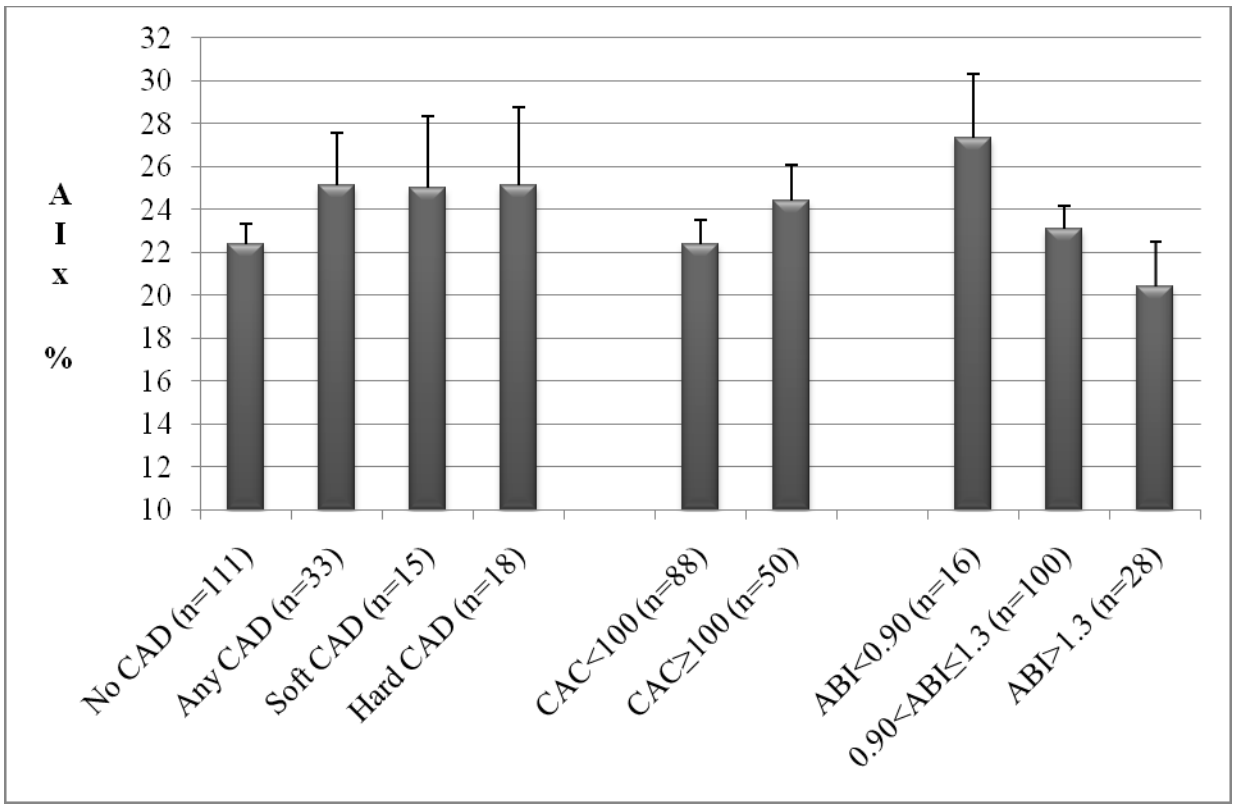

Figure 1. Augmentation Index (mean and standard error) by prevalent coronary artery disease (CAD), coronary artery calcification (CAC) score and ankle-brachial index (ABI) categories in The Pittsburgh EDC Pulse Wave Analysis study population

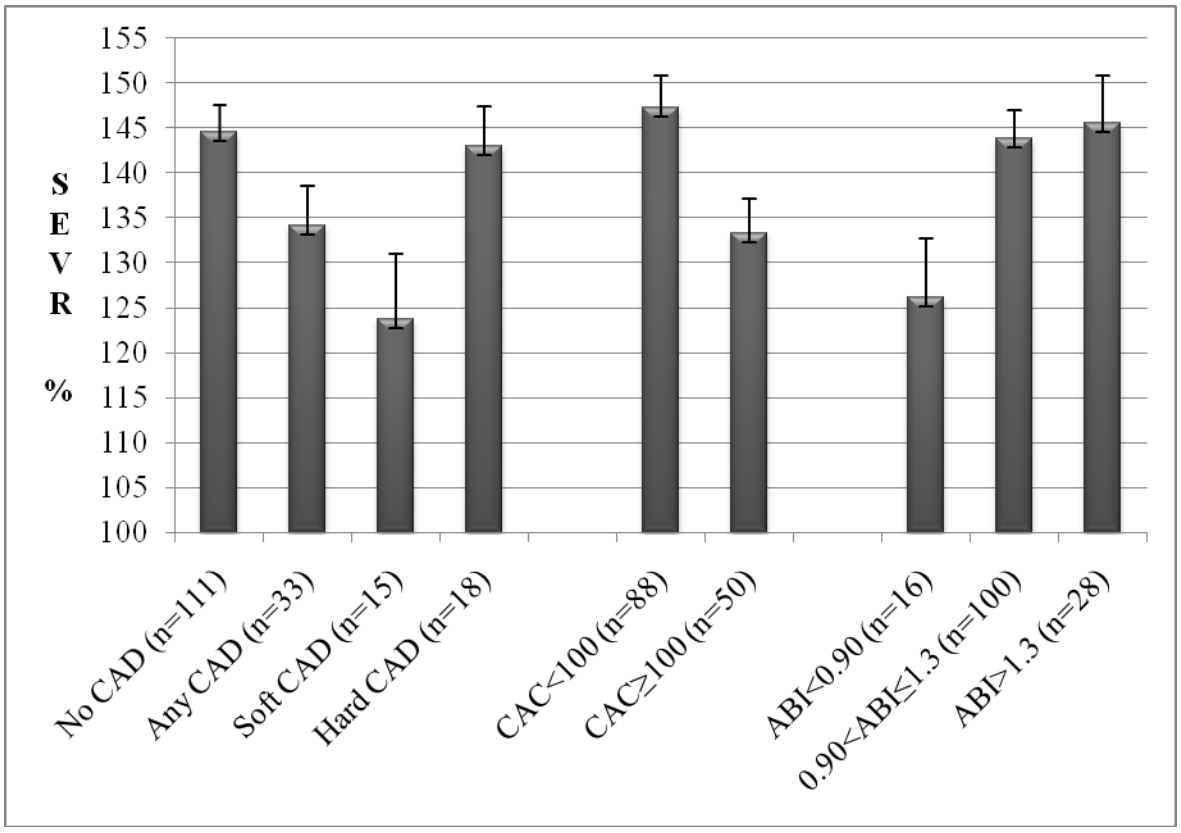

Figure 2. Subendocardial Viability Ratio (mean and standard error) by prevalent coronary artery disease (CAD), coronary artery calcification (CAC) score and ankle-brachial index (ABI) categories in The Pittsburgh EDC Pulse Wave Analysis study population

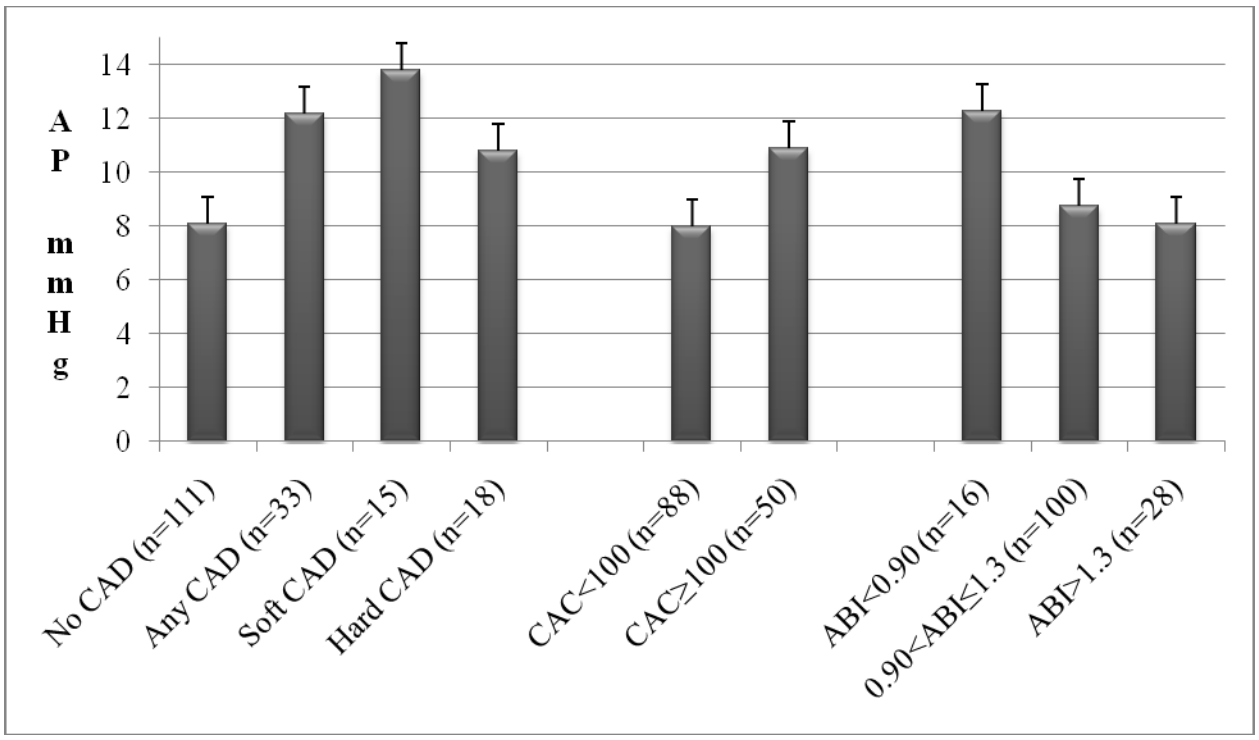

Figure 3. Augmentation Pressure (mean and standard error) by prevalent coronary artery disease (CAD), coronary artery calcification (CAC) score and ankle-brachial index (ABI) categories in The Pittsburgh EDC Pulse Wave Analysis study population

Table 9. Multivariate logistic regression model for high coronary artery calcification score (≥100) in The Pittsburgh EDC Pulse Wave Analysis study population

	OR	95%CI	p
Subendocardial Viability Ratio	0.57	0.31-1.07	.08
Heart Rate	0.73	0.39-1.38	.34
Age	2.29	1.38-3.81	.001
Prevalent Hard CAD	7.03	1.65-29.9	.008

Excludes those reporting nitrate use (n=4). Odds ratios are per standardized unit.
Variables available to the model: subendocardial viability ratio, age, brachial systolic blood pressure, bracial diastolic blood pressure, waist-to-hip ratio, HDL-c, NonHDL-c, sex, HbA1c, anti-lipidemic agent use, PWD use (use of at least one: ACE inhibitor, angiotensin II receptor blocker, calcium channel blocker, beta-blocker), smoking history, albumin excretion rate.
Model adjusted for potential confounder: heart rate

Table 10. Cross-sectional characteristics of The Pittsburgh EDC pulse wave analysis study population by ankle-brachial index category

	ABI<0.9	0.9<ABI<=1.3	ABI>1.3
n (%)	16 (11.1)	100 (69.4)	28 (19.4)
Augmentation Index (%)	27.3±12.0	23.1±10.7	20.4±11.1
Augmentation Pressure (mmHg)	12.3±7.84	8.77±5.83	8.11±7.64*
Subendocardial Viability Ratio (%)	126.2±26.1	143.8±32.1	145.5±28.1*
Heart Rate (bpm)	75.8±12.6	76.9±12.9	80.9±13.8
Age (years)	49.7±7.61	43.5±7.41	46.0±6.00**
Diabetes Duration (years)	41.0±6.07	35.2±6.71	37.9±5.88**
Systolic Blood Pressure (mmHg)	117.6±17.2	114.3±16.8	118.8±15.6
Diastolic Blood Pressure (mmHg)	60.1±8.59	66.8±9.47	69.3±6.86***
Non-HDL-c (mg/dL)	110.2±31.8	115.7±31.9	109.6±36.5
HDL-c (mg/dL)	57.6±18.8	59.5±16.5	58.7±16.5
HbA1c (%)	6.99±1.27	7.41±1.33	7.83±1.52
Waist-to-Hip Ratio	0.89±0.11	0.86±0.09	0.89±0.09*
Body Mass Index (kg/m^2)	29.4±6.48	27.2±4.83	27.2±4.18
Serum Creatinine (mg/dL)	1.39±1.31	1.04±0.35	1.03±0.24
White Blood Cell Count (x10^{-9}/L)	7.52±2.80	6.26±1.82	6.43±1.97
PWD Use (% (n))	81.3 (13)	58.0 (58)	67.9 (19)
ACEI/ARB (% (n))	68.8 (11)	52.5 (99)	19.6 (28)
Beta blocker (% (n))	12.5 (2)	10.1 (10)	14.3 (4)
Calcium Channel Blocker (% (n))	25.0 (4)	12.1 (12)	7.1 (2)
Nitrates (% (n))	12.5 (2)	2.0 (2)	3.6 (1)

Abbreviations:"Pulse Wave Drug" (PWD): Use of at least one of the other medications listed below PWD use. Angiotensin Converting Enzyme Inhibitor, ACEI; Angiotensin II Receptor Blocker (ARB)

*p<.10, ** p<.05, ** p<.01, **** <.001.

Table 11. Multivariate models for low ABI (<0.9), excluding those with high ABI (>1.3) in The Pittsburgh EDC Pulse Wave Analysis in Type 1 diabetes study population

Table 11a: Augmentation Pressure and Low ABI[a]			
Variable	**OR**	**95%CI**	**P**
Augmentation Pressure	1.27	0.67-2.42	.47
Heart Rate	0.91	0.48-1.73	.78
Height	0.80	0.44-1.45	.46
Age	2.38	1.24-4.57	.009
Body Mass Index	1.61	1.24-4.57	.10
Table 11b: Subendocardial Viability Ratio and Low ABI[b]			
	OR	**95%CI**	**P**
Subendocardial Viability Ratio	0.36	0.15-0.86	.02
Heart Rate	0.43	0.19-1.09	.08
Prevalent CAD	6.04	1.73-21.1	.005
Body Mass Index	1.61	0.94-2.75	.08

Odds Ratios are per standardized unit.

[a]Augmentation pressure along with heart rate and height, were forced into a base model.

[b]heart rate entered followed by forward regression allowing for SEVR other variables listed here. Variables available to both models forward regression: age, systolic blood pressure, diastolic blood pressure, body mass index, sex, history of smoking, HDL-c, Non-HDL-c, HbA1c, albumin excretion rate, waist-to-hip ratio, anti-lipidemic agent use. PWD use (use of a "pulse wave drug", 1 or more of the following: ACE Inhibitor, Angiotensin II Receptor Blocker, Calcium Channel Blocker, Beta Blocker, Nitrate).

5.0 ARTICLE 3: AUGMENTATION PRESSURE AND SUBENDOCARDIAL VIABILITY RATIO ARE ASSOCIATED WITH MICROALBUMINURIA AND WITH POOR RENAL FUNCTION IN TYPE 1 DIABETES

To be submitted for publication

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5.1 ABSTRACT

BACKGROUND: Diabetic nephropathy, a major type 1 diabetes (T1D) complication is a potent risk factor for cardiovascular disease (CVD). Known risk factors do not entirely account for its development and progression, and its relationship with CVD is not wholly understood. Microalbuminuria, an early marker of renal damage, is a major predictor of future CV mortality and morbidity in T1D. Therefore, we examined the relationship between arterial stiffness indices, which are shown to predict CVD, and microalbuminuria in T1D and assessed their association with renal function.

METHODS: Pulse wave analysis was measured using the Sphygmocor Px device on 144 participants in The Pittsburgh EDC Study of childhood onset T1D. Arterial stiffness indices augmentation index (AIx) and augmentation pressure (AP) as well as subendocardial viability ratio (SEVR, an estimate of myocardial perfusion) were each analyzed, cross-sectionally, in relation to prevalent microalbuminuria (and degree of albuminuria) and renal function. Albumin excretion rates (AER) were calculated from timed samples; microalbuminuria was defined as AER 20-199 μg/min. Renal function was assessed by two techniques, calculated estimated glomerular filtration rate (eGFR) and by serum Cystatin C.

RESULTS: AP and SEVR were each univariately associated with AER, eGFR and Cystatin C. SEVR was independently related to the presence of microalbuminuria and degree of albuminuria within normo- and micro-albuminuric participants. Reduced SEVR was independently associated with low eGFR (<60 ml/min/1.73m^2) and high Cystatin C (≥1.0 mg/l).

CONCLUSION: Decreased SEVR and increased AP are each related to both early renal damage and poor renal function in T1D. SEVR is a better predictor of AER than traditional brachial

blood pressure measures in those without clinical proteinuria indicating a potential use for early detection, intervention and risk stratification in T1D.

5.2 INTRODUCTION

Diabetic nephropathy (DN) is a major complication of type 1 diabetes (T1D)[35] and is the most common cause of end-stage renal disease (ESRD) [229, 230]. Known risk factors for DN in T1D include poor glycemic control, age and diabetes duration, dyslipidemia [231] and elevated blood pressure (brachial systolic and mean arterial pressure [50, 52]). However, these factors do not entirely explain the risk of nephropathy development on its progression. DN is linked to other complications of T1D such as retinopathy [232, 233] and cardiovascular disease [66, 76, 77] in T1D populations while in the general population reduced renal function has been associated with greater cardiovascular mortality [234-236], increased left-ventricular mass in men [237], and subclinical atherosclerosis[238-240]. Albuminuria (measure of renal damage) is also associated with increased risk of clinical cardiovascular disease (CVD) and mortality in a variety of populations [234, 241-243]. Despite extensive studies, the underlying mechanism relating renal function and/or renal damage to cardiovascular complications are not completely understood.

Augmentation Index (AIx), a index of arterial stiffness, has been linked to progression to ESRD in patients with chronic kidney disease [244] and was recently shown to be associated with GFR in hypertensive patients [245]. Pulse wave velocity (PWV), a measure of arterial stiffness is also shown to be significantly and independently correlated with eGFR (estimated with the Modification of Diet in Renal Disease (MDRD) equation) [246, 247]. PWV is also

shown to increase in a stepwise manner with advancing stage of CKD (stages 1-5) [248]. Measures of arterial stiffness are also associated with left ventricular diastolic function [195], cardiovascular events, mortality [113, 249-251] and risk [195]. Pulse wave analysis (PWA) utilizing applanation tonometry measures variables associated with the forward propagation and reflection of the pulse wave providing indices of arterial stiffness. The pressure wave created by left ventricular contraction propagates forward until meeting sites of resistance which reflect the wave backward. Stiffer artery walls result in earlier wave reflection [168, 171]. When the reflected wave returns during systole rather than diastole, as occurs when there is increased stiffness, systolic pressure is increased or "augmented". Augmentation pressure (AP, in mmHg) is a measure of how much the early reflected wave contributes to central systolic pressure. AIx (in %), represents the level of augmentation measured and is expressed as a percentage of the pulse pressure (PP= systolic – diastolic blood pressure; AIx=(AP/PP)x100). Subendocardial viability ratio (SEVR), the ratio of the diastolic area under the curve (AUC) of an arterial pulse wave, divided by the systolic –AUC [190, 191] is an estimate of myocardial perfusion. The association between PWA measures and measures of renal function and/or renal damage, have yet to be explored in a T1D population. Therefore, the aim of this study is to examine the relationship between PWA measures, AIx, AP and SEVR and measures of renal function (eGFR and Cystatin C) and renal damage (albumin excretion rate (AER)) in a population with childhood onset T1D.

5.3 METHODS

A cross-sectional study at the 18-year follow-up of participants in The Pittsburgh Epidemiology of Complications (EDC) study with childhood-onset (age <17 years) T1D was completed. Individuals were diagnosed with T1D, or seen within 1 year of diagnosis, at Children's Hospital of Pittsburgh between 1950 and 1980 and were on insulin therapy at initial discharge [27, 176]. Initial evaluation in the EDC Study occurred between 1986 and 1988 and biennial reexamination followed for 10 years, then followed up by survey and finally a full reexamination at 18 years, during which pulse wave analysis via applanation tonometry was performed. The study protocol was approved by The University of Pittsburgh Institutional Review Board.

Questionnaires concerning demographic, health care, self-care, and medical history were sent to participants prior to clinic visit. Self-reported smoking history (at least 100 cigarettes in lifetime), current smoker status and medication use were obtained. All medications were coded according to The World Health Organization's Anatomical, Therapeutical, Chemical Classification/Defined Daily Doses (ATC/DDD) codes. Medications with potential effects on pulse wave analysis measures (angiotensinogen converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB), calcium channel blockers (CCB), beta blockers (BB), and nitrates) [165] were of particular interest and use of 1 or more of these medications was categorized as pulse wave drug (PWD) use.

During clinic visits, brachial systolic and diastolic blood pressures (SBP and DBP) were measured using a random zero sphygmomanometer, according to the Hypertension Detection and Follow-Up Program protocol, after a 5-minute rest [180]. Hypertension was defined as a blood pressure ≥130/80 mmHg or the use of antihypertensive medications for the purpose of

lowering blood pressure. Height (in cm) and weight (in kg) were measured and body mass index (BMI) calculated and expressed as kg/m^2. Waist and hip circumferences were measured twice and if not within 0.5 cm, then a 3rd time. Means of waist and means of hip measurements were used to calculate waist-to-hip ratio (WHR).

Total cholesterol was measured enzymatically [181, 182]. High-density lipoprotein cholesterol (HDL-c) levels were determined by a precipitation technique (heparin and manganese chloride) with modification [183] of Lipid Research Clinics method [184]. Non-HDL-c levels were calculated by subtracting HDL-c from total cholesterol. Blood samples were analyzed for hemoglobin A1c (HbA1c) using the DCA 2000 analyzer (Bayer Diagnostics, Tarrytown, NY). Estimated glucose disposal rate (eGDR, a measure of insulin sensitivity) was calculated using a regression equation derived from hyperinsulinemic-euglycemic clamp studies of 24 subjects chosen to represent the full spectrum of insulin resistance, represented by using insulin resistance risk factors: eGDR = 24.4 - 12.97(WHR) - 3.39(HTN) -0.60(HbA1c) [36].

Both urinary and serum albumin were measured by immunonephelometry [186, 187]. Serum creatinine was assayed using an Ectachem 400 Analyzer (Eastman Kodak Co, Rochester, NY). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation [252]: 175 x serum creatinine $(mg/dl)^{-1.154}$ x $age^{-0.203}$ x [0.742 if female] x [1.21 if black]. Renal function categories were created based on MDRD eGFR calculations as follow: No renal insufficiency ($eGFR \geq 90ml/min/1.73m^2$), mild renal insufficiency (eGFR 60-89), moderate insufficiency (30-59), severe insufficiency (15-29) and ESRD (<15). Cystatin C is measured turbidimetrically on an Olympus AU 640 using reagents purchased from DakoCytomation N. America, Inc. (Carpiteria, CA). In brief, samples are incubated at room temperature for 5 minutes with rabbit anti-cystatin C (antibodies coupled to

polystyrene particles). The increase in absorption at 540 nm is measured. Blanks, controls and standards (0.4 to 8.0 mg/L) are run simultaneously with all samples. The intra- and inter-assay coefficients of variation are 1.7% and 2.2%, respectively. Cystatin C was categorized as high if >1.0 and normal if <1.0 mg/l. Albumin excretion rates (AER) were calculated using urinary albumin levels from at least 2 validated timed sample collections. Degree of albuminuria was categorized as normo- (AER<20 μg/min), micro- (20-200 μg/min) or macro-albuminuria (> 200 μg/min). Those reporting a history of renal transplant or dialysis were considered to have a history of renal failure and were excluded from this analysis.

Of those EDC study participants examined at baseline (n=658), 22.7% (n=150) had died by the 18-year follow-up (November 2004 – November 2006), 78 had moved out of area, 101 declined exam, 19 were lost to follow-up leaving 318 eligible for 18 year exam, 309 for which data was available for this analysis. Pulse waveform analysis (PWA) testing began part way through the 18-year examination period (January 2006), after which 189 subjects were seen and 144 (76%) had PWA. The PWA population thus 144 men and women in attendance at the 18-year follow-up clinic visit after January 1 2006. Aortic augmentation index (AIx), aortic augmentation pressure (AP) and subendocardial viability ratio (SEVR) were derived using waveforms measured at the radial artery using the SphygmoCor Vx version 7.01 (AtCor Medical, Sydney, Australia). In brief, a high-fidelity micromanometer with a frequency response of >2 kHz (Millar Instruments, Houston, TX) was placed on the right radial artery, and gentle pressure was applied until a consistent waveform was produced. After at least 20 sequential waveforms had been acquired, measurement was stopped. Central pressure values were estimated from radial measurements using the software's mathematical transfer function [159, 189]; the accuracy and reliability of which have been validated [163, 172]. The pressure wave

created by left ventricular contraction propagates forward until meeting sites of resistance which reflect the wave backward. Stiffer artery walls result in earlier wave reflection [168, 171]. When the reflected wave returns during systole rather than diastole, systolic pressure is increased or "augmented". Augmentation pressure (AP) is a measure of how much the early reflected wave contributes to central systolic pressure. Augmentation index (AIx) represents the level of augmentation measured and is expressed as a percentage of the pulse pressure (AIx = AP/PP). Heart rate is inversely associated with AIx and AP [121]. Subendocardial viability ratio (SEVR), the ratio of the diastolic area under the curve (AUC) of an arterial pulse wave to the systolic-AUC [190, 191] is a ratio of myocardial perfusion (as coronary artery perfusion takes place primarily during diastole) to myocardial contraction, and is a tonometric, non-invasive measure of myocardial perfusion relative to cardiac workload. The SphygmoCor device provides a quality index (QI) which represents reproducibility of the waveform. If PWA produced results with a QI<80 the measure was repeated. Measures with a QI≥80 were included in this study.

Distributional characteristics and normality of variables were assessed. AP, AER, cystatin c were all non-parametric. Student's t-test (for paramentric) and the Mann-Whitney U test (for non-parametric) were used to compare continuous variables between two groups. One-way ANOVA (parametric) or Kruskal-Wallis tests (non-parametrtic) were use for comparisons among >2 groups and the χ^2 test was used for categorical variables. Pearson's and Spearman's correlations were used as appropriate. Linear and logisitic regression were performed in a stepwise manner for continuous and binary outcomes, respectively. A significance level of 0.10 was used as the cut-off for entry into models (due to limited sample size). All continuous variables were standardized by subtracting the mean and dividing the by their standard deviation of the study population. Models were adjusted for potential confounders of PWA measures

(height and heart rate for AIx and AP and heart rate only for SEVR). Age and sex were not available to multivariate models for MDRD eGFR as both of these factors are involved in its calculation. Analyses were completed using SPSS v15 for Windows.

5.4 RESULTS

Of the 144 EDC participants with PWA measures, 11 were excluded from present analyses due to history of renal failure (transplant or dialysis). Mean age±SD and T1D duration for the remaining 133 were 44.3±7.37 and 36.1±6.65. Of the 133, 130 had available AER measurements, variables were available for calculation of MDRD eGFR for 129, and 118 had Cystatin C measures.

5.4.1 Pulse Wave Analysis Measures and Microalbuminuria

Ninety-one of the 130 with AER measures were normoalbuminuric, 25 had microalbuminuria (MA) and 14 had macro. Systolic and diastolic blood pressures increased significantly with increasing albuminuria category as did HbA1c, WHR and, as expected so did serum creatinine, cystatin c and eGFR (Table 12). eGDR decreased with increasing albuminuria category. BMI, although showed a borderline significant, positive linear trend with albuminuria category, did not significantly differ among the groups.

AIx, did not significantly differ by albuminuria category, and did not show a significant linear trend (Table 12 and Figure 4). AP increased with increasing albuminuria category (p=.07) with a significant, positive, linear trend (p=.01) (Table 12 and Figure 5). SEVR differed

significantly by albuminuria category (p=.002) and showed a significant, negative, linear trend (p=.002) (Table 12 and Figure 6). In unadjusted, univariate correlations, only SEVR was correlated, negatively, with AER (r= -.35; p<.001). After adjustment for heart rate (and height for AP and AIx), SEVR remained significantly correlated with LnAER (r= -.22; p=.01) and LnAP was positively correlated (r=.28; p=.001) (Table 13).

To determine if PWA measures were associated with early renal damage, multivariate logistic regression was performed excluding those with macroalbuminuria. AP did not remain significantly associated with presence of MA in multivariate models. It also was not significantly associated with LnAER in linear regression analysis. However, each standardized unit decrease in SEVR was associated, multivariately, with a 65% increased risk for MA (Table 14). In fact, SEVR preferentially entered multivariate models for MA, over brachial SBP and DBP measures. Lower eGDR was also associated with presence of MA, multivariately (OR=0.35; 95%CI: 0.17-0.73; p=.005). Adjustment for PWD use or just ACEI/ARB use, did not significantly alter models and neither was significantly associated with MA. In linear regression for LnAER, among those within the normo- and micro-albuminuric range, SEVR was again significant and entered the model over SBP and DBP. Lower HDL-c was also associated with increased LnAER in this model (Table 15). Factors associated with increased LnAER in the total population (with macroalbuminuric participants included) were increased SBP and Non-HDL-c (data not shown).

5.4.2 Pulse Wave Analysis Measures and Renal Function

Participant characteristics by renal function categories, both eGFR and Cystatin C, are described in Table 16 and Figures 4 through 6 illustrate means (standard error) of AIx, AP and SEVR, respectively by renal function categories.

Modification of Diet in Renal Disease Estimated Glomerular Filtration Rate

AIx, AP and SEVR were all significantly correlated with MDRD eGFR in univariate and in heart rate and height adjusted correlations. (Table 13). Increased AIx (p=.09), AP (p=.03), age, SBP, WHR, AER, serum creatinine and Cystatin C accompanied decreased eGFR category (Table 16). SEVR, although showing a significant borderline linear trend (p=.07), declining with decrease in eGFR category, did not differ significantly between the groups (p=.17). Augmentation pressure showed, negative, linear trend (p=.005), as did AIx (p=.02), increasing with decreasing eGFR category (Table 16, Figure 5 and 4, respectively). A comparison between those low eGFR (<60) and those with normal to mildly impaired renal function (≥60) showed a significant difference in AIx (p=.02), AP (p=.006), but not SEVR (p=.13) between the two groups. However, multivariately, lower SEVR was associated with a 56% increased risk for low eGFR (<60), was selected over brachial SBP and DBP measures, and remained significantly associated with low eGFR when adjusted for heart rate and ACEI/ARB medication use. No other factors were significantly associated with eGFR in this model (Table 16). Adjustment for PWD use instead of ACEI/ARB use did not significantly alter the model. Neither AIx nor AP were significant in multivariate models for low eGFR. Linear regression for eGFR in the total population showed similar results for AIx, AP and SEVR. However, SBP diminished the statistical significance of the SEVR-eGFR relationship in that model.

Cystatin C

Cystatin C was significantly correlated with AP and SEVR, but not with AIx, in univariate and heart rate and height adjusted correlations (Table 13). Those with high Cystatin C

(≥1.0) did however have significantly higher AIx (28.1±12.1 vs. 21.3±10.6; p<.05, Figure 4) and AP (13.6±9.98 vs. 7.71±4.88; p<.01, Figure 5), compared to those with normal Cystatin C levels (<1.0). SEVR, although about 10 units lower in those with higher Cystatin C, was not significantly different between the groups (Figure 6). Other factors univariate associated, with high Cystatin C were older age (but not longer diabetes duration), lower eGDR, higher SBP (but not DBP), and expectedly, higher AER , serum creatinine and eGFR (all p<.001). Results of logistic regression for Cystatin C were similar to those seen for low eGFR. AP, although significant when heart rate and height adjusted only, did not remain significant multivariately. In multivariate models for high Cystatin C with SEVR available; for each standardized unit decrease in SEVR there was a 69% increased risk for high Cystatin C (Table 18). A model with brachial SBP and DBP was also run in which both SEVR and SBP were significantly associated with high Cystatin C. However, the model with hypertension status was more significant that with SBP. Adjustment for PWD use instead of ACEI/ARB use did not substantially change the model. Neither age, nor sex, entered models for Cystatin C.

5.5 DISCUSSION

The prominent findings of the present study are that pulse wave analysis measures, augmentation pressure (an index of arterial stiffness) and subendocardial viability ratio (an estimate of myocardial perfusion) are associated with both renal damage, even at the microalbuminuric level, and with poor renal function, in our Type 1 diabetes population. The relationship SEVR had with both renal function and renal damage remained present in multivariate models, and SEVR was preferred over brachial SBP and DBP as a predictor of albumin excretion rate,

microalbuminuria in those with no to mild renal damage. It was also preferred over SBP and DBP in the multivariate model for high Cystatin C and that for low eGFR.

Univariately, AIx was not associated with AER (continuously or categorically), was only borderline significant in its associated with eGFR (continuously and categorically) and was associated with high Cystatin C compared to normal but not with Cystatin C continuously. In the Hoorn Study, there were significant increases in aortic AIx with increasing albuminuric quartile (measured using urinary albumin-to-creatinine ratio (ACR) in a general population[253]. However, after adjusting for age, sex, glucose tolerance status and mean arterial pressure, AIx was no longer significantly associated with ACR. This study also found no significant relationship between AIx and eGFR (also MDRD calculated), which is consistent with the findings of the present study. It is important to note, however that The Hoorn Study did find an association between other measures of vascular stiffness and both ACR and eGFR. In the present study, higher augmentation pressure was associated with higher AER and higher albuminuric category and with eGFR and Cystatin C. The difference in the associations of AIx and AP with renal damage and renal function may be due to the notion that AIx has limitations in its use as an arterial stiffness index due to its calculation: AP ÷ PP. Simultaneous rises in both AP and PP can result in a stable AIx, thereby reducing its usefulness as a surrogate for change in central pressure waveforms [254]. This limitation is especially apparent in older populations, for which AP may be a more suitable measure of arterial stiffness [202], and may be pronounced in T1D populations due to higher pulse pressures and accelerated vascular aging [153, 254].

To date, no studies have examined the relationship between SEVR and renal function or renal damage. Our finding suggest that, estimated myocardial perfusion is reduced in those with greater damage and reduced function. This is even true when comparing microalbumuric T1D

participants to those within the normal range. This finding is consistent with the fact that albuminuria, even at the micro level, is associated with CAD in T1D and other populations [54, 67, 76, 255, 256].

In our renal function analyses, we looked at both eGFR and Cystatin C due to the current use of eGFR in clinical practice and due to recent findings that Cystatin C may be superior to serum creatinine in assessing GFR, especially in T1D populations[257-259]. AP was associated with eGFR and Cystatin C, but the addition of SBP to the multivariate models eliminated the statistical significance of the association. This is not surprising since AP represents the increase of central systolic blood pressure due to early return of the reflected pulse wave which would be represented in an increase in brachial SBP. In the absence of brachial BP measures, AP remains significantly associated with eGFR and Cystatin C. Yoshida et al. found an association, albeit weak, between increased brachial-ankle PWV, another measure of arterial stiffness, and eGFR in those with normal to mild (MDRD calculated eGFR $\geq$60 but less than 90 ml/min/1.73 m^2) renal function impairment[260]. Wang et al found a stepwise increase in PWV with decreasing CKD category[248]. Although, we also found an association between arterial stiffness as measured with AP, and renal function, the only significant association was found when comparing those with normal and mild impairment to those with moderate to severe impairment. In other regression models, linear in the entire or portions of the population (no to mild impairment, moderate to severe) and logistic for mild impairment compared to normal function, neither AP nor SEVR was associated with the eGFR outcomes.

Chade et al. showed that low eGFR (MDRD calculated <60 ml/min/1.73 m^2) was univariately associated with coronary microvascular dysfunction (defined as coronary flow reserve (CFR) <2.5, evaluated using intracoronary adenosine)[261]. This is consistent with our

finding that SEVR is associated with eGFR. However, in Chade et al's multivariate analysis, the association did not remain significant. The attenuation of the association between renal function and reduced CFR was adjustment for age and gender. In our own multivariate analysis detailed in Table 17, we did not make age or sex available to the eGFR models. Since age and sex are used in the calculation of MDRD eGFR, adjustment for these factors is redundant. When we ran models allowing for these two variables, they did enter the models with over inflated odds ratios. By not allowing age and sex to distort the analysis, we find that SEVR is associated with renal function as measured by eGFR, which is confirmed by the analysis for Cystatin C which did allow for both age and sex and SEVR was significantly related to high Cystatin C.

In summary, higher augmentation pressure and lower subendocardial viability ratio are associated with both renal damage and renal function in T1D. Of great importance is the positive relationship between SEVR in those with no or mild renal damage, during which SEVR was a better predictor of AER than SBP. These findings underscore the importance of measuring and understanding more aspects of the entire pulse wave rather than only brachial SBP and DBP. Pulse wave analysis can be quickly and easily measured in a clinical setting and can provide additional information for renal complication risk stratification than SBP and DBP alone. Early medication intervention to reduce arterial stiffness in those with higher risk such as with ACEI's or ARB's may prevent renal complications in T1D. Additional, prospective research should be completed to confirm these cross-sectional findings.

Table 12. Characteristics of The Pittsburgh EDC Pulse Wave Analysis population by Albuminuria Category

	Normo (n=91)	Micro (n=25)	Macro (n=14)	Total (n=130)
Augmentation Index %	22.1±11.0	21.1±10.1	26.1±12.1	22.9±11.0
Augmentation Pressure (mmHg)[b]**	7.54±4.62	8.84±7.13	13.7±10.4	8.90±6.5
Subendocardial Viability Ratio (%)[b*]**	149.3±26.5	126.7±30.3	131.6±35.6	142.0±31.3
Age (years)	43.3±6.57	46.8±8.69	44.2±7.41	44.5±7.30
Diabetes Duration (years)	35.2±6.09	39.1±7.38	35.1±6.77	36.2±6.71
Sex (% male)	46.2	60.0	57.1	48.9
HbA1c (%)[c*]**	7.33±1.15	8.00±1.70	8.28±1.30	7.56±1.33
eGDR (mg/kg/min)[d**]**	8.33±1.97	6.82±2.27	5.56±2.21	7.74±2.26
Systolic Blood Pressure (mmHg)[d**]**	109.3±11.5	115.7±13.1	139.5±15.5	115.1±15.9
Diastolic Blood Pressure (mmHg) [c**]**	65.4±8.23	63.3±9.12	75.6±11.7	66.4±9.22
Heart Rate (bpm)	76.7±13.0	82.6±12.4	79.9±14.0	77.8±13.0
Non-HDL-c (mg/dL)	111.1±27.3	107.0±34.0	144.9±52.8	114.0±33.0
HDL-c (mg/dL)	60.3±16.7	55.5±15.8	57.115.9	59.2±16.5
Body Mass Index (kg/m^2)[a]	26.7±4.34	27.3±4.89	28.9±3.99	27.1±4.50
Waist-to-Hip Ratio[b*]**	0.85±0.09	0.91±0.09	0.90±0.7	0.87±0.09
Serum Creatinine(mg/dL)[d**]**	0.93±0.17	1.12±0.42	1.32±0.39	1.08±0.54
eGFR (ml/min/1.73 m^2) [d**]**	78.4±15.1	69.7±19.9	56.7±18.0	74.5±17.7
Cystatin C[d**]**	0.80±0.12	0.98±0.34	1.24±0.50	0.95±0.43

Abbreviations: estimated glucose disposal rate, eGDR; estimated glomerular filtration rate, eGFR. *<.10, **<.05, ***<.01, ****<.001.

[a]significant linear trend <.10

[b]significant linear trend <.05

[c]significant linear trend <.01

[d]significant linear trend <.001

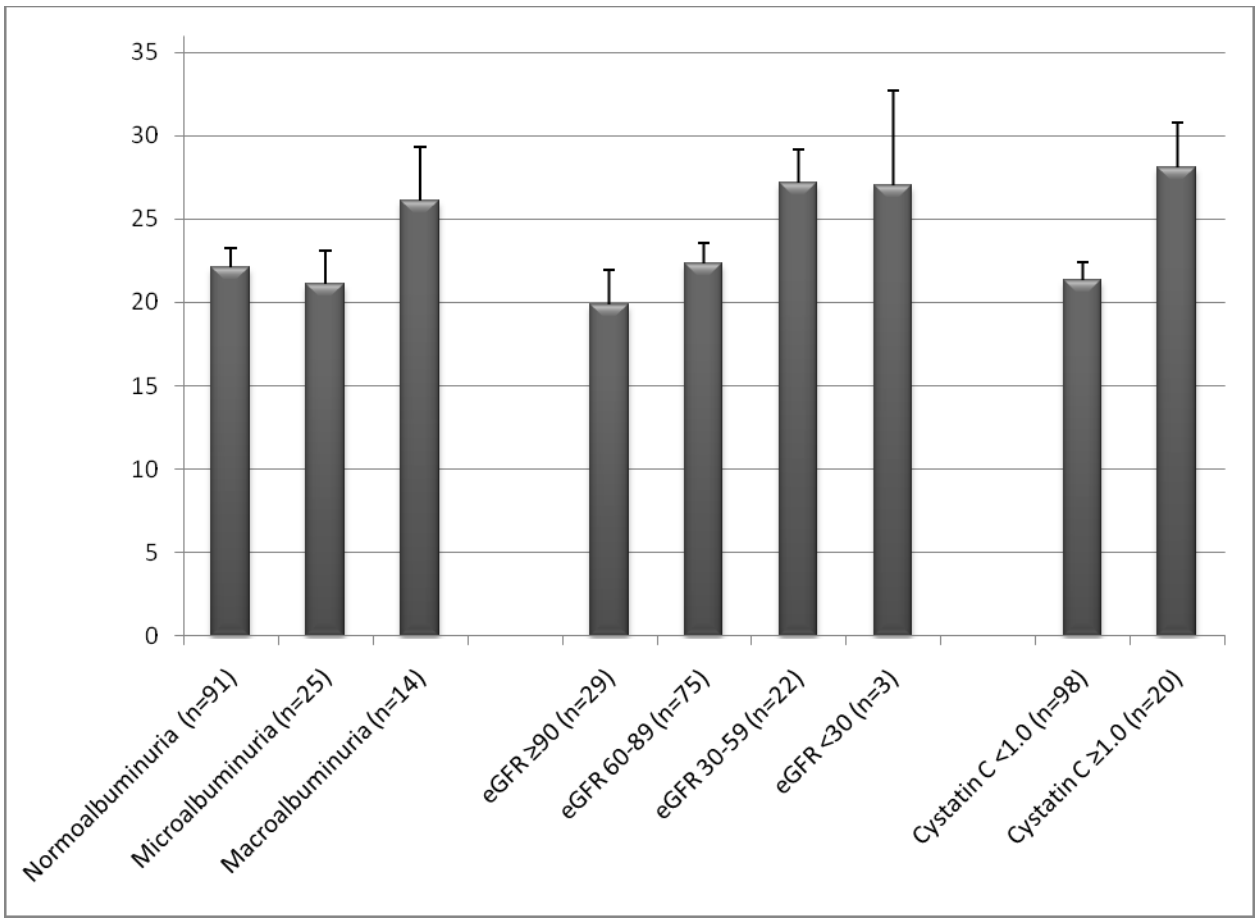

Figure 4. Augmentation Index (mean and standard error) by albuminuria and renal function categories in The Pittsburgh EDC Pulse Wave Analysis Population

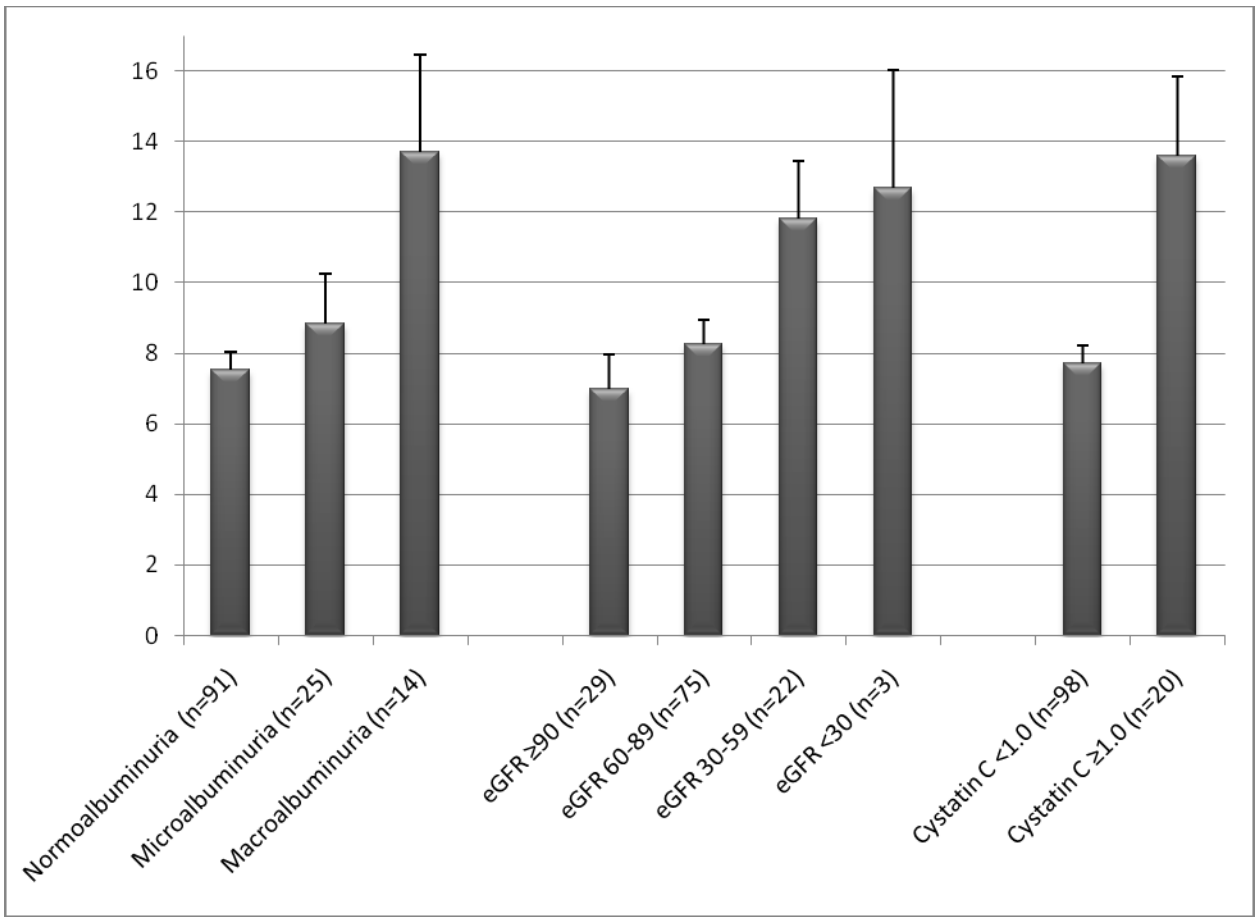

Figure 5. Augmentation Pressure (mean and standard error) by albuminuria and renal function categories in The Pittsburgh EDC Pulse Wave Analysis study population

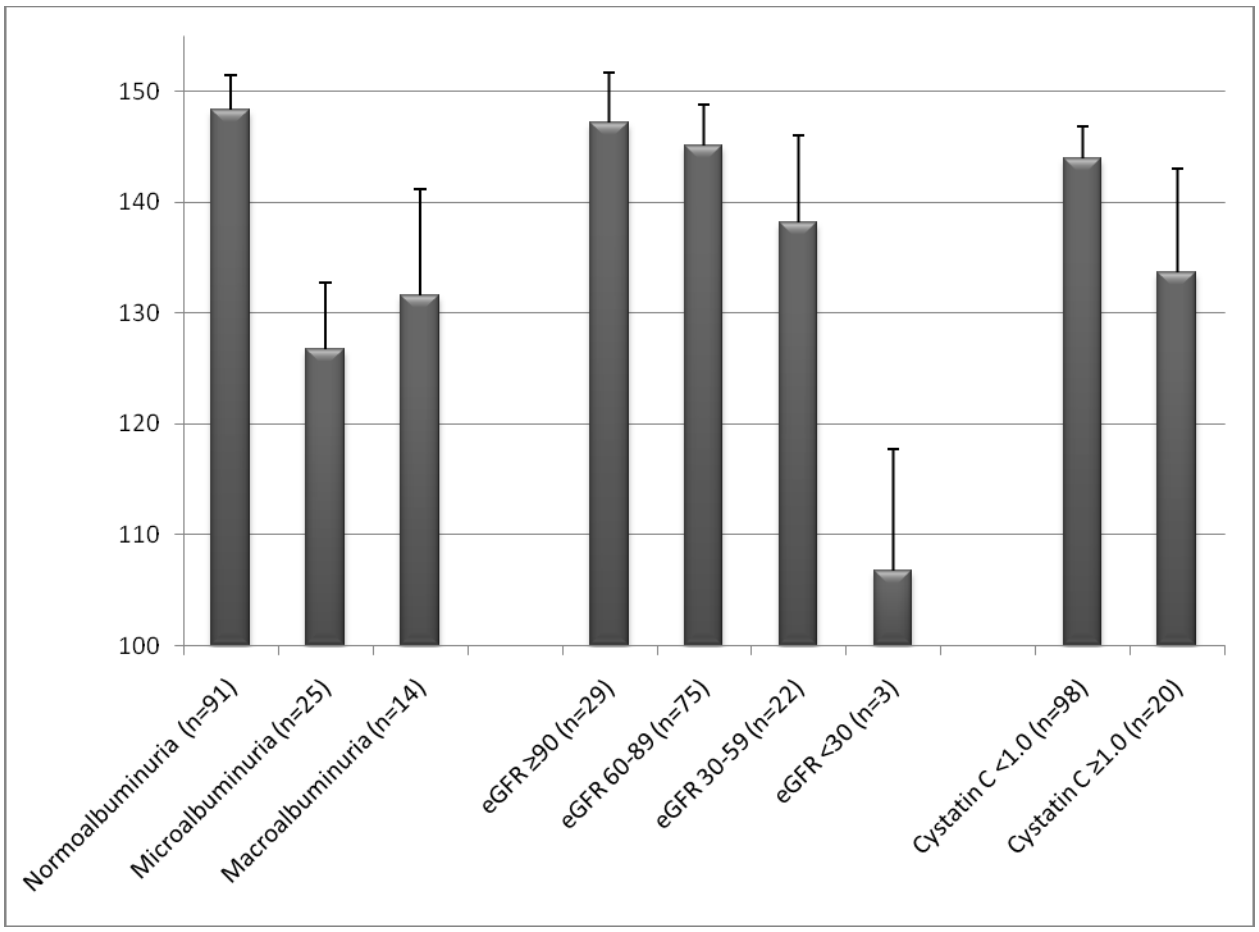

Figure 6. Subendocardial Viability Ratio (mean and standard error) by albuminuria and renal function categories in The Pittsburgh EDC Pulse Wave Analysis study population

Table 13. Bivariate correlations and heart rate adjusted partial correaltions between Augmentation Index, Augmentation Pressure, Subendocardial Viability Ratio and Albumin Excretion rate, Estimated Glomerular Filtration Rate and Cystatin C in The Pittsburgh EDC study

	Albumin Excretion Rate (μg/min)	MDRD eGFR (ml/min/1.73 m^2)	Cystatin C (mg/l)
AIx	-.074	-.210**	.108
AP	.050	-.252***	.209**
SEVR	-.354****	.212**	-.186**
Heart Rate Adjusted Correlations			
	LnAER[b]	**MDRD eGFR**	**Cystatin C**
AIx	.138	-.175**	.170
LnAP[a,b]	.281***	-.249***	.273***
SEVR[a]	-.223**	.257***	.283***

Abbreviations: Modification of Diet in Renal Disease, MDRD; estimated glomerular filtration rate, eGFR; Augmentation Index, AIx; Augmentation Pressure, AP, Subendocardial Viability Ratio, SEVR.

*<.10, **<.05, ***<.01, ****<.001.

[a]also height adjusted, [b] Due to use of Pearson's partial correlations, AER was natural logarithmically transformed because non-parametric

Table 14. Logistic regression model for microalbuminuria in The Pittsburgh EDC Pulse Wave Analysis study population

Variable	OR	95%CI	p
Subendocardial Viability Ratio	0.35	0.17-0.73	.005
Estimated Glucose Disposal Rate	0.52	0.30-0.89	.017
Heart Rate	0.72	0.38-1.38	.32

Odds Ratios are per standardized unit.

Variables available to the model: age, subendocardial viability ratio systolic blood pressure, diastolic blood pressure, waist-to-hip ratio, estimated glucose disposal rate, HDL-cholesterol, Non-HDL-cholesterol, Smoking history, ACE inhibitor/Angiotensin II receptor blocker use, heart rate, body mass index, anti-lipidemic agent use

Table 15. Linear regression model for albumin excretion rate in those within the normal and micro-albuminuric range.

	β	standard error	p
Subendocardial Viability Ratio	-11.3	3.71	.003
HDL-c	-6.56	2.64	.015
Heart Rate	-5.76	3.68	.13

All variables are standardized to the population
Variables available to the model: age, subendocardial viability ratio systolic blood pressure, diastolic blood pressure, waist-to-hip ratio, estimated glucose disposal rate, HDL-cholesterol, Non-HDL-cholesterol, Smoking history, ACE inhibitor/Angiotensin II receptor blocker use, heart rate, body mass index, anti-lipidemic agent use

Table 16. Cross-sectional characteristics by renal function categories in The Pittsburgh EDC Pulse Wave Analysis Study Population

	Estimated Glomerular Filtration Rate				Cystatin c	
	>90 (n=29)	**60-89 (n=75)**	**30-59 (n=22)**	**15-30 (n=3)**	**<1.0 (n=98)**	**≥1.0 (n=20)**
Augmentation Index	19.9±10.8[b*]	22.3±10.8	27.2±9.38	27.0±9.85	21.3±10.6**	28.1±12.1
Augmentation Pressure	7.00±5.11[c**]	8.24±5.97	11.8±7.71	12.7±5.78	7.71±4.88***	13.6±9.98
Subendocardial Viability Ratio	147.2±24.1[b]	143.1±32.2	138.2±36.4	106.7±19.1	143.9±29.4	133.6±42.1
Age	40.6±4.37[b****]	44.2±7.83	49.6±5.98	47.7±6.20	43.7±7.12***	49.2±6.58
Diabetes Duration	33.9±5.34[c***]	35.2±6.70	40.1±7.02	33.2±6.07	36.0±6.33	37.9±7.20
Sex (% male)	58.6[*]	53.3	27.3	33.3	55.1	40.0
HbA1c	7.71±1.62	7.52±1.27	7.41±0.97	7.53±1.32	7.53±1.28	7.56±1.34
Estimated Glucose Disposal Rate	7.88±2.36	7.93±2.09	7.54±2.11	5.37±3.08	7.94±2.09**	6.64±2.51
Systolic Blood Pressure	108.5±13.4[c***]	114.4±14.3	118.2±20.7	136.7±9.50	112.4±14.3**	124.3±19.3
Diastolic Blood Pressure	67.2±7.07	65.7±9.30	65.3±11.0	75.7±16.3	65.6±8.42	66.7±13.9
Heart Rate	78.0±13.1	79.1±13.7	74.7±10.6	86.7±8,08	79.0±12.9	74.2±13.1
Non-HDL-c	111.3±28.1	116.3±33.1	109.6±37.44	100.0±51.1	111.5±31.9	119.2±40.8
HDL-c	59.3±17.2	60.0±16.7	57.5±15.8	47.7±6.43	58.6±16.3	57.2±16.1
Body Mass Index	26.5±4.46[a]	26.9±4.39	28.5±4.86	29.3±3.16	26.7±4.26	27.1±4.20
Waist-to-Hip Ratio	0.86±0.07[b***]	0.86±0.09	0.88±0.10	0.94±0.13	0.87±0.09	0.90±0.10
Albumin Excretion Rate	9.73±12.9[d****]	129.4±442.3	195.4±396.9	646.9±734.8	96.7±385.8****	669.1±1563.1
Serum Creatinine	0.80±0.10[d****]	0.97±0.13	1.24±0.25	2.33±0.51	0.94±0.16****	1.42±0.50
Cystatin C	0.74±0.08[d****]	0.82±0.12	1.09±0.24	1.87±0.25	--	--
MDRD eGFR	--	--	--	--	78.9±14.1****	49.0±14.6

Comparisons between MDRD eGFR categories: *<.10, **<.05, ***<.01, ****<.001.
[a]significant linear trend <.10, [b]significant linear trend <.05, [c]significant linear trend <.01, [d]significant linear trend <.001

Table 17. Multivariate logistic regression model for low estimated glomerular filtration rate (eGFR<60) in The Pittsburgh EDC Pulse Wave Analysis Study

	Odds Ratio	95%CI	p
Subendocardial Viability Ratio	0.44	0.22-0.89	.02
Heart Rate	0.42	0.20-0.88	.02
ACEI/ARB Use	3.57	1.18-10.8	.02

All variables are standardized to the population and odds ratios are expressed as per standardized unit.

Variables available to the model: subendocardial viability ratio, systolic blood pressure, diastolic blood pressure, hypertension status (BP>130/80 or use of medication to reduce blood pressure), waist-to-hip ratio, estimated glucose disposal rate, HDL-cholesterol, Non-HDL-cholesterol, Smoking history, ACE inhibitor/Angiotensin II receptor blocker (ACEI/ARB) use, heart rate, body mass index, anti-lipidemic agent use

Table 18. Multivariate logistic regression model for high Cystatin C (≥1.0 mg/l) in The Pittsburgh EDC Pulse Wave Analysis Study

	Odds Ratio	95%CI	p
Subendocardial Viability Ratio	0.31	0.13-0.73	.007
Heart Rate	0.20	0.07-0.57	.003
Hypertension	6.35	1.88-21.5	.003
ACEI/ARB Use	5.65	1.21-26.4	.03

All variables are standardized to the population and odds ratios are expressed as per standardized unit.

Variables available to the model: subendocardial viability ratio, hypertension status (BP>130/80 or use of medication to reduce blood pressure), waist-to-hip ratio, estimated glucose disposal rate, HDL-cholesterol, Non-HDL-cholesterol, smoking history, ACE inhibitor/Angiotensin II receptor blocker (ACEI/ARB) use, heart rate, body mass index, anti-lipidemic agent use

6.0 FINAL DISCUSSION

6.1 SUMMARY AND CONCLUSIONS

This is the first study to assess potential risk factors (gathered 18 years earlier) for abnormal pulse waveform measures (i.e. augmentation index (AIx) and augmentation pressure (AP)) and decreased subendocardial viability ratio (SEVR). It is also the first to examine the association between these measures and prevalent cardiovascular and renal disease in a T1D population. These data document important associations of autonomic neuropathy with both arterial stiffness (AIx and AP) and estimated myocardial perfusion (SEVR). They also show that the relationships between measures of arterial stiffness and cardiovascular disease are complex due to age, diabetes duration and the pervasive use of various medications. It also showed that renal function and renal damage, even within the normo- and microalbuminuric range, are associated with a reduced SEVR and increased AP.

6.2 GENERAL FINDINGS

6.2.1 Participant Characteristics

The aim of this study was to understand the arterial stiffness measure relationships to complications and risk factors within a population not previously well examined in this regard, those with type 1 diabetes. Therefore, analysis was completed in The Pittsburgh Epidemiology of Diabetes Complications Study, a prospective study in a population with childhood onset Type 1 diabetes that has been followed for approximately 18 years. At the time of pulse wave analysis, the population involved in this aspect of the study was comprised of 73 female and 71 male participants. Males had higher blood pressures, lower estimated glucose disposal rate (insulin sensitivity), lower HDL-c, better renal function (lower eGFR), but more renal damage (higher AER), and a greater percentage with coronary artery disease (Table 19). Compared to the remaining EDC population (those without PWA measures who attended the 18 year follow-up clinic visit), our PWA population had higher mean heart rate, smaller waist-to-hip ratio and lower albumin excretion rate. A greater percentage of those not in the PWA study had CAD compared to those included (30.9% versus 23.1), although this difference did not reach statistical significance. Due to these differences, our PWA may represent a somewhat healthier portion of the overall EDC study population at the 18 year follow-up. An important factor included in the analyses was the reported use of medications potentially affecting PWA measures. ACE inhibitors (ACEI), angiotensin receptor blockers (ARB), beta-blockers, calcium channel blockers and nitrates may exhibit some effect on arterial stiffness indices [179] and coronary artery perfusion[262-264]. Of the 144 PWA participants, 90 (62.5%) were on at least one of the

aforementioned medications, especially use of ACE inhibitors, reported by 55% (n=79). Another 14.7% (n=21) reported ARB use, 5 of which were also using an ACEI resulting in 56% (n=80) of the PWA population taking either an ACEI or ARB. Wide use of ACEI/ARB medications in the present study population is not surprising due to their effectiveness in treating hypertension and the known protective effect against renal damage [265, 266]. Both ACEI and ARB medications are also shown to be effective in reducing arterial stiffness [267]. Both act on the renin-angiotensin-aldosterone system (RAAS), the system responsible for the regulation of blood pressure. ACEI's inhibit the conversion of angiotensin I to angiotensin II (AngII). AngII causes vasoconstriction, stimulates the release of aldosterone (acts on kidney to increase sodium retention and potassium excretion) from the adrenal cortex and stimulates the release of vasopressin (aka: anti-diuretic hormone; acts on kidneys to increase water retention). All of these AngII actions work to increase blood pressure therefore it follows that ACE inhibition would cause BP reductions. ARB's, on the other hand, block the action of AngII thereby reducing blood pressure as well. ACEI have been shown to reduce arterial stiffness independent of BP reduction[268] as has ARB use [269]; use of ACEI in conjunction with ARB shows synergistic and possibly additive effects on systemic arterial stiffness[270] and in coronary artery perfusion [263]. Beyond the aforementioned effect of ACEI on blood pressure, in animal models treatment with ACEI is shown to cause reduction in size of the medial (muscular) layer of arteries due to reversal of smooth muscle hypertrophy [271] and to decrease the collagen (but not elastin) density in arteries [272] both of which contributed to reductions arterial stiffness seen in ACEI treatment. ACEI and ARB are also shown to improve endothelial function by increasing the bioavailability of nitric oxide (NO)[273], a potent vasodilator and regulator of vascular tone.

6.2.2 Risk Factors for Pulse Wave Analysis Measures

Previous studies have suggested that indices of arterial stiffness are significantly higher in those with T1D and SEVR is significantly lower, compared to non-diabetic controls [9, 10, 169]. Duprez et al. studied associations between pulse wave analysis measures and traditional cardiovascular risk factors in a non-diabetic population (69% male; mean age 50±12 years) with low cardiovascular risk (determined via Framingham Risk Score). The study results showed that both AIx and AP were significantly and positively associated with age, SBP, pulse pressure and Framingham Risk Score (comprised of age, sex, smoking history, BP, total and HDL cholesterol levels and blood glucose or history of diabetes) in both men and women. DBP and mean arterial pressure (MAP) as well as total and HDL cholesterol levels were also positively associated with AIx and AP in women, but not in men. Interestingly, in the male participants of Duprez et al study, total cholesterol, LDL-c, and BMI were all significantly and negatively associated with AIx and AP. This was the only published study available that has examined potential risk factors for PWA measures; it was in healthy, non-diabetic persons of middle age (50±12), was cross-sectional in nature and looked only at univariate correlations. In order to understand what factors contribute to increased AIx and AP and decreased SEVR in a T1D population, the present research examined the relationship between factors at baseline and the follow-up measures. We too found that AIx and AP were univariate associated with Non-HDL-c (but not HDL-c), systolic and diastolic BP in cross-sectional analyses (Table 20). However, in the adjusted model for AIx, only ever smoker status, DBP, and estimated glucose disposal rate were significant while only ever smoker status and SBP were independently associated with AP. Of greater interest are the significant, independent associations found between baseline HDL-c, E/I ratio and ever smoker status and both AIx and AP, as well as the positive association between baseline HbA1 and AP

in our adjusted models. Arterial stiffness indices and SEVR are significantly affected by age [104, 274]. They also tend to be higher in women compared to men[275], which is partially, but not entirely, due to shorter stature [276] since height is inversely related to timing of pulse wave reflection [120]. Pulse wave reflection, specifically, is also dependent on heart rate. Wilkinson et al showed that there is an inverse linear relationship between heart rate and AIx/AP. This relationship occurs because faster HR decreases the absolute duration of systole shifting the occurrence of reflected wave return to diastole, reducing central pressure augmentation [121]. Due to the potential confounding effects of age, sex, height and heart rate, the final models relating other potential risk factors were adjusted for these variables. Resulting models showed that the prominent risk for all 3 measures was reduced ECG monitored deep breathing expiration-to-inspiration ratio, a measure of heart rate variability. Lower E/I ratios are indicative of autonomic neuropathy [277]. Ahlgren et al showed similar results relating E/I to ultrasound derived aortic stiffness measures in women with T1D in cross-sectional analyses. Taskiran et al showed that in T1D, the presence of autonomic neuropathy was associated with decreased myocardial perfusion reserve as measured using perfusion reserve index assess from MRI during induced vasodialation. This finding is in agreement with that from the present study showing AN to be associated with SEVR. Autonomic neuropathy involves damage to the nerves that comprise those from the brain and spinal cord to organs such as the heart and to blood vessels. Our finding, that a lower E/I ratio was independently predictive of higher AIx and AP and lower SEVR measured some 18 years later is particularly important as it suggests that neutrally mediated changes in vascular function may underlie the pathophysiology of increased vascular stiffness. However, our findings also show that both having a history of cigarette smoking and poor glycemic control (higher HbA1) at baseline were also predictive of AP and SEVR.

Smoking is a known risk factor for cardiovascular disease [278] and has previously been shown to be associated with arterial stiffness measures[129, 200, 209, 279, 280]. Rehill et al. suggest that the connection between smoking and systemic arterial stiffness is impaired basal arterial tone, an index of endothelial dysfunction [280]. Smoking is associated with reduced bioavailability of nitric oxide (NO), which is synthesized in the endothelium of arteries and causes vasodilatation and contributes to resting arterial tone and smooth muscle cell proliferation [281]. The relationship between AP and history of cigarette smoking therefore is biologically plausible and consistent with findings in prior research. The relationship between SEVR and smoking history is also consistent as AP affects SEVR and according to Guo et al, cigarette smoke affects the wall properties of not only peripheral arteries, but also coronary arteries [282]. Alterations in the extracellular matrix of the media and adventitia of arteries have also been shown to be associated with increased arterial stiffness [283, 284]. These alterations can be due to advanced glycosylation end-product (AGE) accumulation on matrix proteins due to poor glycemic control [285]. Therefore, our finding that higher baseline HbA1 is associated with increased AP may be due to the influence of AGE's . Glycemic control has also been shown to be associated with incident coronary artery disease events in T1D [71, 256], which is in agreement with the association between poorer control at baseline and higher follow-up SEVR in the current study. The present study also showed that lower HDL-c was associated with higher AP and AIx. As low HDL-c is a known risk factor for atherosclerosis, it is therefore reasonable that indices are arterial stiffness are elevated as atherosclerosis contributes to increased stiffness. The results of the present research suggest that risk factors for increased arterial stiffness include those affecting functional (AN) as well as structural (oxidative stress due to smoking, AGE accumulation due to poor glycemic control, and atherosclerosis due to low HDL-c) aspects of

arteries and that these factors, along with increased peripheral arterial stiffness, also contribute to reduced myocardial perfusion.

6.2.3 Pulse Wave Analysis and Prevalent Cardiovascular Disease (CVD)

We are not aware of any study to date that has explored the association between pulse wave analysis measures and prevalent or incident CVD in T1D. Numerous studies have however shown PWA measures to be higher in populations that have greater risk for CVD including those with diabetes [9, 169, 286] and those with renal disease [117, 246, 248]. A few studies have published on the relationship between these measures and CVD outcomes either incident or prevalent. Those that have been done have not been in T1D. Weber et al. completed PWA in male patients undergoing coronary angiography for either diagnosis or exclusion of coronary artery disease (CAD) finding that higher AIx and AP were univariately (4^{th} vs. 1^{st} quartile) associated with presence of CAD. However, multivariate analysis for CAD was completed only with the AIx variable showing that it was also multivariately (controlling for age, height, hypertension status, HDL-c and ACEI, beta-blocker and statin use) associated with the prevalent CAD[211]. Both AP and AIx were also shown to be independent predictors of major adverse cardiovascular events and cardiovascular mortality in those with preexisting CAD [251]. The findings of the present study show that AIx was not significantly associated with prevalent CAD (any, hard or soft) but that increased AP was associated with presence of hard CAD when those reporting nitrate use were excluded. For both AIx and AP as well as for SEVR, the addition of age diminished the statistical significance of their associations with the CAD outcomes. As previously discussed, AP increases with age and SEVR decreases with age. In type 1 diabetes, especially in childhood onset T1D, age and diabetes duration are highly correlated. Type 1

diabetes has been shown to be associated with accelerated vascular aging [153] therefore, age, essentially representing both age and diabetes duration in those with childhood onset T1D, seems to be the prominent factor associated with increased arterial stiffness, reduced coronary artery perfusion and CAD, so much so that it is difficult to decipher the relationship in this cross-sectional analysis. Due to the limited sample size, stratification by shorter and longer duration or older and younger age did not shed any light on this issue. In this T1D population, age may better represent vascular age than in non-diabetic populations. Therefore, the addition of age to multivariate models and the subsequent reduction in statistical significance of AP and SEVR does not deem the relationship between AP and CAD, and that between SEVR and CAD, biologically insignificant.

Another interesting finding in this CAD analysis was the impact of medications. Those with soft CAD had significantly lower SEVR than those without, yet those with hard CAD were comparable to those without CAD. A potential explanation for this is the more pervasive use of medication in those with hard CAD. In fact, the percentage of those with soft CAD on ACEI/ARB (40%) was less than in those without CAD (55.5%). A greater percentage, 61.1%, of those with CAD reported use of at least to different PWD medications compared to 20.0% of those with soft CAD and 12.7% of those without CAD. This finding suggests that a more pervasive use of medications and the use of multiple medications in those with hard CAD are effective in reducing arterial stiffness and improving myocardial perfusion. One type medication that seemed very influential, at least on AP, was nitrate use even though only 5 participants reported use. Those taking nitrate medications had a mean±SD AIx of 20.1±18.1, AP of 8.80±10.2 and SEVR of 142.2±21.9 compared to 12.1±10.7, 9.04±6.397 and 142.2±31.5 in those not on nitrate medication, respectively. These differences did not reach statistical significance of

course, as there were only 5 persons on nitrate medication. However, it is interesting that although 4 of them had Hard CAD the means of the arterial stiffness indices were lower than the remainder of the population and that these means were also comparable than those for the non-CAD participants (AIx: 22.4±9.82; AP:8.09±4.67; SEVR: 144.5±32.4).

The presence of high CAC score was associated with a significant increase in AP (10.9±8.25 vs. 7.98±5.03). Coronary artery calcification was also associated AP and with SEVR univariately however, the relationship between AP and high CAC score did not remain significant multivariate, again due to the addition of age. However, lower SEVR remained significantly associated with high CAC once those taking nitrates were excluded from analyses. This finding is consistent with that by Hata et al that CAC is a predictor of ischemic heart disease [221] and makes biological sense in that the greater the burden of calcification/atherosclerosis in coronary arteries the worse the perfusion of the heart. Finally, both AP and SEVR were associated with presence of low ABI. This finding is consistent with the fact that PWA measures are of reflected waves which are reflected back to the aorta from the periphery. If follows that the presence of vascular disease in the lower extremities would cause earlier timing of the reflected wave.

6.2.4 Pulse Wave Analysis Measures and Renal Damage and Function

As previously mentioned, autonomic neuropathy was associated with all of the PWA measures examined in this study. Interesting, Maguire, et al found that autonomic nerve testing predicted the development of microalbuminuria at 12 year follow up in a cohort of adolescents with T1D[287]. Our finding that AP and SEVR were related to albuminuria is consistent with those of Maguire et al. Chico et al found that autonomic neuropathy was associated with silent

myocardial ischemia especially in the presence of microalbuminuria[288] which is in agreement with the association between AN and SEVR. It also is consistent with the proposed connection between all aspects of this study which will be discussed shortly.

While renal function relates to the ability of the kidneys to filter the blood, renal damage is reflected by the excretion of protein (albumin) in urine at concentrations which would not normally be excreted. Albuminuria is a widely recognized and potent risk factor for coronary artery disease in those with diabetes [54, 67, 255]. It has been shown, that renal damage typically precedes renal function loss in The Pittsburgh EDC population[289], though occasionally reduced renal function is seen without preceding albuminuria. The strongest association found in this renal analysis was the association between SEVR and albuminuria, specifically at the low end. However, SEVR was also related to presence of poor renal function by both methods used to estimate renal function, MDRD eGFR and Cystatin C levels. The pathophysiology behind albuminuria-cardiovascular disease connection may involve arterial stiffness. Greater stiffness could potentially cause increased rate of blood flow to the kidneys resulting in kidney damage. This damage in turn causes an increase in blood pressure which contributes to further increases in arterial stiffness. It may follow that earlier and earlier return of reflected waves due to arterial stiffness cause a decrease in time in diastole, thereby reducing coronary artery perfusion resulting in coronary ischemia and/or infarction.

6.2.5 The Connections

The proposed relationship between autonomic neuropathy, arterial stiffness indices and subendocardial viability ratio, kidney damage and coronary artery are illustrated in Figure 1.

Autonomic neuropathy causes vascular dysfunction reducing the ability to regulate vascular tone. This factor causes an increase in arterial stiffness and perhaps increased or more rapid blood flow to the kidneys causing kidney damage. Kidney damage results in higher blood pressure levels further increasing arterial stiffness. Autonomic neuropathy (AN) is also associated with impaired ability to regulate heart rate. Inability to properly regulate HR is most likely why AN was associated with lower SEVR, because this inability may result in reduce the time in diastole.

The notion that arterial stiffness, due to either functional or structural changes in arteries caused by autonomic neuropathy and smoking, poor glycemic control or hypertension, causes kidney damage which in turn increases arterial stiffness which contributes to even greater increases in blood pressure (particularly systolic), may be the explanation as why AP was related renal outcomes until the addition of SBP to multivariate models. Greater decreases in SEVR, on the other hand, are most likely due to prolonged stiffness that results in isolated systolic hypertension and may be why SEVR maintains its statistical significance.

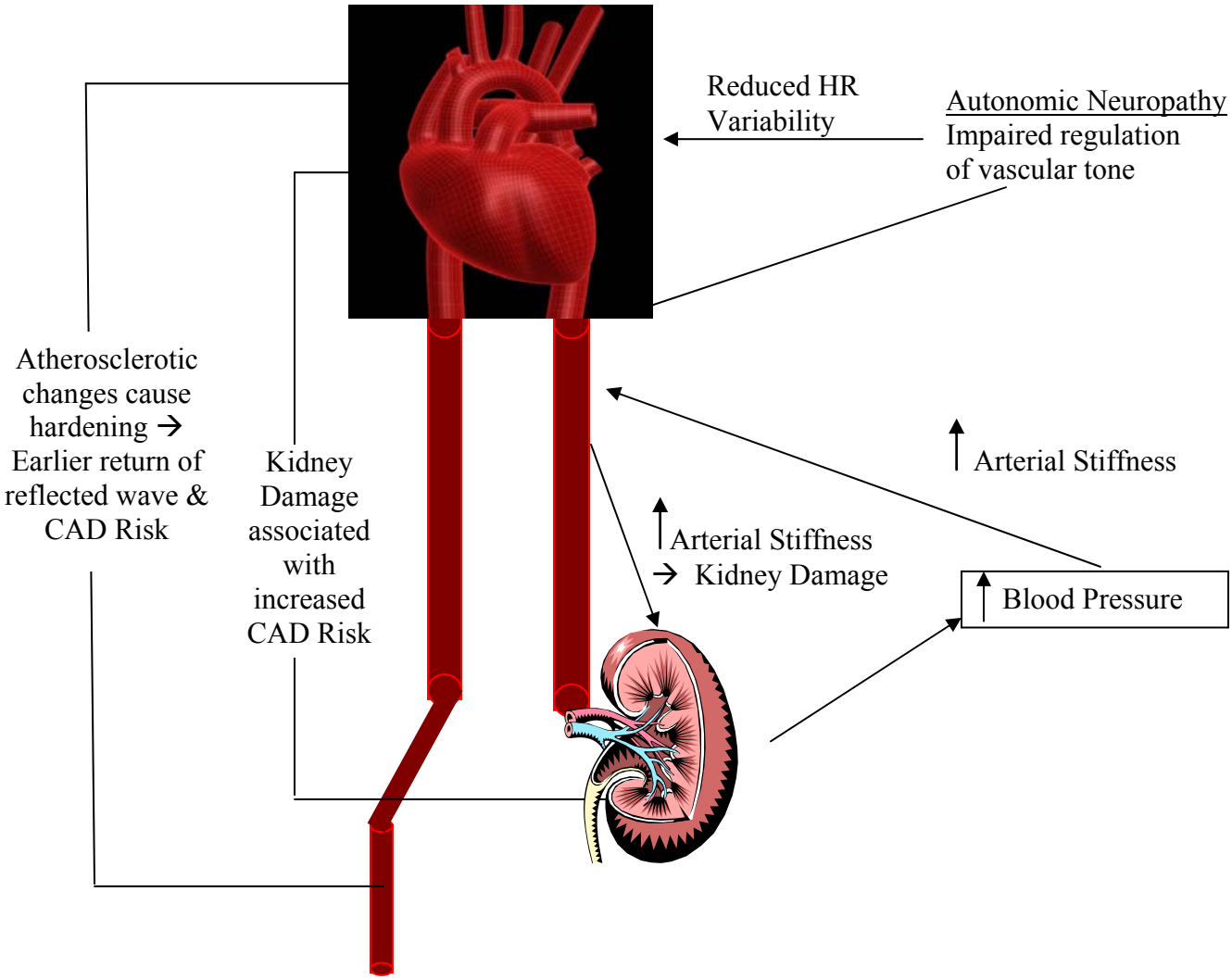

Figure 7. The proposed relationship between autonomic neuropathy, arterial stiffness, renal disease and coronary artery disease in Type 1 diabetes.

6.3 STRENGTHS

This study is one of few that has examined pulse wave analysis in a T1D population, the only that has looked at its relationship with prevalent cardiovascular disease and one of few that as studied these measures in relation to renal function. Subendocardial viability ratio has been one measure obtained through pulse wave analysis that is extremely lacking in the research literature. This study also examined SEVR and illustrating its importance in cardiovascular and renal disease. Perhaps the greatest strength is the ability to related risk factors measured 18 years earlier to current PWA measures. This allows for the evaluation of variables as potential risk factors for later increased arterial stiffness and reduced coronary perfusion.

Finally, another strength of this study was that the majority of the measurements used in this research were obtained by one technician for whom intra-observer reproducibility analysis showed excellent reproducibility.

6.4 LIMITATIONS

This study had a limited sample size of 144 men and women, which did not allow for stratified analyses. The sex-differences in risk factors associated with augmented central pressure or SEVR or their relationship with cardiovascular and renal disease could therefore not be examined properly. The pervasive use of medications, particularly those that may potentially

affect PWA measures, also makes it difficult to be certain what is occurring patho-physiologically. Again, the limited sample size did not allow for assessment within those not using medications as the majority reported use. Additionally, the lack of pulse wave analysis at baseline did not allow for adjustment of baseline stiffness or perfusion measures in the analysis of potential risk factors. Finally, the cross-sectional nature of the complications analysis does not allow for the establishment of a temporal relationship between PWA measures and complications and therefore these relationships must be assessed in a prospective study to evaluate true causality. The cross-sectional design may also have prevented appropriate assessment of the relationship between PWA measures and coronary artery disease in T1D.

Although the fact that this T1D cohort has been followed for so long is a strength, it is also a limitation in that it is essentially a survivor cohort, therefore it excludes those with the worse complications of the disease. Also, pulse wave analysis was not carried out on all EDC participants who returned for the 18 year visit. Cross-sectional analysis shows that although participants were specifically selected, those who ended up in the PWA represent a slightly healthier portion of the 18-year EDC population with lower albumin excretion rates and smaller waist-to-hip ratios.

In renal analysis, our glomerular filtration rate was estimated using the Modification of Diet in Renal Disease formula and not measured directly. However, Cystatin C was also examined showing similar results. Albumin excretion rates and serum creatinine levels (used to calculate eGFR) were not available for all participants in the PWA study population and Cystatin C was available for less than either AER or serum creatinine. Insulin sensitivity was estimated using the calculated estimated glucose disposal rate rather than measured using euglycemic clamp studies.

Finally, also due to the limited sample size, refinement of the relation between both age and duration on arterial stiffness was unable to be assessed. Stratification by older and younger age or shorter and longer duration may have helped to understand how each affected PWA measures and their relationship with complications.

6.5 FUTURE RESEARCH

With regard to repeatability and reproducibility, studies should be completed that examine what factors are associated with variability of PWA measures within an individual. These studies should be of substantial size and in a variety of populations of males and females, within different racial/ethnic groups and various disease states (diabetes, hypertension, renal disease, etc).

Future prospective studies with baseline pulse wave analysis are necessary to confirm the findings in this research. Specifically, the assessment of if PWA measures predict cardiovascular and renal outcomes is of great importance. Studies with larger sample sizes are needed to compare and contrast the relationship between risk factors and complications with PWA measures in males and females. Previous studies have found arterial stiffness differences between males and females [274, 276, 290-293], therefore there may be potential sex-differences in their relationship with disease outcomes. There may also be different risk factors for increase arterial stiffness or reduced coronary perfusion by sex which should also be addressed in future research.

6.6 CLINICAL UTILITY OF PULSE WAVE ANALYSIS

Pulse wave analysis allows for quick, non-invasive, measurement and monitoring of a variety of features of the pulse wave and estimates central pressure changes. Radial tonometry is simple and well-tolerated in a clinical setting, more so than are other techniques for measuring arterial stiffness (i.e. PWV, ultrasonography). Recently, The Conduit Artery Functional Endpoint Study (CAFÉ) study showed that improvement in PWA measures obtained via radial applanation using the Sphygmocor, not brachially measured blood pressures, were associated with reduced risk for cardiovascular outcomes[2]. Systolic and diastolic blood pressures vary throughout the arterial tree, and brachially measured pressures are ineffective in detecting pressures and pressure changes in the aorta due to the reflected pulse wave as brachial pressures are less effected by reflected waves. Reductions in brachial systolic pressures do not necessarily signify reductions at the aorta, essentially where it counts with respect to cardiovascular outcomes. Carotid-femoral pulse wave velocity is currently considered the gold standard due to its direct measurement of factors related to arterial stiffness and primarily due to the large amount of epidemiological data linking it to cardiovascular outcomes. However, if a technique is not used clinically, due to inconvenient implementation, it cannot aid in risk stratification or early intervention. There is a reason that measurement of brachial blood pressures have been the means by which hypertension has been diagnosed and treated for decades even though it has been known for some time that it is suboptimal; it is a matter of convenience. Pulse wave analysis has the potential to be implemented clinically because it requires little set-up, is well tolerated by patients as it only requires application of a tonometer to the inside of their wrist, and measurement is an easily acquired skill require little expertise. Granted, additional studies should be completed, just as they have been for PWV, to more fully understand how PWA measures relate to and predict

clinical outcomes. Guidelines for the methodological issues (i.e. manner in which measurements should be obtained, what factors should be considered at the time of measurement (known factors include height and heart rate, but blood glucose, time since meals or medication may also influence measures) as well as clinical implications, should be published.

6.7 CONCLUSION AND PUBLIC HEALTH SIGNIFICANCE

In conclusion, autonomic neuropathy, history of smoking and poorer glycemic control, are predictive of increased central pressure augmentation an index of arterial stiffness. These factors are also independently associated with poorer coronary perfusion in T1D. Higher central pressure augmentation and lower coronary perfusion are associated with lower-extremity arterial disease, renal function and renal damage in this population. SEVR is also associated with higher coronary artery calcification burden, and is more significantly associated with degree of renal damage than systolic blood pressure in those with no or mild albuminuria. Confirmation of these findings in a larger sample with sex stratified analyses may give rise to better risk stratification for complications in those with T1D. Also, the findings of this research suggest that early treatment of autonomic neuropathy, better glycemic control, improvement of lipid profiles and smoking cessation within those with T1D may result in slower vascular aging and perhaps subsequent cardiovascular outcomes in this already high risk group. Confirmation of the findings regarding renal function and damage in T1D may give rise to better assessment of risk and the use of renal protective medications sooner.

Table 19. Cross-sectional characteristics (and sex comparisons) of The Pittsburgh EDC Pulse Wave Analysis study population and the remaining 18-year EDC follow-up population

	Female N=73	Male[a] N=71	PWA Study Population N=144	Remaining EDC Population[b] N=165
Female	NA	NA	50.7	52.1
Age (years)	43.9±7.12	45.4±7.76	44.7±7.46	45.3±7.58
Diabetes Duration (years)	35.7±7.12	37.1±6.40	36.4±6.78	37.3±7.41
Height (cm)	160.6±8.00	174.6±6.66****	168.1±8.78	167.5±10.1
SBP (mmHg)	112.2±15.5	119.06±16.9**	115.6±16.5	118.2±17.0
DBP (mmHg)	63.9±9.29	69.2±8.38****	66.5±9.21	66.3±12.1
HR (bpm)	75.7±12.7	76.1±12.1	75.9±12.3	73.1±11.1**
HbA1c (%)	7.37±2.10	6.83±2.20	7.44±1.37	7.57±1.44
eGDR (mg/kg/min)	8.53±1.88	7.65±1.76****	7.69±2.31	7.06±2.34**
Non-HDL (mg/dL)	112.8±33.0	115.4±32.5	114.1±32.7	119.5±39.2
HDL (mg/dL)	64.9±16.1	53.2±15.1****	59.2±16.6	58.3±16.2
TG (mg/dL)	80.7±43.9	91.0±54.8	85.8±49.7	88.2±46.6
WHR	0.82±0.09	0.92±0.07****	0.87±0.09	0.90±0.09**
BMI (kg/m2)	27.7±5.14	26.6±5.15	27.2±4.59	27.1±4.50
Serum Creatinine	0.96±0.29	1.21±0.70	1.08±0.54	1.18±0.74
eGFR (mL/min/1.73m^2)	72.3±19.8	80.2±21.6**	76.1±20.1	74.4±26.1
Albumin Excretion Rate	78.5±249.4	351.5±1115.9**	212.1±809.4	653.4±2593.4**
WBC	6.40±2.1	6.50±2.0	6.44±2.01	6.46±1.93
Ever Smoker (%)	37.1	42.9	40.0	31.7
PW Medications[d] (%)	45.6	54.4	48.1	51.9
Hypertension (%)	42.3	57.7	42.6	57.4
E:I Ratio	1.15±0.12	1.13±0.13	1.14±0.12	1.13±0.12
CAD (% with)	16.7	29.6*	23.1	30.9
AIx (%)	24.7(1.38)[c]	21.3(1.36)[c]	23.0±11.0	NA
AP (mmHg)	9.53(0.84)[c]	8.51(0.86)[c]	9.03±6.51	NA
SEVR (%)	138.9(4.06)[c]	145.5(4.13)[c]	142.2±31.1	NA

Abbreviations: Epidemiology of Diabetes Complications, EDC; systolic blood pressure, SBP; diastolic blood pressure, DBP; heart rate, HR; eGDR, estimated glucose disposal rate; high-density lipoprotein, HDL, triglyceride, TG; waist-to-hip ratio, WHR; body mass index, BMI; kilocalories, KCAL; albumin excretion rate, AER; glomerular filtration rate, GFR; white blood cell count, WBC; low-density lipoprotein, LDL; blood pressure, BP; hypertension, HTN; augmentation index, AIx; augmentation pressure, AP; subendocardial viability ratio, SEVR.
Data presented mean±SD or %

[a] females and males compared within PWA population

[b]Those included in PWA study compared to the rest of the Pittsburgh EDC study population.

[c]height adjusted mean(SE)

[d]those potentially affecting PWA measures: calcium channel blockers, ACE inhibitors, angiogensin receptor blockers, beta blockers, nitrates.

* p<0.10; ** p<0.05; *** p<0.01; **** p<0.001

Table 20. Cross-sectional correlations between pulse wave analysis measures (AIx, AP and SEVR) and other factors measured at the 18-year follow-up examination in The Pittsburgh EDC Pulse Wave Analysis study population

	AIx	LnAP	SEVR
Systolic Blood Pressure (mmHg)	.265***	.560****	-.437****
Diastolic Blood Pressure (mmHg)	.396****	.292****	-.040
HbA1c (%)	.161*	.160*	.011
Body Mass Index (kg/m2)	.033	.089	-.161*
Waist-to-Hip Ratio	.159*	.156*	-.168**
HDL-cholesterol (mg/dL)	-.060	-.072	.157*
eGDR	-.160*	-.267***	.203**
White Blood Cell Count	.266***	.289***	-.083
Albumin Excretion Rate	.111	..272***	-.248***
E:I Ratio	-.193**	-.242***	.250***
Ever Smoker (%)	.117	.138	-.284***
PWD Use (%)	-.168**	-.128	.031

* p<0.10; ** p<0.05; *** p<0.01; **** p<0.001

APPENDIX A. REPEATABILITY STUDY

Assessments of the repeatability of measures obtained by the SphymoCor pulse wave analysis device have previously been performed in various populations and are detailed in Appendix B. All of the studies listed found good to excellent intra- and/or inter-observer reproducibility for various measures obtained via applanation tonometry PWA measures.

As part of the present research, a small repeatability study was completed. The parameters studied for repeatability were AIx, AP and SEVR however, data on other variables measured by PWA are included in the tables.

Intra-observer

A small sample of participants from the Epidemiology of Diabetes Complications (EDC) study whom have Type 1 diabetes mellitus to determine the inter- and intra-observer reproducibility of variables obtained via applanation tonometry using the SphygmoCor device (AtCor, Sydney, Australia). For the intra-observer part of the study, 1 observer recorded 2 different measurements, 5 minutes apart on 17 different EDC participants. Table 21 shows descriptive data for SphgymoCor variables obtained during the intra-observer study. The mean intra-observer difference was 0.42±9.51% for AIx, 0.42±2.53 mmHg for AP and 2.59±9.68% for SEVR. Figures 8 through 10 show the Bland-Altman plots for the individual data points. As illustrated in the graphs, all data points fell within 2 SD for AIx and SEVR, and all but 1 for AP. The coefficient of variation (CV) was for 20.3 for AIx, 26.0 for AP, and 7.8 for SEVR (Table 21).

Table 21. Intra-observer repeatability study on participants with type 1 diabetes (n=17) in The Pittsburgh Epidemiology of Diabetes Complications Study

Variable	Grand Mean	S.D.	Mean Difference	S.D. of Difference	Coeff. Of Variation
Aortic SBP	106.0	10.4	0.42	1.6	1.5
Aortic DBP	67.1	9.0	-0.06	1.3	1.9
HR	85.0	16.8	0.77	3.5	4.15
AIX	23.5	10.6	0.42	4.8	20.3
AIXHR75*	27.6	7.9	0.87	4.2	15.2
AP	9.6	5.4	0.41	2.5	26.0
APHR75*	11.6	5.7	0.87	2.2	19.0
ED	286.5	27.2	0.94	7.4	2.6
EDPER	40.1	5.6	0.42	1.3	3.2
SEVR	124.7	35.0	-2.6	9.7	7.8
ESP	94.4	9.5	0.53	2.2	2.33

*Calculated only for those with a HR 55-110 bpm (n=15)

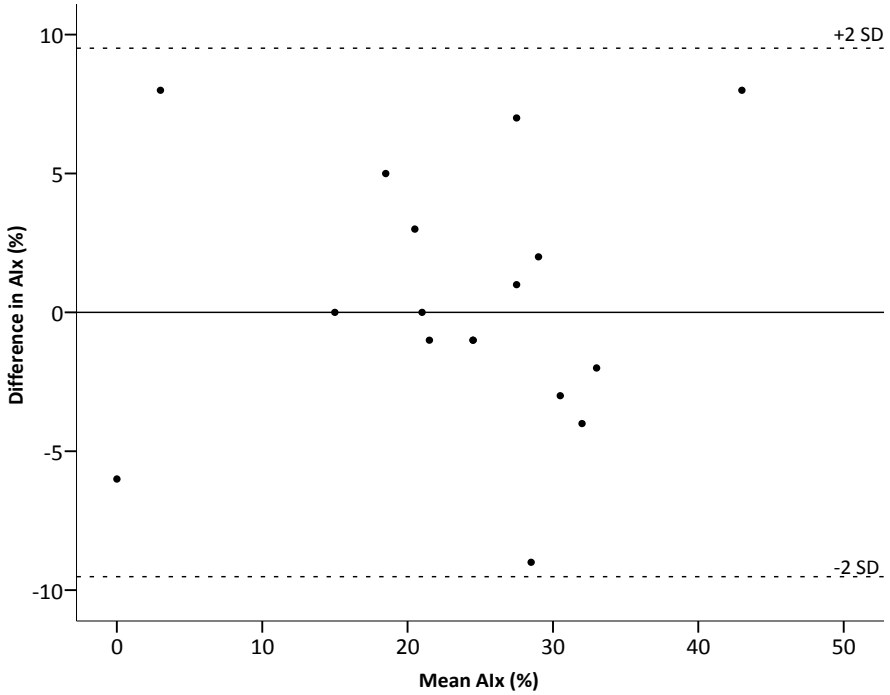

Figure 8. Bland-Altman plot for Augmentation Index (AIx) to show intra-observer reproducibility. Mean±SD difference (n=17): 0.42 ± 4.76%

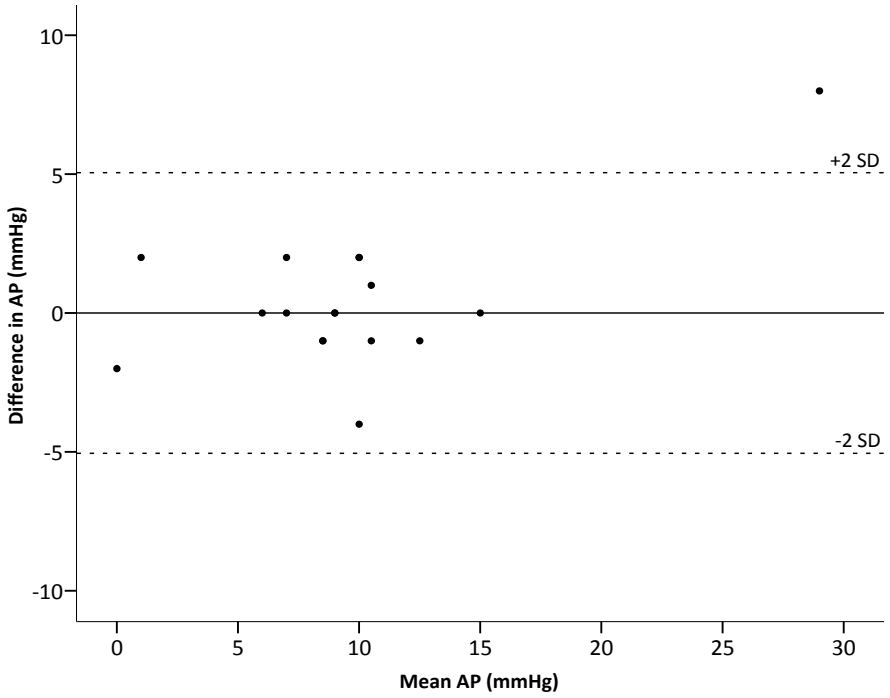

Figure 9. Bland-Altman plot for Augmentation Pressure (AP) to show intra-observer reproducibility. Mean±SD difference (n=17): 0.42 ± 2.53 mmHg

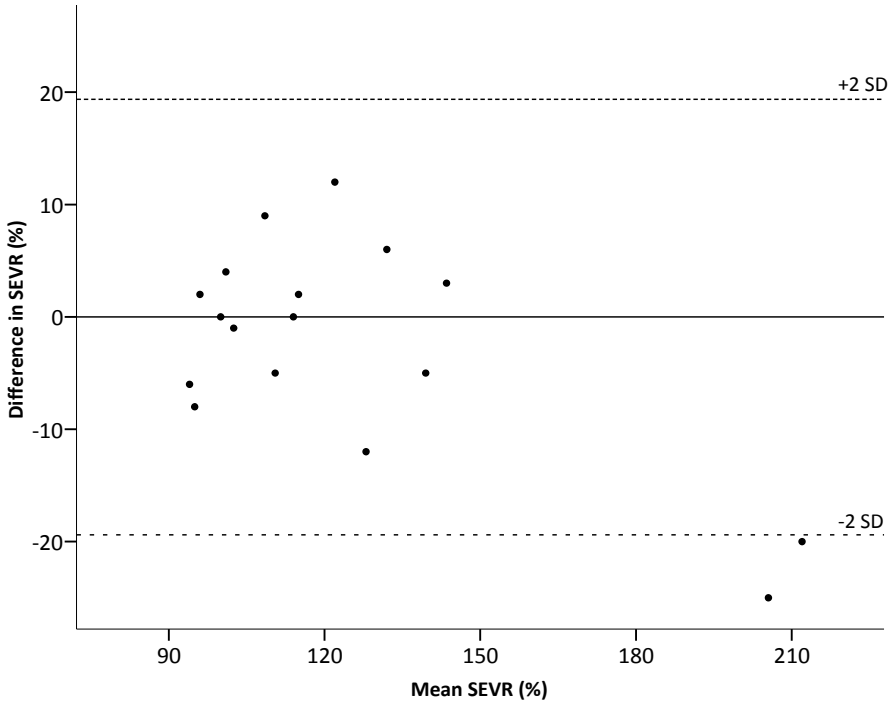

Figure 10. Bland-Altman plot for Subendocardial Viability Ratio (SEVR) to show intra-observer reproducibility. Mean±SD difference (n=17): 2.59 ± 9.68%

Interobserver

Table 22 contains the results of the inter-observer repeatability part of the study. Two different observers obtained SphygmoCor measurements on 7 different EDC participants within 30-45 minutes of each other. Each observer measured radial blood pressure using a cuff prior to completing the applanation tonometry. The mean±SD inter-observer differences were 8.57±11.2% for AIx, 4.57±3.74 mmHg for AP, and 5.43±9.88% for SEVR. Bland-Altman plots for inter-observer reproducibility (Figures 11-13) show that 2 of 7 data points lay outside 2 SD for AIx and AP, and 1 of 7 for SEVR. The coefficients of variation were 44.0, 42.9 and 6.74 for AIx, AP and SEVR, respectively, in the inter-observer analysis (Table 22). A limitation of the inter-observer study is that 3 measurements from one of the two observers were "Inconclusive" with an error indicating that the "Aortic T1 is out of range". Inconclusive measures indicate that some "noise" exists in the measurement and the measurement is not entirely clear. To correct for this error, the measurement should be retaken as recommended by the company. However, the observer with inconclusive measurements was not the primary observer in this research study. The other observer for whom the intra-observer analysis was completed, was the primary PWA technician and completed over 90% of the measurements used in the present study. This observer showed excellent intra-observer reproducibility as previously discussed.

Table 22. Inter-observer repeatability variability in The Pittsburgh EDC Pulse Wave Analysis Repeatability Study

Variable	Grand Mean	S.D.	Mean Difference	S.D. of Difference	Coeff. Of Variation
SBP	127.43	15.9	-6.57	8.14	6.39
DBP	83.43	8.20	3.14	6.20	7.43
Aortic SBP	116.71	12.6	-5.14	5.93	5.08
Aortic DBP	84.29	8.32	2.57	6.27	7.44
HR	80.57	17.2	0.29	3.40	4.22
AIX	25.43	13.1	-8.57	11.2	44.0
AIXHR75*	27.92	10.3	-10.2	10.7	38.3
AP	8.71	5.77	-4.57	3.74	42.9
APHR75*	9.62	4.54	-5.33	3.60	37.4
ED	285.57	33.8	-3.14	7.82	2.74
EDPER	37.64	4.34	-0.43	1.27	3.37
SEVR	146.71	30.0	5.43	9.88	6.74
ESP	108.07	10.0	-3.86	4.95	4.58

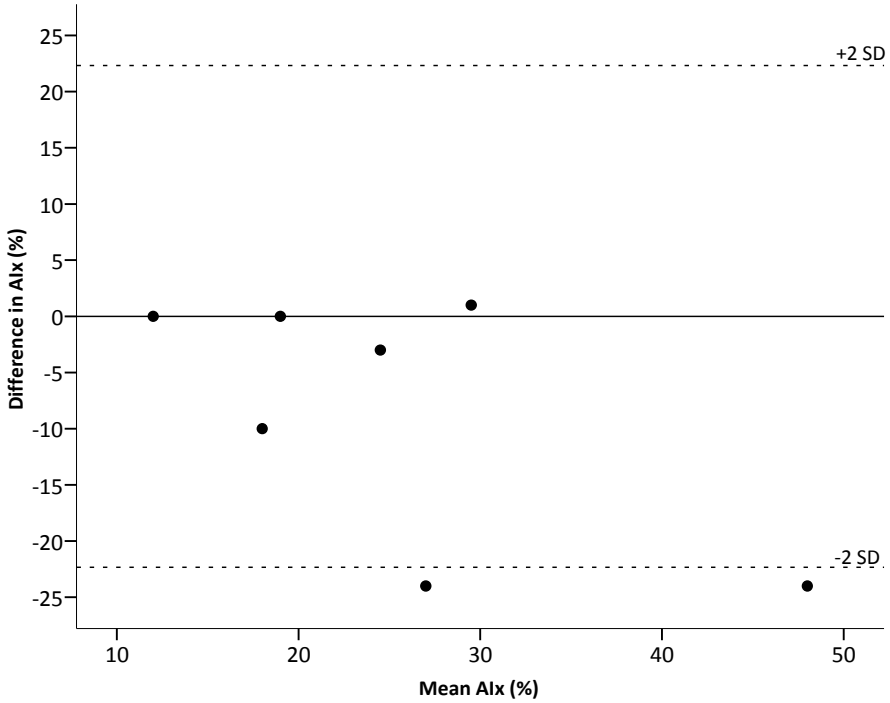

Figure 11. Bland-Altman plot for Augmentation Index (AIx) to show inter-observer reproducibility. Mean±SD difference (n=6): 8.57 ± 11.2%

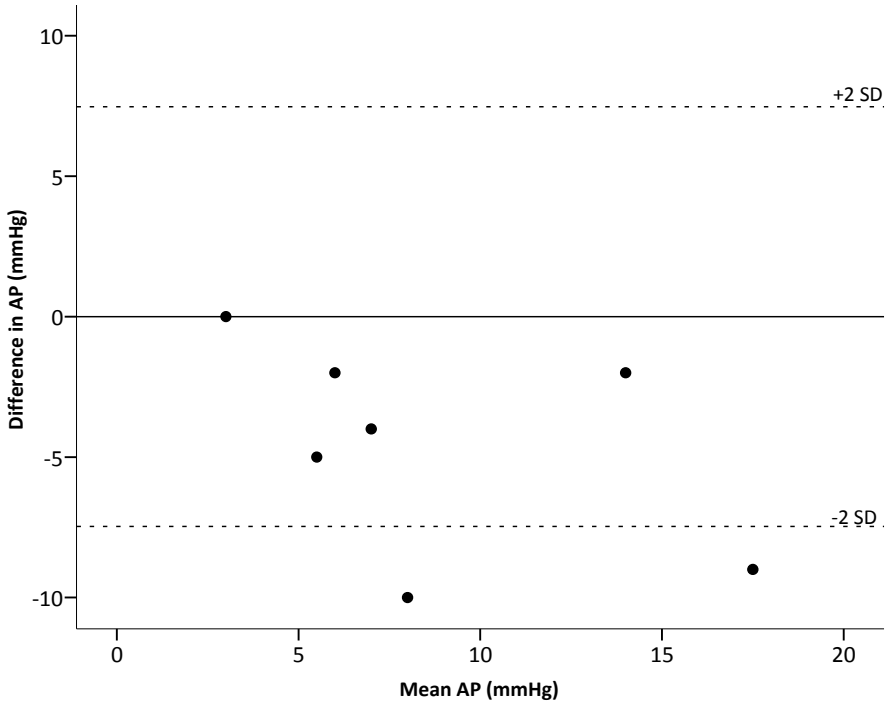

Figure 12. Bland-Altman plot for Augmentation Pressure (AP) to show inter-observer reproducibility. Mean±SD difference (n=6): 4.57 ± 3.74 mmHg

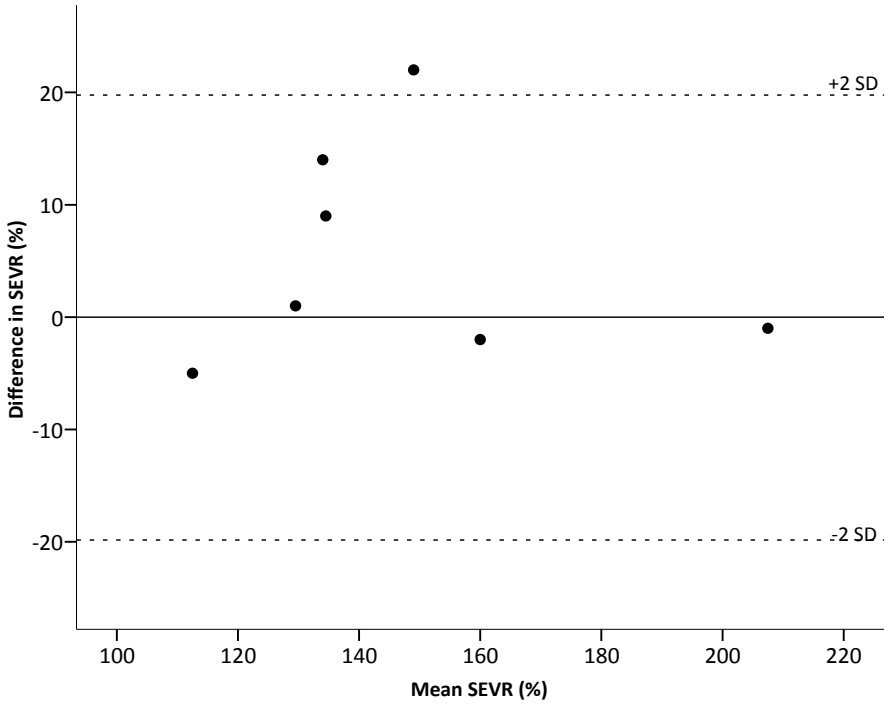

Figure 13. Bland-Altman plot for Subendocardial Viability Ratio (SEVR) to show inter-observer reproducibility. Mean±SD difference (n=6): 5.43 ± 9.88%

Biologic Variability Over Time

In order to study variability over time, two different individuals were selected to have repeated measures on different days at varying times. Since the SEVR variable has a defined cut-off value (150%), potential participants were screened and one with normal SEVR (>150%) and one with low SEVR (<150%) were selected. The person with the normal SEVR was a 26 year old, healthy female without diabetes, no history of cardiovascular or renal disease who was not taking medications at the time and had a BMI of 18.5 kg/m^2. The "abnormal" participant was a 50 year old woman, with a 26.4 kg/m^2 BMI, without diabetes but with a history of hypertension, reporting use of an anti-hypertensive agent. The pulse wave analysis measures for each participant are presented in Table 23. As expected, mean values for AIx and AP were higher in the individual with abnormal screening values measurements compared to the one with normal values, while SEVR was lower. As illustrated in Figures 14 and 15, AIx and AP seemed to vary more over the 6 different time points in the individual with abnormal values. The highest AIx and AP values in this individual were at a BP of 130/68 mmHg and HR of 70 bpm (Measurement 2, Table 23b). By comparing measurement 2 to measurement 1 in this same individual, during which the HR was the same (70 bpm), DBP was very similar (68 mmHg) but SBP was much lower (110 mmHg), it can be seen that it was the increase in SBP that corresponded to the increased AP and AIx as well as a decreased SEVR (Figure 16). SEVR seemed to vary more in the participant with normal screening values as shown in Figure 16. Interestingly, the lowest value in this individual was 145% at measurement 4, less than the 150% cut-off for normal. This SEVR measurement occurred when SBP/DBP were also at their lowest (92/52 mmHg) for this individual. It is worth mentioning that this measurement was taking within less than 10 minutes of this person eating a meal. However, AIx and AP at this

measurement were similar to that in most other measures. AIx and AP in this individual were lowest when this person's blood pressure and heart rate were at their highest, 106/84 mmHg and 70 bpm, respectively. However, the BP was well below the cut-off for hypertension and the measurement corresponded with this individual's lowest pulse pressure (PP=22 mmHg). The highest PP in the abnormal individual (68 mmHg) corresponded with that persons highest AP (13 mmHg) and AIx (27%) measures and her lowest SEVR (107%) and her lowest PP (40 mmHg, which is actually equal to the highest in the normal individual) corresponded to her lowest AP and AIx and highest SEVR.

To summarize, there seems to be variability of PWA measures within an individual at different time points and variability may depend on the degree of arterial stiffness in an individual. Additional studies, with more than two individuals, should be performed to understand what factors affect these measures within an individual.

Table 23. Pulse Wave Analysis measures over time in one normal and one abnormal (based on SEVR) participant.

	SBP	DBP	aSBP	aDBP	HR	AIX	AP	AIX HR75	AP HR75	ED	ED%	SEVR	ESP
21a. Normal subject (SEVR >150% at screening)													
Measure 1	106	84	98	84	70	8	1	6	1	313	37	161	95
Measure 2	104	64	92	65	64	17	4	11	3	303	32	183	86
Measure 3	98	58	86	59	67	13	3	9	2	302	34	169	80
Measure 4	92	52	80	53	70	17	5	14	4	313	37	145	74
Measure 5	104	64	94	65	60	18	5	11	3	336	33	175	88
Measure 6	106	68	94	69	62	14	3	8	2	305	31	193	89
Mean	101.7	65.0	90.7	65.8	65.5	14.5	3.50	9.83	2.50	312.0	34.0	171.0	85.3
SD	5.57	10.86	6.53	10.52	4.18	3.73	1.52	2.79	1.05	12.71	2.53	16.88	7.37
21b. Abnormal Subject (SEVR<150% at screening)													
Measure 1	110	70	97	70	84	14	4	19	5	267	38	141	89
Measure 2	130	68	116	70	88	27	13	33	17	291	43	107	104
Measure 3	128	70	114	71	79	23	10	25	11	299	39	123	102
Measure 4	112	64	97	65	93	16	5	25	9	272	42	114	86
Measure 5	130	76	118	77	85	24	10	29	13	296	42	115	108
Measure 6	128	74	113	76	98	18	7	30	13	266	44	108	102
Mean	123.0	70.3	109.2	71.5	87.8	20.3	8.17	26.8	11.3	281.8	41.3	118.0	98.5
SD	9.36	4.27	9.58	4.42	6.79	5.09	3.43	4.92	4.08	15.14	2.34	12.65	8.85

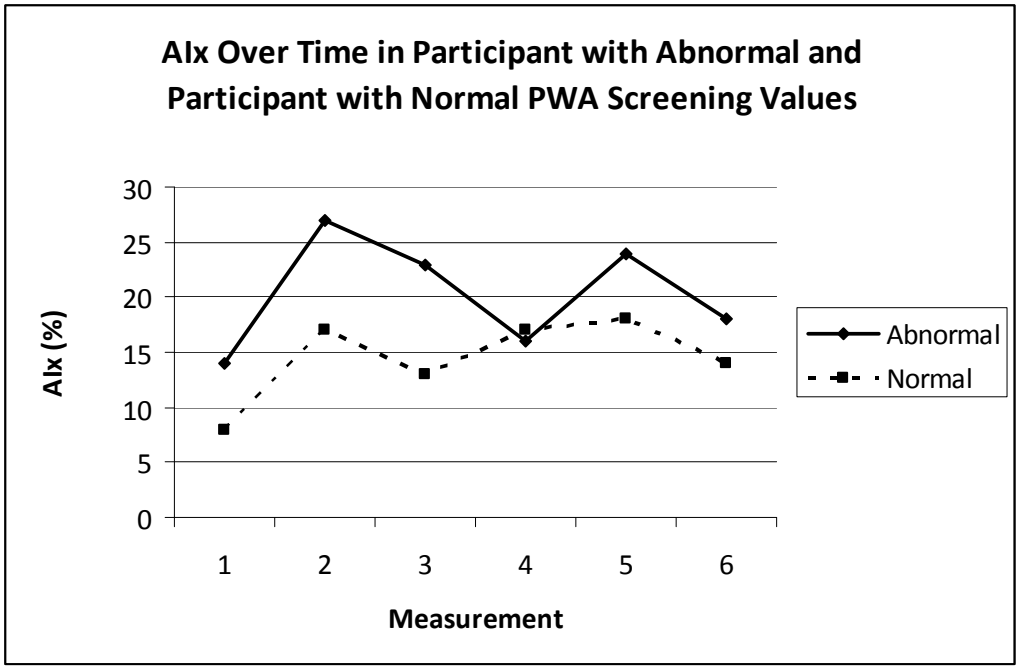

Figure 14. Augmentation Index (AIx) at 6 different time points, over a 2 week period in non-diabetic participants, one with abnormal PWA values at screening and one with normal values

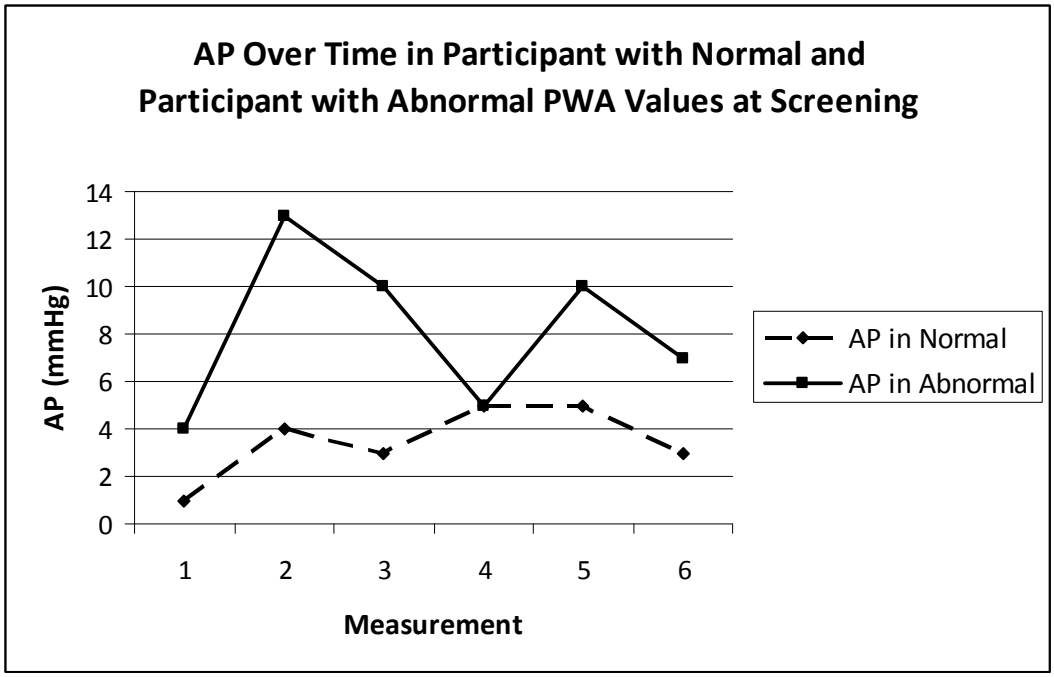

Figure 15. Augmentation Pressure (AP) at 6 different time points, over a 2 week period in non-diabetic participants, one with abnormal PWA values at screening and one with normal values.

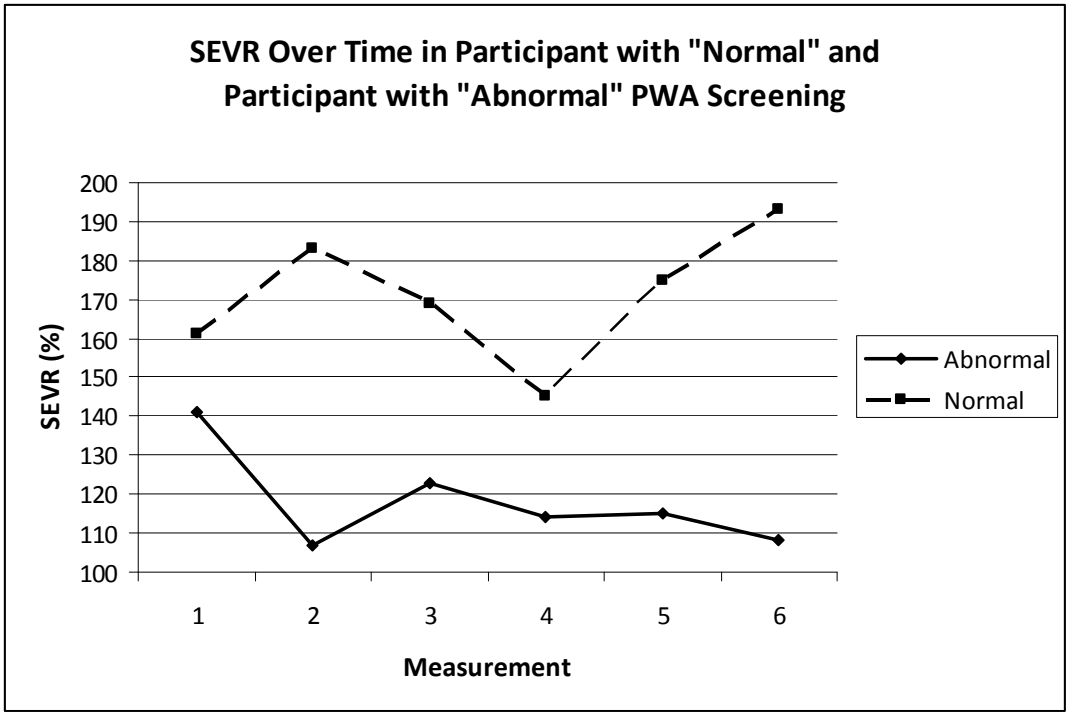

Figure 16. Subendocardial Viability Ratio (SEVR) at 6 different time points, over a 2 week period in non-diabetic participants, one with abnormal PWA values at screening and one with normal values.

APPENDIX B. REPEATABILITY STUDIES FOR THE SPHYGMOCOR DEVICE

Table 24. Repeatability studies for the SphygmoCor Pulse Wave Analysis device

Author	**Primary Aim**	**Study Population**	**Sample Size**	**Indices evaluated**	**Results**
Frimodt-Moller, M et al Nephrol Dial Transplant 2007 Nov 7 epub	Evaluate intra- and inter-observer and day-to-day reproducibility of PWA and PWV	Pre-dialysis patients with CKD stages 3-5 Mean GFR = 25.3ml/min/1.73m(2)	19	AIx SEVR aPP	Mean inter-observer differences: AIx: 0.9±15.8% SEVR: -0.9±15.2% aPP:1.4±13.3mmHg **Mean day-to-day differences:** AIx: 2.6±11.2%, SEVR: -0.4±24.7% aPP:0.3±20.9 mmHg **Mean Intra-observer differences:** AIx: 1.9±10.6%, SEVR: -1.1±17.4% aPP:0.3±4.0 mmHg

Table 24 Continued					
Author	**Primary Aim**	**Study Population**	**Sample Size**	**Indices evaluated**	**Results**
Crilly M et al Vasc Med 2007;12(3):189-197	Assess within- and between-observer repeatability of ED%, AIx@75 and SEVR%	Ambulant patients in sinus rhythm	20 (16 male)	AIx@75 ED% SEVR%	**Inter-observer difference (mean of 2 readings):** ED% - 0.3±2.0 AIx@75 1.0±3.9 SEVR%: 1.7±14.2 **Inter-observer based on 1 measurement:** ED% - 0.3±3.3 AIx@75 1.7±6.9 SEVR%: 0.6±22.6 **Intra-observer difference:** ***Observer 1*** ED% 0.0±5.4 AIx@75 1.5±7.0 SEVR%: 1.7±39.0 ***Observer 2*** ED% 0.1±3.8 AIx@75 0.1±8.0 SEVR%: 0.6±23.3

Table 24 Continued					
Author	**Primary Aim**	**Study Population**	**Sample Size**	**Indices evaluated**	**Results**
Papaioannou TG et al J Clin Monit Comput 2004;18(2):137-44	To assess reproducibility of aortic AIx in patients with low blood pressure	Patients with cardiogenic shock due to acute MI who underwent mechanical assistance with intraaortic balloon pump	19	AIx	**AIx** range:30-184% **Intra-observer difference:** 0.10±5.82%
Savage MT et al Clin Sci (Lond). 2002;103:59-65	To assess the reproducibility of PWA in patients with chronic renal failure	Patients with chronic renal failure (71 pre-dialysis, 67 dialysis and 27 transplant), and 27 healthy controls	188	aMBP AIx SEVR	**Inter-observer difference** AIx:0±3% MBP: 1±4 mmHg SEVR: 1±29% **Intra-observer difference** AIx:0±4% MBP: 0±3 mmHg SEVR: 0±18%
Wilkinson IB et al J Hypertens 1998;16(12 Pt2):2079-84	To determine the reproducibility of pulse wave velocity and AIx measured using PWA	Subjects with and without a range of CV risk factors	24 PWV 33 AIx	PWV AIx	**AIx**: Range = -15.0-+45.0% Mean±SD = 19.6±12.0% **Intra-observer difference:** AIx: 0.49±5.37%

Abbreviations: Pulse-wave analysis (PWA), Pulse-Wave Velocity (PWV), Augmentation Index (AIx), Mean Blood Pressure (MBP), Sub-endocardial Viability Ratio (SEVR), Ejection Duration % (ED%), Augmentation Index at Heart Rate of 75 bpm AIx@HR75, aortic Pulse Pressure

BIBLIOGRAPHY

1. *National diabetes fact sheet: general information and national estimates on diabetes in the United States*, U.S. Department of Health and Human Services and C.f.D.C.a. Prevention, Editors. 2005: Atlanta.
2. Williams, B., et al., *Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study.* Circulation, 2006. **113**(9): p. 1213-25.
3. Shoji, T., et al., *Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease.* J Am Soc Nephrol, 2001. **12**(10): p. 2117-24.
4. Laurent, S., et al., *Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients.* Hypertension, 2001. **37**(5): p. 1236-41.
5. Shokawa, T., et al., *Pulse wave velocity predicts cardiovascular mortality: findings from the Hawaii-Los Angeles-Hiroshima study.* Circ J, 2005. **69**(3): p. 259-64.
6. Willum-Hansen, T., et al., *Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population.* Circulation, 2006. **113**(5): p. 664-70.
7. McEniery, C.M., et al., *Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT).* J Am Coll Cardiol, 2005. **46**(9): p. 1753-60.
8. Berry, K.L., et al., *Systemic arterial compliance is reduced in young patients with IDDM.* Am J Physiol, 1999. **276**(6 Pt 2): p. H1839-45.
9. Brooks, B., L. Molyneaux, and D.K. Yue, *Augmentation of central arterial pressure in type 1 diabetes.* Diabetes Care, 1999. **22**(10): p. 1722-7.
10. Haller, M.J., et al., *Radial artery tonometry demonstrates arterial stiffness in children with type 1 diabetes.* Diabetes Care, 2004. **27**(12): p. 2911-7.
11. London, G.M., et al., *Aortic and large artery compliance in end-stage renal failure.* Kidney Int, 1990. **37**(1): p. 137-42.
12. Shoji, T., et al., *Intermediate-density lipoprotein as an independent risk factor for aortic atherosclerosis in hemodialysis patients.* J Am Soc Nephrol, 1998. **9**(7): p. 1277-84.
13. Kohara, K., et al., *Microalbuminuria and arterial stiffness in a general population: the Shimanami Health Promoting Program (J-SHIPP) study.* Hypertens Res, 2004. **27**(7): p. 471-7.
14. Derchi, L.E., et al., *Mild renal dysfunction and renal vascular resistance in primary hypertension.* Am J Hypertens, 2005. **18**(7): p. 966-71.
15. Genuth, S., et al., *Follow-up report on the diagnosis of diabetes mellitus.* Diabetes Care, 2003. **26**(11): p. 3160-7.
16. Fuller, J.H., et al., *Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study.* Lancet, 1980. **1**(8183): p. 1373-6.
17. Knip, M., et al., *Environmental triggers and determinants of type 1 diabetes.* Diabetes, 2005. **54 Suppl 2**: p. S125-36.

18. DeFronzo, R.A., R.C. Bonadonna, and E. Ferrannini, *Pathogenesis of NIDDM. A balanced overview.* Diabetes Care, 1992. **15**(3): p. 318-68.
19. Ratner, R.E., *An update on the Diabetes Prevention Program.* Endocr Pract, 2006. **12 Suppl 1**: p. 20-4.
20. Alberti, K.G., *Impaired glucose tolerance: what are the clinical implications?* Diabetes Res Clin Pract, 1998. **40 Suppl**: p. S3-8.
21. Chiasson, J.L., *Impaired glucose tolerance: a risk factor for type 2 diabetes and cardiovascular disease.* Diabetes Res Clin Pract, 1998. **40 Suppl**: p. S1-2.
22. Boulton, A.J. and R.A. Malik, *Diabetic neuropathy.* Med Clin North Am, 1998. **82**(4): p. 909-29.
23. Maser, R.E., et al., *Epidemiological correlates of diabetic neuropathy. Report from Pittsburgh Epidemiology of Diabetes Complications Study.* Diabetes, 1989. **38**(11): p. 1456-61.
24. Orchard, T.J., et al., *Prevalence of complications in IDDM by sex and duration. Pittsburgh Epidemiology of Diabetes Complications Study II*. 1990. p. 1116-1124.
25. Knuiman, M.W., et al., *Prevalence of diabetic complications in relation to risk factors.* Diabetes, 1986. **35**(12): p. 1332-9.
26. England, J.D., et al., *Distal symmetric polyneuropathy: a definition for clinical research: report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation.* Neurology, 2005. **64**(2): p. 199-207.
27. Orchard, T.J., et al., *Factors associated with avoidance of severe complications after 25 yr of IDDM. Pittsburgh Epidemiology of Diabetes Complications Study I.* Diabetes Care, 1990. **13**(7): p. 741-7.
28. Forrest, K.Y., et al., *Hypertension as a risk factor for diabetic neuropathy: a prospective study.* Diabetes, 1997. **46**(4): p. 665-70.
29. Tesfaye, S., et al., *Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study.* Diabetologia, 1996. **39**(11): p. 1377-84.
30. Sibal, L., et al., *Cardiovascular risk factors predicting the development of distal symmetrical polyneuropathy in people with type 1 diabetes: A 9-year follow-up study.* Ann N Y Acad Sci, 2006. **1084**: p. 304-18.
31. Sztajzel, J., *Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system.* Swiss Med Wkly, 2004. **134**(35-36): p. 514-22.
32. Vinik, A.I., et al., *Diabetic autonomic neuropathy.* Diabetes Care, 2003. **26**(5): p. 1553-79.
33. Shakespeare, C.F., et al., *Differences in autonomic nerve function in patients with silent and symptomatic myocardial ischaemia.* Br Heart J, 1994. **71**(1): p. 22-9.
34. Vinik, A.I. and D. Ziegler, *Diabetic cardiovascular autonomic neuropathy.* Circulation, 2007. **115**(3): p. 387-97.
35. Pambianco, G., et al., *The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience.* Diabetes, 2006. **55**(5): p. 1463-9.
36. Boulton, A.J., et al., *Diabetic neuropathies: a statement by the American Diabetes Association.* Diabetes Care, 2005. **28**(4): p. 956-62.

37. Stella, P., et al., *Cardiovascular autonomic neuropathy (expiration and inspiration ratio) in type 1 diabetes. Incidence and predictors.* J Diabetes Complications, 2000. **14**(1): p. 1-6.
38. Candido, R. and T.J. Allen, *Haemodynamics in microvascular complications in type 1 diabetes.* Diabetes Metab Res Rev, 2002. **18**(4): p. 286-304.
39. *National Diabetes Fact Sheet: National Estimates and General Information on Diabetes in the United States.* 2003 [cited 2007 September]; Available from: http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2003.pdf.
40. *Supplement 1. American Diabetes Association: clinical practice recommendations 2000.* Diabetes Care, 2000. **23 Suppl 1**: p. S1-116.
41. Wilkinson, C.P., et al., *Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales.* Ophthalmology, 2003. **110**(9): p. 1677-82.
42. Wong, T.Y., et al., *Diabetic retinopathy in a multi-ethnic cohort in the United States.* Am J Ophthalmol, 2006. **141**(3): p. 446-455.
43. Roy, M.S., et al., *The prevalence of diabetic retinopathy among adult type 1 diabetic persons in the United States.* Arch Ophthalmol, 2004. **122**(4): p. 546-51.
44. Molitch, M.E., et al., *Nephropathy in diabetes.* Diabetes Care, 2004. **27 Suppl 1**: p. S79-83.
45. *National Diabetes Statistics*. 2005 [cited 2007 September 10]; Available from: http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm#13.
46. Vannini, P., et al., *Lipid abnormalities in insulin-dependent diabetic patients with albuminuria.* Diabetes Care, 1984. **7**(2): p. 151-4.
47. Klein, R., et al., *The 10-year incidence of renal insufficiency in people with type 1 diabetes.* Diabetes Care, 1999. **22**(5): p. 743-51.
48. Dodson, P.M., D.J. Galton, and A.F. Winder, *Retinal vascular abnormalities in the hyperlipidaemias.* Trans Ophthalmol Soc U K, 1981. **101**(1): p. 17-21.
49. Chase, H.P., et al., *Glucose control and the renal and retinal complications of insulin-dependent diabetes.* JAMA, 1989. **261**(8): p. 1155-60.
50. Coonrod, B.A., et al., *Predictors of microalbuminuria in individuals with IDDM. Pittsburgh Epidemiology of Diabetes Complications Study.* Diabetes Care, 1993. **16**(10): p. 1376-83.
51. Andersen, A.R., et al., *Diabetic nephropathy in Type 1 (insulin-dependent) diabetes: an epidemiological study.* Diabetologia, 1983. **25**(6): p. 496-501.
52. Hovind, P., et al., *Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study.* Bmj, 2004. **328**(7448): p. 1105.
53. Borch-Johnsen, K., P.K. Andersen, and T. Deckert, *The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus.* Diabetologia, 1985. **28**(8): p. 590-6.
54. Hall, W.D., *Abnormalities of kidney function as a cause and a consequence of cardiovascular disease.* Am J Med Sci, 1999. **317**(3): p. 176-82.
55. Schmitz, A. and M. Vaeth, *Microalbuminuria: a major risk factor in non-insulin-dependent diabetes. A 10-year follow-up study of 503 patients.* Diabet Med, 1988. **5**(2): p. 126-34.
56. Cheung, N., et al., *Diabetic retinopathy and the risk of coronary heart disease: the Atherosclerosis Risk in Communities Study.* Diabetes Care, 2007. **30**(7): p. 1742-6.

57. Parfrey, P.S. and R.N. Foley, *The clinical epidemiology of cardiac disease in chronic renal failure.* J Am Soc Nephrol, 1999. **10**(7): p. 1606-15.
58. Nathan, D.M., *Long-term complications of diabetes mellitus.* N Engl J Med, 1993. **328**(23): p. 1676-85.
59. *Lipid and lipoprotein levels in patients with IDDM diabetes control and complication. Trial experience. The DCCT Research Group.* Diabetes Care, 1992. **15**(7): p. 886-94.
60. Idzior-Walus, B., et al., *Factors associated with plasma lipids and lipoproteins in type 1 diabetes mellitus: the EURODIAB IDDM Complications Study.* Diabet Med, 2001. **18**(10): p. 786-96.
61. Laakso, M., et al., *Lipid and lipoprotein abnormalities associated with coronary heart disease in patients with insulin-dependent diabetes mellitus.* Arteriosclerosis, 1986. **6**(6): p. 679-84.
62. Deckert, T., *[The prognosis of insulin-dependent diabetes mellitus].* Z Gesamte Inn Med, 1981. **36**(1): p. 4-8.
63. Laing, S.P., et al., *Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes.* Diabetologia, 2003. **46**(6): p. 760-5.
64. Dorman, J.S., et al., *The Pittsburgh insulin-dependent diabetes mellitus (IDDM) morbidity and mortality study. Mortality results.* Diabetes, 1984. **33**(3): p. 271-6.
65. Lehto, S., et al., *Poor glycemic control predicts coronary heart disease events in patients with type 1 diabetes without nephropathy.* Arterioscler Thromb Vasc Biol, 1999. **19**(4): p. 1014-9.
66. Klein, B.E., et al., *Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin epidemiologic study of diabetic retinopathy.* Arch Intern Med, 2004. **164**(17): p. 1917-24.
67. Soedamah-Muthu, S.S., et al., *Risk factors for coronary heart disease in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study.* Diabetes Care, 2004. **27**(2): p. 530-7.
68. Selvin, E., et al., *Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus.* Ann Intern Med, 2004. **141**(6): p. 421-31.
69. Stettler, C., et al., *Glycemic control and macrovascular disease in types 1 and 2 diabetes mellitus: Meta-analysis of randomized trials.* Am Heart J, 2006. **152**(1): p. 27-38.
70. Orchard, T.J., et al., *Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes: 10-year follow-up data from the Pittsburgh Epidemiology of Diabetes Complications Study.* Diabetes Care, 2003. **26**(5): p. 1374-9.
71. Prince, C.T., et al., *Changes in glycaemic control and risk of coronary artery disease in type 1 diabetes mellitus: findings from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC).* Diabetologia, 2007.
72. Donahue, R.P. and T.J. Orchard, *Diabetes mellitus and macrovascular complications. An epidemiological perspective.* Diabetes Care, 1992. **15**(9): p. 1141-55.
73. Lloyd, C.E., et al., *Coronary artery disease in IDDM. Gender differences in risk factors but not risk.* Arterioscler Thromb Vasc Biol, 1996. **16**(6): p. 720-6.
74. Jensen, T., et al., *Coronary heart disease in young type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy: incidence and risk factors.* Diabetologia, 1987. **30**(3): p. 144-8.
75. Krolewski, A.S., et al., *The changing natural history of nephropathy in type I diabetes.* Am J Med, 1985. **78**(5): p. 785-94.

76. Torffvit, O., et al., *Nephropathy, but not retinopathy, is associated with the development of heart disease in Type 1 diabetes: a 12-year observation study of 462 patients.* Diabet Med, 2005. **22**(6): p. 723-9.
77. Tuomilehto, J., et al., *Incidence of cardiovascular disease in Type 1 (insulin-dependent) diabetic subjects with and without diabetic nephropathy in Finland.* Diabetologia, 1998. **41**(7): p. 784-90.
78. Soedamah-Muthu, S.S., et al., *Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary artery disease in Type 1 diabetes. A prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study.* Diabetologia, 2003. **46**(5): p. 674-82.
79. Borch-Johnsen, K. and S. Kreiner, *Proteinuria: value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus.* Br Med J (Clin Res Ed), 1987. **294**(6588): p. 1651-4.
80. Lee, P., et al., *Prothrombotic and antithrombotic factors are elevated in patients with type 1 diabetes complicated by microalbuminuria.* Diabet Med, 1993. **10**(2): p. 122-8.
81. Janus, E.D., *Apolipoprotein(a) and atherogenesis.* Pathology, 1993. **25**(3): p. 291-3.
82. Jensen, T., S. Stender, and T. Deckert, *Abnormalities in plasmas concentrations of lipoproteins and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased urinary albumin excretion.* Diabetologia, 1988. **31**(3): p. 142-5.
83. Jenkins, A.J., et al., *Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy.* Kidney Int, 2003. **64**(3): p. 817-28.
84. Guglielmi, M.D., et al., *Impaired left ventricular diastolic function and vascular postischemic vasodilation associated with microalbuminuria in IDDM patients.* Diabetes Care, 1995. **18**(3): p. 353-60.
85. Sacks, D., et al., *Position statement on the use of the ankle brachial index in the evaluation of patients with peripheral vascular disease. A consensus statement developed by the Standards Division of the Society of Interventional Radiology.* J Vasc Interv Radiol, 2003. **14**(9 Pt 2): p. S389.
86. Forrest, K.Y., et al., *Are predictors of coronary heart disease and lower-extremity arterial disease in type 1 diabetes the same? A prospective study.* Atherosclerosis, 2000. **148**(1): p. 159-69.
87. Olson, J.C., et al., *Glycemia (or, in women, estimated glucose disposal rate) predict lower extremity arterial disease events in type 1 diabetes.* Metabolism, 2002. **51**(2): p. 248-54.
88. Murabito, J.M., et al., *The ankle-brachial index in the elderly and risk of stroke, coronary disease, and death: the Framingham Study.* Arch Intern Med, 2003. **163**(16): p. 1939-42.
89. Newman, A.B., et al., *Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. The Cardiovascular Health Study Group.* Arterioscler Thromb Vasc Biol, 1999. **19**(3): p. 538-45.
90. Selvin, E. and T.P. Erlinger, *Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National Health and Nutrition Examination Survey, 1999-2000.* Circulation, 2004. **110**(6): p. 738-43.
91. Gregg, E.W., et al., *Trends in the prevalence and ratio of diagnosed to undiagnosed diabetes according to obesity levels in the U.S.* Diabetes Care, 2004. **27**(12): p. 2806-12.
92. *Stroke*. 2007 [cited 2007 September]; Medline Plus Medical Encyclopedia]. Available from: http://www.nlm.nih.gov/medlineplus/ency/article/000726.htm.

93. Fuller, J.H., L.K. Stevens, and S.L. Wang, *Risk factors for cardiovascular mortality and morbidity: the WHO Mutinational Study of Vascular Disease in Diabetes.* Diabetologia, 2001. **44 Suppl 2**: p. S54-64.
94. Fuller, J.H., L.K. Stevens, and S.L. Wang, *International variations in cardiovascular mortality associated with diabetes mellitus: the WHO Multinational Study of Vascular Disease in Diabetes.* Ann Med, 1996. **28**(4): p. 319-22.
95. Laing, S.P., et al., *Mortality from cerebrovascular disease in a cohort of 23 000 patients with insulin-treated diabetes.* Stroke, 2003. **34**(2): p. 418-21.
96. Soedamah-Muthu, S.S., et al., *High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database.* Diabetes Care, 2006. **29**(4): p. 798-804.
97. Janghorbani, M., et al., *Prospective study of type 1 and type 2 diabetes and risk of stroke subtypes: the Nurses' Health Study.* Diabetes Care, 2007. **30**(7): p. 1730-5.
98. McPhee, S.J., Lingappa, Vishwanath R., Ganong, William F., and Lange, Jack D. , *Pathophysiology of Disease: An Introduction to Clinical Medicine*. 1999: McGraw-Hill Medical. 662.
99. Safar, M.E., B.I. Levy, and H. Struijker-Boudier, *Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases.* Circulation, 2003. **107**(22): p. 2864-9.
100. Mackenzie, I.S., I.B. Wilkinson, and J.R. Cockcroft, *Assessment of arterial stiffness in clinical practice.* QJM, 2002. **95**(2): p. 67-74.
101. Laurent, S., et al., *Expert consensus document on arterial stiffness: methodological issues and clinical applications.* Eur Heart J, 2006. **27**(21): p. 2588-605.
102. O'Rourke, M.F., et al., *Clinical applications of arterial stiffness; definitions and reference values.* Am J Hypertens, 2002. **15**(5): p. 426-44.
103. Dart, A.M. and B.A. Kingwell, *Pulse pressure--a review of mechanisms and clinical relevance.* J Am Coll Cardiol, 2001. **37**(4): p. 975-84.
104. Franklin, S.S., et al., *Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study.* Circulation, 1997. **96**(1): p. 308-15.
105. Pauca, A.L., et al., *Does radial artery pressure accurately reflect aortic pressure?* Chest, 1992. **102**(4): p. 1193-8.
106. Franklin, S.S., et al., *Is pulse pressure useful in predicting risk for coronary heart Disease? The Framingham heart study.* Circulation, 1999. **100**(4): p. 354-60.
107. Millar, J.A., A.F. Lever, and V. Burke, *Pulse pressure as a risk factor for cardiovascular events in the MRC Mild Hypertension Trial.* J Hypertens, 1999. **17**(8): p. 1065-72.
108. Safar, M., *[Aging and arterial rigidity].* J Mal Vasc, 2002. **27 Spec No**: p. S34-8.
109. Aronson, D., *Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes.* J Hypertens, 2003. **21**(1): p. 3-12.
110. Van Bortel, L.M., et al., *Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures.* Am J Hypertens, 2002. **15**(5): p. 445-52.
111. Meaume, S., et al., *Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age.* Arterioscler Thromb Vasc Biol, 2001. **21**(12): p. 2046-50.
112. Mattace-Raso, F.U., et al., *Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study.* Circulation, 2006. **113**(5): p. 657-63.

113. Cruickshank, K., et al., *Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function?* Circulation, 2002. **106**(16): p. 2085-90.
114. Tsivgoulis, G., et al., *Common carotid arterial stiffness and the risk of ischaemic stroke.* Eur J Neurol, 2006. **13**(5): p. 475-81.
115. Stork, S., et al., *Carotid artery plaque burden, stiffness, and mortality risk in elderly men: a prospective, population-based cohort study.* Circulation, 2004. **110**(3): p. 344-8.
116. Liao, D., et al., *Arterial stiffness and the development of hypertension. The ARIC study.* Hypertension, 1999. **34**(2): p. 201-6.
117. London, G.M., et al., *Arterial wave reflections and survival in end-stage renal failure.* Hypertension, 2001. **38**(3): p. 434-8.
118. Kelly, R.P., et al., *Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men.* Hypertension, 2001. **37**(6): p. 1429-33.
119. Wilkinson, I.B., et al., *Changes in the derived central pressure waveform and pulse pressure in response to angiotensin II and noradrenaline in man.* J Physiol, 2001. **530**(Pt 3): p. 541-50.
120. Smulyan, H., et al., *Influence of body height on pulsatile arterial hemodynamic data.* J Am Coll Cardiol, 1998. **31**(5): p. 1103-9.
121. Wilkinson, I.B., et al., *The influence of heart rate on augmentation index and central arterial pressure in humans.* J Physiol, 2000. **525 Pt 1**: p. 263-70.
122. Nagai, Y., et al., *Carotid artery intima-media thickness and plaque score for the risk assessment of stroke subtypes.* Ultrasound Med Biol, 2002. **28**(10): p. 1239-43.
123. Jani, B. and C. Rajkumar, *Ageing and vascular ageing.* Postgrad Med J, 2006. **82**(968): p. 357-62.
124. Virmani, R., et al., *Effect of aging on aortic morphology in populations with high and low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities.* Am J Pathol, 1991. **139**(5): p. 1119-29.
125. Avolio, A., *Ageing and wave reflection.* J Hypertens Suppl, 1992. **10**(6): p. S83-6.
126. Crilly, M., et al., *Repeatability of the measurement of augmentation index in the clinical assessment of arterial stiffness using radial applanation tonometry.* Scand J Clin Lab Invest, 2007. **67**(4): p. 413-22.
127. Kingwell, B.A., et al., *Arterial compliance increases after moderate-intensity cycling.* Am J Physiol, 1997. **273**(5 Pt 2): p. H2186-91.
128. Ferreira, I., et al., *The metabolic syndrome, cardiopulmonary fitness, and subcutaneous trunk fat as independent determinants of arterial stiffness: the Amsterdam Growth and Health Longitudinal Study.* Arch Intern Med, 2005. **165**(8): p. 875-82.
129. Kool, M.J., et al., *Short- and long-term effects of smoking on arterial wall properties in habitual smokers.* J Am Coll Cardiol, 1993. **22**(7): p. 1881-6.
130. Simon, A.C., et al., *Evidence of early degenerative changes in large arteries in human essential hypertension.* Hypertension, 1985. **7**(5): p. 675-80.
131. Wilkinson, I.B., et al., *Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia.* J Am Coll Cardiol, 2002. **39**(6): p. 1005-11.
132. Henry, R.M., et al., *Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study.* Circulation, 2003. **107**(16): p. 2089-95.
133. Schram, M.T., et al., *Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study.* Hypertension, 2004. **43**(2): p. 176-81.

134. Oxlund, H., et al., *Increased aortic stiffness in patients with type 1 (insulin-dependent) diabetes mellitus.* Diabetologia, 1989. **32**(10): p. 748-52.
135. Schram, M.T., et al., *Advanced glycation end products are associated with pulse pressure in type 1 diabetes: the EURODIAB Prospective Complications Study.* Hypertension, 2005. **46**(1): p. 232-7.
136. McVeigh, G.E., et al., *Nitric oxide modulation of blood vessel tone identified by arterial waveform analysis.* Clin Sci (Lond), 2001. **100**(4): p. 387-93.
137. Furchgott, R.F. and J.V. Zawadzki, *The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.* Nature, 1980. **288**(5789): p. 373-6.
138. Rubanyi, G.M. and P.M. Vanhoutte, *Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor.* Am J Physiol, 1986. **250**(5 Pt 2): p. H822-7.
139. Tomasian, D., J.F. Keaney, and J.A. Vita, *Antioxidants and the bioactivity of endothelium-derived nitric oxide.* Cardiovasc Res, 2000. **47**(3): p. 426-35.
140. Cai, H. and D.G. Harrison, *Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress.* Circ Res, 2000. **87**(10): p. 840-4.
141. Giugliano, D., et al., *Vascular effects of acute hyperglycemia in humans are reversed by L-arginine. Evidence for reduced availability of nitric oxide during hyperglycemia.* Circulation, 1997. **95**(7): p. 1783-90.
142. Kawano, H., et al., *Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery.* J Am Coll Cardiol, 1999. **34**(1): p. 146-54.
143. Giardino, I., D. Edelstein, and M. Brownlee, *BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells.* J Clin Invest, 1996. **97**(6): p. 1422-8.
144. Baynes, J.W., *Role of oxidative stress in development of complications in diabetes.* Diabetes, 1991. **40**(4): p. 405-12.
145. Nishikawa, T., et al., *Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage.* Nature, 2000. **404**(6779): p. 787-90.
146. Brownlee, M., *Advanced protein glycosylation in diabetes and aging.* Annu Rev Med, 1995. **46**: p. 223-34.
147. Koya, D. and G.L. King, *Protein kinase C activation and the development of diabetic complications.* Diabetes, 1998. **47**(6): p. 859-66.
148. Lee, A.Y., S.K. Chung, and S.S. Chung, *Demonstration that polyol accumulation is responsible for diabetic cataract by the use of transgenic mice expressing the aldose reductase gene in the lens.* Proc Natl Acad Sci U S A, 1995. **92**(7): p. 2780-4.
149. Nigam, A., et al., *Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease.* Am J Cardiol, 2003. **92**(4): p. 395-9.
150. Nakamura, M., et al., *Reduced vascular compliance is associated with impaired endothelium-dependent dilatation in the brachial artery of patients with congestive heart failure.* J Card Fail, 2004. **10**(1): p. 36-42.
151. Jadhav, U.M. and N.N. Kadam, *Non-invasive assessment of arterial stiffness by pulse-wave velocity correlates with endothelial dysfunction.* Indian Heart J, 2005. **57**(3): p. 226-32.
152. Westhoff, T.H., et al., *Small artery elasticity assessed by pulse wave analysis is no measure of endothelial dysfunction.* J Hypertens, 2007. **25**(3): p. 571-6.

153. Ronnback, M., et al., *Altered age-related blood pressure pattern in type 1 diabetes.* Circulation, 2004. **110**(9): p. 1076-82.
154. Ryden Ahlgren, A., et al., *Increased arterial stiffness in women, but not in men, with IDDM.* Diabetologia, 1995. **38**(9): p. 1082-9.
155. Ahlgren, A.R., et al., *Increased aortic stiffness is persistent in type 1 diabetic women: a follow-up study.* Diabetologia, 2005. **48**(4): p. 780-3.
156. Schram, M.T., et al., *Pulse pressure is associated with age and cardiovascular disease in type 1 diabetes: the Eurodiab Prospective Complications Study.* J Hypertens, 2003. **21**(11): p. 2035-44.
157. Gordin, D., et al., *Acute hyperglycaemia rapidly increases arterial stiffness in young patients with type 1 diabetes.* Diabetologia, 2007. **50**(9): p. 1808-14.
158. Westerbacka, J., et al., *Insulin-induced decrease in large artery stiffness is impaired in uncomplicated type 1 diabetes mellitus.* Hypertension, 2000. **35**(5): p. 1043-8.
159. Chen, C.H., et al., *Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function.* Circulation, 1997. **95**(7): p. 1827-36.
160. Karamanoglu, M., et al., *An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man.* Eur Heart J, 1993. **14**(2): p. 160-7.
161. Pauca, A.L., M.F. O'Rourke, and N.D. Kon, *Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform.* Hypertension, 2001. **38**(4): p. 932-7.
162. Savage, M.T., et al., *Reproducibility of derived central arterial waveforms in patients with chronic renal failure.* Clin Sci (Lond), 2002. **103**(1): p. 59-65.
163. Wilkinson, I.B., et al., *Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis.* J Hypertens, 1998. **16**(12 Pt 2): p. 2079-84.
164. Papaioannou, T.G., et al., *Monitoring of arterial stiffness indices by applanation tonometry and pulse wave analysis: reproducibility at low blood pressures.* J Clin Monit Comput, 2004. **18**(2): p. 137-44.
165. Hamilton, P.K., et al., *Arterial stiffness: clinical relevance, measurement and treatment.* Clin Sci (Lond), 2007. **113**(4): p. 157-70.
166. Asmar, R., et al., *Pulse pressure and aortic pulse wave are markers of cardiovascular risk in hypertensive populations.* Am J Hypertens, 2001. **14**(2): p. 91-7.
167. Blacher, J., et al., *Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients.* Hypertension, 1999. **33**(5): p. 1111-7.
168. O'Rourke, M., *Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension.* Hypertension, 1990. **15**(4): p. 339-47.
169. Wilkinson, I.B., et al., *Increased augmentation index and systolic stress in type 1 diabetes mellitus.* Qjm, 2000. **93**(7): p. 441-8.
170. Oliver, J.J. and D.J. Webb, *Noninvasive assessment of arterial stiffness and risk of atherosclerotic events.* Arterioscler Thromb Vasc Biol, 2003. **23**(4): p. 554-66.
171. O'Rourke, M.F., *Wave travel and reflection in the arterial system.* J Hypertens Suppl, 1999. **17**(5): p. S45-7.
172. Yasmin and M.J. Brown, *Similarities and differences between augmentation index and pulse wave velocity in the assessment of arterial stiffness.* Qjm, 1999. **92**(10): p. 595-600.

173. Cameron, J.D., B.P. McGrath, and A.M. Dart, *Use of radial artery applanation tonometry and a generalized transfer function to determine aortic pressure augmentation in subjects with treated hypertension.* J Am Coll Cardiol, 1998. **32**(5): p. 1214-20.
174. Buckberg, G.D., et al., *Subendocardial ischemia after cardiopulmonary bypass.* J Thorac Cardiovasc Surg, 1972. **64**(5): p. 669-84.
175. Astrup, A.S., et al., *Cardiac autonomic neuropathy predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy.* Diabetes Care, 2006. **29**(2): p. 334-9.
176. Orchard, T.J., et al., *Prevalence of complications in IDDM by sex and duration. Pittsburgh Epidemiology of Diabetes Complications Study II.* Diabetes, 1990. **39**(9): p. 1116-24.
177. Wagener, D., et al., *Pittsburgh diabetes mellitus study. II. Secondary attack rates in families with insulin-dependent diabetes mellitus.* Am J Epidemiol, 1982. **115**(6): p. 868-78.
178. Kriska, A.M., et al., *The association of physical activity and diabetic complications in individuals with insulin-dependent diabetes mellitus: the Epidemiology of Diabetes Complications Study--VII.* J Clin Epidemiol, 1991. **44**(11): p. 1207-14.
179. Morgan, T., et al., *Effect of different antihypertensive drug classes on central aortic pressure.* Am J Hypertens, 2004. **17**(2): p. 118-23.
180. *The hypertension detection and follow-up program: Hypertension detection and follow-up program cooperative group.* Prev Med, 1976. **5**(2): p. 207-15.
181. Allain, C.C., et al., *Enzymatic determination of total serum cholesterol.* Clin Chem, 1974. **20**(4): p. 470-5.
182. Bucolo, G. and H. David, *Quantitative determination of serum triglycerides by the use of enzymes.* Clin Chem, 1973. **19**(5): p. 476-82.
183. Warnick, G.R. and J.J. Albers, *Heparin--Mn2+ quantitation of high-density-lipoprotein cholesterol: an ultrafiltration procedure for lipemic samples.* Clin Chem, 1978. **24**(6): p. 900-4.
184. *National Institutes of Health*, D.o.H.E.a.W.L.R.C. Program, Editor. 1975, Washington, DC, US Government Printing Office.
185. Assadi, F.K., et al., *Falsely elevated serum creatinine concentration in ketoacidosis.* J Pediatr, 1985. **107**(4): p. 562-4.
186. Ellis, D. and G.J. Buffone, *New approach to evaluation of proteinuric states.* Clin Chem, 1977. **23**(4): p. 666-70.
187. Ellis, D., et al., *Choice of urine sample predictive of microalbuminuria in patients with insulin-dependent diabetes mellitus.* Am J Kidney Dis, 1989. **13**(4): p. 321-8.
188. Schumer, M., et al., *Diabetic autonomic neuropathy--Part I. Autonomic nervous system data analysis by a computerized central unit in a multicenter trial.* Am J Med, 1988. **85**(5A): p. 137-43.
189. O'Rourke, M.F., A. Pauca, and X.J. Jiang, *Pulse wave analysis.* Br J Clin Pharmacol, 2001. **51**(6): p. 507-22.
190. Buckberg, G.D., et al., *Experimental subendocardial ischemia during left ventricular hypertension.* Surg Forum, 1971. **22**: p. 124-5.
191. Buckberg, G.D., et al., *Experimental subendocardial ischemia in dogs with normal coronary arteries.* Circ Res, 1972. **30**(1): p. 67-81.

192. Ahlgren, A.R., et al., *Increased aortic stiffness in women with type 1 diabetes mellitus is associated with diabetes duration and autonomic nerve function.* Diabet Med, 1999. **16**(4): p. 291-7.
193. Orchard, T.J., et al., *Why does diabetic autonomic neuropathy predict IDDM mortality? An analysis from the Pittsburgh Epidemiology of Diabetes Complications Study.* Diabetes Res Clin Pract, 1996. **34 Suppl**: p. S165-71.
194. Taskiran, M., et al., *Left ventricular dysfunction in normotensive Type 1 diabetic patients: the impact of autonomic neuropathy.* Diabet Med, 2004. **21**(6): p. 524-30.
195. Abhayaratna, W.P., et al., *Relation of arterial stiffness to left ventricular diastolic function and cardiovascular risk prediction in patients > or =65 years of age.* Am J Cardiol, 2006. **98**(10): p. 1387-92.
196. Giannattasio, C., et al., *Early impairment of large artery structure and function in type I diabetes mellitus.* Diabetologia, 1999. **42**(8): p. 987-94.
197. Enderle, M.D., et al., *Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects.* Diabetes Care, 1998. **21**(2): p. 271-7.
198. Duprez, D.A., et al., *Determinants of radial artery pulse wave analysis in asymptomatic individuals.* Am J Hypertens, 2004. **17**(8): p. 647-53.
199. Doyle, J.T., *Risk factors in arteriosclerosis and cardiovascular disease with special emphasis on cigarette smoking.* Prev Med, 1979. **8**(3): p. 264-70.
200. Jatoi, N.A., et al., *Impact of smoking and smoking cessation on arterial stiffness and aortic wave reflection in hypertension.* Hypertension, 2007. **49**(5): p. 981-5.
201. McNulty, M., A. Mahmud, and J. Feely, *Advanced glycation end-products and arterial stiffness in hypertension.* Am J Hypertens, 2007. **20**(3): p. 242-7.
202. Fantin, F., et al., *Is augmentation index a good measure of vascular stiffness in the elderly?* Age Ageing, 2007. **36**(1): p. 43-8.
203. Krolewski, A.S., et al., *Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus.* Am J Cardiol, 1987. **59**(8): p. 750-5.
204. Orchard, T.J., et al., *Lipid and blood pressure treatment goals for type 1 diabetes: 10-year incidence data from the Pittsburgh Epidemiology of Diabetes Complications Study.* Diabetes Care, 2001. **24**(6): p. 1053-9.
205. Maser, R.E., et al., *Cardiovascular disease and arterial calcification in insulin-dependent diabetes mellitus: interrelations and risk factor profiles. Pittsburgh Epidemiology of Diabetes Complications Study-V.* Arterioscler Thromb, 1991. **11**(4): p. 958-65.
206. Janowitz, W.R., et al., *Differences in prevalence and extent of coronary artery calcium detected by ultrafast computed tomography in asymptomatic men and women.* Am J Cardiol, 1993. **72**(3): p. 247-54.
207. Olson, J.C., et al., *Coronary calcium in adults with type 1 diabetes: a stronger correlate of clinical coronary artery disease in men than in women.* Diabetes, 2000. **49**(9): p. 1571-8.
208. Nichols, W.W. and B.M. Singh, *Augmentation index as a measure of peripheral vascular disease state.* Curr Opin Cardiol, 2002. **17**(5): p. 543-51.
209. Mahmud, A. and J. Feely, *Effect of smoking on arterial stiffness and pulse pressure amplification.* Hypertension, 2003. **41**(1): p. 183-7.
210. Hayashi, T., et al., *Reflection in the arterial system and the risk of coronary heart disease.* Am J Hypertens, 2002. **15**(5): p. 405-9.

211. Weber, T., et al., *Arterial stiffness, wave reflections, and the risk of coronary artery disease.* Circulation, 2004. **109**(2): p. 184-9.
212. Nurnberger, J., et al., *Augmentation index is associated with cardiovascular risk.* J Hypertens, 2002. **20**(12): p. 2407-14.
213. Lekakis, J.P., et al., *Arterial wave reflection is associated with severity of extracoronary atherosclerosis in patients with coronary artery disease.* Eur J Cardiovasc Prev Rehabil, 2006. **13**(2): p. 236-42.
214. Covic, A., et al., *Aortic pulse wave velocity and arterial wave reflections predict the extent and severity of coronary artery disease in chronic kidney disease patients.* J Nephrol, 2005. **18**(4): p. 388-96.
215. Altunkan, S., K. Oztas, and B. Seref, *Arterial stiffness index as a screening test for cardiovascular risk: a comparative study between coronary artery calcification determined by electron beam tomography and arterial stiffness index determined by a VitalVision device in asymptomatic subjects.* Eur J Intern Med, 2005. **16**(8): p. 580-4.
216. Haydar, A.A., et al., *Coronary artery calcification and aortic pulse wave velocity in chronic kidney disease patients.* Kidney Int, 2004. **65**(5): p. 1790-4.
217. Khaleghi, M. and I.J. Kullo, *Aortic augmentation index is associated with the ankle-brachial index: a community-based study.* Atherosclerosis, 2007. **195**(2): p. 248-53.
218. Rose, G.A. and H. Blackburn, *Cardiovascular survey methods.* Monogr Ser World Health Organ, 1968. **56**: p. 1-188.
219. Sibal, L., et al., *Predicting the development of macrovascular disease in people with type 1 diabetes: A 9-year follow-up study.* Ann N Y Acad Sci, 2006. **1084**: p. 191-207.
220. Costacou, T., et al., *Progression of coronary artery calcium in type 1 diabetes mellitus.* Am J Cardiol, 2007. **100**(10): p. 1543-7.
221. Hata, N., et al., *Clinical significance of coronary artery calcification.* Int Angiol, 1994. **13**(4): p. 281-5.
222. Gozna, E.R., et al., *Age-related changes in the mechanics of the aorta and pulmonary artery of man.* J Appl Physiol, 1974. **36**(4): p. 407-11.
223. Lambert, J., et al., *Carotid artery stiffness is increased in microalbuminuric IDDM patients.* Diabetes Care, 1998. **21**(1): p. 99-103.
224. Pannier, B.M., et al., *Different aortic reflection wave responses following long-term angiotensin-converting enzyme inhibition and beta-blocker in essential hypertension.* Clin Exp Pharmacol Physiol, 2001. **28**(12): p. 1074-7.
225. Simon, A.C., et al., *Effect of nitroglycerin on peripheral large arteries in hypertension.* Br J Clin Pharmacol, 1982. **14**(2): p. 241-6.
226. Stokes, G.S., et al., *A controlled study of the effects of isosorbide mononitrate on arterial blood pressure and pulse wave form in systolic hypertension.* J Hypertens, 1999. **17**(12 Pt 1): p. 1767-73.
227. Stokes, G.S., et al., *Long-term effectiveness of extended-release nitrate for the treatment of systolic hypertension.* Hypertension, 2005. **45**(3): p. 380-4.
228. Olson, J.C., et al., *Subclinical atherosclerosis and estimated glucose disposal rate as predictors of mortality in type 1 diabetes.* Ann Epidemiol, 2002. **12**(5): p. 331-7.
229. *Incidence of end-stage renal disease among persons with diabetes--United States, 1990-2002.* MMWR Morb Mortal Wkly Rep, 2005. **54**(43): p. 1097-100.
230. *American Diabetes Association, Diabetic Nephropathy.* Diabetes Care, 2002. **25**(Supplement): p. S85-S89.

231. Yishak, A.A., et al., *Novel predictors of overt nephropathy in subjects with type 1 diabetes. A nested case control study from the Pittsburgh Epidemiology of Diabetes Complications cohort.* Nephrol Dial Transplant, 2006. **21**(1): p. 93-100.
232. Kostraba, J.N., et al., *The epidemiology of diabetes complications study. IV. Correlates of diabetic background and proliferative retinopathy.* Am J Epidemiol, 1991. **133**(4): p. 381-91.
233. Romero, P., et al., *Renal and retinal microangiopathy after 15 years of follow-up study in a sample of Type 1 diabetes mellitus patients.* J Diabetes Complications, 2007. **21**(2): p. 93-100.
234. Astor, B.C., et al., *Glomerular filtration rate, albuminuria, and risk of cardiovascular and all-cause mortality in the US population.* Am J Epidemiol, 2008. **167**(10): p. 1226-34.
235. Henry, R.M., et al., *Mild renal insufficiency is associated with increased cardiovascular mortality: The Hoorn Study.* Kidney Int, 2002. **62**(4): p. 1402-7.
236. De Leeuw, P.W., et al., *Prognostic significance of renal function in elderly patients with isolated systolic hypertension: results from the Syst-Eur trial.* J Am Soc Nephrol, 2002. **13**(9): p. 2213-22.
237. Henry, R.M., et al., *Mild renal insufficiency is associated with increased left ventricular mass in men, but not in women: an arterial stiffness-related phenomenon--the Hoorn Study.* Kidney Int, 2005. **68**(2): p. 673-9.
238. Ishizaka, N., et al., *Association between chronic kidney disease and carotid intima-media thickening in individuals with hypertension and impaired glucose metabolism.* Hypertens Res, 2007. **30**(11): p. 1035-41.
239. Maahs, D.M., et al., *Serum cystatin C predicts progression of subclinical coronary atherosclerosis in individuals with type 1 diabetes.* Diabetes, 2007. **56**(11): p. 2774-9.
240. Fox, C.S., et al., *Kidney function is inversely associated with coronary artery calcification in men and women free of cardiovascular disease: the Framingham Heart Study.* Kidney Int, 2004. **66**(5): p. 2017-21.
241. Arnlov, J., et al., *Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study.* Circulation, 2005. **112**(7): p. 969-75.
242. Stehouwer, C.D., et al., *Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus.* Lancet, 1992. **340**(8815): p. 319-23.
243. Hillege, H.L., et al., *Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population.* Circulation, 2002. **106**(14): p. 1777-82.
244. Taal, M.W., et al., *Markers of arterial stiffness are risk factors for progression to end-stage renal disease among patients with chronic kidney disease stages 4 and 5.* Nephron Clin Pract, 2007. **107**(4): p. c177-81.
245. Mule, G., et al., *Inverse relationship between ambulatory arterial stiffness index and glomerular filtration rate in arterial hypertension.* Am J Hypertens, 2008. **21**(1): p. 35-40.
246. Kawamoto, R., et al., *An association between decreased estimated glomerular filtration rate and arterial stiffness.* Intern Med, 2008. **47**(7): p. 593-8.

247. Nakagawa, N., et al., *A newly estimated glomerular filtration rate is independently associated with arterial stiffness in Japanese patients.* Hypertens Res, 2008. **31**(2): p. 193-201.
248. Wang, M.C., et al., *Stepwise increase in arterial stiffness corresponding with the stages of chronic kidney disease.* Am J Kidney Dis, 2005. **45**(3): p. 494-501.
249. Guerin, A.P., et al., *Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure.* Circulation, 2001. **103**(7): p. 987-92.
250. Blacher, J., et al., *Aortic pulse wave velocity index and mortality in end-stage renal disease.* Kidney Int, 2003. **63**(5): p. 1852-60.
251. Chirinos, J.A., et al., *Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease.* Hypertension, 2005. **45**(5): p. 980-5.
252. Levey, A.S., et al., *Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values.* Clin Chem, 2007. **53**(4): p. 766-72.
253. Hermans, M.M., et al., *Estimated glomerular filtration rate and urinary albumin excretion are independently associated with greater arterial stiffness: the Hoorn Study.* J Am Soc Nephrol, 2007. **18**(6): p. 1942-52.
254. Cheng, L.T., et al., *Limitation of the augmentation index for evaluating arterial stiffness.* Hypertens Res, 2007. **30**(8): p. 713-22.
255. Pambianco, G., T. Costacou, and T.J. Orchard, *The prediction of major outcomes of type 1 diabetes: a 12-year prospective evaluation of three separate definitions of the metabolic syndrome and their components and estimated glucose disposal rate: the Pittsburgh Epidemiology of Diabetes Complications Study experience.* Diabetes Care, 2007. **30**(5): p. 1248-54.
256. Nathan, D.M., et al., *Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes.* N Engl J Med, 2005. **353**(25): p. 2643-53.
257. Buysschaert, M., et al., *Comparative performance of serum cystatin-c versus serum creatinine in diabetic subjects.* Diabetes Metab, 2003. **29**(4 Pt 1): p. 377-83.
258. Dharnidharka, V.R., C. Kwon, and G. Stevens, *Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis.* Am J Kidney Dis, 2002. **40**(2): p. 221-6.
259. Laterza, O.F., C.P. Price, and M.G. Scott, *Cystatin C: an improved estimator of glomerular filtration rate?* Clin Chem, 2002. **48**(5): p. 699-707.
260. Yoshida, M., et al., *Relationships among renal function loss within the normal to mildly impaired range, arterial stiffness, inflammation, and oxidative stress.* Clin J Am Soc Nephrol, 2007. **2**(6): p. 1118-24.
261. Chade, A.R., et al., *Mild renal insufficiency is associated with reduced coronary flow in patients with non-obstructive coronary artery disease.* Kidney Int, 2006. **69**(2): p. 266-71.
262. Shimada, Y. and M. Avkiran, *Left and right coronary circulation in left ventricular hypertrophy: effects of angiotensin converting enzyme inhibition.* Circ J, 2002. **66**(12): p. 1168-72.
263. Kitakaze, M., et al., *Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers synergistically increase coronary blood flow in canine ischemic myocardium: role of bradykinin.* J Am Coll Cardiol, 2002. **40**(1): p. 162-6.

264. Galderisi, M. and A. D'Errico, *Beta-blockers and coronary flow reserve: the importance of a vasodilatory action.* Drugs, 2008. **68**(5): p. 579-90.
265. Keane, W.F. and B.E. Shapiro, *Renal protective effects of angiotensin-converting enzyme inhibition.* Am J Cardiol, 1990. **65**(19): p. 49I-53I.
266. Remuzzi, A., et al., *Comparison of the effects of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade on the evolution of spontaneous glomerular injury in male MWF/Ztm rats.* Exp Nephrol, 1996. **4**(1): p. 19-25.
267. Manolis, A.J., et al., *Arterial compliance changes in diabetic normotensive patients after angiotensin-converting enzyme inhibition therapy.* Am J Hypertens, 2005. **18**(1): p. 18-22.
268. London, G.M., et al., *Antihypertensive effects and arterial haemodynamic alterations during angiotensin converting enzyme inhibition.* J Hypertens, 1996. **14**(9): p. 1139-46.
269. Agata, J., et al., *Angiotensin II receptor blocker prevents increased arterial stiffness in patients with essential hypertension.* Circ J, 2004. **68**(12): p. 1194-8.
270. Mahmud, A. and J. Feely, *Favourable effects on arterial wave reflection and pulse pressure amplification of adding angiotensin II receptor blockade in resistant hypertension.* J Hum Hypertens, 2000. **14**(9): p. 541-6.
271. Levy, B.I., et al., *Angiotensin converting enzyme (ACE) inhibitors in experimental hypertension: influence on heart and arteries.* Basic Res Cardiol, 1991. **86 Suppl 1**: p. 43-53.
272. Levy, B.I., et al., *Long-term effects of angiotensin-converting enzyme inhibition on the arterial wall of adult spontaneously hypertensive rats.* Am J Cardiol, 1993. **71**(17): p. 8E-16E.
273. De Gennaro Colonna, V., et al., *Angiotensin-converting enzyme inhibition and angiotensin AT1-receptor antagonism equally improve endothelial vasodilator function in L-NAME-induced hypertensive rats.* Eur J Pharmacol, 2005. **516**(3): p. 253-9.
274. Tomiyama, H., et al., *Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement--a survey of 12517 subjects.* Atherosclerosis, 2003. **166**(2): p. 303-9.
275. Weinberger, M.H., N.S. Fineberg, and S.E. Fineberg, *Effects of age, race, gender, blood pressure, and estrogen on arterial compliance.* Am J Hypertens, 2002. **15**(4 Pt 1): p. 358-63.
276. Smulyan, H., et al., *Comparative effects of aging in men and women on the properties of the arterial tree.* J Am Coll Cardiol, 2001. **37**(5): p. 1374-80.
277. Sundkvist, G., L. Almer, and B. Lilja, *Respiratory influence on heart rate in diabetes mellitus.* Br Med J, 1979. **1**(6168): p. 924-5.
278. Jacobs, D.R., Jr., et al., *Cigarette smoking and mortality risk: twenty-five-year follow-up of the Seven Countries Study.* Arch Intern Med, 1999. **159**(7): p. 733-40.
279. Oren, S., et al., *The influence of smoking cessation on hemodynamics and arterial compliance.* Angiology, 2006. **57**(5): p. 564-8.
280. Rehill, N., et al., *The effect of chronic tobacco smoking on arterial stiffness.* Br J Clin Pharmacol, 2006. **61**(6): p. 767-73.
281. Scott-Burden, T. and P.M. Vanhoutte, *Regulation of smooth muscle cell growth by endothelium-derived factors.* Tex Heart Inst J, 1994. **21**(1): p. 91-7.

282. Guo, X., et al., *Effect of cigarette smoking on nitric oxide, structural, and mechanical properties of mouse arteries.* Am J Physiol Heart Circ Physiol, 2006. **291**(5): p. H2354-61.
283. Glagov, S., et al., *Micro-architecture and composition of artery walls: relationship to location, diameter and the distribution of mechanical stress.* J Hypertens Suppl, 1992. **10**(6): p. S101-4.
284. Tayebjee, M.H., R.J. MacFadyen, and G.Y. Lip, *Extracellular matrix biology: a new frontier in linking the pathology and therapy of hypertension?* J Hypertens, 2003. **21**(12): p. 2211-8.
285. Airaksinen, K.E., et al., *Diminished arterial elasticity in diabetes: association with fluorescent advanced glycosylation end products in collagen.* Cardiovasc Res, 1993. **27**(6): p. 942-5.
286. Romney, J.S. and R.Z. Lewanczuk, *Vascular compliance is reduced in the early stages of type 1 diabetes.* Diabetes Care, 2001. **24**(12): p. 2102-6.
287. Maguire, A.M., et al., *Autonomic nerve testing predicts the development of complications: a 12-year follow-up study.* Diabetes Care, 2007. **30**(1): p. 77-82.
288. Chico, A., A. Tomas, and A. Novials, *Silent myocardial ischemia is associated with autonomic neuropathy and other cardiovascular risk factors in type 1 and type 2 diabetic subjects, especially in those with microalbuminuria.* Endocrine, 2005. **27**(3): p. 213-7.
289. Costacou, T., et al., *Sequence of progression of albuminuria and decreased GFR in persons with type 1 diabetes: a cohort study.* Am J Kidney Dis, 2007. **50**(5): p. 721-32.
290. Hlaing, W.M. and R.J. Prineas, *Arterial stiffness variations by gender in African-American and Caucasian children.* J Natl Med Assoc, 2006. **98**(2): p. 181-9.
291. Ahimastos, A.A., et al., *Gender differences in large artery stiffness pre- and post puberty.* J Clin Endocrinol Metab, 2003. **88**(11): p. 5375-80.
292. Ahlund, C., K. Pettersson, and L. Lind, *Pulse wave analysis on fingertip arterial pressure: effects of age, gender and stressors on reflected waves and their relation to brachial and femoral artery blood flow.* Clin Physiol Funct Imaging, 2008. **28**(2): p. 86-95.
293. O'Rourke, M.F. and C.S. Hayward, *Arterial stiffness, gender and heart rate.* J Hypertens, 2003. **21**(3): p. 487-90.