Cardiovascular Autonomic Neuropathy, HDL Cholesterol, and Smoking Correlate With Arterial Stiffness Markers Determined 18 Years Later in Type 1 Diabetes

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OBJECTIVE — To examine the relationship between cardiovascular autonomic neuropathy and pulse waveform analysis (PWA) measures of arterial stiffness in a childhood-onset type 1 diabetes population.

RESEARCH DESIGN AND METHODS — Cardiac autonomic nerve function was measured in the baseline examination of the Pittsburgh Epidemiology of Diabetes Complications Study of childhood-onset type 1 diabetes by heart rate variability (R-R interval) during deep breathing and expressed as expiration-to-inspiration (E/I) ratio. Other cardiovascular and diabetes factors were also assessed. PWA was performed using SphgymoCor Px on 144 participants at the 18-year follow-up examination. Univariate and multivariate analyses for associations between baseline nerve function and other cardiovascular and diabetes-related factors were performed for augmentation index (AIx), augmentation pressure (AP), and subendocardial viability ratio (SEVR), a surrogate marker of myocardial perfusion.

RESULTS — E/I ratio correlated negatively with both AIx ($r = -0.18$, $P = 0.03$) and AP ($r = -0.32$, $P < 0.001$) and positively with SEVR ($r = 0.47$, $P < 0.001$) univariately. Lower baseline E/I ratio, HDL cholesterol, and a history of smoking were associated with higher follow-up (18 years later) AIx and AP and lower SEVR in multivariate analyses. Higher baseline HbA_1 was also associated with higher AP and lower SEVR multivariately.

CONCLUSIONS — Cardiovascular autonomic neuropathy is associated with increased arterial stiffness measures and decreased estimated myocardial perfusion in those with type 1 diabetes some 18 years later. This association persists after adjustment for potential confounders as well as for baseline HbA_1, HDL cholesterol, and smoking history, which were also associated with these PWA measures.

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Cardiovascular disease occurs earlier and with greater frequency in people with diabetes, a finding particularly striking in women (1). These observations are especially true for young adults with type 1 diabetes, in whom coronary artery disease is increased 10-fold or greater (2). Much of the risk for coronary artery disease in type 1 diabetes lies in the presence and severity of atherosclerosis and its risk factors (dyslipidemia/hyperlipidemia). Blood flow dynamics and arteriosclerosis or arterial stiffening, which are measured in a variety of ways, are also important risk factors for cardiovascular events and mortality (3). Interestingly, in case-control studies, arterial stiffness indexes are shown to be increased generally in those with type 1 diabetes (4). Indexes of arterial stiffness can be measured noninvasively with pulse waveform analysis (PWA) using applanation tonometry (external application of a micromanometer-tipped probe over a peripheral artery) (5). PWA provides a variety of indexes and hemodynamic parameters, including *1*) augmentation pressure (AP) and 2) augmentation index (AIx), both of which provide information about the effects of early wave reflection on central blood pressure, as well as *3*) subendocardial viability ratio (SEVR), an indicator of potential for myocardial ischemia. SEVR is the ratio of the area under the time-pressure curve during diastole (an estimate of myocardial perfusion) to the area under the curve during systole (an estimate of cardiac workload) (6).

Currently, few data are available concerning risk factors for increased arterial stiffness in those with type 1 diabetes. In addition to traditional cardiovascular disease risk factors, autonomic neuropathy (AN), a complication of type 1 diabetes, is of particular interest, as it predicts cardiovascular events and mortality (7). The autonomic nervous system is responsible for regulating heart rate and vascular tone and, therefore, may contribute to increased arterial stiffness in those with type 1 diabetes. Thus, the aim of the present study is to examine the association between cardiovascular autonomic nerve function and arterial stiffness, via AIx and AP, and myocardial perfusion, via SEVR, in a type 1 diabetic population.

RESEARCH DESIGN AND METHODS

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

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dren's Hospital of Pittsburgh between 1950 and 1980 and were on insulin therapy at initial discharge. Baseline examination in the EDC study occurred between 1986 and 1988, after which participants were examined biennially thereafter. At the 18-year examination (November 2004 to November 2006), PWA was performed. The study protocol was approved by the University of Pittsburgh Institutional Review Board.

Of the 658 EDC study participants examined at baseline, 140 (21.3%) had died by the 18-year follow-up and 79 (12%) had moved out of the area, leaving 439 eligible for examination, 72% of whom ($n = 318$) took part. For this analysis, complete data were available for 309 participants. PWA testing began part way through the 18-year examination period (January 2006), after which 189 subjects were seen, 144 (76%) of whom had PWA performed and formed the study population for this analysis.

Physical activity was assessed by survey as previously described (8). Self-reported alcohol consumption (average drinks/week), current and ever-smoker status (at least 100 cigarettes during lifetime), and medication use (coded according to the World Health Organization's Anatomical, Therapeutical, and Chemical Classification/Defined Daily Dose [ATC/DDD] codes) were obtained. Medications of interest in these analyses were those potentially effecting pulse wave reflection indexes (i.e., ACE inhibitors, angiotensin II receptor blockers [ARBs], calcium channel blockers, β-blockers, and nitrates) (9). To account for the potential confounding effect of use of these medications, a dichotomous "pulse wave drug" (PWD) variable was created (any use versus no use).

Systolic and diastolic blood pressure levels were measured after a 5-min rest using a random zero sphygmomanometer, according to a standardized protocol. Hypertension was defined as systolic blood pressure >130 mmHg or diastolic blood pressure >80 mmHg or the use of antihypertensive medication for the purpose of lowering blood pressure. Height was measured in centimeters and weight in kilograms and from these, BMI was calculated (kg/m^2). Waist and hip circumferences were measured two times (a third time only if the first two measures were not within 0.5 cm of each other). These waist and hip measurements were averaged and used to calculate waist-to-hip ratio. Total cholesterol levels were measured enzymatically (10). HDL cholesterol levels were determined using a modified (11) precipitation technique (heparin and manganese chloride) based on the Lipid Research Clinics method. Non-HDL cholesterol was calculated by subtracting HDL cholesterol level from total cholesterol level. Heart rate response to deep breathing, the expiration-inspiration (E/I) ratio, defined cardiovascular autonomic neuropathy. In addition, symptomatic autonomic neuropathy consisted of both an abnormal E/I ratio (<1.1) and at least two positive responses to 21 questions asked during the EDC physician examination (Diabetes Control and Complications Trial clinical protocol) about symptoms of autonomic neuropathy (e.g., gastroparesis, incontinence, impotence, etc.).

Complete blood counts (CBC) were determined using the Coulter S-Plus IV (Beckman Coulter, Fullerton, CA). Urinary and serum creatinine concentrations were measured using an Ectachem 400 Analyzer (Eastman Kodak, Rochester, NY). For the first 18 months, blood samples were analyzed for hemoglobin A_1 (HbA_1) using microcolumn cation-exchange (Isolab, Akron, OH). For the remainder of the baseline clinic visits, automated high-performance liquid chromatography (Diamat; Bio-Rad, Hercules, CA) was performed. The two assays were highly correlated ($r = 0.95$; [Diamat HbA_1] = $-0.18 + 1.00$ [Isolab HbA_1]). Urinary albumin was measured by immunonephelometry (12). Albumin excretion rates (AERs) were calculated using urinary albumin levels from at least two validated timed sample collections.

Aortic AIx, aortic AP, and SEVR were derived using waveforms measured at the radial artery using the SphygmoCor Px version 7.01 (AtCor Medical, Sydney, Australia). In brief, a high-fidelity micromanometer with a frequency response of >2 kHz (Millar Instruments, Houston, TX) was gently pressed on the right radial artery until a consistent waveform was produced. Measurement was stopped after at least 20 sequential waveforms had been acquired. Central pressure values were estimated from radial measurements using the software's mathematical transfer function, which is both accurate and reliable (5). The pressure wave created by left ventricular contraction propagates forward until meeting sites of resistance that reflect the wave backward; thus, stiffer artery walls result in earlier wave reflection (13). When the reflected wave returns during systole rather than diastole, systolic pressure is increased or "augmented." AP is a measure of how much the early reflected wave contributes to central systolic pressure. AIx represents the level of augmentation measured and is expressed as a percentage of the pulse pressure (PP) (AIx = AP/PP). SEVR is a tonometric noninvasive measure of myocardial perfusion (as coronary artery perfusion takes place primarily during diastole) relative to cardiac workload (myocardial contraction). SEVR is measured as the ratio of the diastolic area under the curve of an arterial pulse wave to the systolic area under the curve. The SphygmoCor device provides a quality index, which represents reproducibility of the waveform. PWA measures with a quality index <80 were repeated, and only measures with a quality index ≥80 were included in this study.

Statistical analysis

Distributional characteristics and normality of all variables were assessed. Student's *t* test and one-way ANOVA were used to compare normally distributed variables between groups, whereas Mann-Whitney *U* and Kruskal-Wallis tests were used for non-normally distributed variables (e.g., AP, triglyceride, serum creatinine, AER, white blood cell). The χ^2 test was used to compare categorical variables between groups.

Pearson's and Spearman's correlations were used for bivariate correlations for normal and non-normally distributed variables, respectively. Linear regression models were fit for AIx, AP, and SEVR. All categorical variables were dichotomized, and all continuous variables were standardized to the sample before multivariate regression analyses, to make appropriate comparisons between the PWA and the remaining 18-year populations. Modeling of AP required natural logarithmically transformed AP [Ln(AP)], since regression residuals were not normally distributed. Stepwise forward regression using baseline variables was completed for each PWA measure adjusting for concurrent potential confounders (even if not statistically significant). All statistical analyses were conducted using SPSS 15.0 for Windows (SPSS, Chicago, IL). A minimal level of significance of $P < 0.05$ is used. However, due to the limited sample size, factors with $P < 0.10$ were also considered important.

Table 1—*Baseline characteristics of the Pittsburgh EDC study PWA population compared with the remaining 18-year follow-up participants*

	PWA study population	Remaining 18-year EDC population
n	144	165
Female (%)	50.7 (73)	52.1 (86)
Age (years)	25.9 ± 7.38	26.7 ± 7.50
Diabetes duration (years)	17.6 ± 6.70	18.7 ± 7.34
E/I ratio	1.14 ± 0.12	1.13 ± 0.12
Symptomatic autonomic neuropathy (%)	14.5 (19)	17.3 (27)
Heart rate (bpm)	76.0 ± 12.3	73.1 ± 11.1*
Systolic blood pressure (mmHg)	111.4 ± 13.1	110.2 ± 12.5
Diastolic blood pressure (mmHg)	71.8 ± 10.1	70.6 ± 10.1
Hypertension (%)	22.2 (32)	19.4 (32)
HbA_1 (%)	10.1 ± 1.69	10.1 ± 1.71
Non-HDL cholesterol (mg/dl)	127.2 ± 39.9	128.1 ± 36.7
HDL cholesterol (mg/dl)	54.0 ± 11.0	55.6 ± 14.1
Triglycerides (mg/dl)	72.0 (55.0–101.0)	77.0 (58.3–100.0)
Waist-to-hip ratio	0.82 ± 0.07	0.82 ± 0.07
BMI (kg/m^2)	23.5 ± 3.10	23.4 ± 3.37
Albumin excretion rate (μg/min)	9.76 (6.16–27.2)	12.2 (6.88–67.7)
Serum creatinine (mg/dl)	0.90 (0.70–1.00)	0.80 (0.70–1.00)
White blood cell count ($\times 10^{-9}$/l)	5.85 (5.18–7.00)	5.90 (5.20–7.00)
Energy expenditure (kcal/week)	450.0 (0.0–1,256.3)	425.0 (0.0–1,585.6)
Ever-smoker (%)	34.5 (50)	28.0 (46)
Prevalent coronary artery disease (%)	4.2 (6)	3.6 (6)

Data are means ± SD, medians (IQR), or % (*n*) unless otherwise indicated, *$P < 0.05$.

RESULTS — Characteristics of the PWA study population ($n = 144$, 46.6%) and the remaining EDC population examined at the 18-year follow-up (nonparticipants, $n = 165$, 53.4%) are listed in Table 1. The mean (±SD) age and diabetes duration for the PWA population at baseline were 25.9 ± 7.4 and 17.6 ± 6.7 years, respectively, which did not significantly differ from the nonparticipants. The only significantly different factor at baseline between PWA participants and nonparticipants was mean baseline heart rate (76.0 ± 12.3 vs. 73.1 ± 11.1; $P = 0.04$). At follow-up 18 years later, individuals with PWA measures still had significantly higher mean heart rate than nonparticipants, as well as lower mean waist-to-hip ratio and mean AER (data not shown). PWA participants also had a borderline significantly greater percentage of reported current smokers at follow-up (15 vs. 8%, $P = 0.05$) but not ever-smokers (40 vs. 32%, $P = 0.13$). They also had greater mean alcoholic drinks/week (2.6 ± 6.1 vs. 1.8 ± 5.2, $P = 0.09$) than non-PWA EDC participants.

AIx and AP were highly correlated ($r = 0.90$, $P < 0.001$). Both factors were also significantly correlated with height, heart rate, and age ($P < 0.01$). SEVR was not significantly correlated with AIx ($r = -0.06$, $P = 0.49$), but was negatively correlated with AP ($r = -0.21$, $P = 0.01$). SEVR was also significantly negatively correlated with age ($r = -0.29$, $P < 0.001$) and heart rate ($r = -0.67$, $P < 0.001$) and positively correlated with height ($r = 0.23$, $P < 0.01$). AIx and AP remained significantly correlated with height after adjustment for sex, but SEVR did not.

Unadjusted correlations between PWA measures and baseline variables are described in Table 2. Baseline E/I ratio, heart rate, and energy expenditure in sports activities were all negatively associated with AIx and AP and positively associated with SEVR. This pattern did not change when individuals with symptomatic AN ($n = 19$) were excluded from analysis (data not shown). Both non-HDL cholesterol and AER were positively associated with AP, but not AIx, and negatively with SEVR. BMI was correlated only with AIx. AIx and AP were higher and SEVR was lower in women than in men (Table 2). This same pattern existed between individuals with a history of smoking compared with individuals without.

A total of 90 (62.5%) of the 144 PWA study participants reported medication use that is known to affect arterial stiffness (PWD use). Most (88.9%; $n = 80$) were using an ACE inhibitor and/or an ARB. Of the 16 participants taking β-blockers, 10 (62.5%) were also taking ACE inhibitors/ARBs. The 13 (of 18) reporting calcium channel blocker use, and 4 (of 5) participants reporting nitrate use, were also taking ACE inhibitors/ARBs.

In multivariate analyses relating base-

Table 2—*Correlations (or means ± SD) between baseline factors and 18-year follow-up PWA measures (AIx, AP, and SEVR)*

	AIx	AP	SEVR
E/I ratio	−0.18†	−0.32‡	0.47‡
Heart rate (bpm)	−0.20†	−0.16§	−0.44‡
Systolic blood pressure (mmHg)	−0.09	−0.03	−0.11
Diastolic blood pressure (mmHg)	−0.00	−0.01	−0.03
HbA_1 (%)	0.02	0.06	−0.12
Non-HDL cholesterol (mg/dl)	0.11	0.15§	−0.16§
HDL cholesterol (mg/dl)	0.10	0.05	0.03
Waist-to-hip ratio	−0.04	0.02	0.05
BMI (kg/m^2)	0.19†	0.11	−0.12
Albumin excretion rate (μg/min)	0.10	0.19†	−0.22‖
Serum creatinine (mg/dl)	0.11	0.11	−0.05
White blood cell count ($\times 10^{-9}$/l)	0.11	0.12	−0.07
Energy expenditure (kcal/week)	−0.28‡	−0.29‡	0.20†
Males ($n = 71$)	19.4 ± 11.5	7.66 ± 6.36	149.7 ± 29.8
Females ($n = 73$)¶	26.5 ± 9.18	10.4 ± 6.42	135.8 ± 31.2
Never-smoker ($n = 93$)	22.0 ± 11.5	8.42 ± 6.85	145.8 ± 31.1
Ever-smoker ($n = 49$)#	25.3 ± 9.59	10.3 ± 5.71	135.1 ± 30.6

Data are means ± SD of AIx, AP, and SEVR for categorical variables. †$P < 0.05$; ‡$P < 0.001$; §$P < 0.10$; ‖$P < 0.01$. ¶Males compared with females: AIx: $P < 0.001$; AP: $P = 0.003$; SEVR: $P = 0.01$. #Never-smokers compared with ever-smokers: AIx: $P = 0.09$; AP: $P = 0.01$; SEVR: $P = 0.05$.

Table 3—*Baseline predictors of AIx, Ln(AP), and SEVR in multivariate linear regression analysis after potential confounder adjustment*

	Base model											
	AIx				Ln(AP)				SEVR			
	β	SEM	*P*	r^2	β	SEM	*P*	r^2	β	SEM	*P*	r^2
Concurrent age	0.19	0.08	0.02		0.07	0.01	<0.001		−0.29	0.07	<0.001	
Concurrent heart rate	−0.43	0.07	<0.001		−0.06	0.01	<0.001		−0.67	0.06	<0.001	
Female sex	0.49	0.19	0.01		0.07	0.03	0.03		−0.38	0.15	<0.001	
Concurrent height	−0.32	0.09	0.001		−0.04	0.02	0.01		—	—	—	
PWD use	−0.29	0.14	0.04		−0.04	0.03	0.05		0.08	0.12	0.52	
Base model (r^2)				0.41				0.37				0.60

	Multivariate model											
	AIx				Ln(AP)				SEVR			
	β	SEM	*P*	Δr^2	β	SEM	*P*	Δr^2	β	SEM	*P*	Δr^2
HDL cholesterol	−0.24	0.07	0.001	0.05	−0.05	0.01	0.001	0.05	—	—	—	—
E/I ratio	−0.17	0.08	0.04	0.02	0.04	0.03	0.02	0.05	0.13	0.07	0.06	0.01
Ever-smoker	0.25	0.14	0.07	0.01	0.02	0.05	0.02	0.04	−0.41	0.11	<0.001	0.05
HbA_1	—	—	—	—	0.02	0.03	0.01	0.02	−0.13	0.06	0.02	0.02
Final model (*P*, r^2)			<0.001	0.49			<0.001	0.53			<0.001	0.68

Variables are standardized to the population: (variable − mean)/SD. Base models with concurrent age, sex, height, heart rate, and PWD use (i.e., medications with potential effect on PWA measures, such as ACE inhibitor, ARB, calcium channel blocker, β-blocker, and/or nitrate use). Baseline variables available for forward regression: systolic blood pressure, diastolic blood pressure, non-HDL cholesterol, HDL cholesterol, white blood cell count, albumin excretion rate, serum creatinine, HbA_1, BMI, waist-to-hip ratio, energy expenditure in sports at baseline, and E/I ratio.

line measures while adjusting for follow-up potential confounders (age, sex, height, heart rate, PWD use), lower E/I ratio, lower HDL cholesterol, and a history of smoking were associated with greater follow-up AIx and Ln(AP) (Table 3). Higher baseline HbA_1 was also associated with higher AP. The baseline factors associated with decreased SEVR were lower E/I ratio, higher HbA_1, and having a smoking history. Results were similar when models were adjusted for ACE inhibitors/ARB use instead of PWD use. However, based on *P* values, ACE inhibitors/ARB use was more significantly related to PWA measures than was the more inclusive PWD use variable (data not shown).

In sex-adjusted analyses, E/I ratio was related to all three PWA measures. In sex-stratified analyses (data not shown), E/I ratio remained significantly associated with AP and SEVR in females but not in males.

CONCLUSIONS — The key finding in this study is that baseline cardiovascular autonomic neuropathy (measured by E/I ratio) strongly correlates with both arterial stiffness (measured by both augmentation index and augmentation pressure) and reduced estimated myocardial perfusion (measured by SEVR) in childhood-onset type 1 diabetes some 18 years later. Along with cardiovascular autonomic neuropathy, known cardiovascular risk factors such as reduced HDL cholesterol and cigarette smoking were also predictive of the increased stiffness indexes AIx and AP. Poorer glycemic control (higher baseline HbA_1) in this population was also associated with higher AP and with lower SEVR. However, because we did not have PWA measures at baseline and cannot be certain that these factors are true predictors of incidence, it seems likely that cardiovascular autonomic neuropathy may exert a major pathophysiological role in the development of arterial stiffening.

E/I ratio was predictive, multivariately, of both AIx and Ln(AP). This finding is consistent with the finding by Ahlgren et al. (14) of a significant correlation between E/I ratio and aortic stiffness (measured as aortic distensibility using ultrasonography) in females, but not in males, with type 1 diabetes. However, because of limited sample size in our study, sex-stratified analyses are not conclusive. In our type 1 diabetes population, diabetic autonomic neuropathy has been associated with increased all-cause mortality, and specifically cardiovascular mortality, as well as nonfatal cardiovascular events (7). Cardiovascular autonomic neuropathy has also been shown to be associated with left ventricular hypertrophy and diastolic dysfunction in type 1 diabetes (15). As arterial stiffness indexes are shown to contribute to left ventricular diastolic dysfunction (16), arterial stiffness may be a critical link between AN and cardiovascular disease. Type 1 diabetic patients without neuropathy, nephropathy, or retinopathy have been observed to have preserved vascular function (17), which suggests an intimate relationship between vascular dysfunction and these complications.

Lower HDL cholesterol, a traditional cardiovascular risk factor but not higher non-HDL cholesterol, was independently predictive of AIx and Ln(AP). In a study of healthy subjects, Duprez et al. (18) showed that low HDL cholesterol was significantly correlated to AP and AIx in women but not in men. However, the study examined only univariate correlations. The results of the present study are generally consistent with baseline HDL cholesterol being associated with both AIx and Ln(AP) in partial correlations and in multivariate sex-adjusted models; in sex-stratified analyses, lower HDL cholesterol remained significantly associated with higher AIx and higher AP in women and with higher AP in men.

A history of smoking was significantly associated with all three outcomes. This finding is not surprising, since cigarette smoking is known to be associated with arterial stiffness indexes, particularly in individuals with hypertension (19). A higher baseline HbA_1 was also associated with increased AP and decreased SEVR in the present study. This is consistent with the notion that the formation of advanced glycation end products (AGEs) is one of the primary mechanisms involved in arterial stiffening, especially in individuals with diabetes. Arterial wall exposure to AGEs can cause cross-linking of collagen molecules, which in turn reduces arterial elasticity (20). AGEs are shown to be associated with increased arterial AIx in individuals with hypertension (21). Schram et al. (22) showed that AGEs, specifically, pentosidine, Nepsilon-(carboxymethyl) lysine, and Nepsilon-(carboxyethyl)lysine, were all significantly associated with increased pulse pressure in individuals with type 1 diabetes cross-sectionally, whereas A1C was not. However, Gordin et al. (23) did find a concurrent association between A1C and AIx in a small study of 22 healthy males with type 1 diabetes. In our study, concurrent A1C also was not significantly associated with PWA measures (data not shown). Baseline HbA_1 level may better represent the historic exposure to hyperglycemia that leads to AGE exposure than concurrent A1C.

Many of the factors associated with AIx were also associated with AP in this study. This is not surprising, since AIx is merely AP/PP × 100. AP is the measure of contribution that the wave reflection makes to the systolic arterial pressure, and it is obtained by measuring the reflected wave coming from the periphery to the center. As the reflected wave returns earlier in the cardiac cycle, there is a disproportionate rise in systolic blood pressure and therefore an increase in PP. It has been shown that AIx increases with age in the healthy population until ~55 years of age, when diastolic blood pressure may plateau or fall, contributing to higher PP. AP, however, steadily increases with age without reaching a plateau (24); thus, AP may be a better representation of vascular aging when PP rises, since this will result in lower AIx. This may be true in individuals with type 1 diabetes at a younger age, since type 1 diabetes is associated with accelerated vascular aging (25). Because poorer glycemic control is also associated with increased PP (22), this may explain why baseline HbA_1 was associated multivariately with AP, but not with AIx.

While nearly half (48.6%) of our study population showed evidence of cardiovascular autonomic neuropathy (E/I ratio <1.1) on exam at baseline, a much smaller proportion of the population (14.5%) was experiencing symptomatic autonomic neuropathy at baseline. Also, at baseline, no objective measures of autonomic neuropathy in other end-organs were obtained (e.g., gastroparesis, impaired bladder function, impotence, etc.). Because we did not see any difference in univariate analyses when individuals with symptomatic autonomic neuropathy were excluded, we hypothesize that individuals with autonomic neuropathy are more likely to have increased arterial stiffness irrespective of concomitant symptoms.

This study has significant limitations. For one, the sample size was relatively limited, and, as such, *P* values <0.10 have been reported. However, this study is the largest to date to assess PWA measures (AIx, AP, and SEVR) in type 1 diabetes. Also, this study lacks PWA measurements at baseline. Thus, significant factors in this study can only be considered potential predictors of the arterial stiffness indexes measured and need to be confirmed prospectively in a type 1 diabetes population, before designation as true predictors. Another limitation is that this population is essentially a survivor population in that those who were either deceased or unable to attend at the 18-year follow-up examination because of poor health may represent individuals with greater risk for complications and/or those most affected by increased arterial stiffness. Also, compared with individuals at the 18-year follow-up but without PWA measures, the PWA study group had significantly lower follow-up AER measures and waist-to-hip ratio and therefore may represent a healthier segment of our type 1 diabetes population (data not shown). However, these limitations are more likely to hinder finding significant relationships between baseline factors and follow-up PWA measures than demonstrate false relationships.

Furthermore, this is the first study to examine multiple potential risk factors for arterial stiffness in a type 1 diabetes population. The findings of this study have clinical significance, since we show potentially modifiable risk factor states (notably cardiovascular autonomic neuropathy, cigarette smoking, poor glycemic control, and low HDL cholesterol levels) are associated with increased arterial stiffness and lower estimated myocardial perfusion later in life in type 1 diabetes. The results of this study also confirm that the use of antihypertensive medications, specifically ACE inhibitors/ARBs, is associated with lower arterial stiffness indexes but does not necessarily improve coronary artery perfusion. Thus, early testing and treatment for autonomic neuropathy may be effective in reducing arterial stiffness and, in turn, cardiovascular morbidity and mortality in individuals with type 1 diabetes.

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