I, Laura Anne Young, hereby submit this as part of the requirements for the degree of: Doctorate of Philosophy (Ph.D.) in: Epidemiology

It is entitled: Endothelial Activation in Young Adults with Type 1A Diabetes Mellitus: An Evaluation of Soluble Cellular Adhesion Molecules

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Endothelial Activation in Young Adults with Type 1A Diabetes Mellitus: An Evaluation of Soluble Cellular Adhesion Molecules

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by

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Endothelial Activation in Young Adults with Type 1A Diabetes Mellitus: An Evaluation of Soluble Cellular Adhesion Molecules

INTRODUCTION
Individuals with Type 1A diabetes mellitus (DM) have an elevated risk for the premature development of severe, atherosclerotic disease. Leukocyte adhesion to the vascular endothelium is an important process in the development of atherosclerosis. These interactions are mediated in part by cellular adhesion molecules (CAMs). Soluble CAMs in the circulation reflect the degree of endothelial activation. It is thought that endothelial activation may play a role in diabetic vascular disease.

OBJECTIVES
The purpose of this cross-sectional study is to examine a wide panel of soluble CAMs in young adults with Type 1A DM and compare them to healthy young adult controls. Additionally we will examine the relationships between soluble CAMs and two clinical measurements that are thought to predict the future development of cardiovascular disease, 24-hour ambulatory blood pressure (ABP) and albumin excretion rate (AER).

METHODS
A panel of soluble CAMS were measured using ELISA in 78 normoalbuminuric, non-hypertensive, young adults with Type 1A DM and compared to 94 controls. Overnight albumin excretion rate was measured for all diabetic subjects. In a subset of the cohort, 60 Type 1A diabetics and 74 healthy controls successfully completed 24-hour ambulatory blood pressure monitoring.

RESULTS
We found that P-selectin, E-selectin, VCAM-1 and ICAM-1 were all significantly (p<0.01) elevated in the young adults with Type 1A DM. In a multiple regression model, glycosylated hemoglobin (HgA1c) was a significant independent predictor of ICAM-1 levels. P-selectin and HgA1c were both significant independent predictors of ambulatory systolic day and night blood pressure, while PECAM-1 was a negative independent predictor of systolic day-night blood pressure difference. Using logistic regression, PECAM-1 was found to be a negative independent predictor of albumin excretion.

CONCLUSIONS
In young adult diabetics endothelial activation is elevated and is related to early adverse changes in ambulatory blood pressure. Our findings suggest that that endothelial activation may be occurring even in apparently healthy young adults with Type 1A DM, which may put them at increased risk for the future development of cardiovascular disease.
Acknowledgements

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~My parents and family without whose unconditional love would I have ever made it this to this point.
TABLE OF CONTENTS

Abstract ........................................................................................................................................ iii
Acknowledgments ...................................................................................................................... v
Table of Contents .................................................................................................................... 1
Introduction and Background .................................................................................................. 3
References .................................................................................................................................. 8

Paper #1

*Elevated Soluble Cellular Adhesion Molecules in Young Adults with Type 1A Diabetes Mellitus*

Introduction .......................................................................................................................... 12
Methods ................................................................................................................................. 13
Results ..................................................................................................................................... 18
Discussion ............................................................................................................................... 20
Table 1: Demographic Characteristics of the Study Participants ............................................. 25
Table 2: Median and Interquartile Ranges of the Soluble AM Concentrations ....................... 26
Table 3: Pearson Correlations Between sCAMs ....................................................................... 27
Table 4: Multiple Regression Analysis: E-Selectin ................................................................. 28
Table 5: Multiple Regression Analysis: ICAM-1 ..................................................................... 29
References ............................................................................................................................... 30

Paper #2

*Soluble Cellular Adhesion Molecules: Predictors of Ambulatory Blood Pressure and Albumin Excretion Rate in Young Adults with Type 1A Diabetes Mellitus*

Introduction .......................................................................................................................... 33
Methods ..................................................................................................................................... 34
Results ..................................................................................................................................... 36
Discussion ............................................................................................................................... 40
Table 1: Demographic Characteristics of the Study Participants ............................................. 48
Table 2: 24-Hour Ambulatory Blood Pressure Measurements ............................................... 49
Table 3: Multiple Regression Analysis ..................................................................................... 50
Table 4: Logistic Regression Analysis ....................................................................................... 51
References ............................................................................................................................... 52

Study Strengths, Limitations and Future Directions ............................................................. 56

Conclusions ............................................................................................................................. 60
Appendix

Role in Study

Protocol for Measurement of ICAM-1

Protocol for Measurement of VCAM-1

Protocol for Measurement of PECAM-1

Pearson Correlations: Soluble AMs and Independent Variables

Soluble AM Means and SDs for Gender and Smoking

Average Day and Night Systolic Ambulatory Blood Pressure

Average Day and Night Diastolic Ambulatory Blood Pressure

Average Day-Night Systolic and Diastolic Ambulatory BP Difference

Nursing Protocol

Consent Form

Recruitment Letter

Recruitment Flier
INTRODUCTION AND BACKGROUND

Individuals with Type 1A diabetes mellitus (DM) are at increased risk for development of extensive, premature atherosclerosis, which is often more severe than that observed in the general population. Interestingly, classical risk factors, including hypertension and hyperlipidemia, do not fully account for the increased incidence of atherosclerosis observed in individuals with Type 1A DM (1). While the exact molecular basis of atherosclerotic vascular disease is not clear, it is apparent that adhesion of circulating leukocytes to the endothelial surface and subsequent transendothelial migration are important steps in the process of atherosclerosis (2-4). One proposed explanatory mechanism for increased occurrence of cardiovascular disease in individuals with diabetes is elevated susceptibility to leukocyte adherence to vascular endothelium.

Animal models have demonstrated one of the earliest morphological changes observed in the atherosclerotic process is inflammation of vasculature via focal adhesion of monocytes and T-lymphocytes to the endothelium in regions of arteries that are prone to lesion development (5-8). Human atherosclerotic lesions have also shown similar involvement of macrophages and T-lymphocytes (9-12). Recruitment of leukocytes to perturbed areas of the endothelium is thought to be a “response to injury” mediated in part by glycoproteins called cellular adhesion molecules (AMs) (13,14). This is a highly choreographed process involving several steps mediated by two main classes of AMs, the selectins and the immunoglobulin gene superfamily. The selectins mediate initial leukocyte attraction to and rolling on the endothelium (15,16). The two selectins responsible for these more transient interactions between leukocytes and the endothelium include P-selectin and E-selectin. P-selectin is expressed on a variety of tissues,
including the endothelium and platelets, while E-selectin in only expressed on activated endothelium. Vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and platelet endothelial adhesion molecule-1 (PECAM-1) all belong to the immunoglobulin gene superfamily (16). They bind integrins on the surface of leukocytes. They are expressed by a variety of tissues, but most prominently on the stimulated vascular endothelial surface. VCAM-1, ICAM-1 and PECAM-1 mediate leukocyte firm attachment to endothelium and transendothelial migration. Activation of the endothelium and transmigration of monocytes results in release of hydrolytic enzymes, cytokines, chemokines and growth factors (17-19). Ensuing smooth cell proliferation and formation of a typical atherosclerotic plaque generally follows.

It has been hypothesized that individuals with diabetes are at increased risk for elevated expression of AMs given their altered biochemistry. Elevated glucose, a hallmark of diabetes, has both a direct and indirect effect on expression of AMs. Glucose directly up regulates expression of several adhesion molecules on vascular endothelial cells (20-23). Over time, elevated glucose also results in nonenzymatic glycation of proteins, which leads to formation of irreversible terminal products known collectively as advanced glycation end-products (AGEs). It has been demonstrated, in vitro, that AGEs interact with AGE receptors on the endothelial surface resulting in increased oxidative stress, which subsequently induces the expression of VCAM-1, ICAM-1 and E-selectin on endothelial cells (24-27). Human studies also show a positive relationship between AGE concentration and increased circulating VCAM-1 (28,29).
Upon mediating leukocyte/endothelial cell interactions, soluble cellular adhesion molecules (soluble AMs) are shed into the circulation (30). They can be measured using enzyme-linked immunoassays and are thought to be good markers of endothelial activation in vivo. Clinical data regarding soluble E-selectin, P-selectin, VCAM-1 and ICAM-1 suggest that they are elevated in patients with atherosclerosis (31-35). In addition ICAM-1 and P-selectin have been found to be good predictors of future adverse cardiovascular events (36,37). While PECAM-1 is important in transendothelial migration of leukocytes, less is known about soluble PECAM-1 as a marker for cardiovascular disease.

Numerous studies demonstrate elevated soluble AMs in subjects with Type 2 DM compared to healthy controls (38-41). One of the strongest predictors of increased circulating E-selectin in this population is poor glycemic control (42, 43). Fortunately, it has been shown in Type 2 diabetics that levels of E-selectin can be significantly reduced within a relatively short period of time by improved glycemic control, resulting in deceased leukocyte/endothelium interaction (42, 43).

Less work has gone into characterizing soluble AMs in individuals with Type 1A DM, however several studies in adults suggest increased endothelial activation may be occurring. Fasching et al examined concentrations of soluble AMs in adults with Type 1A DM compared to healthy controls. They discovered both VCAM-1 and ICAM-1 were significantly elevated in the diabetic group compared to controls by about 30%. No significant difference was noted in E-selectin concentrations. VCAM-1 was found to be 17% higher in diabetic patients with clinical evidence of microangiopathy compared to those without. No differences in the ICAM-1 or E-selectin concentrations were observed
based on the presence or absence of microangiopathy (44). Schmidt et al documented that microalbuminuric subjects with Type 1A DM had increased plasma levels of soluble VCAM-1, which were close to 1.5 times greater than diabetic patients without microalbuminuria (45). Additionally, increased VCAM-1, ICAM-1 and E-selectin expression have been documented on retinal and choroidal tissue of subjects with Type 1 DM (46,47). Finally, based on the findings of increased P-selectin expression on platelets and choroidal microvessels in patients with Type 1A DM, Jilma et al examined soluble, circulating levels of P-selectin in a group of Type 1A diabetics. They found P-selectin levels were 20% higher in patients with diabetes compared to individually matched controls (48). Not only do these studies indicate soluble AMs are elevated in adults with Type 1A DM, but they are also suggestive of a relationship between elevated VCAM-1 and increased diabetic vascular disease.

To date, the studies examining soluble AMs in adolescents and young adults with Type 1 DM are limited and have produced conflicting results. The first report by Elhadd found E-selectin and ICAM-1 were elevated in adolescents and young adults with Type 1A DM (49). Neither duration of disease or HgA1c were significantly related to E-selectin or ICAM-1. In an analysis by Skyrme-Jones et al, no difference in levels of VCAM-1 and P-selectin were reported in young adults with Type 1A DM compared to controls (50). In addition, P-selectin and VCAM-1 were directly related to duration of disease, but not HgA1c. Finally, in a recent study of young children with Type 1A DM, E-selectin, but not VCAM-1 or ICAM-1 were reportedly elevated compared to healthy controls (51). E-selectin was positively associated with serum glucose and glycemic control. While these studies do imply that increased endothelial activation may be
occurring early in the disease process, thus far a systematic assessment of a wide panel of soluble AMs evaluating the various interactions between leukocytes and the endothelium in young adults with Type 1A DM, who are free of complications, has not been reported. Additionally, no studies in children or young adults have evaluated potential relationships between the soluble AMs and early physiological changes in variables such as ambulatory blood pressure monitoring or albumin excretion rate that are related to future development of cardiovascular disease.
REFERENCES:  INTRODUCTION AND BACKGROUND


Elevated Soluble Cellular Adhesion Molecules in Young Adults with Type 1A Diabetes Mellitus
INTRODUCTION

Cardiovascular complications are the leading cause of premature morbidity and mortality in individuals with Type 1A diabetes mellitus (DM); however, the enhanced risk for the development of vascular disease is not clearly understood in this population. It has been suggested that increased activation of leukocytes and/or the endothelium with subsequent inflammation may in part explain the elevated risk of atherosclerosis observed in diabetes. Endothelial and leukocyte activation results in adherence of circulating leukocytes to the endothelium and is thought to be one of the earliest steps in the initiation and promotion of atherosclerosis (1-4). This process is mediated by adhesion glycoproteins called cellular adhesion molecules (AMs) (5,6). The selectins, which include P-selectin and E-selectin, mediate rolling and more transient contact of the leukocyte with the endothelium (7,8). Vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule (ICAM-1) and platelet endothelial cellular adhesion molecule (PECAM-1) are members of the immunoglobulin superfamily and mediate adherence of the leukocytes to the endothelium. Upon mediating leukocyte/endothelial cell interactions, soluble forms of the AMs are shed into the circulation (9). Circulating levels of soluble AMs are thought to reflect the degree of endothelial activation. Soluble AMs have been shown to be elevated in individuals with cardiovascular disease and to predict the occurrence of future cardiovascular events (10-15).

It is clear that soluble AMs are elevated in individuals with Type 2 DM, however the picture is less clear for individuals with Type 1A DM (16-18). Data from older adults with Type 1A DM suggests that soluble AMs may be elevated and that glycemic control is the predominant diabetic disease variable related to elevated expression of the AMs.
In adolescents and young adults, the existing studies that have examined levels of soluble AMs report both elevated and normal values (22-25). To date, no study has examined a wide panel of soluble AMs representative of the various stages of leukocyte attachment to the endothelium in normoalbuminuric, young adults with Type 1A DM. Furthermore, the relationships between the soluble AMs and diabetic disease variables including glycemic control, duration of disease, age of disease onset and daily insulin dose are still unclear in young adults with Type 1A DM.

The purpose of the present study is to compare the levels of a panel of soluble AMs, including P-selectin, E-selectin, VCAM-1, ICAM-1 and PECAM-1, between a group of normoalbuminuric, young adults with Type 1A DM and healthy controls. A secondary goal is to characterize relationships between diabetic disease variables and soluble AMs.
METHODS

Study Population

Eighty four subjects (38 F / 46 M) with Type 1A DM were recruited from the patient database at the Diabetes Center at Children’s Hospital Medical Center (CHMC) in Cincinnati, Ohio. In this study a total of 13 subjects were excluded including 2 who had increased microalbuminuria, 2 due to abnormal funduscopic examinations, 8 with inadequate urine collection and 1 because no adhesion molecules were measured. E-selectin, VCAM-1, ICAM-1 and PECAM-1 were measured in 71 young adults with Type 1A DM. In a subgroup of 50 diabetics P-selectin was measured. The control group consisted of ninety-four healthy young adult control subjects (59 F / 35 M) who were recruited from the CHMC campus and from the University of Cincinnati.

All participants were between the ages of 18-30 years. The diabetic subjects all had a duration of disease greater than two years. All of the diabetic subjects had normal albumin excretion rates (< 20 µg/min), none had evidence of clinical neuropathy and no abnormalities were observed on funduscopic eye examination during the physical examination. All of the subjects with diabetes were being treated with insulin and were metabolically stable. None of the participants reported any recent illnesses or had clinical evidence of macrovascular disease.

Study Protocol

This study was approved by the Institutional Review Board of CHMC. Upon obtaining written informed consent, subjects were enrolled in the study. All subjects underwent a standard physical examination and history that included documentation of current smoking status (yes/no). For the diabetic subjects, duration of disease, age of
onset and current dose of insulin was also recorded. Height and weight were measured using a Harpenden Stadiometer (Seritex, Carstadt, NJ) and digital scale (Scale Tronix, White Plains, NY). Fasting venous blood to measure the soluble AMs and glycemic control (HgA1c) was drawn after an overnight fast. Subjects were instructed to abstain from smoking 12 hours prior to the visit.

**Biochemical Tests**

HgA1c levels were measured by the Abbott IMX Glycated Hemoglobin test (Abbott Laboratories, Abbott Park, IL). The inter-assay and intra-assay coefficients of variation were both less than 5.0%. Samples for measurement of the soluble AMs were stored at -80°C. Determination of serum soluble AMs was performed using enzyme linked immunoabsorbent assays (R & D Systems, Minneapolis, MN). Each sample was run in duplicate and an equal number of diabetic and control samples were assayed on the same plate to minimize inter-assay variation. Both the inter-and intra-assay coefficient of variation was ≤9.5% for the various soluble AMs that were measured.

**Statistical Analysis**

In this analysis, soluble AMs were considered the dependent variables, while the independent variables include age, body mass index (BMI), sex, smoking status, and the diabetic disease variables. The distributions of the dependent variables were tested for normality. The soluble AMs were not normally distributed; therefore differences between the diabetics and controls were determined using the non-parametric Wilcoxon Rank Sum Test. Prior to correlation analysis, log transformations were performed for the soluble AMs to achieve a normal distribution. Pearson Correlations were calculated to measure the strength of the association among the soluble AMs. Correlation analysis was
also utilized to test the strength of association between the soluble AMs and the independent variables. Finally, for those soluble AMs that exhibited significant correlations with the independent variables, multiple regression models were constructed. Prior to building the models, the independent variables were tested for multicolinearity. Independent variables were removed from the equations using backward elimination. Statistical significance was judged using a p-value=0.05, while borderline statistical significance is reported as $0.5 < p \geq 0.10$. Statistical analyses were performed using SAS version 8.0.
RESULTS

The demographic data are summarized in Table 1. The two groups were similar on all variables except for age and BMI. Subjects with diabetes were somewhat younger and had slightly greater adiposity. Table 2 shows the differences in the median concentrations of soluble AMs between diabetics and healthy controls. Median values for P-selectin, E-selectin, VCAM-1 and ICAM-1 were elevated in diabetics compared to controls by 16%, 35%, 15% and 9% respectively. There was no statistically significant difference in PECAM-1 levels between the two groups. Bivariate correlations between adhesion molecules are presented in Table 3.

Bivariate Analysis

Correlation coefficients revealed a positive relationship between E-selectin levels and HgA1c ($r=0.26$, $p=0.03$). This suggests that patients with diabetes who had poorer control tended to have higher E-selectin levels than those with better glycemic control. In addition, E-selectin concentrations were higher in young adult males with diabetes compared to young adult females ($1.80 \pm 0.15$ ng/ml vs. $1.71 \pm 0.19$ ng/ml; $p=0.04$).

Levels of ICAM-1 also positively correlated with HgA1c ($r=0.28$, $p=0.02$) and were inversely related to age ($r=-0.29$, 0.01). In addition, circulating levels of ICAM-1 were significantly higher in young adult diabetic smokers compared to non-smoking diabetics ($2.52 \pm 0.14$ ng/ml vs. $2.44 \pm 0.11$ ng/ml; $p=0.05$).

Circulating P-selectin, VCAM-1 and PECAM-1 did not correlate significantly with any of the independent variables.
Multiple Regression Analysis

Using the significant bivariate correlations, multiple regression models were built for E-selectin and ICAM-1. Multicollinearity was not a problem for any of the candidate independent variables. The results of the analysis are shown in Tables 4 and 5.
DISCUSSION

This study demonstrates that in young, normoalbuminuric adults with Type 1A DM concentrations of P-selectin, E-selectin, VCAM-1 and ICAM-1 are significantly elevated compared to healthy controls. PECAM-1 concentrations were not different between the two groups. Our findings suggest that even with normal albumin excretion and no clinical signs of macrovascular disease, young subjects with diabetes are prone to increased interactions between leukocytes and the endothelium compared to healthy controls. Furthermore, poor glycemic control appears to be an important variable promoting leukocyte adhesion to the endothelium. Although only approaching statistical significance, we also find that smoking and gender may be important factors contributing to endothelial activation in young adult patients with diabetes.

P-selectin and E-selectin are both involved in early steps of leukocyte capture and rolling. E-selectin is expressed by endothelial cells exclusively, while P-selectin is expressed by the endothelium and platelets. This study is the first to demonstrate elevated levels of P-selectin in young adults with Type 1A DM. Elevated levels of P-selectin have been documented in older adults with Type 1A DM (21). In an earlier study of 41 young adults with Type 1A DM, mean P-selectin levels were higher in the diabetics compared to the healthy controls, however the difference was not statistically significant (24). We suspect that our larger sample size (n=71) yielded the statistical power to detect differences between diabetic young adults and controls. We did not find any significant relationships between P-selectin concentrations and diabetic disease variables or other independent variables. Our findings confirm previous reports of elevated E-selectin in older adults, young adults and children with Type 1A DM.
Although the multiple regression model demonstrated only borderline significant relationships between HgA1c and male gender with E-selectin (p=0.06, p=0.08 respectively), we believe these relationships are worth noting, especially given the good glycemic control and normoalbuminuric nature of this cohort. In addition, the power of this study may have limited our ability to demonstrate statistically significant associations.

VCAM-1 and ICAM-1 mediate more stable interactions between leukocytes and the endothelium. VCAM-1 is expressed by the endothelium as well as by smooth muscle cells and monocytes, while ICAM-1 is expressed on a variety of cells including endothelial cells, monocytes and lymphocytes. Both VCAM-1 and ICAM-1 are essential for firm adhesion of leukocytes to vascular endothelium. Our findings confirm previous reports of elevated ICAM-1 in both adults and adolescents with Type 1A DM (20,23). We also showed that VCAM-1 was elevated in the diabetics compared to the controls. VCAM-1 is elevated in older adults with Type 1A DM, however in young adults, adolescents and children with Type 1A DM no difference in VCAM-1 concentrations has been previously reported (19,24,25). While VCAM-1 was not statistically correlated with any of independent variables in our study, ICAM-1 was positively associated with glycemic control (p=0.03). ICAM-1 was also related to smoking (p=0.10), although the relationship only approaches statistical significance. To our knowledge this is the first report of elevated VCAM-1 levels in young adults (18-30 years of age) with Type 1A DM.

PECAM-1, along with VCAM-1 and ICAM-1, aid in the transendothelial migration of the leukocytes following endothelial activation. PECAM-1 is primarily
expressed on endothelial cells. We report that PECAM-1 levels in young adult Type 1A diabetics were not different from young adult, healthy controls. Soluble PECAM-1 was also not significantly related to any of the independent variables. Unlike both ICAM-1 and VCAM-1, cytokine stimulation does not regulate PECAM-1 expression on the vascular endothelium (26). This may suggest that cytokine stimulation is an important step in endothelial activation in young adults with Type 1A DM. Further study of these relationships is warranted.

Glycemic control appears to be an important mediator of both transient and more stable interactions between the leukocytes and the endothelium in young adults with Type 1A DM, evidenced by a positive relationship between HgA1c and the adhesion molecules, E-selectin and ICAM-1. While it is beyond the scope of this study to identify the mechanism(s) responsible for the relationship between poor glycemic control and elevated expression of adhesion molecules, we speculate that elevated circulating glucose may have both direct and indirect effects on endothelial expression of E-selectin and ICAM-1. Elevated circulating glucose has been shown to directly induce expression of ICAM-1 on vascular endothelial cells (27,28). Increased levels of glucose are also known to promote production of advanced glycation end products (AGEs) and to create a pro-oxidative state, both of which can increase expression of adhesion molecules (29,30). It is important to note that HgA1c is only a single measurement reflecting blood glucose control for the previous 3 months and may not provide an accurate view of chronic blood glucose control. Good control of blood glucose, early in the disease process, can help delay development of diabetic vascular disease. Our findings suggest that good glycemic control in young adults with Type 1A DM may reduce expression of E-selectin and
ICAM-1, thereby providing a mechanistic explanation for the link between glycemic control and diabetic vascular disease.

Although relationships between smoking and gender with ICAM-1 and E-selectin show only borderline statistical significance in the multivariate model (p=0.10, p=0.08 respectively), these findings are intriguing and appear biologically plausible. In the bivariate analysis, young adult diabetics who smoke had significantly higher levels of ICAM-1 compared to diabetic non-smokers, while female diabetics had significantly lower concentrations of E-selectin compared to male diabetics. Cigarette smoke condensate is thought to increase protein kinase C activity followed by subsequent up regulation of ICAM-1 on endothelial cells (31), while estrogen is thought to have numerous effects on the endothelium, including decreased E-selectin expression (32). Studies of diabetic and healthy subjects show that smokers have dose-dependent elevated levels of circulating ICAM-1 compared to non-smokers (33,34). Healthy women have been shown to have lower levels of E-selectin compared to males, however this relationship in diabetics has not previously been reported (35). While our findings are only of borderline statistical significance, the data are consistent with the following hypotheses: 1) smoking may play a significant role in ICAM-1 induction, thus contributing to increased leukocyte/endothelial adhesion and subsequent initiation/promotion of atherosclerosis, and 2) young adult women with diabetes may still have some degree of cardiovascular protection provided by estrogen, as evidenced by lower E-selectin levels compared to young adult males with Type 1A DM. Further research is necessary to explore the relationships between smoking, gender and AMs in diabetes.
It is possible that soluble AMs could arise from non-endothelial sources, especially since only E-selectin is derived exclusively from the vascular endothelium. While this may pose a problem with specificity, numerous studies have shown a relationship between circulating levels of soluble AMs and development or existence of atherosclerotic disease (10-15). Furthermore, improvement of various cardiovascular disease risk factors appears to attenuate elevated soluble AM levels. Specifically, in adult individuals with diabetes soluble AMs have been shown to be reduced by improved glycemic control over a relatively short period of time (29,36).

Cardiovascular disease is a major health concern for individuals with Type 1A DM. It was once believed that the endothelium displayed no abnormal characteristics before the onset of microalbuminuria. It has been suggested however, that endothelial activation and subsequent dysfunction may begin prior to the onset of increased albumin excretion. Our findings suggest that young individuals with Type 1A DM, who have normal albumin excretion and no known cardiovascular disease, exhibit elevated circulating levels of soluble AMs, including E-selectin, P-selectin, VCAM-1 and ICAM-1. These data suggest augmented endothelial activation with increased leukocyte/endothelial interactions in young, normoalbuminuric diabetic subjects compared to healthy controls. Furthermore, our findings suggest that poor glycemic control, smoking and male gender may be positively related to increased endothelial activation, specifically via E-selectin and ICAM-1 expression.
TABLE 1: Demographic Characteristics of the Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Diabetics (n=71)</th>
<th>Controls (n=94)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (years)</td>
<td>22.9 ± 3.4</td>
<td>25.1± 2.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 ± 3.7</td>
<td>24.7 ± 5.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>12.7</td>
<td>12.8</td>
<td>0.99</td>
</tr>
<tr>
<td>Duration of Disease (years)</td>
<td>13.3 ± 5.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>HgAlc (%)</td>
<td>8.2 ± 1.4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Age of Onset (years)</td>
<td>9.6 ± 4.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Daily Insulin Dose (units)</td>
<td>63.1± 26.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values reported as Means ± SD
N/A=Not applicable
### TABLE 2: Median Soluble Cellular Adhesion Molecule Concentrations with (Interquartile Ranges)

<table>
<thead>
<tr>
<th></th>
<th>Diabetics (n=71)</th>
<th>Controls (n=94)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P-Selectin ng/ml</strong></td>
<td>95.3 (72.1-137.3)</td>
<td>80.1 (51.8-108.2)</td>
</tr>
<tr>
<td><strong>E-Selectin ng/ml</strong></td>
<td>62.1 (45.2-77.9)</td>
<td>40.2 (23.6-56.5)</td>
</tr>
<tr>
<td><strong>VCAM-1 ng/ml</strong></td>
<td>426.7 (352.0-500.0)</td>
<td>360.8 (297.5-432-5)</td>
</tr>
<tr>
<td><strong>ICAM-1 ng/ml</strong></td>
<td>276.3 (242.7-328.0)</td>
<td>250.4 (219.8-281.5)</td>
</tr>
<tr>
<td><strong>PECAM-1 ng/ml</strong></td>
<td>30.0 (22.9-37.0)</td>
<td>31.2 (25.5-37.2)</td>
</tr>
</tbody>
</table>

*Wilcoxon Rank Sum Test p-value < 0.001*
**TABLE 3: Pearson Bivariate Correlations Between Soluble Cellular Adhesion Molecules**

<table>
<thead>
<tr>
<th></th>
<th>LogPsel</th>
<th>LogEsel</th>
<th>LogVCAM</th>
<th>LogICAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogPsel</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LogEsel</td>
<td>0.27 0.06</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LogVCAM</td>
<td>0.28 0.05</td>
<td>0.19 0.11</td>
<td>1.0</td>
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</tr>
<tr>
<td>LogICAM</td>
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<td>0.52 0.01</td>
<td>0.48 0.01</td>
<td>0.50 0.01</td>
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### TABLE 4: Multiple Regression Analysis Results: E-Selectin

<table>
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<tr>
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<th>Standard Error</th>
<th>F Value</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>Log[ESel]*</td>
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<td></td>
<td></td>
</tr>
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<td>Intercept</td>
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</tr>
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<td>HgA1c</td>
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<td>0.01</td>
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<td>Gender (M=0/F=1)</td>
<td>-0.07</td>
<td>0.04</td>
<td>3.12</td>
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* R-Square = 0.11
TABLE 5: Multiple Regression Analysis Results: ICAM-1

<table>
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<tr>
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<th>Coefficient (β)</th>
<th>Standard Error</th>
<th>F Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log[ICAM]*</td>
<td>2.27</td>
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<td>789.73</td>
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</tr>
<tr>
<td>HgA1c</td>
<td>0.02</td>
<td>0.01</td>
<td>4.60</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoking (0=No/1=Yes)</td>
<td>0.07</td>
<td>0.04</td>
<td>2.98</td>
<td>0.10</td>
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</tbody>
</table>

*R-Square = 0.12
REFERENCES


Soluble Cellular Adhesion Molecules: Predictors of Ambulatory Blood Pressure and Albumin Excretion Rate in Young Adults with Type 1A Diabetes Mellitus
INTRODUCTION

A key step in the initiation of atherosclerosis is the adhesion of circulating leucocytes to the vascular endothelium, followed by leukocyte extravasation into the subendothelial space (1-3). This process is mediated in part by glycoproteins called cellular adhesion molecules (AMs) that are induced in response to a variety of stimuli including cytokines, oxidized LDL, glucose and advanced glycation end products (4-12). Once bound, the adhesion molecules can be cleaved, shed into the circulation and subsequently measured in the plasma (13). Circulating levels of soluble AMs are thought to reflect the degree of endothelial activation. Higher concentrations of soluble AMs have been demonstrated in individuals with atherosclerosis and hypertension (14-17). They have also been shown to predict future atherosclerotic events (18,19).

Individuals with Type 1A DM diabetes mellitus (DM) are at increased risk for the premature development of cardiovascular disease. Elevated levels of the soluble AMs have been documented in adults with Type 1A DM (20-23). Both normal and elevated levels of soluble AMs have also been noted in young diabetics compared to healthy controls (24-26). We recently reported that circulating levels of P-selectin, E-selectin, vascular cellular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were elevated in a group of normoalbuminuric, young adults with Type 1A DM compared to healthy controls. There was no difference in soluble platelet endothelial cellular adhesion molecule (PECAM) concentrations for the two groups. These data support the hypothesis that individuals with diabetes are susceptible to increased endothelial activation that begins early in the disease process. It is believed that increased endothelial activation may contribute to the development of endothelial
dysfunction, eventually resulting in the inability of the endothelium to adequately maintain normal physiologic control of blood pressure.

Little is known about the relationship between soluble AMs and early clinical markers for future development of cardiovascular disease in individuals with diabetes. Ambulatory blood pressure (ABP) monitoring has been shown to be superior to office blood pressure measurements in predicting future end organ disease (27-30). Changes in ABP and albumin excretion, both evident in young adults with Type 1A DM, are related to the future development of diabetic vascular disease (31,32). In adults with Type 1A DM relationships between ABP and soluble AMs have not been examined, however it has been reported that increased albumin excretion is related to elevated circulating levels of VCAM-1 and ICAM-1 (21,22). No prior studies have investigated the potential relationships between ABP, albumin excretion and soluble AMs, in children and young adults.

The purpose of this study was to test the hypothesis that ABP is elevated in young subjects with Type 1A diabetes compared to healthy controls, and to test whether differences are related, in part, to endothelial activation reflected by circulating levels of adhesion molecules. A secondary goal was to test the hypothesis that albumin excretion is positively related to concentrations of soluble AMs in young adults with Type 1A DM.
METHODS

Study Population

In this analysis, we studied 60 young adults (26 F / 34 M) with Type 1A DM. This is a subset sample that was recruited from the patient database at the Diabetes Center at Children’s Hospital Medical Center (CHMC) in Cincinnati, Ohio. Inclusion into this subsample required successful overnight urine collection for the determination of albumin excretion rate and a complete 24-hour ABP profile. The control group consisted of eighty-two healthy young adult control subjects (52 F / 30 M), who were randomly recruited from the CHMC campus and from the University of Cincinnati. All subjects were between the ages of 18-30 years. Diabetic subjects all had a duration of disease greater than two years. All diabetic subjects had normal albumin excretion rates (< 20 µg/min), none had evidence of clinical neuropathy and no abnormalities were observed on funduscopic eye examination. None of the participants reported any recent illnesses or had clinical evidence of macrovascular disease. All Type 1A diabetic subjects were being treated with insulin and were metabolically stable.

Study Protocol

This study was approved by the Institutional Review Board of CHMC. Upon obtaining written informed consent, subjects were enrolled in the study. The study protocol required two study visits. On visit one, all subjects underwent a standard physical examination and history. In the diabetic subjects, duration of disease, age of diabetes onset and current dose of insulin were recorded. Height and weight were measured using a Harpenden Stadiometer (Seritex, Carstadt, NJ) and digital scale (Scale Tronix, White Plains, NY). Fasting venous blood for soluble AMs and glycosylated
hemoglobin measurement was drawn following an overnight fast. Subjects were instructed to abstain from smoking 12 hours prior to the visit. All subjects were fitted with a portable, ABP monitor and instructed on its use. During the second visit, study participants returned the ABP monitor and the diabetic subjects provided an overnight urine collection for determination of urinary albumin excretion rate. To eliminate occult urinary tract infection as a cause of increased protein excretion, a fresh, clean-catch urine sample was obtained for culture. If a positive urine culture was reported, subjects completed another timed, overnight urine collection.

**Ambulatory Blood Pressure Measurement**

The Space Labs model 90207 ABP monitor (Redmond, WA) was used to record subjects’ blood pressure during a 24-hour period. The monitor is capable of measuring systolic and diastolic blood pressure as well as heart rate at preset intervals. The appropriate cuff size was chosen according to the size of the subject to cover two thirds of the upper arm length. Before the subjects began the 24-hour monitoring, a trained research nurse instructed subjects on its use. Subjects were instructed to proceed with normal daily activities except for contact sports or very vigorous activities during the 24-hour period. The monitor was programmed to take measurements at 15-minute intervals. Subjects recorded the time that they went to sleep and time they awoke in a sleep diary. The day night intervals were determined from the sleep times recorded in the sleep diary. Mean 24-hour, day and night systolic and diastolic blood pressures were measured. The day/night difference in blood pressure was calculated by subtracting the average sleeping blood pressure from the average awake blood pressure. Subjects whose mean night systolic blood pressure decreased by at least 10% of mean day systolic blood pressure
were classified as “dippers,” while those with less than 10% change were classified as “non-dippers.”

**Biochemical Tests**

HgA1c levels were measured by the Abbott IMX Glycated Hemoglobin Test (Abbott Laboratories, Abbott Park, IL). The inter-assay and intra-assay coefficients of variation were both less than <5%. Samples for measurement of the soluble AMs were stored at -80°C. Determination of serum soluble AMs was performed using enzyme linked immunoabsorbent assays (R & D Systems, Minneapolis, MN). Each sample was run in duplicate and an equal number of diabetic and control samples were assayed on the same plate to minimize interassay variation. The inter-and intra-assay coefficient of variation was ≤ 9.5% for the various soluble AMs measured.

**Statistical Analysis**

For statistical analysis, ABP variables and albumin excretion rate were considered the dependent variables, while independent variables included soluble AMs, age, body mass index (BMI), sex and diabetic disease variables. Distributions of the dependent ABP variables were tested for normality. Blood pressure variables were not normally distributed; therefore differences between the two groups were determined by the non-parametric Wilcoxon Rank Sum Test. Prior to correlation analysis, log transformations were used to achieve normal distributions. Pearson Correlations were calculated to test the strength of association between the blood pressure variables and the independent variables. Multiple regression analysis with backward elimination was used to identify variables that were independently associated with the ABP variables. Pearson bivariate correlations whose p-value was ≤ 0.10 were entered into the multiple regression
equations. Variables were tested for multicollinearity prior to development of the multiple regression models.

Due to a large percentage of albumin excretion rates reported at or below the limit of detection, this variable was dichotomized into a low albumin excretion group \(<1.8 \mu g/min (n=44)\) and slightly elevated albumin excretion group \(\geq1.8 \mu g/min (n=16)\). Student’s t-test and contingency table analysis were used to determine differences between the albumin excretion group on the continuous and categorical independent variables respectively. Logistic regression was used to determine which independent variables were important predictors of albumin excretion. In addition, ABP variables that were significantly different in terms of the albumin excretion groups were also entered into the logistic regression model.

In the multiple regression analyses, \(p \leq 0.05\) was used to judge statistical significance. Analyses were performed using SAS, version 8.0 (SAS Institute, Cary, NC).
RESULTS

Demographic data are displayed in Table 1. The diabetics are slightly younger than the controls. Table 2 illustrates the mean ABP measurements. Mean systolic day blood pressure and mean systolic night ABP were significantly higher in the diabetics compared to the controls. Dipper status was not different between the cases and the controls, with 38.3% of the diabetics and 32.9% of the controls classified as non-dippers.

Bivariate Analysis

Bivariate correlations are reported for those blood pressure variables for which one or more soluble AMs exhibits a relevant statistically significant relationship. For the bivariate analyses a less stringent p-value of $p \leq 0.10$ was considered to be statistically significant. In doing this, we maximize our ability to explore the relationships between soluble AMs and ABP variables. Average systolic day blood pressure was significantly correlated with log P Selectin ($r=0.27$, $p=0.07$), log BMI ($r=0.22$, $p=0.09$) and HgA1c ($r=0.24$, $p=0.06$). Average systolic night blood pressure was significantly related to log P-selectin ($r=0.25$, $p=0.09$) and log BMI ($r=0.23$, $p=0.07$). Systolic day night difference was inversely related only to log PECAM ($r=-0.26$, $p=0.04$). None of the sCAM concentrations were significantly different based upon classification into dipper/non-dipper status.

For albumin excretion, subjects were classified into two groups, those with low albumin excretion (<1.8 µg/min) and those with mildly elevated albumin excretion (≥1.8 µg/min). Log P-selectin, log E-selectin and log ICAM-1 concentrations were not different between the two groups. Log VCAM-1 was higher in the low albumin
excretion group (2.66 ± 0.13 ng/ml vs. 2.56 ± 0.14 ng/ml, p=0.02). Likewise, log
PECAM-1 was elevated in the low albumin excretion group (1.51 ± 0.13 ng/ml vs 1.23 ±
0.33ng/ml, p<0.01). Age, log BMI and gender were not significantly different between
the two albumin excretion groups. Of the diabetic disease variables, HgA1c, onset and
insulin dose did not differ between the two groups. Duration of disease was shorter in the
low albumin excretion group (12.4 ± 4.5 years vs. 16.2 ± 5.5 years, p=0.01). Log of the
diastolic day (1.85 ± 0.04 mmHg vs. 1.87 ± 0.03 mmHg, p=0.03) and log of the diastolic
night BP (1.76 ± 0.05 mmHg vs. 1.80 ± 0.05 mmHg, p=0.03) were less in the lower
albumin excretion group. Log of the systolic day BP, log of the systolic night BP,
systolic day-night BP difference and diastolic day-night BP difference were not different
between the two groups. The frequency of dippers in the low albumin excretion group
was not statistically different from the higher albumin excretion group.

**Multiple Regression Analysis**

To explore the variables independently associated with the log systolic day ABP,
the log systolic night ABP and the day night systolic ABP difference, multiple regression
analysis was performed. To avoid the problem of multicolinearity, the relationships
between the independent candidate variables were assessed. Results of the regression
analyses using backward elimination are reported in Table 3.

Multiple logistic regression was used to identify factors that were independently
associated with having a mildly elevated albumin excretion rate (> 1.8 µg/min but < 20
µg/min). Based upon univariate analyses, the candidate variables for inclusion in the
logistic regression model included VCAM-1, PECAM-1, duration of disease, diastolic
day ABP and the diastolic night ABP. To avoid the problem of multicollinearity, the relationships between the candidate variables were assessed. VCAM-1 and PECAM-1 concentrations were highly correlated, as were the diastolic day ABP and the diastolic night ABP. Entering the correlated independent variables into the logistic regression equation one at a time with duration of disease, VCAM-1 and diastolic day ABP proved to not be significant. In the final logistic regression model, PECAM-1, diastolic night ABP and duration of disease are all independent predictors of albumin excretion. Table 4 illustrates the results of the final logistic model.
DISCUSSION

Change in vascular structure and function are thought to occur long before the development of overt atherosclerotic disease (33-36). These changes are thought to be a result of endothelial activation and inflammation eventually resulting in endothelial dysfunction (37,38). Circulating levels of soluble AMs are thought to reflect the degree of endothelial activation. Unfavorable alterations in ABP and albumin excretion are believed to develop with increasing endothelial dysfunction, and are evident as early as adolescence in young subjects with Type 1A DM. To date the relationship between changes in ABP and soluble AMs has not been explored. While it does appear that soluble AMs are elevated in adults with Type 1A DM with increased albumin excretion, this relationship has not been evaluated in young adults with Type 1A DM.

Elevated systolic blood pressure is a strong predictor of left ventricular hypertrophy and future cardiovascular disease (39,40). In our analysis young patients with diabetes exhibit significantly elevated systolic day and night blood pressure compared to controls. This is consistent with similar findings in adolescents and young adults with Type 1A DM. Results from the multiple regression analysis reveal that P-selectin and glycemic control are positively related to both day and night systolic ABP. P-selectin is an adhesion molecule, released from the endothelium and the platelets, thought to aid in initial capture and rolling of the leukocytes on the endothelium. It has been demonstrated that individuals with malignant hypertension and uncomplicated nonmalignant essential hypertension have elevated P-selectin levels compared to healthy controls (41). Our findings in a group of young diabetic adults, without hypertension, lend further support for a relationship between blood pressure abnormalities and P-
selectin expression. It is important to note that we are unable to distinguish between the platelet and/or endothelial origin of the circulating P-selectin measured in the assay. P-selectin has gained significant support as a marker for platelet activation (42,43). It is possible that the positive linear relationship between P-selectin and systolic blood pressure that we are able to detect, may reflect elevated platelet activation with increasing blood pressure (44). Our findings thus suggest that prior to the development of clinically detectable microvascular disease, slight elevations of systolic day and night ABP are evident in young adults with Type 1A DM and are positively related to increased endothelial and/or platelet activation. Additional research is necessary to further define these relationships.

Sleep is generally associated with a decrease in both systolic and diastolic blood pressure. It has been shown that diabetics are more likely to exhibit less of a decline in blood pressure at night (45,46). Less of a day night blood pressure difference has been associated with elevated albumin excretion, increased diastolic dysfunction and future cardiovascular events (32,47). In this population of normoalbuminuric, young adult diabetics, systolic and diastolic day-night blood pressure differences were similar to those observed in healthy controls. This is not surprising given the normal albumin excretion in these subjects. We and others have shown that less of a day night difference in systolic blood pressure is related to increased albumin excretion (32,47). Of interest, however, is the inverse relationship between systolic day-night blood pressure difference and PECAM-1. Increased endothelial activation may be associated with less of a decline in systolic blood pressure at night, possibly leading to increased risk for the development of diabetic cardiovascular disease.
In a cross-sectional study it is not possible to determine the causal direction of the relationships between soluble AMs and alterations in ABP. It is possible that increased endothelial activation results in elevated endothelial dysfunction, which leads to adverse alterations in blood pressure. On the other hand, it is also possible that elevated blood pressure may increase endothelial sheer stress, which may amplify endothelial activation resulting in elevated levels of circulating soluble AMs. We speculate that these processes may feedback upon each other, eventually resulting in clinically detectable diabetic atherosclerotic vascular disease.

In older adults with Type 1A DM, endothelial activation is thought to escalate with increasing albumin excretion, evidenced by elevated soluble AMs, but this has not been evaluated in young diabetics (21,22). Slightly elevated levels of albumin excretion, even within the normal range, are associated with increased cardiovascular disease. Therefore, in our cohort of normoalbuminuric Type 1A diabetics, we classified the individuals into two groups, those with low albumin excretion (≤1.8 µg/minute) and those with elevated excretion (>1.8 µg/minute but < 20 µg/minute). In a logistic regression analysis, we show that increased albumin excretion is associated with decreased levels of soluble PECAM-1. Presumably, with increased pathological changes, the glomeruli become “leaky” and have less size and charge specificity. Increased proteinuria results, evidenced by increased albumin in the urine. In individuals with urinary albumin excretion between 6.6-150 µg/minute, it has been demonstrated that fractional clearance of IgG, molecular weight 150 KD, is also increased compared to healthy controls (48). We speculate that a similar phenomenon may be occurring,
resulting in increased PECAM-1 filtration with increased albumin excretion rate.

PECAM-1 is a member of the immunoglobulin superfamily and has a molecular weight of 130 KD (49). Our data also does not exclude the possibility that other circulating soluble AMs are also excreted in the urine. While soluble AMs have been measured in urine, little is known about how circulating adhesion molecules are filtered by the kidney, but excretion of cytokines such as TNFα, IL and IL are increased in certain types of renal pathology (50,51). We cautiously suggest that this may explain the failure to detect a difference in PECAM-1 levels between cases and controls. Urinary excretion of PECAM-1 and/or other AMs could mask differences in circulating sCAM levels. Further research is necessary to define the relationships between albumin excretion and endothelial activation measured by soluble AMs.

We have shown that compared to controls, in healthy young adults with Type 1A DM, who have normal albumin excretion, normal blood pressure and no clinical signs of vascular disease, both mean systolic day and night ABP are elevated. The data also suggest that increases in systolic day and night ABP correspond to increased endothelial activation and/or platelet activation as estimated by soluble P-selectin. While there is not a significant alteration in the diurnal blood pressure at this point, we report an inverse relationship between PECAM-1 and systolic day-night difference in ABP. This suggests a relationship between increased endothelial dysfunction and less of a drop in systolic blood pressure at night. This may be a precursor to further development of abnormal day-night differences in blood pressure in patients with diabetes. Finally, we find that longer duration of disease, higher diastolic night blood pressure and lower levels of PECAM-1 predict increased albumin excretion. We speculate that along with increasing
albumin excretion, PECAM-1 and other soluble AMs may also be excreted, resulting in this inverse relationship. Our findings suggest that health care providers caring for young adults with Type 1A DM who have normal random blood pressures and normal albumin excretion rates should be aware of early endothelial activation that may be occurring in these otherwise seemingly healthy individuals. It is possible that these changes could lead to further cardiovascular abnormalities over time.
TABLE 1: Demographic Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Diabetics (n=60)</th>
<th>Controls (n=82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (years)*</td>
<td>22.9 ± 3.2</td>
<td>25.3 ± 2.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 3.5</td>
<td>24.0 ± 4.7</td>
</tr>
<tr>
<td>Duration of Disease (years)</td>
<td>13.4 ± 5.0</td>
<td>N/A</td>
</tr>
<tr>
<td>HgA1C (%)</td>
<td>8.2 ± 1.4</td>
<td>N/A</td>
</tr>
<tr>
<td>Age of Onset (years)</td>
<td>9.5 ± 4.4</td>
<td>N/A</td>
</tr>
<tr>
<td>Daily Insulin Dose (units/kg/day)</td>
<td>0.82 ± 0.32</td>
<td>N/A</td>
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</tbody>
</table>

*Indicates a statistically significant difference (p<0.01)
Table 2: Mean ± SD 24-hour Ambulatory Blood Pressure Measurements for Diabetics and Controls

<table>
<thead>
<tr>
<th></th>
<th>Diabetics (n=60)</th>
<th>Controls (n=82)</th>
</tr>
</thead>
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<tr>
<td><strong>Systolic Blood Pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Day*</td>
<td>121.4 ± 8.4</td>
<td>118.4 ± 7.6</td>
</tr>
<tr>
<td>Average Night*</td>
<td>107.7 ± 8.6</td>
<td>104.7 ± 8.3</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Day</td>
<td>72.6 ± 6.2</td>
<td>72.8 ± 5.8</td>
</tr>
<tr>
<td>Average Night</td>
<td>59.7 ± 7.5</td>
<td>58.6 ± 6.8</td>
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<tr>
<td><strong>Day Night Difference</strong></td>
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<tr>
<td>Systolic</td>
<td>13.7 ± 6.5</td>
<td>13.8 ± 7.6</td>
</tr>
<tr>
<td>Diastolic</td>
<td>12.8 ± 5.7</td>
<td>14.2 ± 6.2</td>
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</tbody>
</table>

* p-value ≤ 0.04
Table 3: Multiple Regression Analysis: Variables Independently Related to Systolic Day BP, Systolic Night BP and Diastolic Day/Night BP Difference

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient (β)</th>
<th>Standard Error</th>
<th>F Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Log Systolic Day BP</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1.96</td>
<td>0.04</td>
<td>2087.66</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Log [P-selectin]</td>
<td>0.04</td>
<td>0.02</td>
<td>4.58</td>
<td>0.03</td>
</tr>
<tr>
<td>HgA1c</td>
<td>0.01</td>
<td>0.002</td>
<td>5.18</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Log Systolic Night BP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1.86</td>
<td>0.05</td>
<td>1559.73</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HgA1c</td>
<td>0.01</td>
<td>0.002</td>
<td>11.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Log P-Selectin</td>
<td>0.04</td>
<td>0.01</td>
<td>5.08</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Systolic Day Night BP Difference</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>24.12</td>
<td>5.07</td>
<td>22.60</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Log PECAM</td>
<td>-7.22</td>
<td>3.48</td>
<td>4.32</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* R-Square = 0.17
**R-Square = 0.25
***R-Square = 0.07
Table 4: Logistic Regression: Variables Independently Related to Increased Albumin Excretion in Normoalbuminuric Type 1A Diabetic Young Adults

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient (β)</th>
<th>Standard Error</th>
<th>Wald χ²</th>
<th>p-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Intercept</td>
<td>-9.32</td>
<td>4.40</td>
<td>4.50</td>
<td>0.03</td>
<td>1.23</td>
<td>1.03-1.48</td>
</tr>
<tr>
<td>Duration</td>
<td>0.21</td>
<td>0.09</td>
<td>5.18</td>
<td>0.02</td>
<td>1.23</td>
<td>1.03-1.48</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>-0.10</td>
<td>0.04</td>
<td>7.53</td>
<td>0.01</td>
<td>0.91</td>
<td>0.84-0.97</td>
</tr>
<tr>
<td>Diastolic Night BP</td>
<td>0.13</td>
<td>0.07</td>
<td>4.07</td>
<td>0.04</td>
<td>1.14</td>
<td>1.00-1.30</td>
</tr>
</tbody>
</table>

Chi Square for the Likelihood Ratio=24.85 w/3 degrees of freedom
P-value <0.001
REFERENCES

12. fdsf


STUDY LIMITATIONS, STRENGTHS AND FUTURE DIRECTIONS

A considerable strength of this study lies in the diversity of the panel of soluble cellular adhesion molecules (soluble AMs) that we measured. Most studies to date in young adults with Type 1A DM characterize at most three adhesion molecules at a time in a population. The wide panel of soluble AMs evaluated in this study allows us to better characterize the nature of the leukocyte/endothelial cell interactions. Additionally, this study has a relatively large sample size compared to previous studies, of which the largest had 51 diabetic subjects and 29 controls. Our ability to recruit a normoalbuminuric population of young adult Type 1A diabetics is also advantageous. Our results are not confounded by the presence of early renal microvascular disease, which could produce misleading results.

The cross-sectional design of this study poses several limitations. As with any cross-sectional study, we are unable to detect any true cause and effect pathways between the soluble AMs and the other variables. Instead, we are only able to identify significant correlations among the variables. Also, the results of a cross-sectional study typically have poor generalizability. One must use caution in relating results from this study to other populations. While limitations of a cross-sectionally designed study do exist, findings from these studies often result in the generation of new hypotheses. We and other researchers will utilize the findings in the planning and design of future longitudinal studies that will characterize the cause and effect pathways of the relationships we have identified. The identification of significant relationships between elevated levels of several soluble AMs and adverse changes in the ambulatory blood pressure profile will
hopefully prompt cardiovascular disease researchers to explore these relationships in other populations, providing results that will have more widespread generalizability.

The use of HgA1c as a marker of glycemic control is a standard method utilized by most researchers in the field of diabetes and endocrinology. However, it is important to point out that it is only representative of glycemic control for the previous 3 months. While acute glycemic control is an important measurement, it is likely that characterization of chronic control and its relationship to endothelial activation will provide additional evidence that can help scientists elucidate the pathophysiological mechanisms resulting in diabetic vascular disease. Future studies should include measurement of both HgA1c, as a marker of acute control, and advanced glycation end products (AGEs), which are markers of chronic control.

The racial composition of the cohort in this study poses limitations. Type 1A DM is a disease that primarily affects Caucasians. Of those in the US with Type 1A DM, 8-9% are African-American. The racial make up of the patients with Type 1A DM, in the Diabetes Patient Database, between 18-30 years of age, at Children's Hospital Medical Center, from which our cohort was recruited is 90% Caucasian, 8.8% African American and 1.2% other ethnic groups. We originally recruited 2 African American females with Type 1 diabetes, however, they are not included in some of the analyses because they were unable to successfully complete the study. We recruited 2 young adults, who were classified as other with respect to ethnic group. In the control group we were more successful with minority recruitment with 3 African Americans and 5 people of other ethnic groups that completed the study. Unfortunately, the small sample sizes did not afford us the opportunity to examine race as a variable. While we acknowledge that
African Americans are under represented in our sample, all ethnic groups were equally recruited during the recruitment process. Recruitment and enrollment of African Americans and other minorities in Cincinnati, as well as nationwide, has historically been difficult. Special recruitment efforts to enroll African Americans and other ethnic minorities would have possibly increased our minority enrollment and will be performed in future studies to attain a more representative population. Few studies to date have evaluated ethnic differences in the expression of AMs. Given the ethnic differences in incidence and prevalence of several cardiovascular diseases, future studies evaluating ethnic variation in endothelial activation are warranted.

The ability to measure blood pressure using 24-hour ambulatory blood pressure monitoring is a major benefit of this study. 24-hour blood pressure has been shown to be a better predictor of future cardiovascular disease compared to serial office measurements. This is a non-invasive measurement that is well tolerated by both adults and children. In addition to providing mean day and night blood pressure intervals, one is able to evaluate the diurnal variation in blood pressure. To date, no study has evaluated the relationships between soluble AMs and ambulatory blood pressure variables. Further exploration in other populations would be interesting.

It has been suggested that oxidized low-density lipoprotein (LDL) within the arterial intima is a key factor in the actual initiation of vascular inflammation. Within the intima, the oxidized LDL is protected from circulating anti-oxidants and has been shown to stimulate monocyte adhesiveness to the endothelium and increase endothelial production of monocyte chemoattractant protein (MCP-1) and macrophage stimulating factor. Upon activation, monocytes and to some extent T-lymphocytes within the
circulation are stimulated to release a variety of cytokines, including TNF, IL-1 and IL-4. These factors are known to stimulate the expression of soluble AMs on the surface of the endothelium. Unfortunately, we are not able to assess the effect of oxidized LDL or cytokines on the expression of AMs. Future studies examining these relationships in young adults with Type 1A DM would be interesting.

Finally, while we do show that endothelial activation appears to be elevated in young adults with Type1A DM who have no vascular complications, there is a need for longitudinal data examining the impact of elevated soluble AMs on the future development of diabetic complications. P-selectin and ICAM-1 in particular have been found to be strong predictors for the development of future cardiovascular disease in non-diabetic populations. Using the baseline data that we have collected, it would be interesting to re-examine this diabetic cohort in several years and evaluate their cardiovascular status. While our findings are interesting and suggest that changes in the vasculature are occurring well in advance of clinically detectable atherosclerotic disease, taking the next step in evaluating these findings in a longitudinal study will provide better answers to the causal relationship between endothelial activation and the genesis and progression of diabetic vascular disease.
CONCLUSIONS

Cardiovascular disease is a significant health issue for individuals living with diabetes. The process of atherosclerosis is thought to begin early in the diabetic disease process. Even with normal albumin excretion and no known cardiovascular disease, young individuals in our study with Type 1A DM exhibit elevated circulating levels of some soluble AMs, including E-selectin, P-selectin, VCAM-1 and ICAM-1, suggesting that portions of the inflammation pathway may be affected to a greater degree than others. Our findings suggest increased endothelial activation with amplified leukocyte/endothelial interactions in young, normoalbuminuric diabetic subjects compared to healthy controls. Furthermore, our findings propose that poor glycemic control, smoking and male gender may be positively related to increased endothelial activation, specifically via E-selectin and ICAM-1 expression. Additionally, we have highlighted important adverse relationships between ambulatory blood pressure alterations and increased endothelial activation. Finally, our findings are suggestive of an inverse relationship between elevated PECAM-1 and decreased albumin excretion. This finding suggests that the circulating levels of AMs may actually be higher, due to increased urinary excretion, than reported in individuals with moderately increased albumin excretion. Further research is necessary to determine causal pathways for the relationships we have identified.
Appendix
Role in the Study

Study Design
This study is part of a larger study, Coronary Atherosclerosis in Early Insulin Dependent Diabetes Mellitus (IDDM), designed by Dr. Lawrence Dolan and Stephen Daniels. Laura Young, with the assistance of Dr. Dolan, Dr. Daniels and Dr. David Nelson, played a role in planning which adhesion molecules would be measured.

Institutional Review Board Approval
Dr. Dolan wrote the IRB proposal and solicited IRB approval.

Subject Recruitment
Debbie Standiford, research coordinator, designed the initial recruitment fliers and letters. Laura Young designed and coordinated the distribution of a second recruitment flier approximately half way through the study.

Data Collection & Study Management
The data collection period for this study was approximately two years. Dr. Dolan performed the history and physicals. Debbie Standiford managed the study and assisted in the collection of the data for the majority of the study duration. While Debbie Standiford was on maternity leave for approximately 5 months, Laura Young managed the study and assisted in the data collection. While the nurses in the treatment center generally administered informed consent document, on occasion Laura or Debbie explained the document to the participants.

Laboratory Measurements
Laura Young measured all of the soluble adhesion molecules in the lab of Dr. Nelson. The Clinical Research Center Laboratory measured HgA1c and albumin excretion rates. The ambulatory blood pressure monitor readings were downloaded by Vicki Anderson Davis, Dr. Daniel’s administrative assistant.

Data Entry
Data entry was performed by Debbie Standiford and Laura Young.

Statistical Analysis
Laura Young performed the statistical analysis under the helpful guidance of Dr. Paul Succop and Dr. Daniels.

Literature Review
Laura Young performed the literature review.

Funding
This study was funded by a grant from the American Heart Association and from contributions by the Cardiology Department at Children’s Hospital Medical Center.
An expansion of this study, to include younger individuals and additional measures of cardiovascular structure and function, was planned by Laura Young with the assistance of Dr. Daniels and Dr. Dolan. Laura Young wrote the IRB proposal and achieved IRB approval for this study. Laura Young also prepared the consent form, nursing protocols and recruitment letter for this second study.
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, Standards and the sICAM-1 Control be assayed in duplicate.

1. Prepare all reagents, working Standards, samples, and Control as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.

3. Add 100 _L diluted Conjugate to each well.

4. Add 100 _L Standard, sICAM-1 Control*, or sample** to each well with sufficient force to ensure mixing.

5. Cover the plate with a plate sealer provided and incubate at room temperature for 1.5 hours.

6. Aspirate or decant each well and wash, repeating the process five times for a total of six washes. Wash by filling each well with Wash Buffer (300 _L) using a multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid after each wash is essential to good performance. After the last wash, aspirate or decant the contents and remove any remaining Wash Buffer by tapping the inverted plate firmly on clean paper towelling.

7. Immediately add 100 _L Substrate to each well. Cover the plate with a new plate sealer and incubate at room temperature for 30 minutes.

8. Add 100 _L of Stop Solution to each well. The Stop Solution should be added to the wells in the same order as the Substrate.

9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 620 or 650 nm. If wavelength correction is not available, subtract readings at 620 or 650 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
ASSAY PROCEDURE
Bring all reagents and samples to room temperature before use. It is recommended that all samples, Standards and the sVCAM-1 Control be assayed in duplicate.

1. Prepare all reagents, working Standards, samples, and Control as directed in the previous sections.

2. Remove excess microplate strips from the frame and store in the resealed foil pouch with the desiccant pack.

3. Add 100 _L diluted Conjugate to each well.

4. Add 100 _L Standard, Control*, or sample** to each well with sufficient force to ensure mixing.

5. Cover the plate with a plate sealer provided and incubate at room temperature for 1.5 hours.

6. Aspirate or decant contents from each well and wash by adding 300 _L of Wash Buffer per well. Repeat the process five times for a total of six washes. After the last wash, aspirate or decant the contents and remove any remaining Wash Buffer by tapping the inverted plate firmly on clean paper towelling.

7. Immediately add 100 _L Substrate to each well. Cover the plate with a new plate sealer and incubate at room temperature for 20 minutes.

8. Add 100 _L of Stop Solution to each well. The Stop Solution should be added to the wells in the same order as the Substrate.

9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 620 nm. If wavelength correction is not available, subtract readings at 620 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, Standards and the sCD31 Control be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 100 _L Standard, Sample,* or Control* to each well in duplicate. Ensure sample addition is uninterrupted and completed within 15 minutes. Cover the plate with a plate sealer provided and incubate at room temperature for 1.75 hours.

4. Add 100 _L of diluted sCD31 Conjugate to each well with sufficient force to ensure mixing. Cover the plate with a new plate sealer and incubate at room temperature for 30 minutes.

5. Aspirate or decant contents from each well and wash by adding 400 _L of Wash Buffer per well. Repeat the process five times for a total of six washes. After the last wash, aspirate or decant the contents and remove any remaining Wash Buffer by tapping the inverted plate firmly on clean paper towelling.

6. Add 100 _L Substrate to each well. Cover the plate with a new plate sealer and incubate at room temperature for 30 minutes.

7. Add 100 _L of Stop Solution to each well. The Stop Solution should be added to the wells in the same order as the Substrate.

8. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 620 or 650 nm. If wavelength correction is not available, subtract readings at 620 or 650 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
Correlation Matrix: Diabetic Disease Variables, Age and BMI with Markers of Inflammation (Paper 1)
DIABETICS ONLY
Pearson Correlation Coefficients and Corresponding p-values

<table>
<thead>
<tr>
<th></th>
<th>Duration</th>
<th>HgA1c</th>
<th>Insulin Dose</th>
<th>Age of Onset</th>
<th>BMI</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOGVCAM (n=71)</td>
<td>-0.10</td>
<td>0.08</td>
<td>-0.04</td>
<td>0.15</td>
<td>&lt;0.01</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.48</td>
<td>0.76</td>
<td>0.22</td>
<td>0.98</td>
<td>0.68</td>
</tr>
<tr>
<td>LOGICAM (n=71)</td>
<td>-0.18</td>
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<td>0.09</td>
<td>-0.02</td>
<td>0.06</td>
<td>-0.29*</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.02</td>
<td>0.47</td>
<td>0.89</td>
<td>0.63</td>
<td>0.01</td>
</tr>
<tr>
<td>LOGPCAM (n=71)</td>
<td>-0.15</td>
<td>0.07</td>
<td>-0.07</td>
<td>0.10</td>
<td>0.03</td>
<td>-0.09</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.58</td>
<td>0.54</td>
<td>0.38</td>
<td>0.78</td>
<td>0.46</td>
</tr>
<tr>
<td>LOGESEL (n=71)</td>
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<td>0.26*</td>
<td>0.08</td>
<td>-0.02</td>
<td>0.08</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>0.67</td>
<td>0.03</td>
<td>0.51</td>
<td>0.85</td>
<td>0.50</td>
<td>0.37</td>
</tr>
<tr>
<td>LOGPSEL (n=50)</td>
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<td>-0.12</td>
<td>&lt;0.01</td>
<td>-0.01</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>0.45</td>
<td>0.42</td>
<td>0.99</td>
<td>0.96</td>
<td>0.35</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* indicates a statistically significant correlation
Mean Log Transformed Soluble Cellular Adhesion Molecule Concentrations: Gender and Smoking Differences (Paper 1)

DIABETICS ONLY
Means ± SD and Corresponding p-values
Differences tested using Student’s T-Test

<table>
<thead>
<tr>
<th></th>
<th>LOG VCAM-1 (ng/ml)</th>
<th>LOG ICAM-1 (ng/ml)</th>
<th>LOG PECAM-1 (ng/ml)</th>
<th>LOG E-SELECTIN (ng/ml)</th>
<th>LOG P-SELECTIN (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GENDER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male n=40</td>
<td>2.63 ± 0.13</td>
<td>2.45 ± 0.13</td>
<td>1.43 ± 0.26</td>
<td>1.79 ± 0.15</td>
<td>2.01 ± 0.20</td>
</tr>
<tr>
<td>Female n=31</td>
<td>2.64 ± 0.13</td>
<td>2.45 ± 0.11</td>
<td>1.38 ± 0.27</td>
<td>1.71 ± 0.19</td>
<td>1.97 ± 0.27</td>
</tr>
<tr>
<td>p-value</td>
<td>0.81</td>
<td>0.97</td>
<td>0.40</td>
<td>0.04</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>SMOKING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES n=9</td>
<td>2.62 ± 0.14</td>
<td>2.44 ± 0.11</td>
<td>1.42 ± 0.18</td>
<td>1.75 ± 0.17</td>
<td>2.00 ± 0.24</td>
</tr>
<tr>
<td>NO n=62</td>
<td>2.69 ± 0.10</td>
<td>2.52 ± 0.14</td>
<td>1.41 ± 0.27</td>
<td>1.79 ± 0.23</td>
<td>1.95 ± 0.16</td>
</tr>
<tr>
<td>p-value</td>
<td>0.15</td>
<td>0.05</td>
<td>0.89</td>
<td>0.53</td>
<td>0.67</td>
</tr>
</tbody>
</table>
## Average Day and Night Systolic Blood Pressure

### Pearson Correlation Analysis

n=60; except for P-selectin n=47

<table>
<thead>
<tr>
<th></th>
<th>Log Systolic Day Blood Pressure</th>
<th>Log Systolic Night Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Log P-selectin</td>
<td>0.27</td>
<td>0.07</td>
</tr>
<tr>
<td>Log E-selectin</td>
<td>0.11</td>
<td>0.42</td>
</tr>
<tr>
<td>Log VCAM-1</td>
<td>-0.05</td>
<td>0.72</td>
</tr>
<tr>
<td>Log ICAM-1</td>
<td>-0.05</td>
<td>0.71</td>
</tr>
<tr>
<td>Log PECAM</td>
<td>-0.21</td>
<td>0.11</td>
</tr>
<tr>
<td>Age</td>
<td>-0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>Log BMI</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>Duration of Disease</td>
<td>0.06</td>
<td>0.65</td>
</tr>
<tr>
<td>HgA1c</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>-0.09</td>
<td>0.51</td>
</tr>
<tr>
<td>Daily Insulin Dose</td>
<td>0.08</td>
<td>0.53</td>
</tr>
</tbody>
</table>
## Average Day and Night Diastolic Blood Pressure

### Pearson Correlation Analysis

n=60; except for P-selectin n=47

<table>
<thead>
<tr>
<th></th>
<th>Log Diastolic Day Blood Pressure</th>
<th>Log Diastolic Night Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r pvalue</td>
<td>r pvalue</td>
</tr>
<tr>
<td>Log P-selectin (n=47)</td>
<td>0.21 0.16</td>
<td>0.16 0.27</td>
</tr>
<tr>
<td>Log E-selectin</td>
<td>-0.05 0.70</td>
<td>-0.03 0.84</td>
</tr>
<tr>
<td>Log VCAM-1</td>
<td>0.05 0.73</td>
<td>0.06 0.67</td>
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<tr>
<td>Log ICAM-1</td>
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<td>0.07 0.57</td>
</tr>
<tr>
<td>Log PECAM</td>
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<td>-0.2 0.12</td>
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<tr>
<td>Age</td>
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<td>0.05 0.72</td>
</tr>
<tr>
<td>BMI</td>
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<td>0.05 0.73</td>
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<tr>
<td>Duration of Disease</td>
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<td>0.04 0.78</td>
</tr>
<tr>
<td>HgA1c</td>
<td>0.08 0.53</td>
<td>0.33 0.01</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>0.12 0.37</td>
<td>-0.01 0.96</td>
</tr>
<tr>
<td>Daily Insulin Dose</td>
<td>-0.21 0.11</td>
<td>0.03 0.84</td>
</tr>
</tbody>
</table>
### Average Day Night Systolic and Diastolic Blood Pressure Difference

#### Pearson Correlation Analysis

*n=60; except for P-selectin n=47*

<table>
<thead>
<tr>
<th></th>
<th>Systolic Day Night Blood Pressure Difference</th>
<th>Diastolic Day Night Blood Pressure Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>r</em></td>
<td><em>p</em></td>
</tr>
<tr>
<td><strong>Log P-selectin</strong></td>
<td>0.01</td>
<td>0.95</td>
</tr>
<tr>
<td><em>(n=47)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log E-selectin</strong></td>
<td>0.07</td>
<td>0.57</td>
</tr>
<tr>
<td><em>(n=60)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log VCAM-1</strong></td>
<td>-0.16</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Log ICAM-1</strong></td>
<td>-0.11</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Log PECAM</strong></td>
<td><strong>-0.26</strong></td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>0.14</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>-0.02</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Duration of Disease</strong></td>
<td>0.03</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>HgA1c</strong></td>
<td>-0.17</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Age of Onset</strong></td>
<td>0.06</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Daily Insulin Dose</strong></td>
<td>-0.03</td>
<td>0.84</td>
</tr>
</tbody>
</table>
NURSING DOCUMENTATION FORM

Coronary Atherosclerosis in Early Insulin-Dependent Diabetes Mellitus - Protocol # 486

Subject Name: _________________________             Date:                               .
CHMCNO: ___________________________             ID no: ___________________________.
___ Consent form signed (give back copy to subject)
___ Reimbursement (patient payment) form signed
___ Urine tests: FEMALES (prior to CAT scan): ___ Pregnancy test – using kit:_______
SUBJECTS WITH IDDM: ___ Culture – send to lab
ALL SUBJECTS: ___ Hoxworth
___ CAT scan
___ Height & weight (record on history & physical form)
___ Fasted x 10 hours
___ Bloodwork: SUBJECTS with IDDM: CONTROL SUBJECTS:
YSI: _______         YSI: _______
Lipid profile (fill 5cc. purple-top) Lipid profile (fill 5cc. purple-top)
Homocysteine level (fill 7cc. purple-top EDTA) Homocysteine level (fill 7cc. purple-top EDTA)
Folate & Vitamin B₁₂ (fill 5cc. SST) Folate & Vitamin B₁₂ (fill 5cc. SST)
- spin, separate, save serum & freeze at (-20°) - spin, separate, save serum & freeze at (-20°)
Hoxworth (fill 4 purple-top 10cc. EDTA) Hoxworth (fill 4 purple-top 10cc. EDTA)
- immediately store on ice - immediately store on ice
HgbA₁c (fill 2cc. purple-top) - send to lab Adhesion Molecules (fill 5cc. SST tube) – spin, separate.
Creatinine (fill 0.7cc green-Li) - spin, separate, save serum in 4 microtubes (small yellow tubes used with YSI ~ cc. serum/ tube) & freeze at (-20°)
Adhesion Molecules (fill 5cc. SST tube) – spin, separate, save serum in 4 microtubes (small yellow tubes used with YSI ~ cc. serum/ tube) & freeze at (-20°)
___ SUBJECTS WITH IDDM: insulin given (usual dose - to be brought in by subject)
___ Breakfast   ___ Echocardiogram   ___ Food questionnaire (self-administered)
___ History & physical (page Dr. Dolan for IDDM) (page D. Standiford for controls)

B/P cuff size: Hawksley site: Average of HgbA₁c:

<table>
<thead>
<tr>
<th>Auscultated B/P</th>
<th>Random Zero</th>
<th>Actual B/P</th>
<th>2nd &amp; 3rd</th>
<th>Culture: _____</th>
<th>Urine Creatinine: _____</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ____________</td>
<td>____________</td>
<td>__________</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. ____________</td>
<td>____________</td>
<td>__________</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. ____________</td>
<td>____________</td>
<td>__________</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Urine Creatinine: _____

Second visit - Date: ____________ - Time: ____________.

___ Ambulatory B/P monitor (sent home with subject) ALL SUBJECTS

---

___ Overnight urine collection kit (sent home with subject) ALL SUBJECTS

Initials: __ Signature: ___________________________ Initials: __ Signature: ___________________________.
Coronary Atherosclerosis in Early Insulin-Dependent Diabetes Mellitus

# 486

1) THE DAY BEFORE VISIT, order a select breakfast with a variety of choices (with coffee). Subject will arrive as out-pt. All forms are in CAC binder. (Page Debbie Standiford (1118-1388) if questions.)


3) Document on Nursing Documentation Form – found in protocol drawer.

4) If IDDM, obtain urine specimen in sterile jar for culture & Hoxworth. Remove 5ml. for Hoxworth. Pour into a falcon tube (tube with blue cap) and label with patient’s medical record number, name, birthdate, and date. Save tube by placing in RANDOM URINE rack in freezer. Label sterile jar with patient’s name, DOB, and date, and send to lab for urine culture with downtime slip using study plate entitled “Dolan, AHA”.

5) If CONTROL, obtain urine specimen in cup. Remove 5ml. for Hoxworth. Pour into a falcon tube (tube with blue cap) and label with patient’s medical record number, name, birthdate, and date. Save tube by placing in RANDOM URINE rack in freezer.

6) If female, obtain urine specimen for pregnancy test. Test urine with pregnancy kit, found in CRC lab & wt. room in Tx. Center #12. Urine test must be done prior to CAT scan. If the test is positive, the patient is ineligible for this study.

7) Send patient to CAT scan at pre-determined time (will usually be 7:30 AM). CAT scan is located in Radiology, 1st floor of Hospital Tower, near security desk. Follow signs to CT/MRI.

7) Obtain height and weight, and record on history & physical sheet - page 2. (May be done before or after CAT scan, depending on time allowances.)

8) Confirm that patient has fasted (except for water) for at least 10 hours prior to the study. Draw the following bloodwork: (Bloodwork may be done before or after CAT scan.)

SUBJECTS with IDDM:
* Check blood glucose on YSI and record on Nursing Documentation Sheet.
* Lipid Profile - fill 5cc. purple-top tube - stays in CRC. Label plastic tube with patient’s name, DOB, and date. Spin, separate, save plasma in plastic tube & freeze in box “Dolan CAC - lipids”.
* Homocysteine Level - fill 7cc. purple-top tube - stays in CRC (same instructions as Lipid Profile). (Box is labeled “Dolan CAC - homocysteine”.)
* Folate & Vitamin B₁₂ – fill 5cc SST tube – stays in CRC (same instructions as Lipid Profile).
  (Box is labeled “Dolan CAC - Folate & Vitamin B₁₂”.)
* Creatinine – fill 0.7cc green Lithium tube – stays in CRC (same instructions as Lipid Profile).  (Box is labeled “Dolan CAC - creatinine”.
* Adhesion Molecules - fill 5cc. SST tube – stays in CRC (same instructions as Lipid Profile except save serum in 4 microtubes – small yellow tubes used with YSI ~ cc. serum/tube).  (Box is labeled “Dolan CAC – Adhesion Molecules”.)
* Hoxworth – fill 4 purple-top 10cc. tubes – immediately store on ice (samples will be picked up at ~ 8:30 AM by someone from Hoxworth
* HgbA₁c - fill 2cc. purple-top tube.  Label with patient’s name, birthdate, and date.  Send to lab with downtime slip using study plate entitled “Dolan - AHA”.

**CONTROL SUBJECTS:**
* Check blood glucose on YSI and record on Nursing Documentation Sheet.
* Lipid Profile - fill 5cc. purple-top tube - stays in CRC.  Label plastic tube with patient’s name, DOB, and date.  Spin, separate, save plasma in plastic tube & freeze in box “Dolan CAC - lipids”.
* Homocysteine Level - fill 7cc. purple-top tube - stays in CRC (same instructions as Lipid Profile).  (Box is labeled “Dolan CAC - homocysteine”).
* Folate & Vitamin B₁₂ – fill 5cc SST tube – stays in CRC (same instructions as Lipid Profile).
  (Box is labeled “Dolan CAC - Folate & Vitamin B₁₂”.)
* Adhesion Molecules - fill 5cc. SST tube – stays in CRC (same instructions as Lipid Profile except save serum in 4 microtubes – small yellow tubes used with YSI ~ cc. serum/tube).  (Box is labeled “Dolan CAC – Adhesion Molecules”).
* Hoxworth – fill 4 purple-top 10cc. tubes – immediately store on ice (samples will be picked up at ~ 8:30 AM by someone from Hoxworth

9) **IDDM SUBJECTS:** Subject may take usual dose of insulin.  (Insulin is to be brought in by subject.)

10) Offer breakfast to subject (anytime after bloodwork).  Patient may have breakfast tray or select from Tx. Center #12 stock - according to their usual meal pattern.  Offer to get coffee for patient if not on tray.

11) Ask subject to complete food questionnaire: give subject questionnaire, instruction sheet, and food model kit.
12) If IDDM, page Dr. Dolan when patient is ready for history & physical. (Pager = 736-2560) If control, page Debbie Standiford for history & physical. (Pager = 1118-1388)

13) Measure resting blood pressure x3, using Hawksley Random Zero Sphygmomanometer, and record on nursing flow sheet. (Take 1st reading after 5 minutes of sitting at rest, and allow 2 minutes of rest between next 2 readings.)

14) Demonstrate use of ambulatory B/P monitor. Send monitor and instruction sheet home with subject. Ask subject to complete form at bottom of instruction sheet, and to return monitor and instruction sheet on 2nd visit.

15) Send subject to Cardiology for echocardiogram at pre-scheduled time (OSB, 4th floor).

16) Explain how to obtain overnight urine collection and send collection home with PEP on overnight urine collection, brown urine collection container, and (if female) urine “hat”. Ask subject to complete tag with “start” and “stop” dates & times, and to return urine collection container on 2nd visit.

17) Set up 2nd visit within 2-3 days - may be scheduled at any time convenient to subject. The purpose of this visit is only to return B/P monitor and urine collection.

18) Patient may be discharged when study completed. Checks for completion of study will be mailed to subjects (allow 2-3 weeks). SUBJECTS WITH IDDM: Results of the HgbA1c test will also be mailed. Keep all forms together until 2nd visit has been completed.

2nd Visit: Date: ____________, Time: ____________.

1) Return ambulatory B/P monitor with instruction sheet – info. on bottom of form completed.
   If subject forgot form, ask them to write on a piece of paper: date & times monitor worn, time subject went to sleep, and time subject awakened.

2) SUBJECTS WITH IDDM: Return overnight urine collection container with “start” and “stop” times written on tag. Obtain total volume of collection by weighing the full urine container and subtracting the weight of the container. Process urine collection: Label 4 falcon tubes (tubes with blue caps) with patient’s medical record number, name, birthdate, and date. Send one tube to lab for timed creatinine with downtime slip using study plate entitled “Dolan – AHA”. (On downtime slip, will need to record “start” and “stop” dates & times, as well as total volume of urine collection.) Save other tubes by placing in racks in freezer marked OVERNIGHT URINE – PROTEIN, OVERNIGHT URINE – HOXWORTH, and OVERNIGHT URINE - MELATONIN.

3) CONTROL SUBJECTS: Return overnight urine collection container with “start” and “stop” times written on tag. Obtain total volume of collection by weighing the full urine container and subtracting the weight of the container. Process urine
collection: Label 3 falcon tubes (tubes with blue caps) with patient’s medical record number, name, birthdate, and date. Send one tube to lab for timed creatinine with downtime slip using study plate entitled “Dolan – AHA”. (On downtime slip, will need to record :start” and “stop” dates & times, as well as total volume of urine collection. Save other tubes by placing in racks in freezer marked OVERNIGHT URINE – HOXWORTH, and OVERNIGHT URINE - MELATONIN.

3) Save all forms for Debbie Standiford (mailbox is in Tx. Center #12 conf. room) - do NOT send to Medical Records. (includes Nursing Documentation form, History & Physical form, food questionnaire, B/P monitor form with sleep times, and copy of all downtime slips)
   verbal order: Lawrence M. Dolan, MD
1. TITLE AND INTRODUCTORY PARAGRAPH

Coronary Atherosclerosis in Early Insulin Dependent Diabetes Mellitus

Before agreeing that I will participate in this study, it is important that I read and understand the following explanation. It describes, in words that can be understood by a lay person, the purpose, procedures, benefits, risks and discomforts of the study and the precautions that will be taken. It also describes the alternatives available and the right to withdraw from the study at any time. It is important to understand that no guarantee or assurance can be made as to the results of the study. It is also understood that refusal to participate will not influence the availability of standard medical treatment.

2. OBJECTIVES OF THE STUDY

I, ___________________________________________ of

______________________________________________, street address

______________________________________________, city state zip code

have been asked to participate in a research study. The purpose of this study is to identify how many individuals with diabetes have fatty deposits (atherosclerosis) in the walls of the blood vessels of the heart and what factors may put individuals with diabetes at risk to develop these fatty deposits. Participants in this study will include subjects with diabetes, as well as subjects without diabetes.

3. PROCEDURES

For my initial visit, I will come to the Clinical Research Center (CRC) at Children's Hospital Medical Center in the morning at a pre-appointed time. I will fast from all foods and liquids, except for water, for 10 hours before my appointment time. During this visit a blood sample will be obtained to measure kidney function (creatinine), protein components (homocysteine, B12, and folate), red blood cell membrane integrity (lipid oxidation), protein production (homocysteine metabolism), adhesion molecules, and blood fat level (cholesterol). If I have diabetes, the blood sample will also be used to measure blood sugar control (hemoglobin A1c).

I will be asked to provide an early morning urine sample to measure creatinine and oxidative stress (8-iso-PGF2a). If I have diabetes, this urine sample will also be cultured to rule out a urinary tract infection. If I am a female, my urine will also be tested to rule out a pregnancy. If my pregnancy test is positive, I will not continue in the study. If my pregnancy test is negative or if I am a male, I will have a CT (computed axial tomography) scan performed in the Radiology Department at Children’s Hospital Medical Center to document fatty deposits in the blood vessels of the heart.

I will also have an echocardiogram (a measure of heart function by sound waves) performed in the echocardiography laboratory of Children’s Hospital Medical Center.
I will have a routine medical history and physical examination; and my blood pressure will be measured 3 times at two-minute intervals. I will be asked to complete a questionnaire describing the types of foods I eat on a regular basis.

I will be instructed in the use of an ambulatory blood pressure monitor; and I will wear the blood pressure cuff and monitor for a 24-hour period. I will also perform an overnight urine collection. I will come for a second visit to the CRC within the next 2 days to return the blood pressure monitor and my overnight urine collection. My urine sample will be tested for melatonin. If I have diabetes, it will also be tested for protein to measure kidney function. If my urine sample needs to be repeated, I will be asked to bring in another overnight urine collection within the next 2 days.

4. BENEFITS

There are no direct benefits to participating in the study. If any abnormalities are found during this study, the abnormality will be discussed with me and I will be referred for appropriate treatment, if applicable. My participation in this study may benefit others who have diabetes by identifying risk factors for the development of fatty deposits (atherosclerosis) of the heart.

5. RISKS, DISCOMFORTS AND PRECAUTIONS

I may experience brief pain when the needle is inserted in my vein for the blood test. I understand that, in a small number of individuals, local bleeding may occur under the skin at the site of the insertion of the needle, which may result in a bruise. The total amount of blood required for these blood tests is 64 ml. (approximately 2 ounces).

During the CT scan I will be exposed to a small amount of radiation. The amount of radiation exposure from the CT scan is equal to the amount of radiation exposure from 2 x-rays of the stomach. To prevent radiation exposure to a developing fetus, a pregnancy test will be performed on a urine sample obtained the morning of the CT scan from all female participants. The urine pregnancy test will detect a pregnancy 2 weeks after conception. If the pregnancy test is positive, the participant will not have the CT scan done. I understand that if I believe I have been injured as a result of participation in biomedical or behavior research, I am to contact Dr. Dolan at (513) 636-4744 or the Director of Social Services to discuss my concerns. Children's Hospital Medical Center follows a policy of making all decisions concerning compensation and/or medical treatment for physical injuries occurring during or caused by participation in biomedical or behavior research on an individual basis.

6. ALTERNATIVES

If an individual decides not to participate in this study, that decision will not affect the standard of care at Children's Hospital Medical Center.

7. CONFIDENTIALITY OF RECORDS

All information gathered during this study will be held in strict confidence. Any publication resulting from participation in the study will not identify me by name.

8. AVAILABILITY OF INFORMATION

If any questions should arise concerning this study, I can call Dr. Dolan in the Division of Endocrinology at (513) 636-4744 or Dr. Daniels in the Division of Cardiology at (513) 636-8265. For information regarding my rights as a research subject, I can call Dr. Light, Chairman of Children’s Hospital Medical Center Institutional Review Board, at (513) 636-8039.

9. THE RIGHT TO WITHDRAW
I may withdraw from this study at any time. Withdrawal from this study will have no effect on my access
to care; nor will it have any effect on the standard of care provided at Children’s Hospital Medical Center.

10. ADDITIONAL ELEMENTS

Upon completion of the required visits, I will receive $50. If all study requirements are fulfilled within a
one-week period, I will receive an additional $25 for a total payment of $75. This payment will serve as
compensation for my cost of travel and the time commitment required for this study.

11. WITNESSING AND SIGNATURES

Based on the information provided above and having had the opportunity to discuss any concerns with the
investigator or his designee, I voluntarily consent to participate in this research study.

________________________________________
subject's signature

________________________________________                          witness as to voluntary signature

________________________________________
investigator

________________________________________
date

11. BASIC ELEMENTS OF INFORMED
CONSENT

The DHS and FDA policies on Protection of Human Subjects list the basic elements of informed consent as
follows:

1. A statement that the study involves research, an explanation of the purposes of the research and the
expected duration of the subject's participation, a description of the procedures to be followed and
identification of any experimental procedures.

2. A description of reasonably foreseeable risks and discomforts.

3. A description of any benefits to the subject or others that can reasonably be expected from the research.

4. A disclosure of appropriate alternative procedures or courses that might be advantageous to the subject.

5. A statement describing the extent, if any, to which confidentiality of records identifying the subject will
be maintained.

6. An explanation of whom to contact for answers to pertinent questions about the research and research
subjects’ right and whom to contact in the event of a research-related injury to the subject.

7. A statement that participation is voluntary, that refusal to participate will not involve loss of benefits and
that subjects may discontinue participation at any time without loss of benefits.

8. A statement as to whether any compensation and medical treatment is available if injury occurs.

This study has been reviewed and approved by the Institutional Review Board of the Children's Hospital
Medical Center (Chairman’s Office telephone number: (513) 636-8039
Dear «FIRSTNAME»:

I am writing to tell you about an important new clinical study that we are beginning at Children’s Hospital Medical Center, funded by the American Heart Association.

**Purpose**

The purpose of this study is to identify how many individuals with diabetes have fatty deposits (atherosclerosis) in the walls of the blood vessels of the heart; and to determine factors that may put individuals with diabetes at risk to develop these fatty deposits.

**Participation**

Participation in this study would require two visits to the Clinical Research Center. The enclosed sheet gives more detailed information regarding study requirements.

**Payment**

We will be able to provide $50 - $75 to each participant to cover the cost of travel and the time required to participate in the study.

**Scheduling**

Please contact Debbie Standiford, MSN, RN, CPNP, at (513) 636-8555 for further information or to schedule an appointment to participate. We look forward to hearing from you.

Sincerely,

Lawrence M. Dolan, MD
Professor of Pediatrics
Division of Endocrinology

cc: Hospital chart, Private chart
1st Visit

Your 1st visit should last 2 ½-3 hours. This visit includes the following: CAT scan of coronary arteries (heart blood vessels), urine & blood samples, history & physical examination, food questionnaire, and echocardiogram. This visit needs to be scheduled on a Tuesday or Friday at 7:30 AM after an overnight fast.

CAT Scan
Your 1st visit begins with a CAT scan at 7:30 AM. The CAT scan requires a small exposure to radiation equal to two x-rays of the stomach. This radiation exposure carries no risk. (A pregnancy test will be performed on urine samples from all female participants on the day of the CAT scan so that a developing fetus is not inadvertently exposed to radiation.)

Urine & Blood Samples
Urine samples will be obtained from all participants to rule out a urinary tract infection and to measure creatinine & oxidative stress. Bloodwork will be obtained for: creatinine (to measure kidney function); homocysteine, vitamin B12, and folate (to measure protein components); lipid oxidation (to measure red blood cell integrity); homocysteine metabolism (to measure protein production); cholesterol (to measure blood fat level); and hemoglobin A1c (to measure blood sugar control). The total amount of blood required for these tests is less than two ounces.

History & Physical Examination
Dr. Dolan or Debbie Standiford will perform a history and physical examination. Nursing personnel will take three blood pressure measurements.

Food Questionnaire
You will be asked to complete a self-administered food questionnaire describing the types of foods you eat on a regular basis.

Echocardiogram
Your visit will also include an echocardiogram, which measures heart function by sound waves.

You will be sent home with a urine collection container and a blood pressure monitor. You will be asked to collect an overnight urine sample (to measure kidney function). You will also be asked to wear the blood pressure monitor for one 24-hour period.
2\textsuperscript{nd} Visit

Your 2\textsuperscript{nd} visit will be scheduled within a few days of your 1\textsuperscript{st} visit. At that time you will return your urine collection and the blood pressure monitor. This visit can be scheduled at your convenience during day or evening hours - any day of the week, including Saturdays and Sundays. Occasionally, urine collections need to be repeated. If your collection needs to be repeated, you will be asked to bring in a second collection within a few days.

Payment

Participants who complete the study will receive $50. If the study requirements are completed within a one-week period, you will receive an additional $25, for a total of $75. Payments and hemoglobin A1\textsubscript{C} results will be mailed to your home.