suPAR level is associated with myocardial impairment assessed with advanced echocardiography in patients with type 1 diabetes with normal ejection fraction and without known heart disease or end-stage renal disease

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Abstract

Aim: Heart disease is a common fatal diabetes-related complication. Early detection of patients at particular risk of heart disease is of prime importance. Soluble urokinase plasminogen activator receptor (suPAR) is a novel biomarker for development of cardiovascular disease. We investigate if suPAR is associated with early myocardial impairment assessed with advanced echocardiographic methods.

Methods: In an observational study on 318 patients with type 1 diabetes without known heart disease and with normal left ventricular ejection fraction (LVEF) (biplane LVEF >45%), we performed conventional, tissue Doppler and speckle tracking echocardiography, and measured plasma suPAR levels. Associations between myocardial function and suPAR levels were studied in adjusted models including significant covariates.

Results: Patients were 55 ± 12 years (mean $\pm$ s.d.) and 160 (50%) males. Median (interquartile range) suPAR was 3.4 (1.7) ng/mL and LVEF was $58 \pm 5\%$. suPAR levels were not associated with LVEF ($P=0.11$). In adjusted models, higher suPAR levels were independently associated with both impaired systolic function assessed with global longitudinal strain (GLS) and tissue velocity s' , and with impaired diastolic measures a' and e'/a' (all $P=0.034$). In multivariable analysis including cardiovascular risk factors and both systolic and diastolic measures (GLS and e'/a'), both remained independently associated with suPAR levels ($P=0.012$).

Conclusions: In patients with type 1 diabetes with normal LVEF and without known heart disease, suPAR is associated with early systolic and diastolic myocardial impairment. Our study implies that both suPAR and advanced echocardiography are useful diagnostic tools for identifying patients with diabetes at risk of future clinical heart disease, suited for intensified medical therapy.

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Introduction

Diabetes is associated with excess cardiovascular morbidity and mortality as well as increased healthcare expenses (1, 2, 3, 4). Moreover, subclinical heart disease is also prevalent in patients with diabetes (5, 6, 7).

Multifactorial treatment reduces risk of developing heart disease in diabetes (8). Detection of early cardiovascular disease (CVD) may improve identification of patients at particular risk who could benefit from preventive treatment, (8, 9) before manifestation of disease.

Newer echocardiographic modalities such as tissue Doppler imaging and speckle tracking echocardiography enable earlier diagnosis of subclinical cardiac impairment, not detected by conventional echocardiography (5, 10). However, echocardiography is not routinely performed in the outpatient assessment of complications in patients with diabetes. Hence, alternative diagnostic tools, which are able to detect subclinical heart disease, could enable early diagnosis of heart failure at a lower cost and without specialist requirements.

Soluble urokinase plasminogen activator receptor (suPAR) is an endothelial-inflammatory marker, previously found to be associated with cardiovascular morbidity and mortality in several populations including patients with diabetes (11, 12, 13, 14, 15).

We hypothesize that suPAR levels are associated with early myocardial impairment assessed with advanced echocardiography. A subgroup of patients from our previous study (Profil Study) (15) participated in an echocardiography study (Thousand & 1 Study). In the current study, we investigated if higher levels of suPAR is associated with subclinical impaired cardiac function assessed with tissue Doppler and speckle tracking echocardiography in patients with type 1 diabetes without known heart disease or end-stage renal disease and with normal left ventricular ejection fraction (LVEF).

Methods

Study population

The cohort consisted of patients who had entered both of two separate studies – The Thousand & 1 Study and the Profil Study – conducted at the Steno Diabetes Center in Denmark.

Both The Thousand & 1 Study and the Profil Study have previously been described in detail (5, 15, 16, 17). Briefly, for The Thousand & 1 Study, between 1 April 2010 and 1 April 2012, patients with type 1 diabetes attending the outpatient clinic at Steno Diabetes Center were invited

to attend the study. Patients were eligible to participate in the study if they were 18 years or older, diagnosed with type 1 diabetes, and without known heart disease. Known heart disease was defined as heart failure; coronary artery disease, including previous myocardial infarction, stable angina, previous percutaneous coronary intervention or coronary artery bypass surgery; atrial fibrillation or atrial flutter; left bundle branch block; congenital heart disease; pacemaker or intracardiac defibrillator implantation, all of which were exclusion criteria.

For the Profil Study, enrollment of patients went from September 2009 to June 2011, and included patients with type 1 diabetes, ≥ 18 years of age, and without end-stage renal disease. End-stage renal disease was defined as dialysis, renal transplantation, or glomerular filtration rate (GFR)/estimated GFR (eGFR) < 15 mL/min/1.73 m².

Hence, the current cohort included 370 patients with type 1 diabetes ≥ 18 years of age, without known heart disease and end-stage renal disease.

The studies were performed in accordance with the second Helsinki Declaration and approved by the regional ethics committee (H-3-2009-139 and PROFIL-H-B-2009-056).

Study visit

Before the echocardiographic examination, all patients received study information, signed the consent form, and filled out a questionnaire on personal lifestyle factors, including smoking and type of medication used. All patients received exogenous insulin. Blood pressure and ECG at rest was recorded in the supine position.

Echocardiography

Echocardiography was performed with a General Electric, Vivid 7 Dimension imaging system device (GE Vingmed Ultrasound AS, Horten, Norway) with a 3.5 MHz transducer in accordance with the recommendations from the European Association of Echocardiography/American Society of Echocardiography (18). Echocardiographic examinations were read and analyzed using General Electric, Vivid 7 Dimension imaging system device (GE Vingmed Ultrasound, Horten, Norway) with a 3.5 MHz transducer. Three consecutive heart cycles were recorded. LVEF was determined by Simpson's biplane method. Left atrial volume was determined by the recommended biplane area-length method, which is $(8/3\pi) \times (A1 \times A2/L)$, and indexed for body surface area. Left ventricular mass was determined by the linear method $((0.8 \times \{1.04[(LV \text{ diameter} + \text{posterior wall thickness} + \text{septal wall$

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thickness) $3-(LV\text{ diameter})^3]+0.6\text{ g}$) and indexed for body surface area. Pulsed-wave Doppler was performed in the apical 4-chamber view with the sample volume placed between the mitral leaflet tips to obtain diastolic mitral early inflow velocity (E). Pulsed-wave early diastolic tissue Doppler velocities (e') were determined by the apical 4-chamber view at the lateral region of the mitral annulus (19), and used to calculate E/e'_{lat} (20). Color Tissue Doppler Imaging velocities were obtained with the highest possible frame rate and analyzed off-line. Tissue velocities were sampled from the lateral mitral annulus. The parameter s' represents the highest longitudinal myocardial tissue contraction velocity during systole, and e' and a' represents the maximal LV early and atrial (late) myocardial tissue relaxation velocities during diastole. Global left ventricular longitudinal strain (GLS) was measured using 2D speckle tracking echocardiography, where deformation of the left ventricle is determined by tracking speckles from frame-to-frame. The method has previously been described in detail (5). GLS was determined as the average of the three apical views, thereby providing a global longitudinal strain measure for the entire left ventricle. Investigators were blinded to levels of suPAR.

Thus, e' , a' , e'/a' , and E/e' were considered diastolic measurements and LVEF, s' , while GLS was considered a systolic measurement.

Normal systolic function was defined as LVEF $>45\%$ (21, 22, 23).

Biochemistry

Levels of hemoglobin A_{1c} (HbA_{1c}) and p -creatinine, and information on albuminuric grade was collected from the electronic patient files at Steno Diabetes Center from the ambulatory visit closest to study inclusion, which was within 4 months of the inclusion date. This information was collected after the study visit and the echocardiographic analysis, ensuring that the investigators were blinded.

Urinary albumin excretion rate (UAER) was measured in 24-h sterile urine collections by enzyme immunoassay. Patients were categorized as normoalbuminuric if UAER, in two out of three consecutive measurements, was $<30\text{ mg/24 h}$, microalbuminuric if UAER was between 30 and 299 mg/24 h, and macroalbuminuric if UAER was $\geq 300\text{ mg/24 h}$. HbA_{1c} was measured by high-performance liquid chromatography (normal range: 21–46 mmol/mol (4.1–6.4%); Variant; Bio-Rad Laboratories) and creatinine concentration by an enzymatic method (Hitachi 912; Roche Diagnostics). Estimated GFR was calculated by the Modification of Diet in Renal Disease MDRD method (22).

At baseline, all participants had blood samples drawn and stored in EDTA plasma at -80°C for future analysis of biomarkers. The median (interquartile range) time interval between blood draw and echocardiographic measurements were 116 (117) days (11, 24). The suPAR levels were measured following one thawing cycle after 2.9 (2.0–3.8) years using a commercially available kit according to manufacturer's manual (suPARnostic kit (validated to measure suPAR levels between 0.6 and 22 ng/mL), ViroGates, Copenhagen, Denmark). Technicians had no access to the clinical patient database behind the plasma samples.

Statistical analysis

All analyses were performed with STATA 12.1 (Statacorp). Categorical variables were analyzed with the χ^2 test and continuous variables with analysis of variance. Continuous variables were reported as means $\pm$ s.d. A total of 1093 patients were included in the Thousand & 1 Study, 676 patients were included in the Profil Study, and 370 patients were included in both studies. Of these, 318 patients had LVEF $>45\%$, and suPAR measurements available and were therefore included in the current analysis.

Multivariable regression models, testing the association between suPAR and LVEF, GLS, s' , a' , e' , e'/a' , and E/e' were performed by including covariates from the baseline characteristics (Table 1) in a backward stepwise selection model with a significance level of 0.1. Variables that reached this significance level in any of the models were included in the final multivariable model. Variables included in the full model included sex, age, diabetes duration, HbA_{1c}, eGFR, use of statins, use of calcium channel blockers, and albuminuria grade; other characteristics from the baseline did not reach the 0.1 significance level in any of the models and were therefore not included in the full final table model. Correlations were analyzed using Spearman's ranked correlation (ρ). A P value of <0.05 was considered to be statistically significant.

Results

The cohort

Patients were 55 ± 12 years of age, 160 (50%) males and 51, 28, and 21% had normo-, micro-, and macroalbuminuria, respectively (Table 1).

As previously presented (15), higher suPAR levels were associated with higher age, diabetes duration, HbA_{1c}, systolic blood pressure, increasing albuminuria

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Table 1 Baseline characteristics and echocardiography measures according to quartiles of suPAR. Data are presented as *n*, (%), mean $\pm$ s.d. or median (IQR).

Variable	All	suPAR				P-value
		1st quartile (≤2.7 ng/mL)	2nd quartile (2.8–3.4 ng/mL)	3rd quartile (3.5–4.4 ng/mL)	4th quartile (>4.5 ng/mL)	
Baseline characteristics						
Number	318	87	75	80	76	
suPAR (ng/mL), median (IQR)	3.4 (1.7)	2.3 (0.5)	3.0 (0.4)	3.9 (0.5)	5.7 (1.6)	NA
Age (years)	55±12	47±12	54±11	61±10	58±11	<0.00
Men	160 (50%)	51 (59%)	41 (55%)	35 (44%)	33 (43%)	0.12
Duration of diabetes (years)	31±16	18±15	30±14	39±15	40±11	<0.001
Body mass index (kg/m²)	25.3±3.7	24.9±2.8	25.6±4.0	25.2±4.0	25.6±4.0	0.56
HbA _{1c} (mmol/mol)	65±12	62±14	64±11	64±11	69±12	0.002
Ever smoker	183 (58%)	40 (46%)	42 (56%)	54 (68%)	47 (62%)	0.034
Systolic blood pressure (mmHg)	135±17	127±14	136±15	138±18	138±17	<0.001
Diastolic blood pressure (mmHg)	72±10	74±9	73±8	69±8	71±12	0.006
eGFR (mL/min/1.73 m²)	82±24	94±17	90±18	78±20	59±22	<0.001
Total P-cholesterol (mmol/L)	4.7±0.9	4.7±0.9	4.7±(0.7)	4.7 (0.8)	4.8±1.2	0.93
Statins	178 (56%)	40 (46%)	35 (47%)	50 (63%)	53 (70%)	0.004
Albuminuria						
Normoalbuminuria	163 (51%)	71 (82%)	44 (59%)	34 (43%)	14 (18%)	<0.001
Microalbuminuria	88 (28%)	14 (16%)	25 (33%)	29 (36%)	20 (26%)	<0.001
Macroalbuminuria	67 (21%)	2 (2%)	6 (8%)	17 (21%)	42 (55%)	<0.001
β-Blockers	25 (8%)	2 (2%)	1 (1%)	11 (14%)	11 (14%)	<0.001
ACE or ATII inhibitors	205 (65%)	35 (40%)	52 (69%)	59 (74%)	59 (78%)	<0.001
Calcium channel blockers	97 (31%)	10 (11%)	20 (27%)	30 (38%)	37 (49%)	<0.001
Diuretics	127 (40%)	14 (16%)	19 (25%)	45 (56%)	49 (64%)	<0.001
Echocardiography measures						
LVEF (biplane) (%)	58±5	57±5	57±5	59±5	58±5	0.079
LV internal diameter (cm)	4.4±0.5	4.6±0.5	4.4±0.5	4.2±0.5	4.2±0.5	<0.001
Septal wall (cm)	0.9±0.2	0.9±0.2	0.9±0.2	0.9±0.2	1.0±0.2	0.018
Posterior wall (cm)	1.0±0.2	1.0±0.2	1.0±0.1	1.0±0.1	1.0±0.2	0.39
LAVI (mL/m³)	30.1±6.6	29.7±5.9	30.0±6.1	29.3±6.1	31.3±8.1	0.27
LVMi (g/m³)	73.4±15	76.1±17	74.6±13	69.4±16	73.3±14	0.036
E velocity (m/s)	0.9±0.2	0.8±0.2	0.8±0.2	0.9±0.2	0.9±0.2	0.13
A velocity (m/s)	0.8±0.2	0.7±0.2	0.8±0.2	0.9±0.2	0.9±0.2	<0.001
E/A ratio	1.2±0.4	1.4±0.5	1.2±0.4	1.0±0.3	1.0±0.3	<0.001
s' (cm/s)	6.4±1.7	6.9±1.6	6.3±1.5	6.2±1.9	6.1±1.5	0.008
a' (cm/s)	7.1±2.1	6.3±2.0	7.0±1.9	7.4±2.3	7.6±1.8	<0.001
e' (cm/s)	8.6±2.5	10.3±2.2	8.6±2.4	7.7±2.0	7.5±2.4	<0.001
e'/a'	1.4±1.3	2.0±2.2	1.4±0.7	1.1±0.5	1.1±0.5	<0.001
E/e'	8.4±3.1	6.4±1.6	8.1±2.8	9.3±3.3	10.0±3.4	<0.001
GLS (%)	−18.2±2.6	−18.8±2.3	−18.2±2.6	−17.9±2.6	−17.9±2.7	0.074

P-values are for unadjusted comparisons (analysis of variance or χ^2) between quartiles of suPAR. ACE, angiotensin-converting enzyme; ATII, angiotensin receptor; NA, not applicable.

grade, more smoking and more frequent use of statins, and antihypertensive therapy ($P=0.034$). Moreover, higher suPAR levels were associated with lower eGFR and diastolic blood pressure ($P=0.006$). suPAR levels were similar in men and women, and were not associated with body mass index or total cholesterol ($P \geq 0.12$) (Table 1).

suPAR and LVEF

In crude analyses, suPAR levels were not associated with LVEF (Table 1 and Fig. 1A). This was unchanged in multivariate adjusted models ($P=0.81$) (Table 2).

suPAR and GLS

As shown in Fig. 1A, there was a significant correlation between suPAR level and systolic function GLS. The association was analyzed in multivariable models, and as shown in Table 2, suPAR remained significantly associated with GLS ($P=0.002$).

suPAR and tissue Doppler imaging

suPAR levels were significantly correlated with all measures of diastolic function (a' , e' , e'/a' , and E/e') and with systolic s' ($P < 0.001$) (Fig. 1B, C, D, E and F). After

Figure 1

(A, B, C, D, E, F and G) suPAR and echocardiographic measures of cardiac function in patients with type 1 diabetes, normal EF, and without known heart disease. A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-15-0986>.

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Table 2 Association between suPAR level and measures of systolic and diastolic function, multivariable model.

	Coefficient	CIs		P-value
LVEF (%)	0.0007	−0.006	0.0049	0.81
s' (cm/s)	0.020	0.0015	0.038	0.034
a' (cm/s)	0.026	0.011	0.041	0.001
e' (cm/s)	−0.013	−0.028	0.0023	0.097
e'/a'	−0.032	−0.057	−0.008	0.009
E/e'	0.0082	−0.0029	0.019	0.146
GLS (%)	0.018	0.0065	0.030	0.002
LAVI (mL/m ²)	0.0034	−0.001	0.008	0.129
LVMI (g/m ²)	−0.0001	−0.002	0.002	0.95

Multivariate adjusted models including significant variables sex, age, diabetes duration, eGFR, albuminuric grade, HbA_{1c}, statin, and calcium channel blocker treatment.

multivariable adjustments, only diastolic tissue velocity a' and the index e'/a' remained significantly associated with suPAR.

In an additional analysis, we investigated if the relationship between suPAR and diastolic function was confounded by a relationship between suPAR and systolic impairment. In this model, we included the most significant measure of diastolic function, e'/a' , and the systolic measure GLS to the full multivariable model. Interestingly, we found that both the diastolic measure e'/a' and the systolic measure GLS were significantly and independently associated with higher suPAR levels (GLS: coefficient: 0.017% per increase in log(suPAR) (0.006; 0.29), $P=0.003$; e'/a' : coefficient: −0.031 (dimensionless) per increase in log(suPAR) (−0.05; 0.006), $P=0.012$).

suPAR and cardiac structure

Quartiles of suPAR were significantly associated with left ventricular mass index (LVMI), but not with left atrial volume index (LAVI). However, in the multivariable analyses neither remained significantly associated with suPAR (Tables 1 and 2).

Discussion

In the current study, we investigated associations between the biomarker suPAR and early echocardiographic measures of diastolic and systolic function in patients with type 1 diabetes with normal LVEF and without known heart disease or end-stage renal disease.

We demonstrate that suPAR levels were significantly associated with early systolic and diastolic myocardial impairment assessed using sensitive echocardiographic methods. Importantly, these associations

were independent of possible confounding factors. Interestingly, there was no association between suPAR levels and conventional echocardiography assessed with LVEF. Although this study is cross-sectional, these findings suggest that suPAR is a sensitive marker of early and discrete myocardial dysfunction detectable by advanced and sensitive echocardiographic measures.

Heart failure is a common complication in type 1 diabetes (25), as is subclinical CVD (7). In fact, heart disease is the most common cause of mortality and morbidity in patients with diabetes (2, 3, 4), indicating a need for earlier detection of and treatment for heart disease.

Previous studies have demonstrated a link between suPAR levels and CVD (11, 12, 13, 14, 15), including previous work from our group showing associations between suPAR and history of CVD, pulse wave velocity, and microvascular complications. Moreover, we and others have previously found that suPAR is a better marker for CVD compared with other markers of inflammation such as high-sensitivity C-reactive protein (13, 15, 26, 27). The reasoning for this may be that suPAR is a marker of vascular inflammation, while C-reactive protein elevations are related to metabolic inflammation (13).

GLS has been developed as an objective measure of systolic function for detection of discrete myocardial dysfunction. In prospective studies, GLS has been shown to be superior to ejection fraction (EF) in predicting mortality in patients with previous CVD or chronic kidney disease (28, 29). In terms of diastolic function, e'/a' ratio is known to be related to stiffness of the left ventricle, and to be impaired in insulin resistance (30) and diabetes (31). Diastolic dysfunction is an entity particularly relevant in patients with diabetes (32).

In this study, we show that suPAR is related to myocardial physiology, that is, systolic and diastolic functions. This study is, to the best of our knowledge, the first to investigate associations between suPAR levels and subclinical myocardial dysfunction, and given the relationship between suPAR and subclinical myocardial dysfunction, suPAR measurements may therefore be useful in the clinic for identifying patients at risk of developing heart disease. Interestingly, suPAR levels were independently associated with impaired GLS, but not with echocardiographic measures associated with cardiac remodeling (LVMI and LAVI). Hence, elevated suPAR levels are present at the very early stages of cardiac dysfunction, even before cardiac morphological changes occur.

suPAR could easily be measured in the clinical setting, and could potentially be used to risk stratify

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and perhaps monitor patients at risk. Recently, in patients with type 1 diabetes and diabetic nephropathy, we showed improved long-term preservation of kidney function and reduced overall mortality following introduction of multifactorial treatment (27). Also, in patients with type 2 diabetes and microvascular disease, intensified medical treatment improve outcome (8). Moreover, there appear to be a benefit of earlier intensified multifactorial treatment on heart disease and mortality in screen-detected type 2 patients with diabetes, who would be expected to have less or even no diabetic complications (9). Hence, even earlier multifactorial treatment may further improve long-term outcome in both type 1 and 2 patients with diabetes, although future studies would have to clarify this.

Today, multifactorial treatment includes improved glucose and blood pressure control, cholesterol lowering, antithrombotic, and anti-inflammatory therapies (33).

Anti-inflammatory therapies may prove to be particularly beneficial in diabetes in whom low-grade inflammation is increased (34), as low-grade vascular inflammation is associated with development of microvascular disease and subsequently CVD (35, 36, 37, 38) and diabetic cardiomyopathy (32). Hence, suPAR, which reflects low-grade vascular inflammation (27), may be useful for identifying patients suited for earlier or increased multifactorial treatment including anti-inflammatory treatments.

Our data show associations between vascular inflammation and myocardial impairment. Interestingly, the present association between suPAR and subclinical myocardial function may provide insights into mechanisms behind the observed myocardial impairment. The findings support the hypothesis that vascular inflammation, or very early vascular disease, is one mechanism behind the observed myocardial impairment (39). This is supported by previous findings verifying links between inflammation and CVD (36, 38) and our own previous findings showing associations between microvascular disease, known as CVD and suPAR in type 1 diabetes (15).

Hence, earlier multifactorial medical treatment for patients with higher suPAR levels may not only prevent classic diabetic complications such as CVD and kidney disease, but may also improve myocardial function. Moreover, multifactorial treatment in these patients could possibly include treatment for heart failure before development of clinical symptoms, and treatment effect could theoretically be monitored through changes in suPAR levels during treatment.

We believe that both suPAR and advanced echocardiography serve as useful diagnostic tools in diabetes for identifying patients at risk of future clinical heart disease, suited for intensified medical therapy, possibly including heart failure treatment. In particular, suPAR – being more convenient and cheaper – may even be useful for monitoring the treatment effect, although longitudinal intervention studies are required to address whether suPAR levels associate with clinical changes in end-organ disease.

Strengths and limitations

In this study, patients with known heart disease or with an LVEF $\leq 45\%$ were excluded. Information on previous heart disease was based on patient recollection and information retrieved from the electronic medical records. However, patients were not examined for subclinical coronary disease before entering the study. A functional test or further studies of possible CAD could have strengthened the findings further.

Echocardiographic examination and blood draw for suPAR measurements were performed at different occasions (median 116 days apart). Moreover, suPAR measurements were performed on frozen blood samples following one freezing–thawing cycle. However, suPAR levels were quite stable, both *in vitro* and *in vivo*. Studies show that suPAR seems to be more stable than other inflammatory biomarkers when kept at room temperature and following freezing–thawing cycles – even after long-term storage (11, 40). Moreover, suPAR levels appear to be stable during the day without circadian fluctuations (24).

Given the cross-sectional design of the study, we were unable to determine causality.

Major strengths are the sample size which makes it possible to determine relationships not previously reported, the cohort being monitored closely in the ambulatory clinic, and the advanced echocardiographic measurements being recorded by the same investigator. Major possible methodological biases are thereby avoided.

Conclusions

In patients with type 1 diabetes with normal EF and without known heart disease or end-stage renal disease, followed at the outpatient clinic at Steno Diabetes Center, suPAR levels are significantly and independently associated with early myocardial impairment assessed with tissue Doppler imaging and GLS by speckle tracking echocardiography.

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suPAR is a novel marker for early diastolic and systolic impairment in patients with type 1 diabetes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S T and M T J researched the data, wrote the manuscript, contributed to the discussion, and edited the manuscript. P R, J E-O, and J S J contributed to the discussion and reviewed the manuscript.

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