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Prevalence and predictors of abnormal arterial function in statin-treated type 2 diabetes mellitus patients

*Sandra J. Hamilton, Gerard T. Chew, Timothy M.E. Davis, Gerald F. Watts**

School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia 6847, Australia

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ABSTRACT

Arterial dysfunction (AD) in type 2 diabetes mellitus (T2DM) predicts cardiovascular events. The objective was to investigate the prevalence and predictors of AD in statin-treated T2DM patients. We measured flow-mediated (FMD) and nitrate-mediated (NMD) brachial artery dilatation in 86 statin-treated T2DM patients. Patients were classified into 2 groups: normal arterial function (FMD ≥3.7% with NMD ≥11.9%) or AD (FMD <3.7% with or without NMD <11.9%). *Endothelial dysfunction without smooth muscle cell dysfunction* (ED) was defined as FMD less than 3.7% with NMD of at least 11.9%, and *endothelial dysfunction with smooth muscle cell dysfunction* (ED/SMD) was defined as FMD less than 3.7% with NMD less than 11.9%. Predictors of arterial function were investigated using linear and logistic regression methods. The prevalence of AD was 33.7% (23.2% with ED and 10.5% with ED/SMD). In multivariate linear regression, history of hypertension ($P < .01$), statin dose ($P < .05$), and estimated glomerular filtration rate (eGFR) ($P = .02$) were significant predictors of FMD. Sex ($P < .01$) and creatinine ($P = .03$) or eGFR ($P = .02$) predicted NMD. In multivariate logistic regression, the independent predictors of AD were history of hypertension (odds ratio [OR], 8.79; 95% confidence interval, 2.14-36.12; $P < .01$), age (OR, 1.08; 1.01-1.17; $P = .03$), and statin dose (OR, 0.33; 0.12-0.87; $P = .02$). A history of hypertension (OR, 8.99; 1.87-43.26; $P < .01$) was the sole independent predictor of ED; eGFR (OR, 0.01; 0.00-0.26; $P < .01$) independently predicted ED/SMD. Our data suggest that one third of statin-treated diabetic patients have residual AD, mainly due to ED alone. Earlier identification and treatment of hypertension and renal impairment may improve AD and further decrease cardiovascular risk in such patients.

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1. Introduction

Type 2 diabetes mellitus (T2DM) markedly increases the risk of all forms of cardiovascular disease (CVD) [1]. Arterial dysfunction (AD) reflects, in part, a decrease in the bioavailability of endothelial nitric oxide and an abnormal vascular smooth muscle cell (VSMC) response to nitric oxide [2,3]. Endothelial dysfunction occurs early in the pathogenesis of atherosclerosis and predicts CVD events [4-6]. Vascular smooth muscle cell dysfunction may also have prognostic significance in individuals at increased CVD risk [2,7,8]. Statins improve endothelial function by both lipid and nonlipid mechanisms, and they have beneficial direct effects on the VSMC [9,10]. However, in clinical trials, the unaddressed residual risk of

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* *Corresponding author*. Tel.: +61 8 92240245; fax: +61 8 92240246.
E-mail address: gerald.watts@uwa.edu.au (G.F. Watts).

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CVD events (the residual risk for cardiovascular events that persists in many patients despite current standards of care) remains high in statin-treated T2DM patients [11-14], suggesting that persistent AD may underlie residual CVD risk in this situation. The primary aim of this study was, therefore, to investigate the prevalence and predictors of abnormal arterial function in statin-treated T2DM patients and to further explore this in relation to endothelial dysfunction without smooth muscle cell dysfunction (ED) and endothelial dysfunction with smooth muscle cell dysfunction (ED/SMD).

2. Methods

2.1. Subjects

We obtained the data for these analyses from the pooled baseline measures of 86 statin-treated patients with T2DM defined by the American Diabetes Association criteria [15]. These patients were recruited, mostly from the community with a small number from Diabetes and Lipid Disorders Clinics, for 3 separate intervention studies [16-18]. The patients were aged 40 to 79 years and had been taking statin therapy at a stable dose for more than 6 weeks. The exclusion criteria, which have been described previously [17], included use of other lipid-regulating therapy, supine blood pressure (BP) greater than 150/90 mm Hg, hemoglobin A_{1c} (HbA_{1c}) greater than 9.0%, serum triglycerides of at least 4.5 mmol/L, serum creatinine greater than 150 μmol/L, and current tobacco use. Therefore, these patients are more representative of well-controlled T2DM patients in the primary care setting in that they have at least moderate control of CVD risk factors. The studies were approved by the Royal Perth Hospital Ethics Committee, all procedures were carried out in accordance with the Declaration of Helsinki, and all participants gave written informed consent.

2.2. Clinical measurements

Following an overnight fast, bloods were drawn for biochemical tests (see below) including serum lipids, glucose, creatinine, alanine transaminase, creatine kinase, and HbA_{1c}. Blood pressure and heart rate were measured using a Dinamap recorder (Model 1846SX, Critikon, Tampa, FL) at 2-minute intervals for 8 minutes. Weight, height, and waist and hip circumferences were recorded; and body mass index (BMI) and waist-hip ratio were calculated.

2.3. Brachial artery ultrasonography

Endothelial function was assessed by measuring flow-mediated dilatation (FMD) of the brachial artery using ultrasonography. The brachial artery was imaged using a 12-MHz transducer connected to an Acuson Aspen ultrasound system (Siemens Medical Solutions, Malvern, PA). After measuring baseline arterial diameter, a pneumatic tourniquet was placed around the forearm and rapidly inflated to 200 mm Hg for 5 minutes to induce local ischemia. During the period of reactive hyperemia following cuff release, scanning was continued for 4 minutes to assess FMD. Estimates of brachial artery blood flow rates at rest and after cuff deflation were derived from pulse wave Doppler flow velocities. Nitrate-mediated (endothelium-independent) vasodilation (NMD) was measured following sublingual administration of glyceryl trinitrate 400 μg as a measure of vascular smooth muscle function. All ultrasound scans were performed by the same skilled operator according to published guidelines, and the images were stored digitally for subsequent analysis using a semiautomated edge detection software system [19,20]. Responses to the vasodilatory stimuli were calculated as maximal percentage change in brachial artery diameter compared with baseline. All images were analyzed by 2 independent observers, with the mean result being reported. The analytical coefficients of variation for our computerized technique are 6.7% (intraobserver) and 14.7% (within-subject) [20]. All scans were of good quality and were therefore used in the analysis.

We used percentage FMD and NMD in our patients to define AD. To define endothelial and smooth muscle cell dysfunction, we have used FMD and NMD cut points that were less than the 25th percentile of age-matched healthy control subjects previously studied in our laboratory (FMD <3.7% and NMD <11.9%) [21]. Patients were classified into 2 groups: normal arterial function (FMD ≥3.7% with NMD ≥11.9%) or AD (FMD <3.7% with or without NMD <11.9%). Subgroup analysis of AD included ED (FMD <3.7% with NMD ≥11.9%) or ED/SMD (FMD <3.7% with NMD <11.9%).

2.4. Laboratory methods

Serum cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were determined by standard enzymatic methods (Hitachi, Tokyo, Japan; Roche Diagnostics, Indianapolis, IN); and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. *Non–HDL cholesterol* was defined as total cholesterol minus HDL cholesterol. Hemoglobin A_{1c} was measured using a high-performance liquid chromatography analyzer (Bio-Rad VARIANT II; Bio-Rad Laboratories, Hercules, CA). Serum glucose, creatinine, alanine transaminase, and creatine kinase were measured using Roche reagents (Roche Diagnostics) and a Hitachi 917 analyzer. The majority of our patients (98%) had an estimated glomerular filtration rate (eGFR) of at least 50 mL/(min 1.73^2). Because of this, we calculated eGFR using the Chronic Kidney Disease Epidemiology Collaboration equations, as these are likely to yield reliable results for eGFR of at least 50 mL/(min 1.73^2) [22].

2.5. Statistical analyses

Data were analyzed using SPSS 15.0 (Chicago, IL) with logarithmic transformation of skewed data where appropriate. Values are reported as mean ± standard deviation (SD) or median (interquartile range). Group differences were compared using independent t tests for continuous variables and the χ^2 test for categorical variables. Multivariate linear and logistic regression analyses, using a manual backward stepwise model, included all variables identified in the bivariate analysis with a P value < .2. Interaction terms were investigated for those variables remaining in the logistic regression

model. To examine serum creatinine and eGFR, 2 linear and 2 logistic regression models were used. Variations in statin use (agent and dose) were accounted for by standardization to an atorvastatin equivalent dose [23,24].

3. Results

3.1. Clinical and biochemical characteristics of the patients

The statin-treated T2DM patients were typically middle-aged (64 ± 8 years [mean ± SD]) and overweight (BMI, 29.5 ± 5.0 kg/m^2) with satisfactory control of serum lipids (LDL cholesterol, 1.9 ± 0.5 mmol/L), BP (systolic, 129 ± 14 mm Hg; diastolic, 71 ± 7 mm Hg), and glycemia (HbA_{1c}, 7.1% ± 1.4% [median and interquartile range]) (Table 1). Median duration of diabetes was 7.5 years (range, 1-30 years), and most (78%) were using hypoglycemic medications: 72% metformin, 41% a sulfonylurea, 10% a thiazolidinedione, and 7.0% nighttime-only insulin combined with oral agents. There were no significant between-group differences in hypoglycemic medication use. Two thirds had a history of hypertension and were using antihypertensive medications: 43% an angiotensin-converting enzyme inhibitor, 24% an angiotensin receptor blocker, 19% a calcium channel blocker, and 16% a β-blocker. When

Table 1 – Clinical and biochemical characteristics of the patients according to the presence and type of arterial function

	All	Normal arterial function	AD	ED alone	ED/SMD
Characteristic					
Male/female (n)	50/36	31/26	19/10	11/9	8/1
Age (y)	64 ± 8	62 ± 9	68 ± 6**	67 ± 6**	69 ± 8
Systolic BP (mm Hg)	129 ± 14	127 ± 15	132 ± 11	134 ± 9*	130 ± 14
Diastolic BP (mm Hg)	71 ± 7	70 ± 7	71 ± 8	72 ± 9	70 ± 6
Pulse pressure (mm Hg)	58 ± 13	57 ± 12	61 ± 14	62 ± 10	60 ± 18
Heart rate (beat/min	63 ± 9	64 ± 8	63 ± 10	64 ± 9	60 ± 12
BMI (kg/m^2)	29.5 ± 5.0	29.7 ± 5.3	29.1 ± 4.2	28.7 ± 4.4	30.1 ± 3.7
Waist circumference (cm)	102 ± 12	102 ± 12	103 ± 13	101 ± 14	107 ± 11
Clinical history					
Duration of diabetes (y)	8.2 ± 5.3	8.1 ± 4.5	8.5 ± 6.6	9.9 ± 6.8	5.5 ± 5.4
Hypertension (%)	64.0	50.9	89.7**	90.0**	88.9
Duration of hypertension (y)	5.0 ± 6.5	3.9 ± 6.4	7.1 ± 6.1*	6.8 ± 6.2	7.9 ± 6.1
CVD (%)	39.0	35.1	48.3	45.0	55.6
Microvascular disease (%)	23.0	17.5	34.5	35.0	33.3
Microalbuminuria	12.0	8.8	22.7	18.8	33.3
Retinopathy	10.0	7.0	17.2	25.0	0.0
Neuropathy	5.0	1.8	10.3	10.0	11.1
Achieved LDL cholesterol <2.5 mmol/L (%)	87.0	86.0	90.0	95.0	78.0
Medications					
Stain dose (atorvastatin equivalent) (mg/d)	20 (30)	20 (20)	20 (20)	20 (10)	20 (30)
Antihypertensive use (%)	70.0	58.0	93.0**	95.0**	88.9
Hypoglycemic use (%)	78.0	75.0	79.0	90.0	55.6
Aspirin (%)	56.0	51.0	65.0	70.0	55.6
Biochemistry					
Glucose (mmol/L)	7.2 ± 1.8	7.2 ± 2.1	7.2 ± 1.3	7.3 ± 1.3	6.8 ± 1.2
HbA_{1c} (%)	7.1 (1.4)	7.1 (1.4)	7.1 (0.9)	7.3 (0.8)	6.5 ± 0.7
Serum creatinine (μmol/L)	77 ± 19	74 ± 18	83 ± 20*	77 ± 15	96 ± 23**
eGFR (mL/[min 1.73^2])	88 (22)	89 (20)	76 (24)**	76 (20)	68 (36)**
Urinary albumin-creatinine ratio (mg/mmol)	0.50 (1.25)	0.50 (1.0)	0.3 (2.65)	0.3 (2.05)	0.35 (10.58)
Cholesterol (mmol/L)	3.9 ± 0.7	3.9 ± 0.7	3.7 ± 0.7	3.8 ± 0.6	3.6 ± 0.9
Triglyceride (mmol/L)	1.4 (1.0)	1.5 (1.3)	1.3 (0.6)	1.1 (0.7)*	1.4 (0.4)
HDL cholesterol (mmol/L)	1.25 ± 0.29	1.25 ± 0.30	1.24 ± 0.28	1.32 ± 0.30	1.07 ± 0.13**
LDL cholesterol (mmol/L)	1.9 ± 0.5	1.9 ± 0.6	1.8 ± 0.4	1.9 ± 0.4	1.8 ± 0.5
Non–HDL cholesterol (mmol/L)	2.60 ± 0.66	2.66 ± 0.67	2.48 ± 0.62	2.46 ± 0.52	2.54 ± 0.85
Total cholesterol to HDL cholesterol ratio (mmol/L)	3.20 ± 0.72	3.25 ± 0.75	3.08 ± 0.64	2.95 ± 0.55	3.38 ± 0.75
Non–HDL to HDL cholesterol ratio (mmol/L)	2.19 ± 0.71	2.24 ± 0.74	2.08 ± 0.64	1.95 ± 0.55	2.38 ± 0.75
LDL to HDL cholesterol ratio (mmol/L)	1.54 ± 0.44	1.54 ± 0.46	1.54 ± 0.39	1.47 ± 0.34	1.69 ± 0.47
Brachial artery					
Baseline diameter (mm)	3.66 ± 0.61	3.55 ± 0.58	3.92 ± 0.65**	3.72 ± 0.62	4.35 ± 0.52***
FMD (%)	5.3 ± 2.9	6.8 ± 2.1	2.2 ± 1.2***	2.2 ± 1.2***	2.2 ± 1.3***
NMD (%)	18.7 ± 6.1	20.4 ± 5.7	15.3 ± 5.5***	18.2 ± 3.8	8.9 ± 2.4***

Values are means ± SD or median (interquartile range). *P* value represents comparison with normal arterial function group.
* $P < .05$.
** $P \leq .01$.
*** $P < .001$.

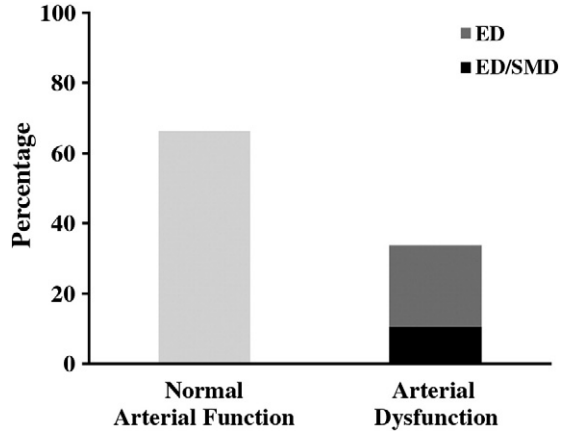

Fig. 1 – The prevalence of abnormal arterial function in statin-treated T2DM patients.

compared with normal arterial function, significantly more patients with ED were using an angiotensin-converting enzyme inhibitor (64% vs 31%, $P = .04$); and significantly more with ED/SMD were using an angiotensin receptor blocker (44% vs 16%, $P = .03$). There was a history of macrovascular disease (cerebrovascular, cardiovascular, or peripheral vascular) in 39% of patients, and 23% had a history of microvascular disease (microalbuminuria [albumin-creatinine ratio >3.0 mg/mmol], retinopathy, or sensory neuropathy). More than half (56%) of the patients were taking aspirin. All participants were treated with a statin, which in decreasing frequency of use was atorvastatin (58%), simvastatin (30%), pravastatin (6%), or rosuvastatin (6%). Median duration of statin use was 3.0 years (range, 1-18 years). There were no significant between-group differences in type or duration of statin use.

3.2. *Prevalence of AD*

Overall, 29 patients (33.7%) had abnormal arterial function, comprising 20 patients (23.2%) with ED and 9 patients (10.5%) with ED/SMD. The remaining 57 patients had normal arterial function (Fig. 1). Within the group of patients with AD, the prevalence of patients with ED (69%) was greater than that of patients with ED/SMD (31%).

Table 2 – Bivariate predictors of variation in arterial function in statin-treated T2DM patients using linear regression methods

	FMD			NMD		
	R^2	β	*P* value	R^2	β	*P* value
Age (y)	0.09	−0.30	<.01	0.06	−0.25	.02
Sex	0.004	−0.06	.55	0.06	−0.24	.03
Baseline brachial artery diameter (mm)	0.12	−0.35	<.01	0.25	−0.50	<.01
History of macrovascular disease	0.04	−0.20	.07	0.01	−0.09	.39
Aspirin use	0.04	−0.23	.06	0.02	−0.15	.18
Diabetes-related factors						
Duration of diabetes (y)	0.004	−0.06	.57	0.003	0.06	.60
Serum glucose (mmol/L)	0.01	0.11	.32	0.000	0.01	.93
HbA_{1c} (%)	0.003	0.05	.61	0.01	0.09	.39
Waist circumference (cm)	0.01	0.07	.52	0.02	−0.13	.24
Serum creatinine (μmol/L)	0.06	−0.25	.02	0.07	−0.26	.02
eGFR (log transformed) (mL/[min 1.73^2])	0.10	0.32	<.01	0.05	0.22	.046
Microalbuminuria	0.00	0.01	.97	0.05	−0.23	.07
Urinary albumin-creatinine ratio (mg/mmol)	0.01	−0.07	.63	0.04	−0.19	.19
Retinopathy	0.01	−0.10	.36	0.05	0.21	.05
Neuropathy	0.00	0.02	.88	0.00	−0.05	.67
Hypertension-related factors						
History of hypertension	0.18	−0.42	<.001	0.04	−0.20	.07
Duration of hypertension (y)	0.09	−0.30	<.01	0.03	−0.17	.11
Antihypertensive use	0.16	−0.40	<.001	0.03	−0.17	.12
Systolic BP (mm Hg)	0.06	−0.25	.02	0.02	−0.14	.19
Diastolic BP (mm Hg)	0.003	−0.06	.60	0.000	−0.01	.96
Pulse pressure (mm Hg)	0.06	−0.24	.02	0.02	−0.15	.17
Heart rate (beat/min)	0.03	0.16	.14	0.12	0.13	.22
Lipid-related factors						
Total cholesterol (mmol/L)	0.000	0.01	.96	0.02	0.13	.22
Triglycerides (log transformed) (mmol/L)	0.03	0.18	.10	0.001	0.03	.76
HDL cholesterol (mmol/L)	0.001	−0.03	.81	0.01	0.12	.27
LDL cholesterol (mmol/L)	0.01	−0.12	.26	0.01	0.10	.36
Non–HDL cholesterol (mmol/L)	0.000	0.02	.87	0.01	0.11	.32
Total cholesterol to HDL cholesterol ratio (mmol/L)	0.001	0.04	.72	0.000	−0.01	.97
Non–HDL to HDL cholesterol ratio (mmol/L)	0.002	0.04	.72	0.000	0.01	.94
LDL to HDL cholesterol ratio (mmol/L)	0.02	−0.13	.23	0.003	−0.06	.60
Statin dose (atorvastatin equivalent) (mg/d)	0.05	0.23	.03	0.01	0.12	.26

3.3. *Predictors of variation in AD using linear regression methods*

Using linear regression methods, the bivariate predictors of variation in FMD were age, baseline artery diameter, serum creatinine, eGFR, statin dose (atorvastatin equivalent), and several hypertension-related factors including history of hypertension, duration of hypertension, antihypertensive use, systolic BP, and pulse pressure. Bivariate predictors of variation in NMD included age, sex, baseline artery diameter, serum creatinine, and eGFR (Table 2).

We assessed 2 models of multivariate linear regression analysis (models 1 and 2), and both were adjusted for baseline artery diameter (Table 3). Independent predictors of variation in FMD were history of hypertension (β = –0.38, $P < .001$) and statin dose (0.19, $P = .048$) (model 1, $R^2 = 0.29$) and history of hypertension (–0.32, $P < .01$), statin dose (0.19, $P = .04$), and eGFR (0.22, $P = .02$) in model 2 ($R^2 = 0.34$). The independent predictors of variation in NMD were serum creatinine (–0.23, $P = .03$) and sex (0.51, $P < .01$) in model 1 ($R^2 = 0.35$) and eGFR (0.21, $P = .02$) and sex (0.41, $P \leq .01$) in model 2 ($R^2 = 0.35$). Glycemia (serum glucose, duration of diabetes, HbA_{1c}), microalbuminuria, and lipids did not predict FMD or NMD.

3.4. *Predictors of AD using logistic regression methods*

Using logistic regression, the bivariate predictors of AD were age, serum creatinine, eGFR, history and duration of hypertension, and antihypertensive medication use (Table 4). Bivariate predictors of ED were age, retinopathy, history of hypertension, and antihypertensive medication use. Bivariate predictors of ED/SMD were baseline artery diameter, serum creatinine, and eGFR (Table 4). Glycemia (serum glucose, duration of diabetes, HbA_{1c}), microalbuminuria, and lipids did not predict AD or its subgroups of ED or ED/SMD.

In multivariate logistic regression analysis, a history of hypertension (odds ratio [OR], 8.79; 95% confidence interval [CI], 2.14-36.12; $P < .01$), age (OR, 1.08; 1.01-1.17; $P = .03$), and statin dose (OR 0.33;0.12-0.87; $P = .02$) were independent predictors of AD (Table 5). A history of hypertension (OR, 8.68;(1.84-40.96; $P < .01$) was the sole predictor of ED. After adjustment for baseline artery diameter, serum creatinine (OR, 1.06; 1.01-1.12; $P = .02$) (model 1) and eGFR (OR, 0.01; 0.00-0.26; $P < .01$) (model 2) were the sole predictors of ED/SMD.

4. Discussion

We provide new data on the prevalence and predictors of AD in T2DM patients receiving anti-CVD therapy, in particular, statins, currently regarded as optimal. In the current study, the prevalence of AD was 33.7%, the prevalence being higher for ED (23.2%) than ED/SMD (10.5%).

4.1. *Predictors of AD*

We found that a history of hypertension predicted AD and ED. In hypertension, increased oxidative stress and release of endothelial-derived constricting factors result in AD [25]. Conversely, a consequence of endothelial dysfunction and increased vessel tone is hypertension, which in turn may further worsen endothelial function [25,26]. The coexistence of diabetes and hypertension has been shown to have an additive deleterious effect on endothelial function in forearm resistance arteries [26]. In that study, both diabetes and hypertension were shown to impair VSMC function; but there was no additive effect [29].

Chronic kidney disease, a common complication of diabetes, is associated with increased risk of CVD events and mortality in the general population and T2DM patients; reduced eGFR and albuminuria are independent predictors of CVD events and mortality [27,28]. Multiple mechanisms may contribute to the pathogenesis of CVD in patients with chronic kidney disease, including dyslipidemia, insulin resistance, inflammation, oxidative stress, hypertension, disturbed calcium/phosphate metabolism, and calcification of VSMCs [29-31].

Endothelial dysfunction and mild renal impairment are associated with, and have been shown to contribute to, cardiovascular mortality [32]. Our results suggest that hypertension and mild renal impairment predict AD, with hypertension predicting ED and renal impairment predicting a later progression towards VSMC dysfunction. Indeed, renal failure, aging, diabetes, hypertension, and osteoporosis are

Table 3 – Multivariate predictors of variation in arterial function in statin-treated T2DM patients using linear regression methods

	FMD			NMD		
	R^2	β	*P* value	R^2	β	*P* value
Model 1	0.29			0.35		
History of hypertension		–0.38	<.001			
Statin dose (atorvastatin equivalent) (mg/d)		0.19	.048			
Serum creatinine (μmol/L)					–0.23	.03
Sex					0.51	<.01
Model 2	0.34			0.35		
History of hypertension		–0.32	<.01			
Statin dose (atorvastatin equivalent) (mg/d)		0.19	.04			
eGFR (log transformed) (mL/[min 1.73^2])		0.22	.02		0.21	.02
Sex					0.41	<.01

Both models 1 and 2 are adjusted for baseline artery diameter (millimeters). Model 1 includes serum creatinine. Model 2 includes eGFR.

Table 4 – Bivariate predictors of abnormal arterial function in statin-treated T2DM patients using logistic regression methods

	AD			ED alone			ED/SMD		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Age (y)	1.09	1.02-1.16	<.01	1.08	1.01-1.16	.03	1.10	1.00-1.21	.06
Sex	1.59	0.63-4.02	.32	1.02	0.37-2.85	.96	6.71	0.79-57.21	.08
Baseline brachial artery diameter (mm)	2.72	1.25-5.95	.12	1.66	0.69-3.97	.26	13.12	2.38-72.30	<.01
History of macrovascular disease	1.73	0.70-4.29	.24	1.51	0.544-4.26	.43	2.31	0.56-9.59	.25
Aspirin use	1.83	0.73-4.63	.20	2.25	0.76-6.69	.14	1.21	0.29-4.96	.79
Diabetes-related factors									
Duration of diabetes (y)	1.02	0.93-1.10	.71	1.06	0.97-1.17	.19	0.86	0.71-1.05	.14
Serum glucose (mmol/L)	1.00	0.77-1.26	.92	1.03	0.79-1.35	.80	0.89	0.60-1.32	.56
HbA1c (%)	0.98	0.63-1.52	.92	1.23	0.75-2.02	.41	0.54	0.24-1.20	.13
Waist circumference (cm)	1.01	0.97-1.04	.70	1.00	0.96-1.04	.87	1.03	0.97-1.10	.28
Serum creatinine (μmol/L)	1.03	1.002-1.055	.03	1.01	0.98-1.04	.42	1.05	1.01-1.09	<.01
eGFR (log transformed) (mL/[min 1.73^2])	0.05	0.01-0.56	.02	0.09	0.01-1.48	.09	0.03	0.002-0.517	.02
Microalbuminuria	2.0	0.51-7.87	.32	1.57	0.33-7.52	.57	3.40	0.49-23.65	.22
Urinary albumin-creatinine ratio (mg/mmol)	1.22	0.79-1.89	.36	1.27	0.75-2.16	.37	1.21	0.62-2.36	.57
Retinopathy	2.76	0.68-11.20	.15	4.42	1.05-18.54	.04	–	–	–
Neuropathy	6.46	0.64-65.13	.11	6.22	0.53-72.72	.14	7.00	0.40-123.35	.18
Hypertension-related factors									
History of hypertension	8.37	2.27-30.80	<.01	8.69	1.84-40.96	<.01	7.72	0.91-65.83	.06
Duration of hypertension (y)	1.08	1.01-1.16	.04	1.06	.99-1.15	.09	1.08	0.98-1.18	.10
Antihypertensive use	9.82	2.13-45.32	<.01	13.82	1.73-110.44	.01	5.82	0.68-49.66	.11
Systolic BP (mm Hg)	1.03	0.99-1.07	.11	1.04	0.99-1.08	.08	1.01	0.96-1.06	.65
Diastolic BP (mm Hg)	1.01	0.95-1.08	.72	1.02	0.95-1.10	.53	0.98	0.88-1.09	.74
Pulse pressure (mm Hg)	1.03	0.99-1.07	.13	1.04	0.99-1.09	.11	1.02	0.96-1.08	.51
Heart rate (beat/min)	0.99	0.94-1.04	.65	1.01	0.95-1.07	.80	0.94	0.86-1.03	.20
Lipid-related factors									
Total cholesterol (mmol/L)	0.68	0.35-1.31	.25	0.75	0.35-1.58	.44	0.55	0.20-1.56	.26
Triglycerides (log transformed) (mmol/L)	0.43	0.16-1.13	.09	0.35	0.10-0.98	.05	0.83	0.20-3.42	.80
HDL cholesterol (mmol/L)	0.91	0.20-4.28	.91	2.20	0.40-12.85	.38	0.07	0.003-1.511	.09
LDL cholesterol (mmol/L)	0.87	0.37-2.04	.76	0.95	0.37-2.46	.92	0.76	0.22-2.63	.66
Non–HDL cholesterol (mmol/L)	0.65	0.31-1.33	.24	0.58	0.25-1.39	.22	0.76	0.27-2.20	.62
Total cholesterol to HDL cholesterol ratio (mmol/L)	0.71	0.37-1.36	.30	0.52	0.23-1.15	.11	1.26	0.50-3.17	.63
Non–HDL to HDL cholesterol ratio (mmol/L)	0.72	0.37-1.38	.32	0.52	0.23-1.16	.11	1.28	0.50-3.27	.60
LDL to HDL cholesterol ratio (mmol/L)	1.01	0.36-2.82	.98	0.70	0.21-2.31	.56	2.07	0.43-9.89	.36
Statin dose (atorvastatin equivalent) (mg/d)	0.45	0.20-1.04	.06	0.41	0.15-1.12	.08	0.49	0.14-1.69	.26

There were no subjects in the ED/SMD group with retinopathy.

all risk factors for calcification in the tunica media of the vessel wall [33]. Impaired brachial artery NMD has been associated with the presence and quantity of coronary artery calcium in adults without a prior cardiovascular history, but the mechanisms underlying this association were not clear [8]. It is possible that VSMC dysfunction could represent, among other processes (eg, oxidative stress), early calcification of the vessel media and that mild renal impairment may be an early marker of this disease process.

Endothelial and VSMC dysfunction as well as carotid intima media thickness are associated with albuminuria in

Table 5 – Independent predictors of abnormal arterial function in statin-treated T2DM patients using logistic regression methods

	AD			ED Alone			ED/SMD		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
	Models 1 and 2			Models 1 and 2			Model 1		
History of hypertension	8.79	2.14-36.12	<.01	8.69	1.84-40.96	<.01			
Age (y)	1.08	1.01-1.17	.03						
Statin dose (atorvastatin equivalent) (mg/d)	0.33	0.12-0.87	.02						
Serum creatinine (μmol/L)							1.06	1.01-1.12	.02
							Model 2		
eGFR (log transformed) (mL/[min 1.73^2])							0.01	0.00-0.26	<.01

Both models 1 and 2 are adjusted for baseline artery diameter (millimeters). Model 1 includes serum creatinine. Model 2 includes eGFR.

T2DM patients [34,35]. We did not find that microalbuminuria predicted AD or its subgroups ED and ED/SMD; but overall, the number of patients with microalbuminuria was small, and overall median urinary albumin-creatinine ratios were low (Table 1). Recent reports suggest that 20% to 25% of T2DM patients have a reduced eGFR (<60 mL/[min 1.73^2]) but that only half of these patients have albuminuria [36,37]. Therefore, in the current study, increased serum creatinine or reduced eGFR levels may have detected mild renal impairment before progression to microalbuminuria.

We have also demonstrated that statin dose was an inverse predictor of AD but serum lipoproteins were not, suggesting that this association is a direct effect of clinically relevant statin doses on endothelial and vascular smooth muscle cells. A recent study has demonstrated that treatment with rosuvastatin 10 mg/d or atorvastatin 40 mg/d inhibited Rho/Rho kinase pathway activity and that this inhibition was associated with improvement in brachial artery FMD but not reductions in LDL cholesterol [9].

4.2. *Study strengths and limitations*

The selection of this group of well-characterized statin-treated T2DM patients, most (87%) of whom were treated to a target LDL cholesterol of less than <2.5 mmol/L, may limit the generalizability of our findings. Owing to the cross-sectional design of our study, we are unable to establish a causal association between hypertension, renal impairment, and ED or ED/SMD; this will require longitudinal and intervention studies. We were unable to establish whether the patients' prior CVD status was the reason for their AD or, conversely, if AD was the basis for their CVD. However, a history of macrovascular disease did not predict FMD or NMD, or ED or ED/SMD. Our patients were treated with different statins at different doses, reflecting usual clinical practice; and this required standardization to an atorvastatin equivalent dose for our analysis. We report clinic-measured BPs (systolic and pulse pressure) as bivariate predictors of variation in FMD, but they were not independent predictors in multivariate regression analysis. This may reflect the fact that the reliability of clinic BP measurements is not ideal [38]. It is possible that our results may have differed had we measured 24-hour ambulatory BP.

4.3. *Clinical significance and implications*

A third of our statin-treated T2DM patients had residual AD and, by implication, a residual increased risk of CVD. Low-density lipoprotein cholesterol lowering with intensified statin therapy improves endothelial function and reduces cardiovascular events in patients with coronary artery disease, with or without T2DM [14,39]. In the current study, we have demonstrated statin dose to be an inverse predictor of AD, reinforcing the value of optimizing statin dose (±ezetimibe) to achieve aggressive LDL cholesterol lowering. Statins have been shown to improve cardiovascular outcomes in patients with chronic kidney disease, but the evidence for benefit on renal function is inconsistent [40,41]. Therefore, combination therapy with statins and renin-angiotensin-aldosterone system inhibitors may be required to lower BP, provide renal protection, and reduce overall cardiovascular risk.

Our results suggest that the early detection and treatment of hypertension and mild renal impairment in T2DM patients may prove beneficial to CVD outcomes by limiting the development of AD and its sequelae. Clinical trial evidence demonstrates that early BP control translates into improved CVD outcomes [42]. However, evidence supporting the benefit of aggressive BP lowering to below recommended target levels in T2DM patients is inconsistent [43-46]. Therefore, because of a lack of benefit in certain populations, intensification of antihypertensive therapy requires a tailored approach in each individual patient.

Therapies that inhibit the renin-angiotensin-aldosterone system not only lower BP but also improve endothelial function (both brachial artery and renal endothelium) and provide renal protection in T2DM patients [47-50]. However, recent evidence suggests that BP and albuminuria response to angiotensin receptor blockade is variable and discordant. Therefore, to achieve optimal cardiovascular benefit, monitoring of both BP and renal responses is required, with treatment adjustments as necessary [51].

5. Conclusion

Our data suggest that one third of statin-treated diabetic patients have residual AD, mainly due to ED alone. The predictors of residual AD were hypertension and mild renal impairment, suggesting that these should also be major targets of early and intensive therapy to further decrease risk of CVD.

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Conflicts of Interest

SJH and GTC have no relationships to industry to disclose. TMED has received honoraria for Advisory Boards, attendance at national and international meetings, lectures, or investigator-initiated research from Pfizer, GlaxoSmithKline, Novo Nordisk, Servier, Sanofi Aventis, Eli Lilly, Alphapharm, Bristol-Myers Squibb, Merck, Novartis, Solvay-Fournier, Roche, and Mepha Pharmaceuticals. GFW has received honoraria for Advisory Boards and lectures from Pfizer, Merck Sharp and Dohme, Solvay, AstraZeneca, GlaxoSmithKline, Sanofi-Aventis.

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