Original Article

Diabetes & Vascular Disease Research
May-June 2023: 1–14

DOI: 10.1177/14791641231183634
journals.sagepub.com/home/dvr
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Cardiometabolic risk factors, peripheral arterial tonometry and metformin in adults with type 1 diabetes participating in the REducing with MetfOrmin Vascular Adverse Lesions trial

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Abstract

Background: Peripheral arterial tonometry (PAT) provides non-invasive measures of vascular health. Beneficial effects of metformin on vascular function have been reported in youth with type 1 diabetes (T1D). In the REducing with MetfOrmin Vascular Adverse Lesions (REMOVAL) trial in adults with T1D and high cardiovascular risk, we examined: (i) the extent to which routinely-measured cardiometabolic risk factors explain variance in baseline PAT; and (ii) the effects of metformin on PAT measures.

Methods: Cross-sectional univariable and multivariable analyses of baseline reactive hyperaemia index (RHI) and augmentation index (AI) (EndoPAT® (Itamar, Israel); and analysis of 36-months metformin versus placebo on vascular tonometry.

Results: In 364 adults ((mean ± SD) age 55.2 ± 8.5 years, T1D 34.0 ± 10.6 years, HbA1c 64.5 ± 9.0 mmol/mol (8.1 ± 0.8%)), RHI was 2.26 ± 0.74 and AI was 15.9 ± 19.2%. In an exhaustive search, independent associates of (i) RHI were smoking, waist circumference, systolic blood pressure and vitamin B12 (adjusted R^2 = 0.11) and (ii) AI were male sex, pulse pressure, heart rate and waist circumference (adjusted R^2 = 0.31). Metformin did not significantly affect RHI or AI.

Conclusion: Cardiometabolic risk factors explained only a modest proportion of variance in PAT measures of vascular health in adults with T1D and high cardiovascular risk. PAT measures were not affected by metformin.

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Keywords
Augmentation index, reactive hyperaemia index, tonometry, type 1 diabetes, vascular function, metformin

Key messages

- In adults with T1D and high cardiovascular risk, non-invasive PAT measures of vascular health (RHI and AI) were associated with cardiometabolic risk factors, Vitamin B12 and diabetes complications
- However, these factors accounted for only a modest proportion of the variance in PAT measures
- Metformin did not affect PAT measures in the REMOVAL trial

Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in people with Type 1 diabetes (T1D).[1] Endothelial dysfunction and arterial stiffness are biomarkers that predict CVD[2,3] often adversely affected in people with T1D[4,5] and are also associated with microvascular complications.[6–8] Abnormal vascular function has been reported to develop soon after T1D diagnosis.[5,9] A range of non-invasive measures of vascular function are available that vary in cost, operator dependency and reproducibility.

The EndoPAT system (Itamar Medical, Caesarea, Israel) detects plethysmographic pressure changes in fingertips caused by the arterial pulse and translates this to peripheral arterial tone (PAT). This provides measurements of digital reactive hyperaemia index (RHI) following release of arterial occlusion (a marker of endothelial function) and augmentation index (AI) (an integrated marker of cardiovascular dysfunction related to arterial stiffness). With appropriate quality control and basic training, it can be used across sites by multiple operators and is hence suitable for assessing surrogate vascular outcomes in multicentre clinical trials. RHI by EndoPAT has been shown to correlate with flow mediated dilatation (FMD)[10–12] and to predict CVD in stable but high-risk individuals.[13,14] Some pharmaceutical agents and supplements including rosuvastatin,[15] ezetimibe,[16] ivabradine,[17] sildenafil[18] and L-arginine with B group vitamins[19] have been shown to improve RHI. However, the extent to which baseline values of these surrogate measures of vascular function are determined by simpler routinely-measured cardiometabolic risk factors (e g. blood pressure (BP), pulse pressure (PP), lipids) is uncertain, particularly in people with T1D. In addition, there are few data on the effects of adjunct metformin therapy on vascular tone, and specifically on RHI and AI in people with T1D, particularly adults.

REducing with MetfOrmin Vascular Adverse Lesions in type 1 diabetes (REMOVAL) was a multicentre, international placebo-controlled trial that randomised 428 adults with T1D at high cardiovascular risk to receive metformin or placebo for 36 months. Metformin did not significantly affect the primary outcome of mean carotid intima media thickness (cIMT),[20] but did reduce it in non-smokers.[21] RHI (a PAT secondary outcome measured in 85% of participants) was unchanged.[20] In a prior randomised controlled trial of 40 T1D subjects, metformin significantly improved RHI and FMD.[22] In the present analyses, we assessed: (i) the extent to which routinely-measured cardiometabolic risk factors explain baseline variance in PAT parameters; and (ii) the effect at 3-years of metformin versus placebo on previously-unreported PAT measures from the trial (including AI).

Methods

Study design and participants

REMOVAL trial participants were adults aged ≥40 years with ≥ 5-years T1D. The study protocol was approved by the West of Scotland Research Ethics Service and all site ethics committees. All participants provided written informed consent. The study design and major findings are published elsewhere.[20,21,23]

Vascular function

Vascular function was assessed using EndoPAT® (Itamar Medical, Caesarea, Israel) in 85% of study participants by trained personnel in 14 of 23 sites. This system measures PAT changes in the microvasculature of the fingertips pre-, during and post-brachial artery occlusion. As per manufacturer's recommendations and trial-specific standard operating procedures, subjects were acclimatised for 20 min in a quiet, dimmed 21–24°C room before testing. A BP cuff was applied to the non-dominant arm and plethysmographic probes were attached to the index fingers of both hands of the supine participant, with any jewelry, watches and heavy or tight-fitting clothing removed. EndoPAT output was observed until there was ≥1-min of adequate signal, then

after 5-min of baseline measurements, the BP cuff was rapidly inflated to 60 mmHg above SBP, or inflated further in 50 mmHg increments (up to 300 mmHg) until complete occlusion of the brachial artery was achieved as measured by the EndoPAT software. Following 5-min of measurements, the cuff was released and the post-occlusive response measured for 10-min.

EndoPAT measures were calculated by integrated software. All readings were individually quality-assured by the manufacturer (masked to treatment allocation) before entry on to the trial database. RHI, a measure of endothelial function (arbitrary units), was determined using the post-to-pre occlusion PAT signal ratio in the occluded arm, normalised to the control side and baseline vascular tone. Framingham RHI (fRHI), presented by the Framingham Heart study, was the natural log transformation of a similar index to RHI, but did not correct for baseline vascular tone and used shorter post-occlusion times (1.5- to 2-min). AI, an integrated marker of cardiovascular dysfunction reflecting arterial stiffness, was determined by analysis of baseline PAT signals. AI was also normalised to a heart rate (HR) of 75 beats per minute to calculate AI @75.

Kidney function

Estimated glomerular filtration rate (eGFR) was calculated from locally-measured serum creatinine using the Modification of Diet in Renal Disease (MDRD) Study equation.[24] Albuminuria was assessed using at least two separate urine specimens and routinely available assays.[23]

Statistical analysis

In this exploratory *post-hoc* analysis of the REMOVAL trial, baseline characteristics are presented for all participants with PAT data and categorised into tertiles based on our data distribution for: (a) baseline RHI; and (b) baseline AI. Comparisons were made across each of the ordered groups using Mantel-Haenszel ordered test for categorical variables, linear trend tests (via simple linear regression) for normally distributed continuous variables and the Jonckheere trend test for non-normally distributed continuous variables. Continuous baseline RHI and AI measures were also compared across various baseline patient characteristic sub-groups, using linear regression with a linear trend test and two-sample t-tests as required, dependent on the number of levels in the sub-group.

Relationships between baseline participant characteristics and baseline vascular function were assessed using univariable linear regression. All variables were included as candidates in an exhaustive search (branch-and-bound algorithm) and random forest (Boruta algorithm) procedures to select variables for inclusion in the final multivariable model. Bayesian Information Criterion (BIC) was used for the best model selection. The main effect of metformin versus placebo on PAT measures of vascular function at 36 months post-randomisation was determined using repeated measures ANCOVA for the change in PAT measure from baseline, adjusted for baseline measure and visit. The model was then extended to include an interaction between treatment and visit.

Statistical inferences were drawn with a 2-sided p-value of 0.05. Results are presented unadjusted for multiple comparisons. SAS (version 9.3; SAS Institute Inc., Cary, NC, USA), SPSS (SPSS Inc, Chicago IL) and R (version 4.0.5; R Core Team, Vienna, Austria) software were used.

Results

Subjects

Of 367 participants who underwent PAT studies at baseline, data were available from 364 (99.2%) after external quality assurance. Baseline characteristics are presented in Tables 1 and 2. In brief, participants had a mean age of 55.2 ± 8.5 years, 34.0 ± 10.6 years of diabetes and mean HbA1c of 64.5 ± 9.0 mmol/mol (8.1 ± 0.8%). Almost 60% were male and nearly all were of Caucasian ethnicity. As shown, traditional risk factors (BP, lipids) were adequately controlled with the majority of participants taking BP-lowering and lipid-lowering agents. Over one third were users of insulin pumps, which are associated with fewer complications and lower mortality.[25,26]

Statistical descriptors of baseline vascular function

Mean RHI and AI were 2.26 ± 0.74 AU and 15.9 ± 19.2% respectively. Sub-group analyses of statistical descriptors of baseline RHI and AI are shown (Figure 1). RHI was lower (adverse) in males, ex- and current smokers, participants with higher body mass index (BMI) and those on multiple daily injections (MDI) insulin therapy versus insulin pump users. AI was higher (adverse) in females, those with lower BMI, insulin pump users and those prescribed statin therapy.

Associations of baseline vascular function with baseline CVD and microvascular complications status

As shown in Figure 1, participants with existing CVD had higher AI by 41% (p = 0.04), than those without CVD. Subjects with prior MI or stroke had higher AI by 61% (p = 0.02) compared to those without a history of these conditions. However, there was no difference in RHI by prior CVD status.

Lower eGFR was associated with higher AI (trend $p < 0.001$) and worsening retinopathy was associated

Table 1. Baseline clinical and demographic characteristics according to baseline RHI tertiles.

		Reactive hyperaemia index (RHI)			Trend test *p* value
	Overall (*n* = 364)	Tertile 1 RHI <1.86 (*n* = 120)	Tertile 2 1.86 ≤ RHI <2.53 (*n* = 122)	Tertile 3 RHI ≥2.53 (*n* = 122)	
General characteristics					
Age (years)	55.2 ± 8.5	55.0 ± 8.8	54.2 ± 7.8	56.5 ± 8.8	0.09
Male	212 (58.2%)	79 (65.8%)	71 (58.2%)	62 (50.8%)	**0.02**
Caucasian	354 (97.3%)	118 (98.3%)	119 (97.5%)	117 (95.9)	0.25
Diabetes duration (years)	34.0 ± 10.6	33.7 ± 11.4	32.7 ± 9.7	35.6 ± 10.6	0.10
C-peptide (nmol/L)	33.0 (30.0–100.0)	33.0 (33.0–100.0)	51.0 (30.0–100.0)	33.0 (30.0–100.0)	0.75
HbA1c					
Absolute (mmol/mol)	64.5 ± 9.0	63.5 ± 9.3	65.8 ± 7.9	64.3 ± 9.7	0.13
% Units	8.05 ± 0.83	7.96 ± 0.85	8.17 ± 0.73	8.03 ± 0.89	—
Vitamin B12 (pmol/L)	335 (255–442)	309 (239–413)	347 (264–463)	362 (270–474)	**0.01**
BMI (kg/m2)	28.5 ± 4.4	28.9 ± 4.0	28.8 ± 4.4	27.8 ± 4.7	0.08
BMI categories	—	—	—	—	**0.03**
Normal	80 (21.7%)	16 (13.3%)	29 (23.8%)	35 (28.7%)	—
Overweight	167 (45.9%)	66 (55.0%)	48 (39.3%)	53 (43.4%)	—
Obese	116 (31.9%)	38 (31.7%)	45 (36.9%)	33 (27.0%)	—
Overweight and obese	283 (77.7%)	104 (86.7%)	93 (76.2%)	86 (70.5%)	—
Waist circumference (cm)	96.8 ± 12.0	99.2 ± 12.7	96.8 ± 10.8	94.5 ± 12.1	**0.01**
Elevated waist circumference[a]	181 (49.7%)	69 (57.5%)	60 (49.2%)	52 (42.6%)	**0.02**
Systolic blood pressure (mmHg)	128.7 ± 14.8	125.9 ± 13.5	129.0 ± 14.9	131.2 ± 15.4	**0.02**
Diastolic blood pressure (mmHg)	72.0 ± 10.1	71.0 ± 10.6	73.2 ± 9.3	71.7 ± 10.4	0.25
Pulse pressure (mmHg)	56.8 ± 13.2	54.9 ± 12.2	55.8 ± 13.7	59.5 ± 13.5	**0.02**
Mean arterial pressure (mmHg)	90.9 ± 10.1	89.3 ± 10.1	91.8 ± 9.5	91.5±10.6	0.10
Smoking history	—	—	—	—	**<0.001**
Current	46 (12.6%)	23 (19.2%)	14 (11.5%)	9 (7.4%)	—
Former	124 (34.1%)	45 (37.5%)	44 (36.1%)	35 (28.7%)	—
Never	194 (53.3%)	52 (43.3%)	64 (52.5%)	78 (63.9%)	—
Insulin dose (units/kg/day)	0.65 ± 0.28	0.71 ± 0.31	0.63 ± 0.24	0.61 ± 0.27	**0.02**
Insulin regimen	—	—	—	—	0.08
Twice daily	10 (2.9%)	5 (4.3%)	3 (2.6%)	2 (1.7%)	—
Basal bolus	205 (58.9%)	68 (58.1%)	79 (67.5%)	59 (50.9%)	—
Insulin pump	134 (38.3%)	44 (7.6%)	35 (29.9%)	55 (47.4%)	—
Estimated glucose disposal rate (eGDR)[b]	5.61 ± 1.69	5.35 ± 1.72	5.53 ± 1.47	5.95 ± 1.82	**0.03**
Clinical history					
Metabolic syndrome[c]	184 (50.5%)	63 (52.5%)	61 (50%)	60 (49.2%)	0.61
Existing CVD	44 (12.1%)	12 (10.0%)	16 (13.1%)	16 (13.1%)	0.46
MI or stroke	24 (6.6%)	4 (3.3%)	11 (9.0%)	9 (7.4%)	0.21
Retinopathy	—	—	—	—	0.05
None	29 (8.0%)	10 (8.3%)	7 (5.7%)	12 (9.8%)	—
Non-proliferative	232 (63.7%)	72 (60%)	81 (66.4%)	79 (64.8%)	—
Inactive proliferative	69 (19.0%)	21 (17.5%)	25 (20.5%)	23 (18.9%)	—
Active proliferative	31 (8.5%)	17 (14.2%)	8 (6.6%)	6 (4.9%)	—
History of impaired monofilament sensation	46 (14.6%)	13 (13.0%)	14 (13.1%)	19 (17.4%)	0.36
Renal function					
eGFR (mL/min/1.73 m^2)	93.3 ± 21.7	94.2 ± 23.3	94.6 ± 19.9	91.1 ± 22.0	0.38
Renal	—	—	—	—	0.71
Normal	220 (60.4%)	72 (60.0%)	77 (63.1%)	71 (58.2%)	—
Stage 1 CKD	—	—	—	—	—

(continued)

Table 1. (continued)

	Overall (n = 364)	Reactive hyperaemia index (RHI)			Trend test p value
		Tertile 1 RHI <1.86 (n = 120)	Tertile 2 1.86 ≤ RHI <2.53 (n = 122)	Tertile 3 RHI ≥2.53 (n = 122)	
Microalbuminuria	22 (6.0%)	4 (3.3%)	9 (7.4%)	9 (7.4%)	—
Macroalbuminuria	17 (4.7%)	5 (4.2%)	5 (4.1%)	7 (5.7%)	—
Stage 2 CKD	94 (25.8%)	33 (27.5%)	29 (23.8%)	32 (26.2%)	—
Stage 3a CKD	11 (3.0%)	6 (5.0%)	2 (1.6%)	3 (2.5%)	—
Lipids					
Total cholesterol (mmol/L)	3.99 ± 0.91	3.91 ± 0.81	4.05 ± 0.99	4.02 ± 0.93	0.43
HDL cholesterol (mmol/L)	1.63 ± 0.58	1.56 ± 0.52	1.62 ± 0.60	1.71 ± 0.61	0.12
LDL cholesterol (mmol/L)	2.21 ± 0.71	2.18 ± 0.64	2.27 ± 0.79	2.19 ± 0.71	0.50
Triglycerides (mmol/L)	1.02 ± 0.65	1.10 ± 0.68	0.98 ± 0.49	0.99 ± 0.76	0.29
Other					
Metformin allocation	183 (50.3%)	58 (48.3%)	63 (51.6%)	62 (50.8%)	0.87
Averaged mean cIMT (mm)	0.774 ± 0.148	0.776 ± 0.159	0.780 ± 0.144	0.767 ± 0.141	0.77
Averaged maximal cIMT (mm)	0.910 ± 0.174	0.920 ± 0.185	0.913 ± 0.167	0.895 ± 0.170	0.52
C-reactive protein (mg/L)	1.39 (0.62–3.06)	1.57 (0.65–3.10)	1.44 (0.76–2.52)	1.23 (0.51–3.34)	0.59
Drugs					
Antihypertensive drugs					
Any	271 (74.5%)	96 (80.0%)	83 (68.0%)	92 (75.4%)	0.42
ACE inhibitor	181 (49.7%)	73 (60.8%)	57 (46.7%)	51 (41.8%)	**0.003**
ARB	85 (23.4%)	25 (20.8%)	25 (20.5%)	35 (28.7%)	0.15
Calcium channel blocker	55 (15.1%)	17 (14.2%)	19 (15.6%)	19 (15.6%)	0.76
Beta blocker	36 (9.9%)	14 (11.7%)	7 (5.7%)	15 (12.3%)	0.85
Statins	300 (82.4%)	103 (85.8%)	98 (80.3%)	99 (81.1%)	0.34
Antiplatelet drugs					
Aspirin	128 (35.2%)	46 (38.3%)	45 (36.9%)	37 (30.3%)	0.21
Clopidogrel	13 (3.6%)	2 (1.7%)	7 (5.7%)	4 (3.3%)	0.50

Lower RHI corresponds with worse endothelial function (adverse).
Data are expressed as mean ± SD, n (%) or median (IQR).
Variables were compared across the three tertiles using the Mantel-Haenszel ordered test for categorical variables, linear regression for normally distributed continuous variables and Jonckheere trend test for non-normally distributed continuous variables.
Following variables had missing data (number of missing data in brackets): history of impaired monofilament sensation (48), diabetes duration (5), insulin dose (4), triglycerides (4), retinopathy (3), waist circumference (2), BMI (1), aspirin (1), beta blocker (1), clopidogrel (1).
Pearson chi square for metformin allocation.
[a]Defined as ≥88 cm in females and ≥102 cm in males.
[b]GDR = exp (4.64,725 – 0.02,032 (waist cm) – 0.09,779 (HbA1c %) – 0.00,235 (TG) mg/dl).
[c]Defined as having ≥2 of the following four factors (i) hypertension as SBP ≥130 mmHg, DBP ≥85 mmHg, or use of anti-hypertensive medications for any reason; (ii) waist ≥88 cm (females) or ≥102 cm (males); (iii) HDL cholesterol <50 mg/dl (females) or <40 mg/dl (males); (iv) fasting triglycerides ≥150 mg/dl.

with lower RHI. No other associations were detected between baseline RHI or AI and prior microvascular complications.

Associations with baseline RHI and AI

Of note, baseline RHI and AI correlated with each other (r = 0.27, p < 0.001). Table 3 shows their respective univariable and multivariable associates.

Significant univariable associates of RHI were male sex (negative), log (Vitamin B12), BMI (negative), waist circumference (WC) (negative), HR (negative), systolic BP, PP, smoking history (negative for both current and former smokers), insulin dose (negative), insulin pump use, active proliferative retinopathy (negative) and high-density lipoprotein cholesterol (HDL-C) (all p < 0.05).

Significant univariable associates of AI (adverse) were age, male sex (negative), diabetes duration, eGFR (negative), log (vitamin B12), BMI (negative), WC (negative), HR (negative), diastolic BP (DBP) (negative), PP, insulin dose (negative), insulin pump use, existing CVD, active proliferative retinopathy (negative), HDL cholesterol and beta blocker use (all p < 0.05).

Table 2. Baseline clinical and demographic characteristics according to baseline AI tertiles.

	Augmentation index (AI)			Trend test *p* value
	Tertile 1 AI ≤5.80 (*n* = 121)	Tertile 2 5.80 < AI ≤20.57) (*n* = 122)	Tertile 3 AI >20.57 (*n* = 121)	
General characteristics				
Age (years)	52.0 ± 7.5	55.7 ± 8.4	57.9 ± 8.5	**<0.001**
Male	94 (77.7%)	70 (57.3%)	48 (33.9%)	**<0.001**
Caucasian	118 (97.5%)	118 (96.7%)	118 (97.5%)	1.00
Diabetes duration (years)	31.6 ± 10.2	34.8 ± 10.8	35.6 ± 10.5	**0.009**
C-peptide (pmol/L)	33.0 (31.5–100.0)	33.0 (30.0–100.0)	55.0 (33.0–100.0)	0.41
HbA1c				
Absolute (mmol/mol)	63.3 ± 8.8	66.5 ± 8.8	63.8 ± 9.2	**0.01**
% Units	7.95 ± 0.80	8.23 ± 0.81	7.98 ± 0.84	—
Vitamin B12 (pmol/L)	329 (244–427)	344 (259–459)	335 (258–445)	0.36
BMI (kg/m^2)	29.3 ± 4.2	28.5 ± 4.3	27.8 ± 4.5	**0.02**
BMI categories	—	—	—	**<0.001**
Normal	13 (10.7%)	28 (23.0%)	38 (31.4%)	—
Overweight	61 (50.4%)	54 (44.3%)	52 (43.0%)	—
Obese	47 (38.8%)	39 (32.0%)	30 (24.8%)	—
Overweight and obese	108 (89.3%)	93 (76.2%)	82 (67.8%)	—
Waist circumference (cm)	100.4 ± 11.2	97.2 ± 12.3	92.9 ± 11.4	**<0.001**
Raised waist circumference	65 (53.7%)	60 (49.2%)	56 (46.3%)	0.25
Systolic blood pressure (mmHg)	125.8 ± 12.2	128.6 ± 15.1	131.8 ± 16.2	**0.005**
Diastolic blood pressure (mmHg)	75.0 ± 9.6	71.1 ± 9.7	69.7 ± 10.5	**<0.001**
Pulse pressure (mmHg)	50.7 ± 10.2	57.4 ± 12.3	62.1 ± 14.4	**<0.001**
Mean arterial pressure (mmHg)	91.9 ± 9.4	90.3 ± 10.2	90.4 ± 10.7	0.25
Smoking history	—	—	—	0.86
Current	15 (12.4%)	21 (17.2%)	10 (8.3%)	—
Former	33 (27.3%)	50 (41.0%)	41 (33.9%)	—
Never	73 (60.3%)	51 (41.8%)	70 (57.9%)	—
Insulin dose (units/kg/day)	0.72 ± 0.30	0.66 ± 0.28	0.57 ± 0.23	**<0.001**
Insulin regimen	—	—	—	**0.02**
Twice daily	4 (3.4%)	3 (2.6%)	3 (2.6%)	—
Basal bolus	79 (66.4%)	67 (58.3%)	60 (51.7%)	—
Insulin pump	36 (30.3%)	45 (39.1%)	53 (45.7%)	—
Estimated glucose disposal rate (eGDR)	5.20 ± 1.49	5.45 ± 1.52	6.17 ± 1.88	**<0.001**
Clinical history				
Metabolic syndrome[a]	62 (51.2%)	62 (50.8%)	60 (49.6%)	0.78
Existing CVD	8 (6.6%)	15 (12.3%)	21 (17.4%)	**0.010**
MI or stroke	5 (4.1%)	7 (5.7%)	12 (9.9%)	**0.070**
Nephropathy	5 (4.1%)	3 (2.5%)	9 (7.4%)	0.22
Retinopathy	—	—	—	0.46
None	8 (6.6%)	8 (6.6%)	13 (10.7%)	—
Non-proliferative	83 (68.6%)	78 (63.9%)	71 (58.7%)	—
Inactive proliferative	16 (13.2%)	23 (18.9%)	30 (24.8%)	—
Active proliferative	14 (11.6%)	12 (9.8%)	5 (4.1%)	—
Impaired monofilament sensation	9 (8.5%)	15 (14.3%)	22 (21.0%)	**0.010**
Renal function				
eGFR (mL/min/1.73 m^2)	97.1 ± 20.1	94.8 ± 22.8	88.1 ± 21.3	**0.003**
Renal	—	—	—	**<0.001**
Normal	80 (66.1%)	84 (68.9%)	56 (46.3%)	—
Stage 1 CKD	—	—	—	—

(continued)

Table 2. (continued)

	Augmentation index (AI)			Trend test *p* value
	Tertile 1 AI ≤5.80 (*n* = 121)	Tertile 2 5.80 < AI ≤20.57) (*n* = 122)	Tertile 3 AI >20.57 (*n* = 121)	
Microalbuminuria	9 (7.4%)	5 (4.1%)	8 (6.6%)	—
Macroalbuminuria	5 (4.1%)	3 (2.5%)	9 (7.4%)	—
Stage 2 CKD	26 (21.5%)	26 (21.3%)	42 (34.7%)	—
Stage 3 CKD	1 (0.8%)	4 (3.3%)	6 (5.0%)	—
Lipids				
Total cholesterol (mmol/L)	3.94 ± 0.86	3.94 ± 1.00	4.09 ± 0.88	0.34
HDL cholesterol (mmol/L)	1.51 ± 0.45	1.61 ± 0.59	1.78 ± 0.65	**0.001**
LDL cholesterol (mmol/L)	2.28 ± 0.74	2.17 ± 0.73	2.19 ± 0.67	0.49
Triglycerides (mmol/L)	1.06 ± 0.66	1.01 ± 0.53	1.00 ± 0.76	0.73
Other				
Metformin allocation	62 (51.2%)	62 (50.8%)	59 (48.8%)	0.92
Averaged mean cIMT (mm)	0.743 ± 0.141	0.791 ± 0.151	0.788 ± 0.148	**0.02**
Averaged maximal cIMT (mm)	0.884 ± 0.167	0.929 ± 0.179	0.915 ± 0.173	0.11
C-reactive protein (mg/L)	1.47 (0.67–2.88)	1.32 (0.60–2.84)	1.39 (0.55–3.24)	0.86
Drugs				
Antihypertensive drugs				
Any	95 (78.5%)	85 (69.7%)	91 (75.2%)	0.56
ACE inhibitor	69 (57.0%)	56 (45.9%)	56 (46.3%)	0.10
ARB	28 (23.1%)	26 (21.3%)	31 (25.6%)	0.65
Calcium channel blocker	17 (14.0%)	16 (13.1%)	22 (18.2%)	0.37
Beta blocker	6 (5.0%)	12 (9.8%)	18 (14.9%)	**0.010**
Statins	101 (83.5%)	95 (77.9%)	104 (86.0%)	0.61
Antiplatelet drugs				
Aspirin	41 (33.9%)	43 (35.2%)	44 (36.4%)	0.69
Clopidogrel	2 (1.7%)	4 (3.3%)	7 (5.8%)	0.08

Higher AI corresponds with increased arterial stiffness (adverse).
Data are expressed as mean ± SD, *n* (%) or median (IQR).
Variables were compared across the three tertiles using the Mantel-Haenszel ordered test for categorical variables, linear regression for normally distributed continuous variables and Jonckheere trend test for non-normally distributed continuous variables.
Pearson chi square for metformin allocation.
[a]Defined as having ≥2 of the following four factors (i) hypertension as SBP ≥130 mmHg, DBP ≥85 mmHg, or use of anti-hypertensive medications for any reason; (ii) waist ≥88 cm (females) or ≥102 cm (males); (iii) HDL-cholesterol <50 mg/dl (females) or <40 mg/dl (males); (iv) fasting triglycerides ≥150 mg/dl.
[b]Defined as ≥88 cm in females and ≥102 cm in males.
[c]GDR = exp (4.64,725 – 0.02,032 (waist cm) – 0.09,779 (HbA1c %) – 0.00,235 (TG) mg/dl).

In an exhaustive search (Table 3), independent associates of RHI were smoking (negative for both current and former smokers), WC (negative), systolic BP (SBP) and vitamin B12 (adjusted $R^2 = 0.11$).

Independent associates of AI were male sex (negative), PP, HR (negative) and WC (negative) (adjusted $R^2 = 0.31$).

Supplementary Tables 1 and 2 compare the independent statistical associates of RHI and AI selected using exhaustive search (best models selected based on lowest BIC, lowest Malow Cp and highest adjusted R^2 selection criterions), random forest classification (Boruta algorithm, for linear and logistic (RHI tertile 1 vs. RHI tertile 3) and stepwise regression. Overall, our exhaustive search model showed the lowest AIC and BIC values (Table 3). All approaches resulted in the selection of similar variables set, but some produced overfitted models.

Effects of metformin on vascular function

As previously reported for RHI,[21] treatment with metformin (vs. placebo) for 36 months had no significant effect on any previously-unreported PAT parameter (fRHI, AI and AI @75) (Table 4).

Discussion

In these novel data from the REMOVAL trial in adults with T1D at high CVD risk, we examined: (i) cross-sectional associations of PAT measures (RHI and AI)

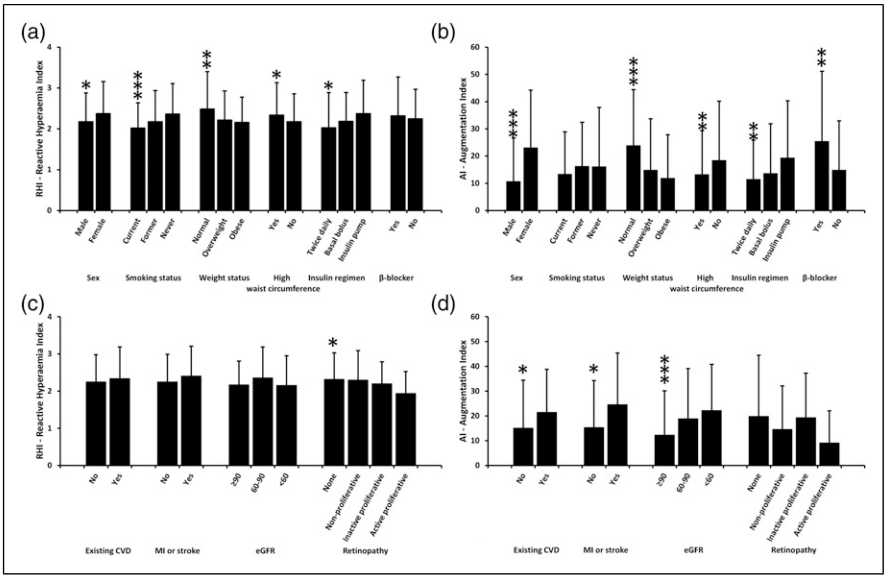

Figure 1. Baseline RHI (panel A) and AI (panel B) are reported in subgroups based on sex, body habitus, insulin regimen and β-blocker use. Baseline RHI (panel C) and AI (panel D) are also shown by prior chronic complications: Cardiovascular Disease (CVD), myocardial infarction (MI) or stroke, estimated Glomerular Filtration Rate (eGFR) and diabetic retinopathy status. Data are expressed as mean ± SD. Variables are compared across subgroups using two-sample t-test for binary variables and linear regression for ordered categorical variables. There was no significant difference in RHI or AI by metabolic syndrome status, prior microalbuminuria, neuropathy status, or statin, antihypertensive, ACE inhibitor, ARB, or calcium channel blocker use (data not shown). *p≤0.05, **p≤0.01, ***p≤0.001.

with other subject characteristics at baseline; and (ii) whether metformin treatment versus placebo affected PAT measures over 3 years of treatment. We found moderate associations of RHI and AI with several cardiometabolic risk factors and diabetes complications but these accounted for only a modest proportion of their variance. In addition, there was no significant effect of metformin (relative to placebo) on AI or any other previously-unreported PAT parameter measured in the trial following 36 months of treatment.

Numerous methods exist to measure endothelial function ranging from non-invasive procedures, such as FMD, to more invasive tests (e g. infusion of the coronary or peripheral circulation with endothelial-dependent vasodilators).[27] However, many of these techniques are costly, operator dependent or both. EndoPAT is an easy-to-use, non-invasive and minimally operator dependent device that reports RHI as a measure of endothelial function and AI as an integrated marker of cardiovascular function (dependent on cardiac and vascular structure and function but particularly reflecting arterial stiffness). In previous studies, RHI has been shown to correlate with coronary endothelial function[28] and brachial artery ultrasound,[10–12] and to be reproducible.[29,30] AI has been associated with ventricular-vascular coupling[31] and correlated with radial artery tonometry (SphygmoCor),[32] although there have been few studies to validate AI with other parameters, and none have been in people with T1D.

The correlates of vascular function we noted in people with T1D are mostly consistent with the existing literature in non-T1D groups. The Framingham study, a general population-based study, and one of the largest to examine RHI (using EndoPAT), also found that higher HR and BMI, lower SBP and HDL-cholesterol, and smoking were associated with abnormal endothelial function.[33] Moreover, in a recently published study of 1809 youth with T1D, increased diabetes duration, HbA1c, adiposity, BP and non-HDL were important correlates of AI and pulse wave velocity (PWV) as measured using SphygmoCor.[34] Our study in older adults did not find any association between vascular function and HbA1c, in contrast to previous studies of youth with T1D.[34,35] We found that AI was higher in females compared to males, which is consistent with findings from the Bogalusa Heart Study that used a different non-invasive device to derive AI.[36]

Our finding of a positive relationship between RHI and AI contrasts with previous studies reporting an inverse correlation between FMD and arterial stiffness (PWV).[37,38] This may reflect site of measurement as EndoPAT assesses tone in digital arteries whereas FMD and PWV focus on conduit arteries, usually radial and brachial. Another major difference between our study and those in other populations is that REMOVAL trial participants were treated with pharmacologic doses of subcutaneous insulin and almost all were treated with as antihypertensive and lipid-lowering agents.

Table 3. Relationship between baseline variables and baseline RHI and AI.

	Baseline RHI		Baseline AI	
	Univariable	Exhaustive search	Univariable	Exhaustive search
General characteristics				
Age (years)	0.01 (0.00, 0.02)	—	**0.53*** (0.32, 0.75)**	—
Male	**−0.19* (-0.34, -0.03)**	—	**−11.45*** (-15.07, -7.84)**	**−11.09*** (-14.65, -7.52)**
Caucasian	0.01 (−0.46, 0.48)	—	−0.49 (−11.90, 10.92)	—
Diabetes duration (years)	<0.01 (0.00, 0.01)	—	**0.21* (0.03, 0.39)**	—
log (C-peptide (pmol/L))	−0.03 (−0.10, 0.04)	—	0.06 (−1.61, 1.73)	—
HbA1c (mmol/mol)	<0.01 (−0.01, 0.01)	—	−0.05 (−0.26, 0.16)	—
eGFR (mL/min/ 1.73 m^2)	<0.01 (0.00, 0.00)	—	**−0.12** (-0.20, -0.03)**	—
log (C-reactive protein (mg/L))	−0.01 (-0.06, 0.04)	—	−0.88 (−2.07, 0.30)	—
log (Vitamin B12 (pmol/L))	**0.27** (0.10, 0.44)**	**0.25** (0.09, 0.43)**	**5.23* (1.07, 9.39)**	—
BMI (kg/m^2)	**−0.02* (-0.04, 0.00)**	—	**−0.75*** (-1.18, -0.33)**	—
Waist circumference (cm)	**−0.01** (-0.02, 0.00)**	**−0.01*** (-0.02, 0.01)**	**−0.46*** (-0.61, -0.31)**	**−0.26*** (-0.40, -0.11)**
Heart rate (bpm)	**−0.01* (-0.01, 0.00)**	—	**−0.49*** (-0.65, -0.33)**	**−0.41*** (-0.56, -0.26)**
Systolic blood pressure (mmHg)	**0.01** (0.00, 0.01)**	**0.01*** (0.01, 0.02)**	0.12 (−0.01, 0.25)	—
Diastolic blood pressure (mmHg)	<0.01 (−0.01, 0.01)	—	**−0.39*** (-0.57, -0.21)**	—
Pulse pressure (mmHg)	**0.01** (0.00, 0.02)**	—	**0.39*** (0.26, 0.53)**	**0.44*** (0.32, 0.57)**
Smoking history				
Current	**−0.35** (-0.58, -0.11)**	**−0.32** (-0.55, -0.09)**	−2.27 (−8.11, 3.57)	—
Former	**−0.20* (-0.37, -0.03)**	—	0.31 (−3.80, 4.43)	—
Insulin dose (units/kg/day)	**−0.41** (-0.68, -0.13)**	—	**−15.81*** (-22.36, -9.25)**	—
Insulin regimen				
Twice daily	−0.20 (−0.69, 0.30)	—	−0.99 (−12.99, 11.01)	—
Insulin pump	**0.17* (0.01, 0.34)**	—	**5.88** (1.96, 9.80)**	—
Other	0.07 (−0.33, 0.47)	—	5.02 (−4.71, 14.75)	—
Clinical history				
Existing CVD	0.05 (−0.19, 0.28)	—	**6.01* (0.20, 11.82)**	—
Retinopathy	—	—	—	—
Non proliferative	−0.02 (−0.30, 0.26)	—	−5.33 (−12.27, 1.61)	—
Inactive proliferative	−0.12 (−0.44, 0.20)	—	−0.65 (−8.45, 7.14)	—
Active proliferative	**−0.38* (-0.76, -0.01)**	—	**−10.88* (-19.98, -1.78)**	—
History of impaired monofilament sensation				
Yes	0.06 (−0.18, 0.29)	—	5.11 (−0.61, 10.82)	—
Unknown	−0.13 (−0.36, 0.09)	—	1.68 (−3.88, 7.24)	—
Lipids				
Total cholesterol (mmol/L)	0.02 (−0.06, 0.10)	—	1.29 (−0.75, 3.34)	—

(continued)

Table 3. (continued)

	Baseline RHI		Baseline AI	
	Univariable	Exhaustive search	Univariable	Exhaustive search
HDL-cholesterol (mmol/L)	**0.20** (0.07, 0.33)**	—	**6.55*** (3.38, 9.72)**	—
LDL-cholesterol (mmol/L)	−0.05 (−0.15.0.06)	—	−1.47 (−4.09, 1.15)	—
Triglycerides (mmol/L)	−0.09 (−0.21, 0.02)	—	−1.73 (−4.59, 1.14)	—
Drugs				
Antihypertensive drugs				
Any	0.03 (−0.14, 0.21)	—	−0.26 (−4.56, 4.04)	—
ACE inhibitor	−0.11 (−0.27, 0.04)	—	−1.13 (−4.88, 2.61)	—
ARB	0.09 (−0.09, 0.27)	—	−0.55 (−5.00, 3.90)	—
Calcium channel blocker	−0.02 (−0.23, 0.20)	—	0.29 (−4.92, 5.50)	—
Beta blocker	0.07 (−0.19, 0.33)	—	**7.67* (1.39, 13.96)**	—
Statins	−0.01 (−0.21, 0.19)	—	−0.39 (−5.30, 4.51)	—
Antiplatelet drugs				
Aspirin	−0.03 (−0.20, 0.13)	—	1.71 (−2.22, 5.63)	—
Clopidogrel	0.01 (−0.40, 0.43)	—	6.64 (−3.40, 16.68)	—
Adjusted R square	—	0.11	—	0.31

All variables were included as candidates in an exhaustive search (brand-and-bound algorithm) and random forest (Boruta algorithm) procedures to select variables for inclusion in the final multivariable model. BIC criterion was used for the best model selection.
*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$.

Table 4. Effect of metformin versus placebo on RHI and previously-unreported EndoPAT parameters (repeated measures analysis over 36 months).

	Change (ANCOVA)[a]	Main effect p value	Visit interaction p value[b]
RHI[1]	−0.0645 (−0.1872, 0.0582)	0.30	0.57
fRHI	−0.0169 (−0.0895, 0.0558)	0.65	0.22
AI	0.6515 (−1.9730, 3.2760)	0.63	**0.008**
AI @75	0.7955 (−1.5664, 3.1573)	0.51	**0.01**

Treatment effect and corresponding 95% CIs are provided for metformin compared with placebo
[a]Repeated measures ANCOVA for the change from baseline, adjusted for baseline value and visit.
[b]Repeated measures model extended to include visit-by-treatment interaction.
[c]reported in Petrie et al.[23]

While prior cross-sectional studies in T1D have demonstrated inverse correlations between FMD and cardiac dysfunction[39] and kidney impairment,[6,40] we did not detect significant associations between RHI and these diabetes complications measured at baseline. This may also reflect intrinsic differences in the methods of vascular assessment or the populations included (e.g. age), but it should be noted that the REMOVAL cohort was larger than many other studies. In addition, data were collected by trained operators across multiple sites and were subject to external quality assurance.

An exhaustive search was used to determine independent associates of vascular function. Only a small proportion of the variance in these baseline parameters was explained by the concurrent demographics and biomarkers, although AI was more strongly associated with these factors than RHI. This was consistent across different statistical models (included in the main table and supplementary material). Our findings are mostly consistent with the existing literature but extend to a high CVD risk group of adults with T1D. The positive association between PP and RHI and negative association between WC and AI contrast with previous studies,[41,42] which used other tests of vascular function (i.e. FMD, PWV) and were conducted in different study populations. As both RHI and AI were positively associated

with PP and negatively with WC, we speculate that higher PP increases the PAT signal while increased adiposity decreases the PAT signal. These factors may contribute to the observed positive correlation between RHI and AI.

Our PAT results (with both RHI and AI) are consistent with epidemiological data that women with T1D are at higher risk of vascular dysfunction.[1] They also can be taken to reinforce the importance of early recognition and treatment of cardiometabolic risk factors in T1D to prevent subsequent CVD. However, given that only a moderate proportion of variance in vascular function was explained by traditional risk factors, other factors such as novel biomarkers and genetics are also likely to be important. Further studies are merited to identify these factors, which may be prognostic markers or potential therapeutic targets for the prevention and treatment of CVD in T1D.

In our study, insulin pump use was associated cross-sectionally with higher RHI (better endothelial function) compared to MDI. This finding is consistent with a previous small observational study of children with T1D which reported better endothelial function after switching from MDI to insulin pump therapy,[43] and a large observational study in which insulin pump therapy was associated with reduced cardiovascular mortality compared to MDI for comparable HbA1c levels.[26] It has been suggested that reduced glycaemic variability[44] and lower exogenous insulin doses associated with insulin pump use (vs. MDI) may mediate improved endothelial function, reducing long-term risk of chronic complications. In contrast, in our study insulin pump therapy was associated with higher AI. Further longitudinal data are required to corroborate the apparent benefits of long-term insulin pump use on vascular function and clinical complications.

Consistent with existing literature, vitamin B12 was a positive independent determinant of endothelial function. Vitamin B12 deficiency can lead to hyperhomocysteinaemia, which induces endothelial dysfunction through reducing nitric oxide bioavailability.[45] Supplementation with vitamin B12 and folate has been shown to improve endothelial function in people with metabolic syndrome and CVD,[46,47] and folate levels were independently correlated with endothelial function in a T1D study.[48] Further investigations on the effects of folate and vitamin B12 supplementation on vascular health in people with T1D are merited.

There was no significant effect of metformin on any PAT parameter following 36 months of treatment, when compared to placebo. Given previous positive findings,[22,49] it is possible that there is less capacity to improve poor vascular function in longer duration T1D and with high CVD risk. A prior randomised controlled trial of 40 people with T1D with mean age 43 years (approximately 12 years younger than in REMOVAL) found that metformin significantly improved FMD and RHI but did not affect arterial stiffness.[22] Unlike in the present trial (REMOVAL), this study did not select subjects based on high CVD risk; hence, as well as somewhat younger age, their arterial systems may have been less severely affected by atherosclerosis and arteriosclerosis. In a study of 48 adolescents with T1D, metformin reduced arterial stiffness measured by phase-contrast MRI.[50] In another study of 90 children with T1D, metformin improved GTN-mediated dilatation but not FMD.[51] While the type of test and the age and vascular health of participants is key in determining the effects of interventions on vascular function, future studies should include larger sample sizes and robust measures of vascular function subject to external quality assurance programs.

The REMOVAL trial is the largest and longest trial of metformin in T1D to date and the first to include a CVD endpoint, albeit a surrogate measure. Study strengths include use of high-quality trial data, high rates of technical acceptability, multiple measures of vascular health and important clinical questions given that vascular health is known to be impaired soon after T1D diagnosis. Study limitations are that while simple and suitable for use across sites in multi-centre trials, EndoPAT is only one of many available techniques for measuring vascular function.

In conclusion, in this novel study in T1D adults at high CVD risk, we demonstrated: (i) modest associations between PAT measures of vascular health and several cardiometabolic risk factors and complications; (ii) metformin treatment for 36 months did not affect any PAT parameter. Further studies are merited to identify novel genetic and other factors which may explain the variance in vascular function unaccounted for by traditional risk factors in adults with T1D, and to better understand the effects of metformin on vascular health.

Acknowledgements

We thank Merck Germany KGaA (Darmstadt, Germany) for donating study medication and placebo and shipping it to study sites; and Itamar Medical (Caesarea, Israel) for donating EndoPAT equipment and providing central quality assurance services for the reactive hyperaemia index measurements.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: JRP has received research grants from JDRF for the present work. He has also received personal fees and travel support from Novo Nordisk, research grants and personal fees from Sanofi Aventis, Quintiles and Janssen unrelated to the present work, non-financial support (donation of study medication for the present trial) from Merck (Germany), personal fees from ACI Clinical unrelated to the present work and non-financial

support (donation of EndoPAT equipment, reading services, and quality assurance support for the present trial) from Itamar Medical. Related to the REMOVAL trial, AJJ has received grants from JDRF International and from JDRF Australia. Unrelated to this trial, she has received grants from Medtronic, the NHMRC (Australia) and Medical Research Future Fund, JDRF Australia, JDRF International, the Helmsley Trust, Sanofi-Aventis, Mylan and Abbott. DC, NG, KD, TF, DMC, ADH, ASJ and CDAS declare no competing interests.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the REMOVAL trial was funded by Juvenile Diabetes Research Foundation International (JDRF) (New York, NY, USA; SRA 17- 2011-272).

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Supplemental Material

Supplemental material for this article is available online.

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Appendix

Abbreviations and acronyms

ACEI	Angiotensin-converting enzyme inhibitor
AI	Augmentation index
ARB	BIC Angiotensin II receptor blocker Bayesian Information Criterion
BMI	Body mass index
BP	Blood pressure
cIMT	Carotid intima media thickness
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eGFR	Estimated glomerular filtration rate
FMD	Flow mediated dilatation
fRHI	Framingham reactive hyperaemia index
HDL-C	High-density lipoprotein cholesterol
HR	Heart rate
MDI	Multiple daily injections
PAT	Peripheral arterial tonometry
PWV	Pulse wave velocity
PP	Pulse pressure
REMOVAL	REducing with MetfOrmin Vascular Adverse Lesions in type 1 diabetes
RHI	Reactive hyperaemia index
SBP	Systolic blood pressure
T1D	Type 1 diabetes
WC	Waist circumference