ARTERIAL STIFFNESS AND ENDOTHELIAL DYSFUNCTION IN CHILDREN WITH TYPE 1 DIABETES

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FUFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2006

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By

Michael James Haller

To the children who face the challenges of diabetes every day.

ACKNOWLEDGMENTS

I thank the members of my supervisory committee for their mentoring, the many participants in my research studies, and the Children's Miracle Network (CMN) and the Diabetes Action Research and Education Foundation (DARE) for financial support. Additional funding was provided by NIH grants 42288-05 and 39250-06 and GCRC grant MO1-RR00082. I thank the Florida Camp for Children and Youth with Diabetes (FCCYD) and Benton Pediatrics for allowing patient recruitment, and Kelvin Lee and Jennifer Stein for performing radial tonometry and Endo-PAT®. I thank my parents for providing unwavering support of my intellectual curiosity and academic interests. I thank my wife for filling my life with joy and meaning every day.

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of the University of Florida in Partial Fulfillment of the
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December 2006

Chair: Marian C. Limacher
Major Department: Medical Sciences--Clinical Investigation

To determine if children with type 1 diabetes mellitus (T1D) have increased arterial stiffness and endothelial dysfunction, we performed two separate case-control studies using radial artery tonometry and augmentation index to estimate arterial stiffness and peripheral artery tonometry (PAT) and Endo-PAT® score to estimate endothelial function. In our arterial stiffness study we studied children aged 10-18 years, 98 with T1D and 57 healthy controls matched for age, sex, race, and BMI, generating 43 matched pairs. Radial artery tonometry was performed under basal resting conditions, immediately before a fasting blood sample was collected for analysis of fasting lipids, HbA1c, glucose, and cytokines on all children. In our endothelial dysfunction studies, 44 children with T1D (age 14.6 ± 2.7 years; duration of diabetes 6.01 ± 4 years; range of diabetes duration 1-16 years; HbA1c 8.34% ± 1.2) and 20 control children (age 14.1 ± 1.5 years) underwent endothelial function testing after an overnight fast using the Endo-PAT® finger tip device. Each child had height, weight, BMI, blood pressure, fasting lipid profile, and glucose determinations. All children with T1D underwent a second Endo-PAT® study 4 weeks after their initial study in order to determine the intra-patient variability of the technique.

We determined that children with diabetes had a significantly higher augmentation index corrected to a heart rate of 75 (AI_{75}) than their matched controls. Mean AI_{75} in T1D subjects was 1.11±10.15 versus -3.32±10.36 in controls. The case-control difference was 5.20±11.02 (p=0.0031). Children with T1D had endothelial dysfunction as evidenced by lower mean Endo-PAT® scores (1.63 vs 1.95, p=0.01) when compared to control children. The mean intra-patient standard deviation of Endo-PAT® score in the children with T1D was 0.261. Children with T1D had higher High Densisty Lipoprotein (HDL) cholesterol (p= 0.0001), mean systolic BP (p=0.02), and mean total cholesterol (p=0.03) than control children. No significant differences in age, body mass index (BMI), diastolic blood pressure, Low Density Lipoprotein (LDL) cholesterol, or triglycerides were observed between the children with T1D and control children.

Using radial artery tonometry derived measures of arterial stiffness and Endo-PAT® derived measures of endothelial function, we determined that children with T1D have increased arterial stiffness and endothelial dysfunction compared to matched controls. Such early arterial abnormalities likely contribute to accelerated progression to cardiovascular disease. Future studies will be able to use these noninvasive techniques to assess the impact of specific interventions on arterial health in children with T1D. Radial artery tonometry and Endo-PAT® are promising noninvasive techniques that can be used to assess arterial stiffness and endothelial dysfunction in children with T1D. Non-invasive measures like radial artery tonometry and Endo-PAT® may provide additional risk stratification data needed to justify more aggressive primary prevention of CVD in children with T1D.

CHAPTER 1
INTRODUCTION

Type 1 diabetes (T1D) is a well established risk factor for the development of premature cardiovascular disease (CVD)(1). Despite advances in medical practice over the last 25 years, the incidence of early CVD in the T1D population remains disproportionately high (2-5). The Diabetes Control and Complications Trial (DCCT) and its longitudinal follow up, the Epidemiology of Diabetes Interventions and Complications Study (EDIC), have demonstrated that the risk of T1D-related microvascular and macrovascular complications is related to long-term glycemic control (6; 7).

Unfortunately, even with intensive insulin therapy, the majority of children with T1D are unable to maintain near-normal glycemia. Children in the intensive arm of the DCCT were only able to achieve an average HbA1c of 8.1% (8). However, glycemic control is only one of several important risk factors in defining CVD risk. Minimizing the long term risks for CVD in patients with T1D may require early and aggressive management of other important CVD risk factors such as blood pressure and lipids (9; 10). Many adult studies have demonstrated that the incidence of cardiovascular events can be lowered through reduction of plasma cholesterol levels and optimal management of hypertension. Unfortunately, the majority of patients who are being treated aggressively have already manifested cardiovascular complications.

Because children rarely experience cardiovascular events, surrogate markers of CVD are needed to provide the additional risk stratification needed to justify and monitor the effects of more aggressive therapy (11). Brachial artery reactivity is a technique that measures the endothelium-dependent dilation of the brachial artery in response to reactive hyperemia. In patients with endothelial dysfunction, the ability of the artery to dilate is impaired. Endothelial dysfunction, as measured by decreased brachial reactivity, and arterial stiffness, as measured by

pulse wave analyses, have both been shown to be independent predictors of cardiovascular events (12). Impaired brachial reactivity has been demonstrated in adults and in a small group of children with T1D (13; 14). Unfortunately, standard brachial reactivity studies are difficult to perform, require expensive equipment and involve subjective analysis of the results. Several new methods for measuring endothelial function and arterial stiffness are now available and may be more applicable for everyday clinical use.

To date, studies of arterial stiffness and endothelial function in children with T1D using these newer techniques have not been performed. Because radial tonometry and PAT can be performed in nearly any clinic setting, are easy and affordable to perform, and because they provide the user with an instant analysis of the patient's arterial stiffness or endothelial function, tonometry and PAT have a potential advantage over standard brachial reactivity and carotid IMT as clinically useful tools. To test the utility of radial tonometry and PAT, we studied whether children with T1D had increased arterial stiffness and endothelial dysfunction when compared to healthy controls.

CHAPTER 2
MATERIALS AND METHODS

Arterial Stiffness Studies

Subjects

We evaluated arterial stiffness in 98 children with type 1 diabetes and 57 control volunteers using radial artery tonometry. Children were recruited from the Florida Diabetes Camp, the University of Florida diabetes and primary care clinics, and general pediatrics practices in the area. Children with T1D were recruited by letters sent to the parents of all children registered for diabetes camp offering free lipid and HbA1c analyses in return for participation. Controls were recruited from general pediatrics practices by letters sent to the parents of clinic patients offering free analyses of lipids, glucose, and HbA1c in return for participation. While children with T1D would likely have been routinely tested for lipids, glucose, and HbA1c, healthy controls would not. Thus, excluding diabetes, there may have been differences in background cardiovascular risk between cases and controls as controls whose physician or family perceived them as being at increased risk may have been more inclined to participate. Inclusion criteria for both children with diabetes and controls were age between 10 and 18 years, no known cardiovascular disease, and no history of using anti-hypertensive or lipid lowering medications. Children with diabetes were included only if they had been diagnosed for at least one year. Children were classified as having T1D based on a history of acute onset of polyuria, polydipsia, polyphagia, weight loss, and ketosis. When the history was not clear, islet cell, glutamic acid decarboxylase, or insulin autoantibody status was used to confirm T1D. There were no children with diabetes included that did not have either a well documented history or at least one positive diabetes-related autoantibody. From the total group, 43 matched pairs were

identified who met all inclusion criteria. The groups were matched for age (±2yrs), sex, race, and BMI (±3kg/m^2).

The study was approved by the Institutional Review Board of the University of Florida. Subjects' parents provided written consent before their child was enrolled in the study and the subjects provided assent. Subjects' parents completed a brief questionnaire that included age, race, medications, family history, and level of exercise. Family history was specified in the questionnaire as pertaining to only 1st and 2nd degree relatives of the child, and definitions of hypercholesterolemia, hypertension, and early heart disease were provided. Level of exercise was graded on a 1 to 4 scale (1 = no exercise, 2 = minimal exercise, 3 = moderate exercise, and 4 = extreme exercise). The most recent HbA1c, duration of diagnosis, and history of recent illnesses was obtained from the medical record.

Study Protocol

Radial artery tonometry was performed and blood samples were obtained between 6 am and 10 am on the same day, with the child supine and relaxed. Study subjects were required to fast after midnight and to abstain from caffeine for 24 hours before the study. Height was measured on a wall mounted, calibrated stadiometer (Genentech, San Francisco, California) and weight was read from a calibrated digital scale. After a 5 minute rest in the supine position, the subjects had their blood pressure measured with a digital oscillometric device (Omron model HEM-739, Omron Healthcare Inc., Vernon Hills, Illinois). Radial tonometry was then performed. Following the completion of the radial tonometry, a venous blood sample was obtained for glucose, HbA1c, total cholesterol, LDL, HDL, triglycerides, hsCRP, IL-1β, and IL-6. Subjects with T1D had their morning insulin injection postponed until all studies were completed.

Measurement of Arterial Stiffness by Radial Tonometry

Augmentation index (AI) and augmentation index corrected to a heart rate of 75 (AI_{75}) were measured using the SphygmoCor Vx version 7.01 (AtCor Medical, Sydney, Australia). In brief, a high-fidelity micromanometer with a frequency response of > 2kHz (Millar Instruments, Houston, Texas) was placed on the right radial artery and gentle pressure was applied until a consistent waveform was produced. After 10-20 sequential waveforms had been acquired, the integrated software was used to generate an averaged peripheral and corresponding central waveform that was used for the determination of the AI and AI_{75} (Figure 2-1).

The algorithm used to convert the radial pulse wave to an aortic wave form was derived from invasive arterial pressure and flow data obtained by cardiac catheterization and has been validated in several adult studies (15-17). Validation studies are underway to confirm that the same algorithm can be applied in children. A quality index is displayed and represents the reproducibility of the waveform. A value greater than 70 is considered to demonstrate excellent waveform consistency. For this study, only measurements with a quality index above 80 were accepted. Two acceptable measurements were obtained on each subject. AI is defined as the difference between the first and second peaks of the central arterial waveform, expressed as a percentage of the pulse pressure and measures the contribution that the wave reflection makes to the arterial pressure waveform. The amplitude and timing of the reflected wave depends largely on the stiffness of the small and large arteries. Thus, AI provides a measure of systemic arterial stiffness. AI_{75} allows for a true comparison of the augmentation of central pressure between study subjects by discounting differences related to heart rate variation (18). An elevated or positive AI suggests stiffer arteries than a low or negative AI (Figure 2-2).

Measurement of Lipids, HbA1c, Glucose

Serum was collected from study participants using standard venipuncture techniques and Vacutainer® serum separator tubes (BD Biosciences, San Diego, CA, USA). After collection, samples to be analyzed for lipid, HbA1c, and glucose were immediately refrigerated and transported to the Shands Hospital laboratory at the University of Florida. Samples were analyzed in the clinical laboratory using standard technique.

Measurement of Cytokines

Serum for cytokine and autoantibody analysis was separated into serial aliquots and frozen at -80° C within 1 hour of the blood draw. All serum analyses were conducted following a single freeze-thaw cycle. Cytokine measurements from serum were performed using a commercially available multiplexed kit (Beadlyte® Human Multi-Cytokine Detection System 3, Upstate, Lake Placid, NY) and the Luminex^{100} LabMAP^{TM} System. Quantitative evaluation of the serum cytokines IL-1β and IL-6 was performed. Serum samples were subjected to a 1:2 dilution in serum diluent provided by the manufacturer in order to reduce the effects of interfering heterophile species (19; 20). High sensitivity CRP (Alpco, Windham NH, USA) levels were measured by standard sandwich ELISA techniques according to manufacturer's instructions. Serum analyte concentrations were calculated using 4-parameter analysis utilizing SoftMax Pro Software, Ver. 2.2.1 (Molecular Devices Corp., Sunnyvale, CA, USA).

Measurement of Autoantibodies

Autoantibodies against two T1D-associated autoantigens were tested from serum obtained from all study participants including those against GAD65 and IA-2. Assays were performed as previously described (20). The investigators are regular participants in workshops and proficiency tests sponsored by the Immunology of Diabetes Society and CDC to validate

assay performance. At the most recent effort (DASP 2003), our performance for GADA assay indicated 80% sensitivity/95% specificity for type 1 diabetes, while our IA-2A assay provided 64% sensitivity/100% specificity.

Statistical Considerations

As described above, the study was planned as a matched pair design. However, if matching factors are treated as covariates all conclusions qualitatively remained the same when analysis was performed on all entrants, independent of match availability. When analyzing the matched pairs, case-control comparisons were assessed with one sample paired t-tests for the following dependent variables: AI and AI_{75} (primary) and total cholesterol, HDL, LDL, triglycerides, blood pressure, HbA1c, and glucose (secondary). All p-values were two-sided. The original study was planned for a sample size of 100 matched pairs. We were unable to recruit the anticipated numbers of matched controls within the planned time-frame due to strict matching criteria and unwillingness of control subjects to participate in a blood draw, However, the matched variations in the variables AI and AI_{75} were smaller than originally anticipated, and a retrospective power calculation, using matched standard deviations of 11.6 and 11.0 respectively, demonstrates that the actual sample size of 43 matched pairs yields sensitivity to differences in the paired means of 5.6 and 5.3 respectively at p=0.025 two-sided and 80% power.

As a secondary objective, separate analyses for associations with AI and AI_{75} were conducted within controls (n=57) and T1D cases (n=98). Due to the potential for outliers in hsCRP and the qualitative nature of some of the variables, Spearman's correlation was utilized to examine the relationship between AI, AI_{75} and total cholesterol, LDL, HDL, triglycerides, blood pressure, HbA1c, glucose, hsCRP, IL-1 IL-6, family history, and exercise regimen.

Endothelial Dysfunction Studies

Subjects

Forty-four children (22 Male, 22 Female) with T1D for at least 1 year were recruited from the University of Florida pediatric endocrinology clinic for this study. Twenty control children (12 Male, 8 Female) were recruited from the Mayo Clinic, Rochester, MN. Control subjects were non-smokers and community based, and did not have any co-existing medical conditions or any family history of premature cardiovascular disease or hyperlipidemia. All T1D and control patients who participated in this study were Caucasian.

Study Protocol

Following an overnight fast and using identical protocols, endothelial function was assessed in all children using the Endo-PAT® device (Itamar Medical Ltd, Caesarea, Israel). Height, weight, and blood pressure were recorded before Endo-PAT® testing. Fasting blood work was performed immediately following the Endo-PAT® assessment. Following Endo-PAT® testing, blood was obtained for lipid profile and glucose determinations in all subjects and also for HbA1c determination in children with T1D. Laboratory analysis of the blood samples was performed at the separate sites using identical laboratory platforms. Four weeks after their initial test, T1D subjects had repeat Endo-PAT® testing to determine intra-patient variability.

Measurement of Endothelial Function

Endo-PAT® is a non-invasive device that combines the traditional flow mediated dilatation technique with pneumatic finger-tip probes to measure arterial pulse wave amplitude and provide an objective measure of endothelial function. The Endo-PAT® device is an operator independent device that allows affordable and objective measurements of endothelial function and eliminates the need for an ultrasound technician or interpretation of the ultrasound signal.

The Endo-PAT® system uses the identical arterial occlusive procedure used to induce reactive hyperemia in standard brachial reactivity measurements of endothelial function. However, instead of measuring brachial artery diameter, Endo-PAT® uses finger-tip plethysmography probes to measure the changes in pulse wave amplitude observed before and after the period of reactive hyperemia. An Endo-PAT® score is then calculated to provide a measure of endothelial function. In adults, the Endo-PAT® score has shown excellent correlation with measures of coronary and peripheral endothelial dysfunction (21; 22). Correlations of Endo-PAT® score with invasive measures of endothelial function have not been performed in children.

Only one previous study has used the Endo-PAT® device to demonstrate endothelial dysfunction in children with T1D(11). This study was designed to confirm the usefulness of Endo-PAT® as a surrogate measure of CVD risk in children with T1D. We expanded the scope of the initial trial by studying a larger group of children with T1D and healthy controls and measuring the reproducibility of Endo-PAT® with serial testing. We hypothesized that children with T1D would have endothelial dysfunction (decreased Endo-PAT® score) when compared to healthy controls and that Endo-PAT® would have acceptable intra-patient variability.

To perform Endo-PAT®, the patient sits in a reclining chair with the hands at heart level and propped in a comfortable position such that the fingers are hanging freely. Fingertip probes are placed on both index fingers and pulse wave amplitudes are recorded for the duration of the study. After 5 minutes of baseline measurement, arterial flow to the non-dominant arm is occluded for 5 minutes using a blood pressure cuff inflated to 40mmHg above systolic pressure. After the 5 minute occlusion, the cuff is rapidly deflated to allow for reactive or flow-mediated hyperemia. Pulse wave amplitudes are recorded for at least 5 minutes after the cuff is deflated. The Endo-PAT® system compares the ratio of arterial pressure in the two fingers before and after

the occlusion to calculate the Endo-PAT® score. The Endo-PAT® score is calculated as the ratio of the average pulse wave amplitude measured over 60 seconds starting 1 minute after cuff deflation divided by the average pulse wave amplitude measured at baseline. This ratio is normalized to the concurrent signal from the contra-lateral finger to correct for changes in systemic vascular tone (Figure 2-3).

Statistical Considerations

The primary endpoint for this study was the Endo-PAT® score. HbA1c, LDL, HDL, total cholesterol, triglycerides, glucose, systolic and diastolic blood pressure, and BMI were analyzed as secondary endpoints. The two groups of patients (TID and control) were compared using a two-sided two sample t-test. Intra-patient standard deviations for Endo-PAT® were estimated from the repeated measures and averaged over the patients within each subgroup. With a sample of 44 TID patients and 20 controls, a two sided, two-sample t-test had 80% power at P=0.05 to detect a difference of 0.78 standard deviations between the two groups. Assuming a standard deviation of 0.4, this corresponds to sensitivity to an Endo-PAT® score difference of 0.32 units.

Figure 2-1. Radial artery tonometry. A high fidelity tonometer is placed on the radial artery until a consistent waveform is generated by the attached software program.

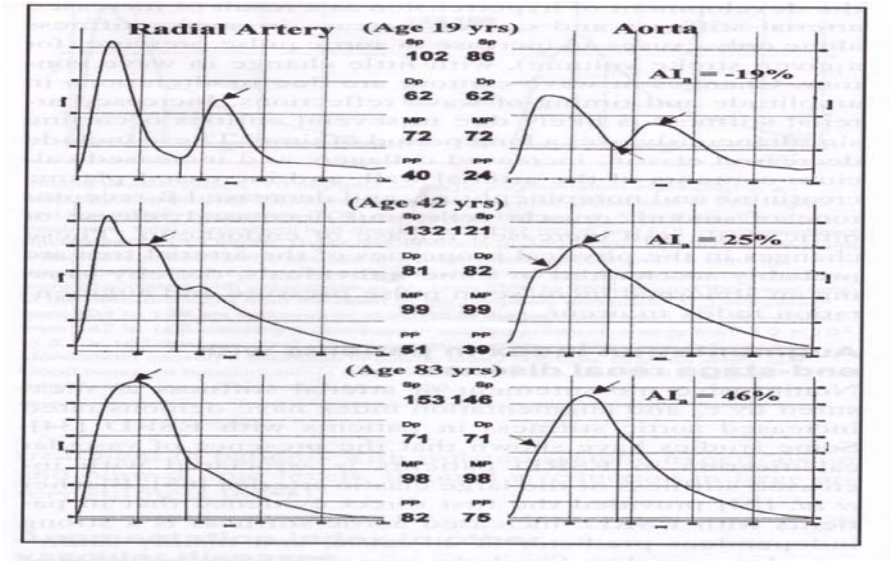

Figure 2-2. Radial artery and corresponding aortic pulse waves. Augmentation index increases with age as arteries stiffen.

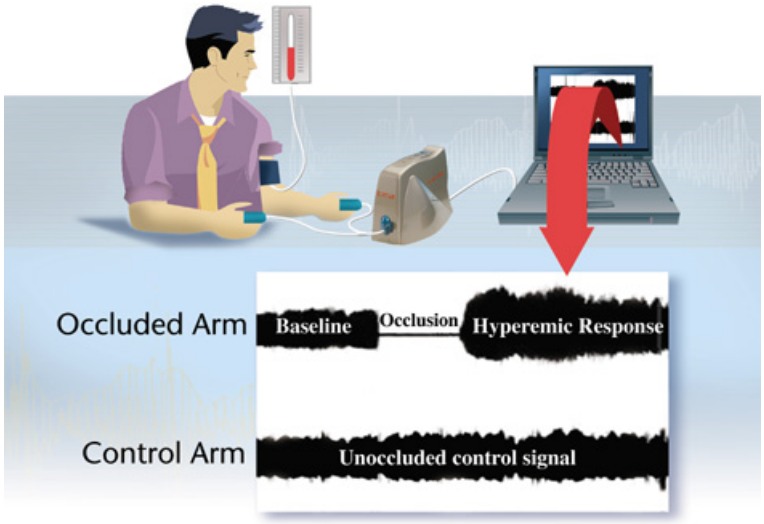

Figure 2-3. Schematic of Endo-PAT® in use. This figure demonstrates the set up and use of the Endo-PAT®. The probes are placed on the index fingers of both arms. A reactive hyperemia procedure is performed by occluding the brachial artery of one arm for 5 minutes. Data are automatically analyzed by the software package and the Endo-PAT® score is provided. (With permission from Itamar Medical, Ltd.)

CHAPTER 3
ATERIAL STIFFNESS FINDINGS

Augmentation Index (AI)

Laboratory and tonometry characteristics of the matched diabetes and control groups are shown in Table 3-1. Spearman correlations of AI and AI_{75} are shown in Table 3-2. T1D was associated with increased arterial stiffness as evidenced by the higher $AI_{75.}$ The mean AI_{75} in T1D subjects was 1.89±10.75 whereas the AI_{75} for controls was -3.32± 10.36. Using a paired difference two-sided t-test, the AI_{75} demonstrated a case-control difference of 5.2±11.0 (p=0.003). There was no significant difference in the uncorrected AI (p=0.37).

Lipids

Total cholesterol, LDL, and HDL analyses did not reveal any significant case-control differences. Triglyceride levels, however, were significantly higher in the control population with a case-control difference of -25.0±62.6 (p=0.012). Even when lipid values among only the T1D subjects were analyzed, no significant associations were noted between total cholesterol, LDL, HDL, or triglycerides and either AI or AI_{75} (Table 2-2).

Blood Pressure

Systolic and diastolic blood pressure analysis revealed significantly higher values in the controls, with differences of -5.2±14.6 (p=0.025) and -3.67±9.9 (p=0.019), respectively. However, no significant associations were noted between systolic or diastolic blood pressure and AI or AI_{75} (Table 2-2).

Length of Diabetes and Control

Duration of diabetes, HbA1c, and blood glucose did not demonstrate a significant association with AI or AI_{75} (Table 2-2). Due to the lack of substantive variation in ages, the

study was not adequately powered to determine if the difference in AI or AI_{75} between controls and T1D subjects became apparent at a specific age.

Cytokines

There were no significant associations between AI or AI_{75} and IL-1, IL-6, or hsCRP (Table 2-2).

Exercise

Level of exercise was graded on a 1 to 4 scale (1 = no exercise, 2 = minimal exercise, 3 = moderate exercise, and 4 = extreme exercise). Differences in the reported exercise level between T1D cases (2.89) and controls (2.79) were not statistically significant. Reported exercise level among T1D cases failed to demonstrate a significant correlation with AI or AI_{75}. Reported exercise level among controls alone did, however, reveal a significant association with both AI ($r = -0.44$ $p<.001$) and AI_{75} ($r = -0.33$ $p=0.015$)(Table 2-2).

Family History

Among T1D cases, family history failed to demonstrate a significant correlation with either AI or AI_{75}. Among controls significant associations were seen between AI and family history of hypercholesterolemia ($r = 0.29$, $p = 0.030$ for AI) and early heart disease ($r = 0.38$, $p = 0.004$ for AI, $r = 0.42$, $p = 0.001$ for AI_{75}), but not family history of hypertension (Table 2-2).

Gender

There were 26 male matched pairs and 17 female matched pairs for a total of 43 matched pairs in the case-control analysis. The case-control difference among paired females demonstrated a significant difference in mean AI_{75}. Case-control difference of mean AI_{75} among paired males did not reveal any significant differences. Although the case-control difference between AI_{75} in matched females reached significance and the difference between matched

males did not, there was no significant difference between males and females. Hence, the overall conclusion should take precedence over the gender-specific conclusion.

Discussion

This study confirms that children as young as 10 years of age with T1D have increased arterial stiffness when compared to matched controls. Although several recent studies present similar findings (14; 23-25), this is the first study to use radial artery tonometry and augmentation index to demonstrate increased arterial stiffness in children with T1D.

Arterial stiffness as a result of endothelial dysfunction is an early sign of cardiovascular disease occuring more often and at an earlier age in patients with T1D compared to those without diabetes. Endothelial dysfunction in T1D is primarily due to increased production of advanced glycation end products (AGEs) causing decreased production and action of nitric oxide and a concomitant decrease in arterial compliance (26). The resultant endothelial dysfunction allows for premature integration of lipid laden macrophages in arterial walls. In addition, the hyperglycemic environment results in qualitative changes in LDL particle size, oxidation, and glycation that have been implicated in early increases in carotid artery IMT and endothelial dysfunction (27; 28). Despite the fact that most T1D patients in reasonable metabolic control have normal cholesterol profiles, decreasing these modified LDLs may be a plausible intervention to reduce cardiovascular disease in T1D. Decreasing LDL values in relatively young T1D patients (average age 34) with normal initial LDL values resulted in improved endothelial function after just 6 weeks of statin therapy (29).

Because management of hyperglycemia is the cornerstone of therapy in T1D, optimal glucose control has been suggested as the primary target to minimize the risk of macrovascular disease. However, even the DCCT's intensive therapy group (average HbA1c of 7.2%), while

showing improved carotid IMT when compared to the conventional treatment group (average HbA1c 9.1%), had significantly increased carotid IMT compared to controls (30; 31). This difference correlated significantly with blood pressure and cholesterol levels (32). Optimal management of all co-morbidities is needed to minimize risk.

Nevertheless, many clinicians believe that diabetes related cardiovascular risk is not sufficiently affected by metabolic derangements in childhood to pursue optimal management of lipids and hypertension in children with T1D. Data from the Pittsburgh Epidemiology of Complications Study, a 10 year follow up of patients who developed T1D before the age of 17, showed that high blood pressure and increased LDL were independent risk factors for microvascular disease, macrovascular disease, and mortality (3). Autopsy studies of over 3000 children found that children have evidence of aortic fatty streaks as early as 3 years of age and raised fibrous plaques as early as 8 years of age (33; 34).The Muscatine Heart Study and the Bogalusa Heart Study have demonstrated that cardiovascular risk factors apparent in childhood predict future coronary artery disease (35; 36).

Radial artery tonometry is an easily learned, affordable, noninvasive, reproducible and accurate technique that can be used to monitor arterial stiffness and, therefore, future cardiovascular risk in both high-risk children and adults (13; 37-39). Radial artery tonometry has been shown to have excellent intra-observer and intra-patient reproducibility and to provide a reliable assessment of endothelial dysfunction (40; 41). In adults with T1D, augmentation index is increased prematurely (38).

This is the first study to evaluate radial artery tonometry in children with T1D. Although we were unable to show any significant correlations between AI in children with T1D and established cardiovascular risk factors, it is possible that the T1D children with the most elevated

AIs might have demonstrated correlations if the study had been adequately powered to evaluate these relationships. Nevertheless, we did demonstrate a clear difference in AI_{75} between controls and subjects with T1D. While the exact mechanisms remain unclear, the observed difference in AI_{75} documents that there are identifiable abnormalities in vessel stiffness in children with T1D.

Using a combination of flow-mediated dilatation and carotid IMT, Järvisalo, et al. and Singh et al., demonstrated endothelial dysfunction in children with T1D (14; 23). Both studies revealed correlations with endothelial dysfunction and LDL cholesterol. The two studies disagreed, however, when describing the relationship between increased carotid IMT and T1D. While the disparate findings may be explained by differences in technique, it is possible that the differences are related to the age and pubertal status of the patient populations. The average age of the T1D children in the two studies was 11 and 15 years, respectively. Although endothelial dysfunction is present in young children with T1D, perhaps the correlations with endothelial function and other cardiovascular risk factors do not become apparent after puberty.

In our study, the average age of the T1D children was 12.9 years. The differences in age and pubertal status, in addition to the different methods used to measure endothelial function, could explain differences in between various studies. Unfortunately, the age distribution of our study population was too narrow to determine at what age the difference between controls and children with T1D becomes apparent. Studies with broader age distributions are needed to determine the effects of age and puberty.

Adult and pediatric studies of endothelial dysfunction in diabetes patients have demonstrated significant associations with high LDL, low HDL, hypertension, glycemic control, duration of disease, folic acid status, exercise regimen, and gender (13; 23; 24; 42). Although we did demonstrate that T1D was associated with increased arterial stiffness, this study did not find

any significant associations with lipids, blood pressure, glycemic control, cytokines, family history, or gender in the children with diabetes. The inability to demonstrate a correlation between established cardiovascular risk factors and increased AI_{75} in children with diabetes is most likely related to the small sample size, narrow age range and lack of longitudinal follow-up. Nonetheless, the risk factors measured are well established as being relevant to the development of atherosclerosis. In children, however, it may be necessary to measure variables that may be more directly related to arterial stiffness such as AGEs, qualitative lipids, nitrite, and superoxide dismutase.

In control children, we found a significant association between elevated AI and family history of hypercholesterolemia. This may have represented a volunteer bias as the control families who volunteered for the study were more likely to have a positive family history of heart disease. This could explain why the control children had significantly higher triglycerides and blood pressures than the T1D subjects. A bias towards increased cardiovascular risk in controls would have decreased the difference in AI_{75}, between controls and children with diabetes. Nonetheless, T1D children demonstrated greater arterial stiffness than controls. That higher exercise levels were associated with decreased arterial stiffness in the controls but not in the T1D subjects suggests that the metabolic derangements of diabetes obscure some of the advantages of exercise.

Despite the novel findings provided by this study, there are several important limitations. The Sphygmocor Vx software used to analyze the wave reflections has not been fully validated in children. The transfer function used to calculate the aortic pressure wave was validated using directly measured aortic and radial pressure waves in adults. While age and AI are linearly associated in adults, such that the same transfer function could be used at all ages, no childhood

data are yet available to confirm the transfer function's validity. Although case-control comparisons eliminated the need for known age adjusted normal values, the argument could be made that the transfer function may not accurately determine aortic pressure waves in children. In a recent study of adults with type 2 diabetes, Hope et al. suggested that generalized transfer functions may not be valid at all in patients with diabetes (43). While their concerns about the applicability of transfer functions to specific populations is valid, they also found no significant differences between directly measured and transfer function derived AI in their diabetes population.

The strict matching criteria that were employed resulted in a lower than expected total number of matches. With a larger study, associations between AI and lipids, blood pressure, and cytokines might have been uncovered. Another weakness of this study was the omission of Tanner staging which may have resulted in significant pubertal differences between age-matched pairs. Pubertal status may be an important determinant in deciphering when children with T1D develop increased arterial stiffness and should be included in all future studies. Finally, the failure to include albuminuria as a study variable is a recognized limitation. Several other studies have demonstrated the importance of microalbuminuria in predicting atherosclerosis in T1D patients (44).

Despite these limitations, the study does provide additional evidence that children with T1D have endothelial dysfunction. Further studies are needed to confirm the relationship between arterial stiffness and other modifiable cardiovascular risk factors. Future interventional studies should be developed to provide longitudinal comparisons of optimal blood pressure, lipid, and glucose control with endothelial function in T1D children.

Table 3-1. Matched T1D Subjects and Controls in AI Study. (n=43) Means, standard deviations, paired differences of the means, and p values are shown.

	T1D	Controls		
	Mean ± SD	Mean ± SD	Paired Difference ± SD	P value
AI	1.11 ± 10.15	-0.47 ± 9.79	1.59 ± 11.61	0.37
AI75	1.88 ± 10.75	-3.31 ± 10.36	5.20 ± 11.02	0.0031
HbA1c	8.41 ± 1.29	5.2 ± 0.25	3.17 ± 1.23	<0.0001
T Cholesterol	151.19 ± 29.46	152.66 ± 36.1	-5.12 ± 35.72	0.35
Triglycerides	61.05 ± 26.32	89.36 ± 54.72	-25.0 ± 62.60	0.012
HDL	56.67 ± 8.2	53.09 ± 12.26	4.05 ± 15.43	0.093
LDL	85.18 ± 34.1	86.02 ± 25.25	-4.09 ± 32.31	0.41
Glucose	159.67 ± 68.78	85.22 ± 9.58	75.89 ± 70.18	<0.001
SBP	109.93 ± 13.6	115.02 ± 10.31	-5.09 ± 14.51	0.025
DBP	67.45 ± 8.8	71.18 ± 7.96	-3.73 ± 9.80	0.015

Table 3-2. Spearman Correlations in T1D Subjects and Controls in AI Study. Spearman values and p-values are shown for AI and AI75 in all T1D (n=98) subjects and Controls (n=57).

	T1DM (n=98)				Controls (n=57)			
	AI	(p value)	AI_{75}	(p value)	AI	(p value)	AI_{75}	(p value)
HbA1c	0.076	(0.46)	0.089	(0.39)	0.060	(0.66)	0.094	(0.49)
T Cholesterol	0.055	(0.60)	0.056	(0.59)	0.150	(0.27)	0.198	(0.14)
Triglycerides	0.096	(0.35)	0.061	(0.55)	0.018	(0.89)	0.046	(0.73)
HDL	-0.027	(0.80)	0.086	(0.41)	0.192	(0.15)	0.126	(0.35)
LDL	0.062	(0.54)	-0.013	(0.89)	0.071	(0.16)	0.076	(0.58)
Glucose	0.164	(0.11)	0.132	(0.20)	0.004	(0.97)	-0.0004	(0.997)
SBP	-0.132	(0.20)	-0.144	(0.15)	-0.289	(0.03)	-0.169	(0.21)
DBP	0.094	(0.36)	0.094	(0.35)	-0.059	(0.66)	0.058	(0.67)
IL-1β	-0.190	(0.06)	-0.079	(0.44)	0.210	(0.12)	0.010	(0.46)
IL-6	-0.150	(0.14)	-0.126	(0.22)	-0.152	(0.26)	-0.074	(0.59)
hsCRP	-0.005	(0.96)	0.004	(0.96)	0.059	(0.66)	0.133	(0.33)
FH of HTN	0.120	(0.24)	0.149	(0.14)	-0.114	(0.44)	-0.066	(0.63)
FH of Early CVD	-0.031	(0.44)	-0.078	(0.45)	0.345	(0.0086)	0.381	(0.0034)
FH of ↑ Cholesterol	-0.132	(0.20)	-0.101	(0.33)	0.303	(0.02)	0.218	(0.10)
Exercise	0.027	(0.79)	0.032	(0.76)	-0.346	(0.0083)	-0.449	(0.0005)
Years of Diabetes	0.041	(0.44)	0.080	(0.44)	N/A		N/A	

CHAPTER 4
ENDOTHELIAL DYSFUNCTION FINDINGS

Endo-PAT® Score

The summary statistics for the comparisons are presented in Table 4-1. The mean intra-patient standard deviation of Endo-PAT® in the T1D patients was 0.261. Children with T1D (n=44) had endothelial dysfunction as evidenced by lower mean Endo-PAT® scores (1.63 vs 1.95, p=0.01) when compared to control children (n=20). However, the range of Endo-PAT® scores was wide. Children with T1D had a range of Endo-PAT® scores of 1.01 to 3.01 while control children had a range of 1.5 to 2.5 (Figure 4-1). Children with T1D had higher mean systolic BP (p=0.02), mean total cholesterol (p=0.03), and mean HDL (p=0.0001) than control children. It was not clear why there were such large differences in blood pressure or HDL between the two groups. No significant differences in age, BMI, diastolic blood pressure, LDL, or triglycerides were observed between the children with T1D and control children.

Discussion

This study confirms that Endo-PAT® can reliably evaluate endothelial function in children with T1D. Significant differences in Endo-PAT® score, consistent with endothelial dysfunction, were observed between children with T1D and controls.

Several characteristics of our study population warrant further comment. The two study populations compared were of similar age and gender, racially homogeneous, and tested using an identical protocol and technique despite the fact that they derived from different locations in North America (Florida and Minnesota). Our T1D population had higher systolic BP, HDL, and total cholesterol. In addition to the hyperglycemia associated with T1D, these important CVD risk factors could explain some of the difference in Endo-PAT® score between the two groups. We must accept, therefore, that the difference in endothelial dysfunction seen in the two groups

may be partially explained by selection bias and not by CVD risk associated solely with the disease state of T1D. In addition, our study was not designed to determine the relative importance of glycemic control, blood pressure, cholesterol, BMI, family history, or any other CVD risk factors on Endo-PAT® score. Additional studies will be needed to further investigate the relative importance of other traditional CVD risk factors on Endo-PAT score. Finally, Endo-PAT® has not yet been used to evaluate the risk of future cardiovascular events in adult or pediatric populations.

Despite the limitations of this particular study, we believe that Endo-PAT® has several potential advantages over traditional reactive hyperemia measurements. Specifically, Endo-PAT® testing is affordable, reproducible, and operator independent. Therefore, the procedure is not subject to the subjective interpretations of blood vessel diameter associated with brachial artery ultrasound imaging methods. While larger studies are needed to confirm the association between low Endo-PAT® score and traditional measures of endothelial dysfunction, Endo-PAT® may be useful as both a research and clinical tool in assessing endothelial function in populations at high risk for developing premature CVD.

Recent Epidemiology of Diabetes Interventions and Complicaitons (EDIC) study data demonstrate that intensive diabetes management significantly decreases cardiovascular events amongst patients with T1D (7). Unfortunately, the overall risk of CVD in T1D patients remains disproportionately high. Because T1D is still associated with a 2-3 fold increased risk of premature CVD, further efforts are needed to accurately identify and treat those patients at high risk for CVD. CVD is a process rooted in early childhood. A multitude of studies now demonstrate that risk factors present in childhood predict cardiovascular event rates in adulthood (33; 35). Furthermore, autopsy studies have shown that permanent plaques associated with CVD

appear in the arteries of children as young as 8 years of age (36). Still, outside of striving for tight glycemic control in children with T1D, many pediatric endocrinologists struggle with which measures to adopt to appropriately identify and manage comorbidities associated with CVD risk (45).

The average HbA1c in our T1D study population (8.34%) approached the HbA1c of the adolescents in the intensive therapy arm of the DCCT and yet endothelial dysfunction was easily identified in our T1D study population. Thus, until technological advances provide for marked improvements in glycemic control for all children with T1D, we are likely to continue to observe average HbA1c levels well above those that eliminate risk of complications even in relatively compliant patients.

Similarly, while slightly higher than those seen in the control population, the average LDL cholesterol (LDL) in our T1D population (87.6 mg/dl) and systolic blood pressure (BP) (109 mmHg) easily met American Diabetes Association and American Heart Association goals. Recent data have demonstrated better endothelial function in adults with CVD who have LDL levels < 80 when compared to those with LDL between 80-100mg/dl (46). Thus, adequate primary prevention of CVD in patients with T1D may require both tight glycemic control and aggressive (i.e. pharmacologic) management of lipids and blood pressure to levels lower than those currently labeled as "normal." Furthermore, to truly minimize the risk of CVD in patients with T1D, initiation of statins and ACE inhibitors may be needed shortly after diagnosis regardless of cholesterol and blood pressure values.

While prospective studies are needed to determine if more aggressive management of lipids and blood pressure can decrease the CVD risk associated with T1D, non-invasive measures of vascular function such as Endo-PAT® may provide the additional level of risk

stratification needed to justify early initiation of pharmacologic lipid and blood pressure lowering therapy in children with T1D.

Table 4-1. Laboratory and Endo-PAT® characteristics of controls and T1D subjects.

Characteristic	Diabetes (n=44)	Controls (n=20)	P Value
Endo-PAT® Score	1.64 ± 0.5	1.95 ± 0.3	0.01
Mean intra-subject SD	0.261	--	--
Age (years)	14.6 ± 1.5	14.1 ± 1.5	0.41
BMI (kg/m2)	22.6 ± 2.7	21.5 ± 2.6	0.34
Systolic BP (mmHg)	109.5 ± 10	103.9 ± 6.3	0.02
Diastolic BP (mmHg)	69.6 ± 9.6	69.3 ± 4.9	0.87
Glucose (mg/dl)	200.1 ± 89.7	86.4 ± 11.5	0.0001
HbA1c (%)	8.34 ± 1.2	--	--
Total cholesterol (mg/dl)	166.4 ± 35	147.7 ± 20	0.03
LDL(mg/dl)	87.6 ± 26	78.1 ± 21	0.16
HDL (mg/dl)	61.7 ± 12.9	38.9 ± 11.1	0.0001
Triglycerides (mg/dl)	85.5 ± 58.7	68.6± 25.8	0.22

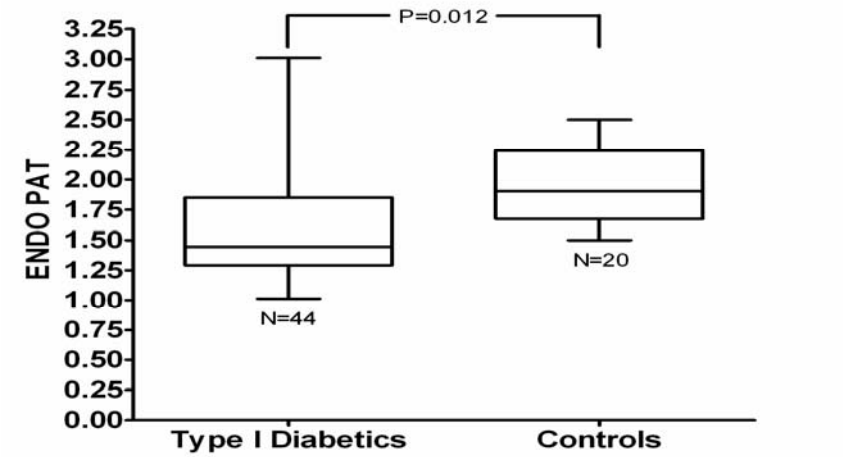

Figure 4-1. Box Plot of Endo-PAT® Score in Controls and Subjects with Type 1 Diabetes. ThisThe box plot demonstrates a significantly lower Endo-PAT® score in the 44 children with type 1 diabetes when compared to the 20 control children. Although there was a wide range of Endo-PAT® scores, the lower Endo-PAT® score is indicative of relative endothelial dysfunction in the cohort with type 1 diabetes.

CHAPTER 5
CONCLUSIONS

The risk of recurrent cardiovascular events is higher in T1D patients than in nondiabetic patients. Well established data document that the early anatomical signs of atherosclerosis appear in childhood. Nevertheless, current guidelines for treating lipids and blood pressure in children with T1D may be underestimating the utility of more aggressive primary prevention. Non-invasive techniques such as radial tonometry and Endo-PAT® can be used to demonstrate abnormal arterial stiffness and endothelial function in children with T1D. These non-invasive techniques may provide the extra tier of cardiovascular risk stratification information needed to design optimal cardiovascular risk reduction strategies in children with T1D.

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BIOGRAPHICAL SKETCH

Michael James Haller, M.D., was born on March 28, 1975, in Gainesville, Florida. He graduated from Gainesville High School and then pursued his undergraduate degree at Duke University. After completing his B.S. in Biology at Duke University in 1996, Michael returned to Gainesville to attend medical school at the University of Florida. After completing his medical school training in 2000, Michael completed both a pediatrics residency and a pediatric endocrinology fellowship at the University of Florida before accepting a position in 2006 as an assistant professor of pediatric endocrinology at the University of Florida. Michael's current research focuses on the prevention and cure of Type 1 diabetes and the primary prevention of cardiovascular disease in children with Type 1 diabetes. Michael is married to Allison Sherman Haller, M.D.