Arterial Stiffness Is Associated With Cardiovascular, Renal, Retinal, and Autonomic Disease in Type 1 Diabetes

SIMONE THEILADE, MD[1]
MARIA LAJER, PHD[1]
FREDERIK PERSSON, MD[1]

CHRISTEL JOERGENSEN, MD[1]
PETER ROSSING, DMSC[1,2]

OBJECTIVE—In patients with type 1 diabetes, we investigated the association between arterial stiffness and diabetes complications.

RESEARCH DESIGN AND METHODS—This was a cross-sectional study including 676 Caucasian patients with type 1 diabetes (374 [55%] men, aged 54 ± 13 years [mean ± SD]) and 51 nondiabetic controls (28 [55%] men, aged 47 ± 13 years). Aortic pulse wave velocity (PWV) was measured with SphygmoCor (AtCor Medical, Sydney, Australia) for 635 patients and all 51 controls.

RESULTS—PWVs (mean ± SD) in patients and controls were 10.4 ± 3.4 and 7.6 ± 1.9 m/s, respectively ($P < 0.001$). After multivariate adjustment, PWV correlated with age, diabetes duration, urinary albumin excretion rate, heart rate, and blood pressure ($P < 0.05$ for all). ANCOVA was used for comparisons between groups and adjusted for gender, age, estimated glomerular filtration rate, heart rate, HbA$_{1c}$, and 24-h mean arterial pressure. PWVs in normoalbuminuric, microalbuminuric, and macroalbuminuric patients were 9.5 ± 3.2, 11.0 ± 3.6, and 11.4 ± 3.0 m/s, respectively (adjusted $P < 0.001$). PWV in patients with previous cardiovascular disease, versus patients without, was 12.1 ± 3.5 vs. 10.0 ± 3.2 m/s, respectively (adjusted $P < 0.001$). PWVs in patients with high (≥140/90 mmHg) versus intermediate (130–40/80–89 mmHg) and low (<130/80 mmHg) blood pressure were 11.8 ± 3.6, 10.0 ± 3.0, and 9.8 ± 3.3 m/s, respectively (adjusted $P < 0.001$). Furthermore, PWV increased with increasing degree of retinopathy: 8.0 ± 2.5 m/s (nil), 10.0 ± 2.8 m/s (simplex), 12.1 ± 3.5 m/s (proliferative), and 12.7 ± 2.4 m/s (blind), respectively (adjusted $P < 0.001$). Finally, PWV increased with abnormal heart rate variability: 11.5 ± 3.3 m/s vs. 10.1 ± 3.1 m/s (borderline) and 8.1 ± 2.1 m/s (normal) (adjusted $P = 0.027$).

CONCLUSIONS—Arterial stiffness increased with presence and duration of type 1 diabetes. Furthermore, PWV increased with all the investigated diabetes complications (cardiovascular, renal, retinal, and autonomic disease) independently of other risk factors.

Arterial stiffness predicts cardiovascular disease (CVD) events in the general population (1), in hypertension (2,3), and in diabetes (4,5). A recent meta-analysis by Vlachopoulos et al. (6) showed pulse wave velocity (PWV) to predict both CVD and all-cause mortality. The gold standard for measurement of arterial stiffness is aortic PWV measurements (7,8), which have shown to be reproducible in the general population (9) and in patients with chronic kidney disease (10).

In hypertension, PWV is a marker of subclinical organ damage. Recent studies have shown PWV to be predictive of future changes in systolic blood pressure (SBP) development of hypertension (11) to potentially improve CVD risk scoring (12,13), and also to be higher among patients less responsive to antihypertensive medication (AHT) (14).

Furthermore, in patients with end-stage renal disease, elevated PWV is an independent predictor of all-cause mortality (15), and lowering PWV by AHT reduces the mortality independently of the blood pressure (BP) reduction (16).

In type 1 diabetes, PWV has been shown to be associated with cerebral microvascular disease, cardiac (17) and renal function (18), and in type 2 diabetes with microalbuminuria (19). These associations are suggestive of PWV being related to vascular dysfunction (20) and large artery alterations, thereby possibly engaging in the development of kidney impairment (21). Arterial stiffness also is associated with autonomic neuropathy in type 1 diabetes (22,23) and retinopathy in type 2 diabetes (24), further illustrating the association between arterial stiffness and microvascular disease.

The current study is the first in a relatively large and diverse group of patients with type 1 diabetes to investigate the association between PWV and a range of diabetes complications, including albuminuria, CVD, elevated BP, retinopathy, and autonomic neuropathy.

RESEARCH DESIGN AND METHODS

Patients

From September 2009 until June 2011, Caucasian patients with type 1 diabetes attending the outpatient clinic at Steno Diabetes Center were invited to enter a cross-sectional study investigating the associations between BP, arterial stiffness, and diabetes complications. Of 1,285 patients invited, 676 patients were included in the study. The cohort was selected to include patients with a wide range of albuminuria; hence, 316 patients with normoalbuminuria, 169 with microalbuminuria, and 191 with macroalbuminuria were included. A control group of 51 persons without diabetes was also included in the study for comparison with patients with normoalbuminuria and short duration of diabetes to investigate if presence of diabetes influenced PWV.

The study conformed to the Declaration of Helsinki and was approved by the Danish National Committee on Biomedical Research Ethics (2009-056;

NCT01171248). All patients gave written informed consent.

Baseline clinical and laboratory methods

Arterial stiffness was measured as carotid-femoral (aortic) PWV by the SphygmoCor (AtCor Medical, Sydney, Australia) device. Participants were placed in supine position and measurements were performed after 15 min of rest by trained laboratory technicians. Three PWV measurements were recorded, and the two measurements closest to each other were averaged and used in the analyses. A PWV ≥12 m/s was regarded as elevated according to current guidelines (25).

Urinary albumin excretion ratio (UAER) was measured in 24-h sterile urine collections by enzyme immunoassay. Patients were stratified as normoalbuminuric if they, in two out of three consecutive measurements, had persistent normoalbuminuria with UAER <30 mg/24 h and were considered microalbuminuric or macroalbuminuric if UAER was between 30 and 300 mg/24 h or >300 mg/24 h, respectively.

The estimated glomerular filtration rate (eGFR) was calculated by the four-variable Modification of Diet in Renal Disease formula (26). Patients with end-stage renal disease, defined as receiving dialysis or renal transplantation, or GFR/eGFR <15 mL/min/1.73 m^2 were not included in the study.

Electrocardiographs were recorded with Cardiosoft version 6.51 (GE Healthcare).

All 24-h ambulatory BPs (AMBPs) were recorded with a validated tonometric watch-like device (BPro; HealthStats, Singapore), which captures radial pulse wave reflection and calculates AMBP (27,28). The BPro device measures BP every 15 min during a 24-h period. In the current study, the BPro device was calibrated with an oscillometric device (UA 787; A&D Medical) (29) before BP measuring. The AMBP data were adequate if ≥14 and ≥7 BP recordings were obtained during day and night, respectively, according to present guidelines (30). The mean number of successful measurements in our cohort was 45 ± 7 and 18 ± 4 during the day and the night, respectively. Dipping was calculated as the percentage decrease in night (11:00 P.M.–7:00 A.M.) compared with day systolic BP (SBP) (7:00 A.M.–11:00 P.M.). Dipping <10% was classified as abnormal. Although the reproducibility of abnormal

dipping is imperfect, abnormal dipping has been shown to be predictive of CVD events (31).

Heart rate variability (HRV) was measured during paced deep breathing (32). Abnormal HRV is one of the earliest signs of cardiac autonomic neuropathy and is highly reproducible (33,34). A HRV ≥15 was classified as normal, HRV of 11–14 was classified as borderline, and HRV <11 bpm was classified an abnormal value.

Retinopathy status was obtained from medical records. All patients attending Steno Diabetes Center have regular ophthalmological examinations, where retinopathy is assessed from retinal photographs taken through dilated pupils and graded as nil, simple, proliferative, or blind based on the worst eye.

HbA$_{1c}$ was measured by high-performance liquid chromatography (normal range: 4.1–6.4%, [21–46 mmol/mol]; Variant; Bio-Rad Laboratories, Munich, Germany) and serum creatinine concentration by an enzymatic method (Hitachi 912; Roche Diagnostics, Mannheim, Germany).

Based on standardized questionnaires, current users of ≥1 cigarettes or cigars or pipes per day were classified as smokers and all others were classified as nonsmokers. Previous CVD was history of myocardial infarction, coronary intervention, stroke, or peripheral arterial disease based on standardized World Health Organization questionnaires and patient records.

Statistical analysis

Normally distributed variables are given as mean ± SD. Non-normally distributed variables are given as median (range) and log10-transformed before analysis. Comparisons between groups were performed by unpaired Student t test or ANOVA. The χ^2 test was used to compare noncontinuous variables. Univariate linear regression models were used to compare PWV with covariates. All covariates independently associated with PWV ($P <$ 0.05) from the univariate analyses were entered in a multivariate backward stepwise selection regression model. ANCOVA was used for multivariable adjustment when comparing groups. In all tests, a two-tailed $P \leq 0.05$ was considered statistically significant.

In the ANCOVA, adjustments were made for gender, age, eGFR, HbA$_{1c}$, heart rate (HR), and 24-h mean arterial pressure (MAP). Covariates used in the

ANCOVA were those that were significantly associated with PWV in the multiple linear backward stepwise regression analysis. eGFR was used instead of logUAER, because albuminuric groups were compared, and 24-h MAP was used instead of 24-h pulse pressure (PP) to not adjust for arterial stiffness. In additional models, we substituted 24-h MAP with 24-h SBP (Table 2). Finally, age was chosen at the expense of diabetes duration to not exclude the controls in the multivariate analysis. However, in additional adjustment models (Table 2), we substituted age with diabetes duration because age also is included in the equation for eGFR. When comparing patients with controls, HbA$_{1c}$ was not included because it would entail a degree of adjustment for diabetes. All statistical calculations were performed using SPSS for Windows, version 15.0 (SPSS, Chicago, IL).

RESULTS

Baseline characteristics

Attainment of three PWV measurements on all participants was attempted, but only 635 (94%) patients and 51 (100%) controls had PWV measurements available. Overall, participants with successful PWV measurements were younger, with shorter diabetes duration, lower HR, lower 24-h PP, lower BMI, higher eGFR, had less previous CVD, and received less AHT ($P < 0.05$ for all) versus patients without PWV measurements. In contrast, gender distribution, 24-h SBP, DBP, MAP, albuminuria group, UAER, HbA$_{1c}$, total cholesterol, smoking status, retinopathy status, and prevalence of autonomic neuropathy were similar among participants with and without PWV measurements ($P > 0.05$ for all).

In 13 patients, only one PWV measurement was obtained. These patients were included in the calculations, using the single measurement as mean value. However, if excluding the 13 patients from the calculations, similar results were obtained.

Baseline characteristics of the 635 patients and 51 controls with PWV measurements are shown in Table 1. Patients with PWV measurements were 54 ± 13 years old, with 32 ± 16 years of diabetes duration, and 349 (55%) were men. Overall, 304 (48%), 152 (24%), and 179 (28%) had normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively.

Table 1—Baseline characteristics for patients according to albuminuria group for the 635 patients and 51 controls

	Controls n = 51	Normo-albuminuria, short duration n = 93	Normo-albuminuria, long duration n = 211	Normo-albuminuria, all n = 304	Micro-albuminuria n = 152	Macro-albuminuria n = 179	All patients n = 635	P Normo-albuminuria, short vs. controls	P Normo-albuminuria, short vs. long	P Normo-albuminuria, all vs. controls	P Normoalbuminuria vs. microalbuminuria	P Normoalbuminuria vs. macroalbuminuria
Sex, men/women, %	55/45	59/41	47/53	50/50	60/40	58/42	55/45	0.293	0.033	0.441		0.072
Age, y	47 ± 13	44 ± 15	56 ± 11	53 ± 13	57 ± 13	54 ± 10	54 ± 13	0.258	<0.001	<0.001	<0.001	0.001
Diabetes duration, y	Not available	6 ± 3	37 ± 11	28 ± 17	35 ± 15	38 ± 11	32 ± 16	Not available	<0.001	Not available	<0.001	<0.001
HbA$_{1c}$, %	5.4 ± 0.3	7.9 ± 1.3	7.7 ± 0.9	7.8 ± 1.0	8.1 ± 1.2	8.3 ± 1.1	8.0 ± 1.1	<0.001	0.201	<0.001	<0.001	<0.001
HbA$_{1c}$, mmol/mol	35 ± 3	63 ± 9	61 ± 10	62 ± 11	65 ± 13	68 ± 13	64 ± 13	<0.001	0.193	<0.001	<0.001	<0.001
Total cholesterol, mmol/L	5.0 ± 0.7	4.7 ± 0.8	4.7 ± 0.7	4.7 ± 0.8	4.7 ± 0.8	4.6 ± 0.9	4.7 ± 0.9	0.014	0.593	0.024		0.379
BMI, kg/m^2	25.6 ± 3.8	24.6 ± 3.1	24.8 ± 3.7	24.7 ± 3.5	25.5 ± 4.0	26.2 ± 9.2	25.3 ± 5.8	0.096	0.740	0.247		0.022
p-Creatinine, μmol/L	74 ± 11	72 ± 14	74 ± 14	72 ± 14	87 ± 43	121 ± 59	90 ± 44	0.311	0.181	0.367	<0.001	<0.001
eGFR, mL/min/1.73 m^2	95 ± 14	103 ± 22	90 ± 19	94 ± 21	85 ± 27	64 ± 29	83 ± 28	0.022	<0.001	<0.001	<0.001	<0.001
UAER, mg/24 h	8 (2–157)	11 (3–44)	7 (2–236)	8 (2–236)	33 (3–4,512)	131 (4–8,271)	17 (2–8,271)	0.030	0.002	0.016	<0.001	<0.001
*Previous CVD, %	4	3	12	10	28	29	19	0.582	0.008	0.145	<0.001	<0.001
Smoking, %	6	23	18	18	17	24	19	0.007	0.190	0.012		0.216
AHT, %	8	25	54	45	89	98	71	<0.001	<0.001	<0.001	<0.001	<0.001
Renin angiotensin aldosterone system, %	4	25	50	42	81	94	66	<0.001	<0.001	<0.001	<0.001	<0.001
Calcium blocker, %	0	9	19	16	45	44	31	0.027	0.018	<0.001	<0.001	<0.001
Calcium blocker 1 or calcium blocker 3	0	13	7	8	9	7	8	0.177	0.001	<0.001	0.982	<0.001
Beta blocker	2	3	9	7	13	17	11	0.555	0.139	0.346	0.003	0.003
Statin, %	6	33	44	41	67	84	59	0.047	0.022	0.022	<0.001	0.021
†Antiplatelets, %	6	18	36	30	66	75	52	<0.001	<0.001	<0.001	<0.001	<0.001
Insulin dose, IU	0	50 ± 24	45 ± 47	47 ± 41	47 ± 24	48 ± 34	48 ± 34	<0.001	0.479	<0.001	0.371	0.328
HR, bpm	65 ± 10	69 ± 11	70 ± 12	70 ± 11	71 ± 12	76 ± 12	72 ± 12	0.031	0.552	<0.001	<0.001	<0.001
24-h AMBP SBP, mmHg	130 ± 21	125 ± 14	129 ± 14	128 ± 15	128 ± 16	131 ± 15	129 ± 15	0.063	0.022	<0.001	0.054	0.046
24-h AMBP DBP, mmHg	82 ± 14	77 ± 10	77 ± 10	77 ± 10	73 ± 9	75 ± 10	75 ± 10	0.018	0.552	0.003	0.003	0.003
24-h AMBP PP, mmHg	48 ± 11	48 ± 9	53 ± 11	51 ± 11	55 ± 12	56 ± 12	53 ± 12	<0.001	<0.001	<0.001	<0.001	<0.001
24-h AMBP MAP, mmHg	98 ± 15	93 ± 11	94 ± 10	94 ± 11	94 ± 10	94 ± 11	93 ± 11	0.024	0.496	0.028	0.028	0.028
Dipping, %	10.9 ± 6.0	11.0 ± 6.0	9.5 ± 5.2	9.9 ± 5.5	9.5 ± 5.5	7.8 ± 5.6	9.2 ± 5.6	0.929	0.026	0.047	0.047	0.001
HRV, bpm	19 ± 8	18 ± 8	11 ± 7	13 ± 8	9 ± 7	7 ± 5	11 ± 8	0.381	<0.001	<0.001	<0.001	<0.001
Pulse wave velocity, m/s	7.6 ± 1.9	7.8 ± 1.9	10.2 ± 3.3	9.5 ± 3.2	11.0 ± 3.6	11.4 ± 3.0	10.4 ± 3.4	0.656	<0.001	<0.001	0.008	<0.001
Pulse wave velocity >12 m/s, %	4	2	24	17	32	39	27	0.008	<0.001	0.008	<0.001	<0.001

Data are percentage (%) and mean ± SD or median (range). Some patients with previous microalbuminuria or macroalbuminuria receiving antihypertensive treatment had values <30 mg/24 h at time of investigation. *Previous CVD events were myocardial infarction, coronary revascularization, stroke, or peripheral vascular disease. †Antiplatelets are acetylsalicylic acid and clopidogrel.

Correlations between PWV and covariates in patients

In the patients, PWV correlated positively with age ($r = 0.63$), diabetes duration ($r = 0.54$), 24-h PP ($r = 0.50$), HRV ($r = 0.48$), 24-h SBP ($r = 0.33$), UAER ($r = 0.24$), HR (0.21), and dipping ($r = 0.10$), and inversely correlated with eGFR ($r = 0.33$; $P < 0.001$ for all) and, to a lesser degree, with 24-h DBP ($r = 0.10$; $P = 0.021$) and 24-h MAP ($r = 0.06$; $P = 0.07$). PWV was higher in men, patients with previous CVD, and patients receiving AHT ($P < 0.05$ for all) (Table 2). In contrast, PWV was similar in smokers and nonsmokers (Table 2) and did not correlate with total daily insulin dose, total cholesterol, BMI, height, or weight (data not shown). The final model after selection of significant covariates showed that PWV correlated with age, diabetes duration, gender, logUAER, HR, and 24-h PP ($P < 0.05$ for all).

Patients with normoalbuminuria versus controls

Mean $\pm$ SD PWV in normoalbuminuric patients ($n = 304$) versus controls ($n = 51$) was 9.5 ± 3.2 vs. 7.6 ± 1.9 m/s, respectively ($P < 0.001$). After adjustment, PWV remained significantly higher in patients versus controls ($P < 0.001$) (Table 1). Entering use of AHT in the adjusted analysis did not alter the result (data not shown).

Overall, 52 (17%) normoalbuminuric patients compared with 2 (4%) controls had elevated PWV (≥ 12 m/s) ($P = 0.008$) (Table 1). Patients with increased PWV

were more often male, older, had longer diabetes duration, higher 24-h SBP, higher UAER, and higher total cholesterol, lower eGFR, received more often AHT, and had more previous CVD ($P < 0.001$ for all). HbA$_{1c}$, BMI, and smoking status were similar in patients with and without elevated PWV ($P > 0.05$ for all).

Patients with normoalbuminuria and short versus long duration of diabetes

Among the normoalbuminuric patients, 93 (31%) had short diabetes duration (<10 years), whereas 211 had long duration (≥ 10 years). PWVs in normoalbuminuric patients with short versus long duration were 7.8 ± 1.9 and 10.2 ± 3.3 m/s, respectively ($P < 0.001$) (Table 1). The difference attenuated after multivariate adjustment (adjusting for age, gender, eGFR, HbA$_{1c}$, HR, and 24-h MAP) ($P = 0.082$); however, if substituting age with diabetes, the difference was sustained ($P < 0.001$) (Table 2). Overall, 2 (2%) patients with short duration compared with 50 (24%) with long duration had elevated PWV (≥ 12 m/s) ($P < 0.001$) (Table 1).

Patients with different degrees of albuminuria

PWVs in patients with normoalbuminuria, microalbuminuria, and macroalbuminuria were 9.5 ± 3.2 m/s, 11.0 ± 3.6 m/s, and 11.4 ± 3.0 m/s, respectively ($P < 0.001$) (Table 1). For the different albuminuria groups, PWV tended to

increase with both albuminuria and age. This finding is consistent with earlier vascular aging in patients with increasing albuminuria. After adjustment, PWV still increased with albuminuria group ($P < 0.001$). Entering use of AHT in the adjusted analysis did not alter the result (data not shown). In addition, PWV was increased (≥ 12 m/s) in 52 (17%), 49 (32%), and 70 (39%) of patients with normoalbuminuria, microalbuminuria, and macroalbuminuria ($P < 0.001$).

Patients with or without previous CVD

Previous CVD was present in 121 (19%) patients, and the PWV was 12.1 ± 3.5 compared with 10.0 ± 3.2 m/s in patients without previous CVD ($P < 0.001$). Patients with previous CVD were older, had longer diabetes duration, higher HbA$_{1c}$, higher UAER, higher 24-h DBP, and received more AHT. However, gender distribution, total cholesterol, BMI, 24-h SBP, and smoking status were similar between groups. After multivariate adjustment, PWV remained higher in patients with previous CVD ($P = 0.017$). Entering use of statins, total cholesterol, or AHT in the adjusted analysis or excluding patients receiving class 1 or 3 calcium blockers or beta blockers did not alter the results (data not shown).

Patients with different levels of BP

A total of 623 patients had both PWV and 24-h AMBP measurements available.

Table 2—Comparison of PWV between groups in all 635 patients

	PWV (m/s)	P Unadjusted	P Adjusted model 1*	P Adjusted model 2†	P Adjusted model 3‡	P Adjusted model 4¶
Gender (male vs. female)§	10.7 ± 3.5 vs. 10.0 ± 3.2	0.006	0.002	<0.001	<0.001	<0.001
Smoking (yes vs. no)	10.2 ± 3.1 vs. 10.4 ± 3.4	0.555	0.139	0.355	0.243	0.270
Normoalbuminuria, short vs. long	7.8 ± 1.9 vs. 10.2 ± 3.3	<0.001	0.082	<0.001	<0.001	<0.001
Normoalbuminuria vs. microalbuminuria vs. macroalbuminuria	9.5 ± 3.2 vs. 11.0 ± 3.6 vs. 11.4 ± 3.0	<0.001	<0.001	0.181	0.151	0.008
Previous CVD (yes vs. no)	12.1 ± 3.5 vs. 10.0 ± 3.2	<0.001	0.017	0.006	0.009	<0.001
AHT (yes vs. no)	11.2 ± 3.3 vs. 8.4 ± 2.5	<0.001	0.015	0.001	0.001	<0.001
BP (low, intermediate, and high)	9.8 ± 3.3, 10.0 ± 3.0, and 11.8 ± 3.6	<0.001	0.001	<0.001	0.015	<0.001
HRV (normal, borderline, and abnormal)	8.1 ± 2.1, 10.1 ± 3.1, and 11.5 ± 3.3	<0.001	0.027	<0.001	<0.001	<0.001
Dipping (normal vs. abnormal)	9.8 ± 3.3 vs. 11.0 ± 3.3	<0.001	0.174	0.144	0.287	0.007
Retinopathy (nil, simplex, proliferative, and blind)	8.0 ± 2.5, 10.0 ± 2.8, 12.1 ± 3.5, and 12.7 ± 2.4	<0.001	<0.001	0.01	0.01	<0.001

Previous CVD events were myocardial infarction, coronary revascularization, stroke, or peripheral vascular disease. BP groups defined as low <130/80, intermediate 130–139/80–89, and high ≥140/90 mmHg. HRV defined as normal ≥15, borderline 11–14, and abnormal <11 bpm. *Model 1: adjusted for age, gender, eGFR, HbA$_{1c}$, HR, and 24-h MAP. †Model 2: same as model 1 but adjusted for diabetes duration instead of age. ‡Model 3: same as model 2 but adjusted for 24-h SBP instead of 24-h MAP. ¶Model 4: same as model 1 but excluding age because it is also a factor in the equation for eGFR. §Adjustments did not include gender.

These patients were divided into three groups based on 24-h AMBP, resulting in 280 (45%) with low (<130/80 mmHg), 192 (31%) with intermediate (130–139/80–89 mmHg), and 151 (24%) patients with high (>140/90 mmHg) BPs. Mean ± SD 24-h AMBP measurements of the three groups were 116 ± 9/68 ± 7, 132 ± 6/79 ± 7, and 147 ± 8/84 ± 9 mmHg, respectively ($P < 0.001$). In comparison, PWVs in patients with low, intermediate, and high BP were 9.8 ± 3.3 m/s, 10.0 ± 3.0 m/s, and 11.8 ± 3.6 m/s, respectively ($P < 0.001$). Patients with the highest BP had lower eGFR, higher PP, and were more often smokers, but had similar gender distribution, age, diabetes duration, CVD, HbA$_{1c}$, BMI, total cholesterol, UAER, and previous CVD. After multivariate adjustment, patients with higher BP still had significantly higher PWV than patients with intermediate and low BP ($P = 0.001$), whereas PWV was similar in patients with low and intermediate BP ($P = 0.174$). This did not change if adjusting for statins, total cholesterol, or AHT, or if excluding patients receiving class 1 or 3 calcium blockers or beta blockers (data not shown).

Patients with different degrees of autonomic dysfunction

Of the 635 patients with PWV measurements, 617 also had HRV measured, whereas the 623 patients with 24-h AMBP measurements had information on dipping status available. Patients with normal, borderline, and abnormal HRV had PWV of 8.1 ± 2.1 m/s, 10.1 ± 3.1 m/s, and 11.5 ± 3.3 m/s, respectively ($P < 0.001$). After adjustment, PWV remained higher in patients with abnormal HRV ($P = 0.027$). Entering use of statins, total cholesterol, or AHT in the adjusted analysis or excluding patients receiving class 1 or 3 calcium blockers or beta blockers did not alter the results (data not shown).

Of the 623 patients with both dipping and PWV measurements available, 304 (49%) had normal dipping, whereas 319 (51%) had abnormal dipping. Patients with normal dipping had lower PWV compared with patients with abnormal dipping 9.8 ± 3.2 m/s and 11.0 ± 3.3 m/s, respectively ($P < 0.001$). However, this was not significant after multivariate adjustment ($P = 0.174$).

Patients with different degrees of retinopathy

A total of 632 patients had both PWV measurements and information on retinopathy status available. Of these patients, 136 (21%) had no retinopathy, 265 (42%) had simple retinopathy, 215 (34%) had proliferative retinopathy, and 16 (3%) patients were blind. PWV increased with degree of retinopathy 8.0 ± 2.5 m/s, 10.0 ± 2.8 m/s, 12.1 ± 3.5 m/s, and 12.7 ± 2.4 m/s, respectively ($P < 0.001$), which remained significant after multivariate adjustment ($P < 0.001$). Entering use of statins, total cholesterol, or AHT in the adjusted analysis or excluding patients receiving class 1 or 3 calcium blockers or beta blockers did not alter the results (data not shown). We measured arterial stiffness by PWV in an observational cross-sectional study including 635 patients with type 1 diabetes and 51 nondiabetic control subjects. PWV was independently associated with gender, age, diabetes duration, BP, HR, and kidney function. Patients with increased PWV were male, older, with longer diabetes duration, higher 24-h SBP, higher total cholesterol, higher UAER, lower eGFR, received more often AHT, and had more previous CVD.

PWV was significantly higher in normoalbuminuric patients compared with controls, even after adjustment for covariates, suggesting early arterial stiffening associated with the presence of diabetes. Overall, 17% of normoalbuminuric patients and 4% of controls had abnormal PWV ≥12 m/s.

PWV predicted degree of albuminuria, previous CVD, 24-h AMBP ≥140/90 mmHg, retinopathy, and autonomic neuropathy, even after adjustment for covariates.

PWV is a measure for arterial stiffness, which previously has been shown to be associated with some microvascular and macrovascular complications in type 1 diabetes and to predict mortality and CVD outcome in various populations including patients with diabetes (1–4,6,35).

Previously, we and others have shown PP and augmentation index, other indirect measures of arterial stiffness, to predict CVD, decline in kidney function and mortality (5), and presence of microvascular and macrovascular complications (35) in type 1 diabetes. However, this study is the first to investigate the association between PWV and kidney function, previous CVD, different BP levels, retinopathy, and autonomic dysfunction in type 1 diabetes. Complications with diabetes often co-occur, although the overlap is incomplete and presence of, e.g., CVD does not entail, e.g., kidney disease.

Complications with diabetes are likely brought on by a complicated array of factors, and identifying single markers predictive of all or most complications could be beneficial. PWV may be one such marker, because we now show it to be elevated in the presence of several different complications to diabetes.

PWV has been shown to be associated with albuminuria in type 2 diabetes (19). We now demonstrate the same association for type 1 diabetes, which accords with evidence of arterial damage and endothelial dysfunction being involved in development of microalbuminuria, impaired kidney function, and CVD (20,21,36).

In the current study, we find PWV to be higher in patients with previous CVD, independently of AHT and BP. This finding is suggestive of lasting pathological arterial damage after CVD, which is either only partially reversible by treatment or persistent because of incomplete treatment.

PWV was similar in patients with 24-h AMBPs <130/80 vs. 130–139/80–89 mmHg. However, patients with 24-h AMBPs ≥140/90 mmHg had significantly higher PWV as compared with patients with 24-h AMBPs <140/90 mmHg. Thus, AMBPs in the range of 130–139/80–89 mmHg is associated with increased arterial stiffness, whereas AMBPs ≥140/90 mmHg are more likely to be pathological.

Others have shown PWV to predict outcome in various populations, and we now demonstrate an association between PWV and baseline characteristics in type 1 diabetes. Prospective follow-up studies on this current cohort potentially could prove interesting, with regard to PWV as predictor of outcome.

Because arterial stiffness is involved in the development of diabetes complications, debating if PWV reduction may prevent or even counteract progression of diabetes complications is warranted. Reversal of arterial stiffness is achieved by altering arterial morphology, endothelial activity, and/or smooth muscle tone. The impact of AHT on arterial stiffness is by reduction of smooth muscle tone and HR. However, few studies have shown AHT to reduce adverse outcome by reduction in arterial stiffness. The REASON trial found different effects of calcium blockers and beta blockers on central and peripheral PP (37), suggesting differences in the AHT impact on arterial stiffness. The CAFÉ study, a substudy of the ASCOT trial, also found calcium blockers versus beta

blockers to have different effects on central and peripheral PP, without similar differences in brachial BP. Furthermore, central and peripheral PP did predict outcome, suggesting an outcome benefit from iatrogenic reduction in arterial stiffness (38). However, PWV measured in a subgroup of the patients (n = 114 [5%]) was not different in patients with compared with without adverse outcome, which makes conclusions on outcome benefits from reduction in arterial stiffness in this cohort ambiguous, although this merely could be related to statistical power. However, recently Guerin et al. (16) supported the theory of outcome benefits from reversal of arterial stiffness by showing a mortality benefit from PWV reduction by AHT in patients with end-stage renal disease.

The association between autonomic dysfunction and arterial stiffness in patients with type 1 diabetes has been demonstrated by others in smaller studies (22,23) and is confirmed by our findings of PWV being associated with autonomic neuropathy. It is possible that autonomic dysfunction impacts arterial stiffness (22), whereas preservation of normal autonomic function may prevent an increase in arterial stiffness. Autonomic dysfunction is frequent in diabetes (33) and may camouflage the cause of arterial stiffness (atherosclerosis, advanced glycation end products [AGEs], neurogenic vasoconstriction, and others) and reduce the effect of treatment of arterial stiffness.

Other causes of arterial stiffness and potential sites of intervention are reduction of cholesterol levels, vascular calcification, inflammation, AGEs, activation of the renin-angiotensin-aldosterone system, and increased exercise (39,40). However, as mentioned, outcome studies investigating the benefit of intervention are still lacking.

Thus, future studies are required to investigate the outcome effect of preventing or reversing arterial stiffness. If future studies suggest a benefit from reduction of PWV, then treatment also should be directed toward PWV reduction rather than just BP control.

Measurements of PWV are easily obtainable and reproducible (7–10), and possibly could be included as a standard clinical measure in the management of diabetes.

Strengths and limitations
Patients included in the current study were recruited from a pool of approximately 3,500 patients with type 1 diabetes attending the outpatient clinic at Steno Diabetes Center. Thus, almost 20% of patients followed-up at Steno Diabetes Center were investigated, representing a broad segment of the Steno population, which covers an unselected population of adult type 1 diabetic patients in the region. However, to ensure a broad spectrum of albuminuria, we sought to include a substantial number of patients with impaired kidney function. Therefore, patients with microalbuminuria and macroalbuminuria were overrepresented, and this may explain why the cohort, on average, had such a high PWV.

PWV measurements were performed by four different laboratory technicians. It would have been optimum if they had been performed by the same investigator. However, all four laboratory technicians were trained to obtain measurements with similar quality-control measures, and measurements were performed at a single center under uniform conditions.

All 24-h AMBPs were measured with a tonometric device (BPro). We have previously shown BP measurements obtained with tonometry by BPro to correlate well with measurements obtained with standard sphygmomanometric devices used at Steno Diabetes Center (28). Furthermore, because the arterial pulse wave changes morphology depending on site of recording and body position, the calculated BP will represent the brachial BP regardless of arm or body position. Moreover, others have validated it for 24-h ambulatory measurements (27). Furthermore, because PWV is measured by tonometry, BP measured by tonometry would only be favorable.

No restrictions to current medications were made, and thus some patients were using AHTs and others were using cardiovascular medication. Patients were not fasting before PWV measurements because of their diabetes.

CONCLUSIONS—In summary, in the present cohort of patients with type 1 diabetes, we found PWV to be independently associated with age, diabetes duration, gender, UAER, BP, and HR. Arterial stiffness was higher in patients as compared with controls, and in patients with increased kidney impairment and previous CVD, independently of covariates. Furthermore, arterial stiffness was increased in patients with 24-h AMBPs ≥140/90 mmHg, and increased with retinopathy and autonomic neuropathy. Thus, type 1 diabetes, kidney impairment, CVD, elevated BP, retinopathy, and autonomic neuropathy are all associated with increased arterial stiffness.

References
1. Willum-Hansen T, Staessen JA, Torp-Pedersen C, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 2006;113:664–670
2. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation 2006;113: 657–663
3. Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001;37:1236–1241
4. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 2002;106:2085–2090
5. Theilade S, Lajer M, Jorsal A, Tarnow L, Parving HH, Rossing P. Arterial stiffness and endothelial dysfunction independently and synergistically predict cardiovascular and renal outcome in patients with type 1 diabetes. Diabet Med 2012;29: 990–994
6. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55: 1318–1327
7. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension 1995;26:485–490
8. Laurent S, Cockcroft J, Van Bortel L, et al.; European Network for Non-invasive Investigation of Large Arteries. Expert

consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27:2588–2605

9. Wilkinson IB, Fuchs SA, Jansen IM, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 1998;16:2079–2084

10. Frimodt-Møller M, Nielsen AH, Kamper AL, Strandgaard S. Reproducibility of pulse-wave analysis and pulse-wave velocity determination in chronic kidney disease. Nephrol Dial Transplant 2008;23:594–600

11. Najjar SS, Scuteri A, Shetty V, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. J Am Coll Cardiol 2008;51:1377–1383

12. Muiesan ML, Salvetti M, Paini A, et al. Pulse wave velocity and cardiovascular risk stratification in a general population: the Vobarno study. J Hypertens 2010;28:1935–1943

13. Sehestedt T, Jeppesen J, Hansen TW, et al. Risk prediction is improved by adding markers of subclinical organ damage to SCORE. Eur Heart J 2010;31:883–891

14. Protogerou A, Blacher J, Stergiou GS, Achimastos A, Safar ME. Blood pressure response under chronic antihypertensive drug therapy: the role of aortic stiffness in the REASON (Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind) study. J Am Coll Cardiol 2009;53:445–451

15. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation 1999;99:2434–2439

16. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. Circulation 2001;103:987–992

17. van Elderen SG, Brandts A, Westenberg JJ, et al. Aortic stiffness is associated with cardiac function and cerebral small vessel disease in patients with type 1 diabetes mellitus: assessment by magnetic resonance imaging. Eur Radiol 2010;20:1132–1138

18. van Elderen SG, Westenberg JJ, Brandts A, et al. Increased aortic stiffness measured by MRI in patients with type 1 diabetes mellitus and relationship to renal function. AJR Am J Roentgenol 2011;196:697–701

19. Smith A, Karalliedde J, De Angelis L, Goldsmith D, Viberti G. Aortic pulse wave velocity and albuminuria in patients with type 2 diabetes. J Am Soc Nephrol 2005;16:1069–1075

20. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage. The Steno hypothesis. Diabetologia 1989;32:219–226

21. Mourad JJ, Pannier B, Blacher J, et al. Creatinine clearance, pulse wave velocity, carotid compliance and essential hypertension. Kidney Int 2001;59:1834–1841

22. Liatis S, Alexiadou K, Tsiakou A, Makrilakis K, Katsilambros N, Tentolouris N. Cardiac autonomic function correlates with arterial stiffness in the early stage of type 1 diabetes. Exp Diabetes Res. 2011:957901

23. Secrest AM, Marshall SL, Miller RG, Prince CT, Orchard TJ. Pulse wave analysis and cardiac autonomic neuropathy in type 1 diabetes: a report from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes Technol Ther 2011;13:1264–1268

24. Yun YW, Shin MH, Lee YH, Rhee JA, Choi JS. Arterial stiffness is associated with diabetic retinopathy in Korean type 2 diabetic patients. J Prev Med Pub Health 2011;44:260–266

25. Mancia G, De Backer G, Dominiczak A, et al.; Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2007;25:1105–1187

26. Levey AS, Coresh J, Greene T, et al.; Chronic Kidney Disease Epidemiology Collaboration. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 2006;145:247–254

27. Nair D, Tan SY, Gan HW, et al. The use of ambulatory tonometric radial arterial wave capture to measure ambulatory blood pressure: the validation of a novel wrist-bound device in adults. J Hum Hypertens 2008;22:220–222

28. Theilade S, Joergensen C, Persson F, Lajer M, Rossing P. Ambulatory tonometric blood pressure measurements in patients with diabetes. Diabetes Technol Ther 2012;14:453–456

29. Longo D, Toffanin G, Garbelotto R, Zaetta V, Businaro L, Palatini P. Performance of the UA-787 oscillometric blood pressure monitor according to the European Society of Hypertension protocol. Blood Press Monit 2003;8:91–95

30. O'Brien E, Asmar R, Beilin L, et al.; European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. J Hypertens 2003;21:821–848

31. Cuspidi C, Vaccarella A, Leonetti G, Sala C. Ambulatory blood pressure and diabetes: targeting nondipping. Curr Diabetes Rev 2010;6:111–115

32. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. Diabetes Care 2003;26:1553–1579

33. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation 2007;115:387–397

34. Risk M, Bril V, Broadbridge C, Cohen A. Heart rate variability measurement in diabetic neuropathy: review of methods. Diabetes Technol Ther 2001;3:63–76

35. Gordin D, Waden J, Forsblom C, et al. Arterial stiffness and vascular complications in patients with type 1 diabetes: The Finnish Diabetic Nephropathy (FinnDiane) Study. Ann Med 2012;44:196–204

36. Stehouwer CD, Henry RM, Dekker JM, Nijpels G, Heine RJ, Bouter LM. Microalbuminuria is associated with impaired brachial artery, flow-mediated vasodilation in elderly individuals without and with diabetes: further evidence for a link between microalbuminuria and endothelial dysfunction—the Hoorn Study. Kidney Int Suppl 2004;(Suppl. 92):S42–S44

37. London GM, Asmar RG, O'Rourke MF, Safar ME; REASON Project Investigators. Mechanism(s) of selective systolic blood pressure reduction after a low-dose combination of perindopril/indapamide in hypertensive subjects: comparison with atenolol. J Am Coll Cardiol 2004;43:92–99

38. Williams B, Lacy PS, Thom SM, et al.; CAFE Investigators; Anglo-Scandinavian Cardiac Outcomes Trial Investigators; CAFE Steering Committee and Writing Committee. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation 2006;113:1213–1225

39. Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. J Am Coll Cardiol 2011;57:1511–1522

40. Chue CD, Townend JN, Steeds RP, Ferro CJ. Arterial stiffness in chronic kidney disease: causes and consequences. Heart 2010;96:817–823