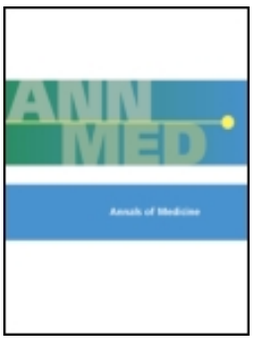

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ORIGINAL ARTICLE

Arterial stiffness and vascular complications in patients with type 1 diabetes: The Finnish Diabetic Nephropathy (FinnDiane) Study

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Abstract

Introduction/aims. While patients with type 1 diabetes (T1D) are known to suffer from early cardiovascular disease (CVD), we examined associations between arterial stiffness and diabetic complications in a large patient group with T1D.
Methods. This study included 807 subjects (622 T1D and 185 healthy volunteers (age 40.6 ± 0.7 versus 41.6 ± 1.2 years; P = NS)). Arterial stiffness was measured by pulse wave analysis from each participant. Furthermore, information on diabetic retinopathy, nephropathy, and CVD was collected. The renal status was verified from at least two out of three urine collections.
Results. Patients with T1D without signs of diabetic nephropathy had stiffer arteries measured as the augmentation index (AIx) than age-matched control subjects (17.3% ± 0.6% versus 10.0% ± 1.2%; $P < 0.001$). Moreover, AIx (OR 1.08; 95% CI 1.03–1.13; $P = 0.002$) was associated with diabetic laser-treated retinopathy in patients with normoalbuminuria in a multivariate logistic regression analysis. The same was true for AIx and diabetic nephropathy (1.04 (1.01–1.08); $P = 0.004$) as well as AIx and CVD (1.06 (1.00–1.12); $P = 0.01$) in patients with T1D.
Conclusions. Arterial stiffness was associated with microvascular and macrovascular complications in patients with T1D.

Key words: *Arterial stiffness, augmentation index, blood pressure, cardiovascular disease, diabetic nephropathy, diabetic retinopathy, pulse pressure*

Introduction

Patients with type 1 diabetes are at increased risk of cardiovascular disease, and diabetic nephropathy increases this risk markedly (1). Increased arterial stiffness has been shown to be an independent predictor of cardiovascular disease in selected patient groups (2). A few rather small-scale studies have reported stiffer arteries in patients with type 1 diabetes than in non-diabetic subjects (3,4). Interestingly, the stiffening of the arteries seems to be present before any clinically detectable signs of microvascular or macrovascular disease (5). Microalbuminuria, reflecting an early phase of diabetic nephropathy, has in patients with type 1 diabetes been associated with arterial stiffness, as estimated by ultrasonography (6). A recent paper by Laugesen et al. showed that ambulatory arterial stiffness index was increased in microalbuminuric

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Key messages

- Arterial stiffness in patients with type 1 diabetes without signs of diabetic nephropathy associated with laser-treated retinopathy.
- Arterial stiffness was present both in men and women with type 1 diabetes and diabetic nephropathy as well as cardiovascular disease.

Abbreviations

AHT	antihypertensive medication
AIx	augmentation index
CVD	cardiovascular disease
DBP	diastolic blood pressure
ESRD	end-stage renal disease
FinnDiane study	Finnish Diabetic Nephropathy Study
PP	pulse pressure
PWA	pulse wave analysis
SBP	systolic blood pressure
SEVR	subendocardial viability ratio
UAER	urinary albumin excretion rate
Tr	time to return of reflected wave
T1D	type 1 diabetes

patients with type 1 diabetes (7). Notably, data regarding diabetic retinopathy and arterial stiffness are scarce in patients with type 1 diabetes (8). In type 2 diabetes, stiffer arteries have been associated with both an impaired renal function and other microvascular complications (9,10).

Pulse pressure (PP), a crude estimate of arterial stiffness, is increased in patients with type 1 diabetes in the Finnish Diabetic Nephropathy (FinnDiane) Study (11). In the EURODIAB prospective complications study it was shown that pulse pressure was associated with diabetic complications and further that pulse pressure was associated with incident cardiovascular disease at follow-up (12). However, blood pressure measured in the peripheral circulation is not the best estimate of the central pressure because of the amplification of the pulse pressure between the central and the peripheral arteries (13). Pulse pressure does not provide accurate estimates of the arterial stiffness in young individuals (14). In contrast, pulse wave analysis (PWA) is a well described method using applanation tonometry in order to estimate arterial stiffness (15). Thus, arterial stiffness assessed by tonometry may give additional information of the process, especially in the young.

The present study explored the association between diabetic retinopathy, diabetic nephropathy, cardiovascular disease, and arterial stiffness in a large group of patients with type 1 diabetes.

Subjects and methods

Patients with type 1 diabetes were recruited from the FinnDiane Study, a nation-wide multicentre study with the aim to identify genetic and clinical risk factors for diabetic complications (16). All patients seen at the Helsinki University Central Hospital have undergone measurements of arterial stiffness by applanation tonometry since 2001. The study protocol was in accordance with the Declaration of Helsinki as revised in 2000 and approved by the local Ethics Committee. Written informed consent was obtained from each participant. Diagnostic criteria for type 1 diabetes included age at onset < 35 years and transition to insulin treatment within 1 year of onset.

The present study has a cross-sectional design. Complete data on arterial stiffness were available on 622 patients with type 1 diabetes, and 185 non-diabetic control subjects were included in this study. Based on their urinary albumin excretion rate (UAER) in two out of three consecutive overnight or 24-h urine collections, 578 patients could be classified as follows: 401 patients had a normal UAER (UAER $<$ 20 μg/min, or $<$ 30 mg/24 h), 67 patients had microalbuminuria (20 μg/min $\leq$ UAER $<$ 200 μg/min, or 30 mg/24 h $\leq$ UAER $<$ 300 mg/24 h), and 89 patients had macroalbuminuria (UAER $\geq$ 200 μg/min, or UAER $\geq$ 300 mg/24 h). Patients ($n = 21$) who were on renal replacement therapy (dialysis or kidney transplantation) were considered to have end-stage renal disease (ESRD). Patients with either microalbuminuria or macroalbuminuria were pooled to represent diabetic nephropathy in the analysis. Glycaemic control was assessed based on one A1C measurement and classified as good (A1C $<$ 7.5%), intermediate (7.5%–9.0%), or poor ($>$ 9.0%).

Data on medication, cardiovascular status, and diabetic complications were registered by a standardized questionnaire, which was completed by the patient's attending physician and thus verified from the medical files. Retinal laser treatment was used as an indication of diabetic retinopathy. Coronary heart disease was defined as diagnosed myocardial infarction or coronary revascularization. Stroke was defined as cerebral infarction or intracerebral haemorrhage. Peripheral vascular disease was defined as vascularization of a peripheral artery or limb amputation. Cardiovascular hard endpoints included diagnosed myocardial infarction, coronary revascularization, stroke, or peripheral vascular disease.

Arterial stiffness by applanation tonometry

Pulse wave analysis. Applanation tonometry (SphygmoCor; Atcor Medical, Sydney, Australia) is a widely used non-invasive method to estimate arterial stiffness by analysing arterial pressure waveforms (17). The pulse wave was recorded from the radial artery of the right arm with a high-fidelity micromanometer (SPT-301; Millar Instruments, Texas, USA). A model of the central pressure waveform was synthesized by the SphygmoCor software using a validated generalized mathematical transformation as previously described (18). The augmentation index (AIx) has been shown to be a reliable parameter of arterial stiffness (15). Measurements were performed three times for each patient, and the median was used in the analyses. The standard deviation for the intra-individual measurements were 2.7% ± 0.9% (mean ± SEM) for AIx and 0.7 ± 0.4 mmHg for aortic PP. AIx was adjusted for heart rate.

The availability of the central aortic waveform also allows the calculation of the subendocardial viability ratio (SEVR), an estimate of coronary perfusion (19). SEVR is calculated according to the formula: (diastolic time × pressure) / (systolic time × pressure).

The time to return of reflected wave (Tr) was calculated as the time from the beginning of the derived aortic systolic pressure waveform to the inflection point. Tr can be used as a substitute for pulse wave velocity (20).

Assays

Fasting blood samples were drawn and analysed for HbA_{1c}, creatinine, C-reactive protein (CRP), and serum lipids and lipoproteins. HbA_{1c} was determined by standardized assays (normal range 4.0%–6.0%), and serum lipid and lipoprotein concentrations were measured at the research laboratory of the Helsinki University Central Hospital by automated enzymatic methods using a Cobas Mira analyser (Hoffman-LaRoche, Basel, Switzerland). Serum creatinine was measured by routine methods and CRP by radioimmunoassay. Urinary albumin excretion rate was assessed from an overnight or a 24-h urine collection by immunoturbidimetry.

Statistical analyses

All analyses were performed with SPSS 15.0 (SPSS, Chicago, IL, USA). Base-line characteristics are presented as means ± SEM for normally distributed values and as median with interquartile range for non-normally distributed values, and percentages. For categorical variables the chi-square test was used when appropriate. Normally distributed variables were tested using ANOVA or Student's *t* test, and non-normally distributed with the Kruskal-Wallis or Mann-Whitney test. Correlation coefficients were calculated using the Pearson's or Spearman's tests when appropriate. Logistic regression analysis was used to adjust for confounding factors. P values < 0.05 were considered significant.

Results

The clinical characteristics of the subjects are described in Table I. Patients with normal UAER did not differ from the control subjects regarding gender, age, and height. They had a higher body mass index (BMI) and HbA_{1c}. No statistical difference was observed between the groups in brachial systolic blood pressure (SBP), although brachial (peripheral) PP was higher in the normoalbuminuric patients than in the non-diabetic control subjects. AIx as well as aortic (central) PP were higher, whereas Tr and SEVR were lower in the patients with type 1 diabetes than in the control subjects (Table II).

There was no difference in age between women and men within the patient group with type 1 diabetes (40.5 ± 1.0 versus 40.7 ± 1.0 years; P = NS) and the control subject group (43.2 ± 1.6 versus 39.7 ± 1.7 years; P = NS).

In the patients with normal UAER, AIx was positively associated with BMI ($r = 0.18$; $P < 0.001$), SBP ($r = 0.15$; $P < 0.01$), diastolic blood pressure (DBP) ($r = 0.13$; $P < 0.01$), age ($r = 0.35$; $P < 0.001$), duration of diabetes ($r = 0.46$; $P < 0.001$) and negatively with height ($r = –0.42$; $P < 0.001$). In the same group of patients aortic PP correlated positively with SBP ($r = 0.88$; $P < 0.001$), DBP ($r = 0.29$; $P < 0.001$), total cholesterol ($r = 0.12$; $P < 0.05$), age ($r = 0.43$; $P < 0.001$), duration of diabetes ($r = 0.51$; $P < 0.001$), and height ($r = 0.12$; $P < 0.05$). No other significant associations were found between variables in Table I and markers of arterial stiffness in normoalbuminuric patients with type 1 diabetes.

Patients with normal UAER and antihypertensive medication (AHT) had a higher AIx (21.1% ± 1.0% versus 15.2% ± 0.8%; $P < 0.01$) and aortic PP (51 ± 2 mmHg versus 40 ± 1 mmHg; $P < 0.01$) than those without antihypertensive medication, also after adjustments of gender, age, and height.

Arterial stiffness and diabetic nephropathy

As seen in Figure 1, clear differences in AIx could be observed in patients with type 1 diabetes and different stages of diabetic nephropathy. The association between AIx and diabetic nephropathy remained significant after adjustments for confounding factors

Table I. Clinical characteristics of patients with type 1 diabetes versus non-diabetic controls.

	Non-diabetic controls (n = 185)	Patients with type 1 diabetes			Overall P value
		Normoalbuminuria (n = 401)	Microalbuminuria (n = 67)	Macroalbuminuria (n = 89)	
Gender (% males)	43.8	46.7	41.8	52.9	0.45
Age (years)	41.6 ± 1.2	40.5 ± 0.9	43.8 ± 2.0	45.5 ± 1.5	<0.001
Duration (years)	–	24.7 ± 0.7	31.3 ± 1.6[c]	33.5 ± 1.1	<0.001
Height (cm)	172 ± 1	170 ± 1	166 ± 3	169 ± 2	0.68
BMI (kg/m^2)	24.2 ± 0.3	26.3 ± 9.7[b]	26.7 ± 1.3	25.8 ± 0.5	0.73
HbA_{1c} (%)	5.6 ± 0.0	8.2 ± 0.1[a]	8.5 ± 0.2	8.4 ± 0.2	0.61
Serum total cholesterol (mmol/L)	4.8 ± 0.1	4.5 ± 1.0[a]	4.7 ± 1.2	4.5 ± 1.0	0.74
Serum triglycerides (mmol/L)	0.9 (0.70–1.27)	0.8 (0.7–1.2)	1.3 (0.8–1.6)[d]	1.5 (1.0–1.8)	<0.001
Serum creatinine (μmol/L)	69 ± 2.2	81 ± 2[b]	88 ± 11	85 ± 4	0.38
Urinary albumin excretion rate (μg/min versus mg/24 h)	1 (0–3)	2 (1–6)	68 (20–82)	461 (40–490)	–
Serum CRP (mg/L)	2.3 ± 0.4	3.7 ± 0.4[a]	4.8 ± 1.3	5.5 ± 1.1	0.29
Brachial SBP (mmHg)	130 ± 1	133 ± 1	134 ± 3	150 ± 3[e]	<0.001
Brachial DBP (mmHg)	77 ± 1	73 ± 1[b]	73 ± 2	76 ± 2	0.60
Brachial PP (mmHg)	53 ± 1	60 ± 1[a]	66 ± 2[d]	74 ± 2[f]	<0.001
Current smoker (%)	13.8	14.7	27.4	20.7	0.10
Antihypertensive treatment (%)	9.3	33.4[a]	80.3	95.5	<0.001
CVD (%)	1.6	5.0[b]	16.4[d]	32.6	<0.001
Retinal laser treatment (%)	–	16.3[a]	56.7	83.1	–

Data are mean ± SEM, median (interquartile range), or %.
[a]$P < 0.001$; [b]$P < 0.05$ for normoalbuminuria compared to healthy controls.
[c]$P < 0.001$; [d]$P < 0.05$ for microalbuminuria compared to normoalbuminuria.
[e]$P < 0.001$; [f]$P < 0.05$ for macroalbuminuria compared to microalbuminuria.
BMI = body mass index; CRP = C-reactive protein; SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; CVD = cardiovascular disease.

in a logistic regression analysis (Table III). When brachial SBP was substituted with AHT in Model 3, AIx remained independently associated with diabetic nephropathy in the logistic regression analysis (1.04 (95% CI 1.01–1.07); $P = 0.008$).

Arterial stiffness and diabetic retinopathy

Patients with type 1 diabetes and normal UAER but with laser-treated diabetic retinopathy had higher AIx and aortic PP than patients without retinopathy (Figure 2). The association remained significant for the AIx but not for the aortic PP in a multivariate regression analysis (Table III). Brachial SBP was independently associated with diabetic retinopathy, while brachial PP was not.

Arterial stiffness and cardiovascular disease

Patients with cardiovascular disease (CVD) had higher AIx (27.1 ± 1.1 versus 16.2 ± 0.5 mmHg;

Table II. Indices of arterial stiffness in patients with type 1 diabetes versus non-diabetic controls.

	Non-diabetic controls	Patients with type 1 diabetes			Overall P value
		Normoalbuminuria	Microalbuminuria	Macroalbuminuria	
Aortic SBP (mmHg)	116 ± 1	121 ± 1[b]	124 ± 2	138 ± 3[e]	<0.001
Aortic DBP (mmHg)	78 ± 1	78 ± 0	78 ± 1	82 ± 1[f]	0.18
Aortic PP (mmHg)	39 ± 1	44 ± 1[b]	46 ± 3	57 ± 2[e]	0.001
AIx (%)	10.0 ± 1.2	17.3 ± 0.6[a]	20.7 ± 0.8[d]	25.9 ± 1.0[f]	<0.001
HR (bpm)	61 ± 1	68 ± 2[a]	72 ± 2	70 ± 1	0.92
Tr (ms)	154 ± 2	144 ± 1[a]	138 ± 2[d]	135 ± 1	<0.001
SEVR (%)	169 ± 2	148 ± 2[a]	128 ± 4[c]	125 ± 3	<0.001

Data are mean ± SEM.
[a]$P < 0.001$; [b]$P < 0.05$ for normoalbuminuria compared to healthy controls.
[c]$P < 0.001$; [d]$P < 0.05$ for microalbuminuria compared to normoalbuminuria.
[e]$P < 0.001$; [f]$P < 0.05$ for macroalbuminuria compared to microalbuminuria.
SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; AIx = augmentation index; HR = heart rate; Tr = time to return of reflected wave; SEVR = subendocardial viability ratio.

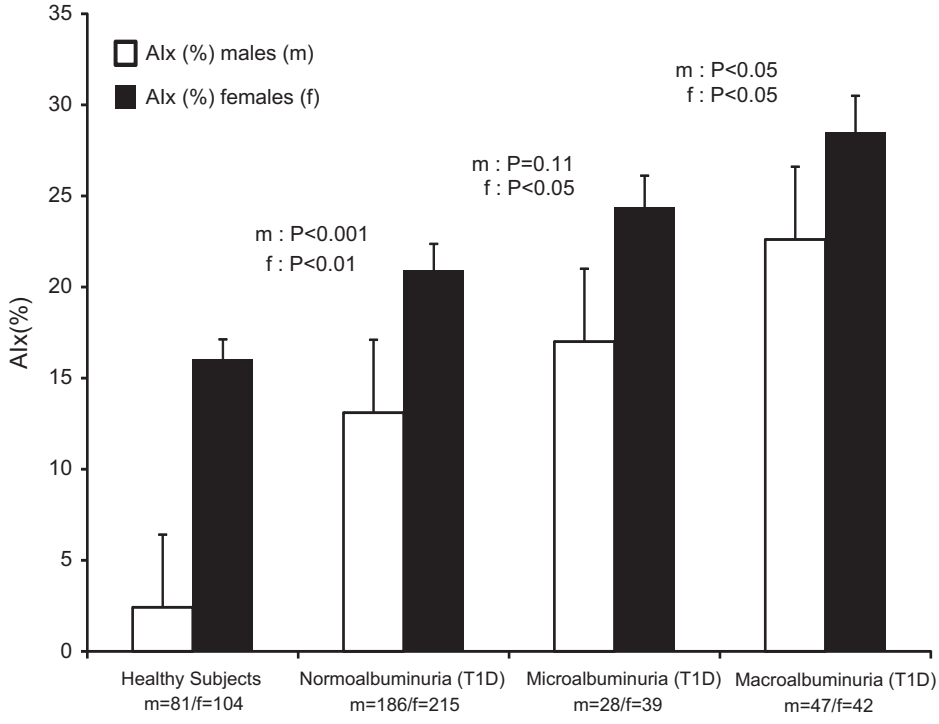

Figure 1. AIx by albuminuria status in patients with type 1 diabetes.

$P < 0.001$) and aortic PP (67 ± 2 versus 42 ± 1 mmHg; $P < 0.001$) than patients without CVD. The same was true for brachial PP and SBP (data not shown). The associations were independent of other known CVD risk factors in a logistic multivariate analysis (Table IV). Diabetic nephropathy was, however, strongly associated with CVD (OR (95% CI) 5.21 (2.48–10.93); $P < 0.001$) in this patient population. After substituting brachial SBP with AHT in Model 3 both AIx (1.07 (1.02–1.12); $P = 0.006$) and aortic PP (1.06 (1.04–1.09); $P < 0.001$) remained independently associated with CVD.

The effect of glycaemic control on arterial stiffness

An association between glycaemic control and arterial stiffness was observed in the macroalbuminuric patient group, where AIx was higher in patients with poor glycaemic control compared to patients with good glycaemic control (29.5% versus 23.9%; $P < 0.05$). No differences in AIx were observed in the

Table III. Multivariate logistic regression analysis of patients with type 1 diabetes with diabetic nephropathy and diabetic retinopathy (normoalbuminuric patients) as dependent variables.

	AIx OR (95% CI); *P* value	Aortic PP OR (95% CI); *P* value	Brachial PP OR (95% CI); *P* value
Diabetic nephropathy:			
Model 1	1.06 (1.04–1.09); $P < 0.001$	1.03 (1.02–1.05); $P < 0.001$	1.04 (1.02–1.05); $P < 0.001$
Model 2	1.04 (1.02–1.07); $P = 0.001$	0.98 (0.98–1.04); $P = 0.86$	0.99 (0.97–1.03); $P = 0.89$
Model 3	1.04 (1.01–1.08); $P = 0.004$	1.01 (0.98–1.04); $P = 0.69$	0.99 (0.97–1.03); $P = 0.97$
Model 4	1.03 (1.00–1.06); $P = 0.044$	0.98 (0.95–1.02); $P = 0.26$	0.99 (0.96–1.02); $P = 0.44$
Diabetic retinopathy:			
Model 1	1.10 (1.06–1.14); $P < 0.001$	1.05 (1.03–1.07); $P < 0.001$	1.04 (1.02–1.06); $P < 0.001$
Model 2	1.09 (1.05–1.14); $P < 0.001$	1.05 (1.01–1.10); $P = 0.008$	1.03 (0.99–1.07); $P = 0.14$
Model 3	1.08 (1.03–1.13); $P = 0.002$	1.03 (0.98–1.08); $P = 0.31$	1.03 (0.99–1.07); $P = 0.18$
Model 4	1.06 (1.01–1.12); $P = 0.025$	0.97 (0.91–1.03); $P = 0.35$	1.01 (0.97–1.07); $P = 0.57$

Model 1 adjusted for age, height, and gender.
Model 2 adjusted for age, height, gender, brachial SBP, and HbA_{1c}.
Model 3 adjusted for age, height, gender, brachial SBP, HbA_{1c}, triglycerides, and current smoking.
Model 4 adjusted for age, duration of diabetes, height, gender, brachial SBP, HbA_{1c}, triglycerides, presence of CVD, and current smoking.

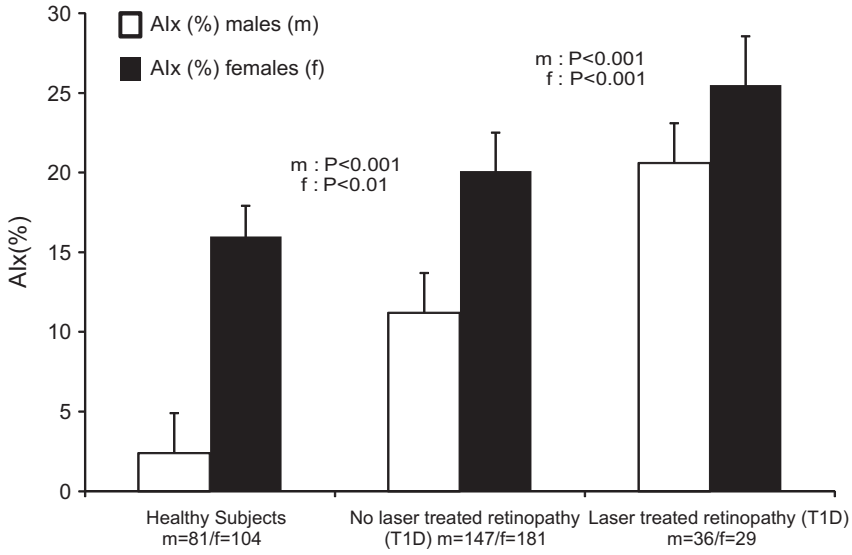

Figure 2. AIx in subjects with type 1 diabetes and normal urinary albumin excretion rate with and without laser treatment.

groups of patients with normo- or microalbuminuria due to glycaemic control (data not shown).

Discussion

The present study showed a novel independent association between diabetic retinopathy and arterial stiffness in patients with type 1 diabetes and normal UAER. Furthermore, a relationship between arterial stiffness and different stages of diabetic nephropathy could be observed for both genders. Finally, patients that had no signs of diabetic nephropathy disease had nevertheless stiffer arteries than the healthy control subjects. These findings imply that the measurement of arterial stiffness may be a useful tool to assess imminent vascular complications in patients with type 1 diabetes.

A positive relationship between diabetic retinopathy and arterial stiffness was recently observed in patients with type 2 diabetes (9,10), but Tryfonopoulos et al. (8) did not find any association between diabetic retinopathy and AIx in patients with type 1 diabetes. However, it is important to point out that the number of patients with type 1 diabetes was small (n = 31), and only women were studied. The present study showed that there was an independent association between arterial stiffness and diabetic retinopathy, although the patients with type 1 diabetes had no signs of diabetic nephropathy. This suggests that a generalized stiffening of the arteries may be involved in the pathogenesis of diabetic retinopathy independently of diabetic nephropathy disease.

In type 2 diabetes the relationship between arterial stiffness and diabetic nephropathy is rather well established (10). Studies in type 1 diabetes in turn have mostly focused on microalbuminuric patients (6,7). However, Prince et al. could in a very recent study not observe differences in AIx between UAER groups in patients with type 1 diabetes, which may be due to a lower number of patients in their study (21). In contrast, we observed that the arteries were stiffer the more severe diabetic nephropathy the

Table IV. Multivariate logistic regression analysis of patients with type 1 diabetes with cardiovascular disease as dependent variable.

	AIx OR (95% CI); P value	Aortic PP OR (95% CI); P value	Brachial PP OR (95% CI); P value
Model 1	1.10 (1.05–1.15); $P < 0.001$	1.06 (1.04–1.09); $P < 0.001$	1.05 (1.03–1.07); $P < 0.001$
Model 2	1.07 (1.02–1.11); $P = 0.006$	1.09 (1.05–1.14); $P < 0.001$	1.04 (1.00–1.08); $P = 0.04$
Model 3	1.06 (1.00–1.12); $P = 0.01$	1.11 (1.06–1.16); $P < 0.001$	1.04 (1.00–1.09); $P = 0.045$
Model 4	1.04 (0.99–1.09); $P = 0.129$	1.07 (1.03–1.12); $P = 0.003$	1.02 (0.98–1.06); $P = 0.352$

Model 1 adjusted for duration of diabetes, height, and gender.
Model 2 adjusted for duration of diabetes, height, gender, brachial SBP, and HbA_{1c}.
Model 3 adjusted for duration of diabetes, height, gender, brachial SBP, HbA_{1c}, total cholesterol, current smoking, and diabetic nephropathy.
Model 4 adjusted for age, duration of diabetes, height, gender, brachial SBP, HbA_{1c}, total cholesterol, current smoking, and diabetic nephropathy.

patients had. It is of note that inclusion of a larger number of patients with various degrees of diabetic nephropathy allowed us to assess arterial stiffness over a wide range of diabetic nephropathy disease.

Several studies have documented that women have higher AIx than men in non-diabetic populations (22,23), and separate analyses of data in men and women may be appropriate (24). Consequently, it was reported that women but not men with type 1 diabetes have increased arterial stiffness compared to healthy subjects (25,26). In the present study, we observed a difference between normoalbuminuric patients with type 1 diabetes and age-matched healthy subjects in both genders. Therefore it cannot be ruled out that the reason for the conflicting results may be due to the larger power of our study, although differences in for example methodology and age distribution may play a role. Furthermore, no differences in gender-specific associations between AIx and diabetic complications could be observed after testing for interaction between gender and AIx (data not shown). Thus, the associations between AIx and the vascular complications seem to be similar regardless of gender.

It is of note that sex-related differences exist in the response of the left ventricle to arterial loading conditions. Notably, women seem to develop more severe left ventricle hypertrophy than men despite a similar load of hypertension (27). Thus hypertension may be a more important risk factor for the development of heart failure in women than in men (28). This may to some extent explain why women with type 1 diabetes carry an increased risk for cardiovascular disease compared to men, but the mechanisms are still largely unknown (29). Interestingly, Janner et al. recently reported separate reference values for the AIx measurements in non-diabetic men and women, and it may be that different reference values are also needed for men and women in patients with type 1 diabetes (30).

A few studies have investigated the role of chronic glucose exposure on arterial stiffness by comparing patients with type 1 diabetes and non-diabetic control subjects. Haller at al. observed an increased AIx in children with type 1 diabetes (31). In a sample of 35 young adult patients with type 1 diabetes and no signs of cardiovascular disease that were compared with 35 non-diabetic control subjects, Wilkinson et al. found AIx to be higher in the patients with type 1 diabetes (32). In this study HbA_{1c} correlated with arterial stiffness in macroalbuminuric patients with type 1 diabetes. These data suggest that diabetes per se is associated with stiffer arteries and this is probably due to the direct effects of glucose on the endothelium and the vasculature. We have previously shown that acute hyperglycaemia increases not only the AIx but also the brachial pulse wave velocity (PWV) in patients with uncomplicated type 1 diabetes (33).

A number of methods have been used to measure arterial stiffness as described by Parati et al. (34). It is therefore of note that aortic PWV, considered the current gold standard of arterial stiffness, was not measured in this study (35). However, McEniery et al. compared AIx and PWV in different age groups and showed that AIx increased steeply with age in younger individuals ($<$ 50 years), whereas aortic PWV did not, suggesting that AIx and PWV provide different information (14). The finding that aortic PWV determines the stiffness of the large elastic arteries whereas PWA estimates systemic arterial stiffness from the reflected waveforms from arterial branching points and resistance arteries (muscular arteries and the arterioles) is in line with the finding that changes in the central elastic arteries occur over a period of time, while changes in the muscular arteries and the arterioles occur earlier (36). Thus, PWA may be preferred in combination with central PP in the population studied here, since the average age was no more than 45 years. Importantly, central PP has been shown to be a better marker of cardiovascular disease than the peripheral PP. Furthermore, PWA cannot distinguish whether diabetes influences the stiffness of the peripheral or the central arteries. It is further of note that PWA is a reproducible method even between different observers due to its internal quality control (17).

As an estimate of PWV the Tr was calculated from the reflected waveform. We observed a relationship between Tr and the degree of diabetic nephropathy in patients with type 1 diabetes. However, as Tr is only a substitute of the PWV we cannot conclusively manifest the finding, although the association is rather likely. Furthermore, the SEVR as an estimate of the coronary perfusion was calculated. The results suggest that estimated myocardial perfusion is reduced in those with microalbuminuria compared to normoalbuminuria. The findings are in line with those by Prince et al. (21).

The AIx was more strongly related to diabetic retinopathy and diabetic nephropathy than aortic PP, while the opposite was observed for cardiovascular disease. One reason why AIx was associated with microvascular complications may be that AIx is particularly reflected by the changes in endothelial function and smooth muscle cell tone, while aortic PP reflects later occurring structural (atherosclerotic) changes (14). The structural changes have been suggested to be due to an increased collagen-elastin ratio and calcification of the arterial intima (37). These findings are also in line with the fact that microvascular complications often occur earlier than cardiovascular disease.

This study cannot provide the ultimate mechanisms behind the relationship between arterial stiffness and diabetic complications. However, one explanation may be overproduction of superoxide and oxidative stress as the consequence of hyperglycaemia (38). Direct evidence that links oxidative stress to arterial stiffness is, however, scarce. Another important player may be endothelial dysfunction and its interplay with the intimal smooth muscle cell layer. In a recent article, Münzel et al. reviewed the pathophysiology of endothelial dysfunction and provided a link to oxidative stress and other factors such as chronic inflammation (39). Markers of chronic inflammation have in turn been associated with arterial stiffness and cardiovascular disease (40,41). CRP was in the current study higher in normoalbuminuric patients with type 1 diabetes than in healthy subjects. Furthermore, recent data show associations between chronic inflammation, diabetic complications, and vascular calcification, factors probably present already at an early stage of diabetic complications (42).

This study has some limitations. The diagnosis of diabetic retinopathy was based on a history of laser therapy rather than on examination of fundus photographs. Therefore patients with macular oedema or proliferative retinopathy could not be separated from one another. However, the need to treat patients with laser therapy suggests that the patient has a severe form of diabetic retinopathy that in the majority of cases in this study was equal to proliferative retinopathy.

In conclusion, as a novel finding, an independent association was observed between arterial stiffness and laser-treated retinopathy in normoalbuminuric patients with type 1 diabetes. Furthermore, arterial stiffness was shown to be present both in men and women with type 1 diabetes and diabetic nephropathy.

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