# Myocardial flow reserve assessed by cardiac $^{82}$Rb positron emission tomography/computed tomography is associated with albumin excretion in patients with Type 1 diabetes

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| **Aims** | To evaluate myocardial flow reserve (MFR) and coronary artery calcium (CAC) in persons with Type 1 diabetes with or without albuminuria and in non-diabetic controls. MFR reflects the function of large epicardial arteries and myocardial microcirculation. CAC represents structural aspects of atherosclerosis. In addition, we evaluated the association of MFR and CAC with retinopathy, another microvascular complication. |
| **Methods and results** | Cross-sectional study in Type 1 diabetes, stratified by normoalbuminuria (NORMO; $n = 30$) and macroalbuminuria (MACRO; $n = 30$), and in non-diabetic controls ($n = 30$). MFR (pharmacological stress flow/rest flow) was evaluated by cardiac $^{82}$Rb positron emission tomography/computed tomography. MFR was similar in patients with NORMO and controls ($3.1 \pm 0.79$ vs. $3.0 \pm 0.79$; $P = 0.74$). Patients with MACRO had lower (impaired) MFR when compared with NORMO ($2.1 \pm 0.92$ vs. $3.1 \pm 0.79$; $P < 0.0001$). The CAC score [median (interquartile range)] was higher in NORMO when compared with controls [72 (22–247) vs. 0 (0–81), $P = 0.03$], and comparable between MACRO and NORMO. MFR was comparable in patients with diabetes and simplex or no retinopathy ($n = 24$ and $n = 12$, $2.8 \pm 0.84$ vs. $3.3 \pm 0.77$, $P = 0.11$), but lower in proliferative ($n = 24$) compared with simplex retinopathy ($2.1 \pm 0.97$ vs. $2.8 \pm 0.84$, $P = 0.02$). The CAC score was comparable between groups of retinopathy. |
| **Conclusion** | Myocardial microvascular function was comparable in non-diabetic controls and patients with Type 1 diabetes and NORMO; but impaired in the presence of microvascular complications (MACRO and proliferative retinopathy). Coronary calcification was elevated in diabetes, however, not explained by albuminuria. |
| **Keywords** | cardiovascular disease • coronary artery calcium score • myocardial flow reserve • macroalbuminuria • cardiac PET/CT • Type 1 diabetes |

## Introduction

Approximately 35% of persons with Type 1 diabetes will develop albuminuria. Deckert et al.[1] proposed in 1989 in the Steno hypothesis that albuminuria reflects widespread vascular damage. The hypothesis links leaky renal vessels to a general impaired vascular endothelial function. Many subsequent studies have confirmed the association between albuminuria, retinopathy, and risk of cardiovascular disease[2,3] supporting the hypothesis of widespread vascular damage in Type 1 diabetes.

Major advances in non-invasive imaging enable the investigation of new aspects of the microcirculation. Among these methods is

quantitative cardiac positron emission tomography (PET) which allows the measurement of myocardial blood flow at rest and during pharmacologically induced hyperaemic conditions. The ratio between resting and maximal induced myocardial blood flow is termed the myocardial flow reserve (MFR) and reflects to what extend the flow can increase during stress. MFR mirrors the function of the large epicardial arteries and the microcirculation of the myocardium.[4] A hybrid scanner can combine cardiac PET with computed tomography (CT) to estimate coronary artery calcium (CAC), a specific marker of atherosclerosis.

Knowledge about cardiac microvascular function in Type 1 diabetes is scarce. Only two small studies have reported impaired MFR in young men with Type 1 diabetes as compared to healthy controls.[5,6]

In a recent study in persons with Type 2 diabetes free of overt cardiovascular disease, we demonstrated lower (impaired) MFR and higher CAC score (increased calcification) to be associated with concomitant albuminuria.[7] It is not clear to what extent these findings can be extrapolated to Type 1 diabetes.

Taking advantages of similar cardiac PET/CT imaging as in our study of persons with Type 2 diabetes, we undertook a cross-sectional study in healthy controls and persons with Type 1 diabetes stratified by albumin excretion. We aimed to gain information of the prevalence and predictors of reduced MFR and increased CAC in persons with Type 1 diabetes (with or without albuminuria) while comparing them to non-diabetic controls, and in addition, we evaluated the association of MFR and CAC with diabetic retinopathy, another microvascular complication.

# Methods

## Study population

We included 60 persons with Type 1 diabetes according to the WHO criteria. A priori, we decided to include 30 participants with normoalbuminuria (NORMO) (<30 mg/24 h or 30 mg/g creatinine) and 30 participants with a history of macroalbuminuria (MACRO) (≥300 mg/24 h or 300 mg/g creatinine in two out of three consecutive urine collections; $n = 30$). Persons with NORMO and MACRO were matched on age and sex. Persons classified with NORMO did not have any history of microalbuminuria or MACRO prior to enrolment in the study. The participants were recruited from the Steno Diabetes Center Copenhagen among subjects participating in a cross-sectional study focusing on detailed phenotyping of Type 1 diabetes patients with or without progressive renal complications. None of the participants included were symptomatic for angina and the reason of the cardiac PET/CT was exclusively research. Exclusion criteria were (i) non-diabetic kidney disease; (ii) renal failure [estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 m$^2$], dialysis, or kidney transplantation; (iii) change in renin–angiotensin aldosterone system blocking treatment during the last month; and (iv) contraindications for cardiac PET/CT. Moreover, 30 healthy non-diabetic persons studied earlier with an identical protocol using the same equipment at our centre in 2013[7] were used as controls.

Power calculation was performed using the power statement implemented in the SAS software, version 9.3. Calculations were based on our previous results on MFR in persons with Type 2 diabetes performed in the same centre using the same equipment.[7] In the assumption of a mean difference in MFR of 0.6 [and a standard deviation (SD) of 0.8] between persons with NORMO vs. MACRO, a total of 54 persons (27 in each group) were needed to provide 80% power for a Type 1 error of 5%. We included 30 persons in each group to account for technical difficulties and incomplete investigations.

The study was performed from August 2016 to January 2018. The study was conducted in accordance with the Helsinki protocol and all participants gave informed written consent and the protocol was approved by the local ethics committee.

## Clinical measurements

HbA$_{1c}$ was measured by high performance liquid chromatography. Plasma creatinine was measured by an enzymatic method (Hitachi 912, Roche Diagnostics, Mannheim, Germany). The CKD-EPI equation was used to calculate eGFR.[8] Urinary albumin creatinine rate (UACR) was measured by an enzyme immunoassay in three consecutive morning urine samples. Measurements of 24-h blood pressure were recorded using a cuff-device (Takeda, TM2430, Japan[9]) programmed to measure blood pressure every 15 min between 7 am and 10 pm and every 30 min between 10 pm and 7 am. Standard resting 12-lead electrocardiogram was obtained. Height and weight was measured, and body mass index was calculated as weight/height (kg/m$^2$). A detailed medical history including treatment and previous cardiovascular disease was obtained from all participants and cross referenced with electronic patient records. Current smoking was defined as one or more cigarettes/cigars/pipes a day.

For the control subjects, all clinical measurements were assessed and defined as described above with the exceptions that (i) urinary albumin excretion rate (UAER) was measured in two 24-h urine collections by an enzyme immunoassay; and (ii) 24-h blood pressure was recorded using BPro (HealthStats, Singapore), a tonometric wrist-device that records brachial blood pressure derived from radial pulse waves.[10] The device captured the blood pressure every 15 min for 24 h.

## Hybrid cardiac PET/CT imaging

A dynamic, gated cardiac PET/CT study was performed using a hybrid PET/CT scanner in 3D mode (Siemens Biograph mCT 128, Siemens, Munich, Germany) after administration of 1100 MBq $^{82}$Rb (CardioGen-82, Bracco Diagnostics, Monroe Township, NJ, USA). The procedure has previously been described in detail.[7] Cardiac PET/CT was performed at rest and at stress after adenosine was infused at 140 µg/kg/min for 6 min to induce maximum myocardial hyperaemia. Myocardial blood flow was automatically calculated using the Siemens Syngo MBF 2.3 (Siemens Medical Solutions, Malvern, PA, USA) with one-compartment tracer kinetic models for $^{82}$Rb[11] and the extraction curve from Lortie et al.[12] Myocardial perfusion abnormalities were assessed semi quantitative by two experienced operators. Before the examination, phosphodiesterase 5 inhibitors were discontinued for 72 h, dipyridamole-containing medications were discontinued for 36 h, and nitrates were discontinued for 12 h. Moreover, all subjects refrained from caffeine-containing beverages for 18 h before examination, and theophylline-containing medications were discontinued for 24 h. Angiotensin converting enzyme inhibitors were not discontinued,[13,14] and we did not set therapy with beta-blocker on hold due to possible discomfort for the patients and it has been demonstrated that beta-blocker can be continued without loss of the essential interpretation of the results.[15] CAC score was calculated as the sum of CAC content in the three main coronary arteries using the method described by Agatston et al.[16] and semiautomated commercially available software (Corridor4DM, INVIA, Ann Arbor, MI, USA).

Different cut-off for MFR has been applied depending on the characteristic of the study population; and a cut-off of 2.5 has been suggested in patients without obstructive coronary artery disease (CAD).[17,18] We, therefore, prespecified a cut-off of 2.5. An elevated CAC was defined as an Agatston score >300.[19]

**Table I**    Clinical characteristics of participants

| Characteristics | Controls (n = 30) | Normoalbuminuria (n = 30) | Macroalbuminuria (n = 30) | *P*-value controls vs. normoalbuminuria | *P*-value normoalbuminuria vs. macroalbuminuria |
|---|---|---|---|---|---|
| Female (%) | 40 | 40 | 43 | 1.0 | 0.79 |
| Age (years) | 59.8 ± 9.9 | 59.8 ± 9.1 | 58.2 ± 9.9 | 0.99 | 0.52 |
| Known diabetes duration (years) | | 32.6 ± 12.7 | 41.4 ± 13.3 | | 0.01 |
| Body mass index (kg/m$^2$) | 24.7 ± 3.4 | 25.6 ± 4.1 | 27.2 ± 4.2 | 0.38 | 0.15 |
| HbA$_{1c}$ (mmol/mol) | 35.8 ± 1.9 | 61.3 ± 8.3 | 66.3 ± 11.7 | <0.0001 | 0.70 |
| HbA$_{1c}$ (%) | 5.4 ± 0.17 | 7.8 ± 0.76 | 8.2 ± 1.07 | <0.0001 | 0.70 |
| LDL cholesterol (mmol/L) | 3.4 ± 0.7 | 2.3 ± 0.7 | 2.1 ± 0.8 | <0.0001 | 0.32 |
| Total cholesterol (mmol/L) | 5.5 ± 0.7 | 4.5 ± 0.8 | 4.4 ± 0.9 | <0.0001 | 0.73 |
| eGFR (mL·min$^{-1}$ 1.73·m$^{-2}$) | 82.8 ± 13.1 | 89.1 ± 10.4 | 62.5 ± 23.1 | 0.043 | <0.0001 |
| Urinary albumin creatinine rate (mg/g)[a] | 6 (5–10.5) | 3 (3–5) | 121 (53–283) | 0.0002 | <0.0001 |
| Smokers (%) | 13 | 14 | 14 | 0.96 | 1.0 |
| Alcohol (beverages/week) | 8.5 (4–14) | 7 (4–18) | 6.5 (1–14) | 0.87 | 0.91 |
| Antihypertensive treatment (%) | 10 | 57 | 100 | 0.0001 | <0.0001 |
| RAAS inhibition treatment (%) | 10 | 47 | 97 | 0.002 | <0.0001 |
| Beta-blocker treatment (%) | 0 | 3 | 27 | 1.0 | 0.026 |
| Aspirin treatment (%) | 3 | 37 | 63 | 0.0012 | 0.039 |
| Lipid-lowering treatment (%) | 0 | 70 | 80 | <0.0001 | 0.37 |
| Retinopathy (no/simplex/proliferative) (%) | | 37/53/10 | 3/27/70 | | |
| Known coronary artery disease (%) | 0 | 0 | 12 | | 0.01 |

Data are expressed as *n* (%), mean ± SD, or geometric mean (IQR). *P* values from independent samples *t*-test and $\chi^2$ test or Fisher's exact test.

eGFR, estimated glomerular filtration rate; RAAS, renin–angiotensin aldosterone system.

[a]Urinary albumin excretion rate (mg/24 h) for the 30 controls.

## Retinopathy

Retinopathy status was obtained from medical records for the diabetic participants. All persons attending the outpatient clinic at Steno Diabetes Center Copenhagen have regular ophthalmology examinations (approximately every 1–2 years) where retinal photographies are taken through a dilated pupil by certified eye nurses. Retinopathy was graded as nil, presence of or historical simplex, proliferative, or blind based on the worst eye.

## Statistical analysis

For continuous variables, the normal distributed are given as mean and SD, and the non-normal distributed (CAC, UACR, and alcohol intake) as median with interquartile range (IQR). Categorical variables are provided as total numbers in percent. When analysing differences between two groups, we applied independent samples *t*-test when comparing continuous variables, and the $\chi^2$ test or Fisher's exact test as appropriate when comparing categorical variables. The non-normal distributed variables were log$_2$ transformed in all analyses. However, the CAC score distribution was highly skewed and hence not amenable to transform into normal distribution and was analysed using the Mann–Whitney *U* test. When analyzing differences in MFR and CAC in adjusted analysis, analysis of covariance was applied, ensuring normal distribution of the residuals for CAC after log$_2$ transformation. Linear regression was used to analyse correlations between MFR, CAC, and other covariates. We provide $R^2$ to present the proportion of variability in the dependent variable explained by the model and the *F*-test was applied to determine whether this relationship was statistically significant.

Participants with a history of percutaneous coronary intervention (*n* = 5) were excluded from the analyses of CAC. Results were considered to be significant at a two-tailed *P*-value <0.05. Statistical analyses were performed using SAS software (version 9.3; SAS Institute).

## Rationale for selection of covariates

We adjusted for traditional cardiovascular risk factors based on prior evidence. In analysis comparing the level of MFR and CAC between controls, patients with diabetes and NORMO or MACRO we adjusted for sex, age, 24-h systolic blood pressure, eGFR, and smoking. The groups were pre-categorized according to UACR, HbA$_{1c}$, and diabetes duration and these covariates were therefore omitted as was medical treatment due to risk of bias by indication. We did not adjust for low density lipoprotein (LDL) cholesterol, since the level was higher in controls when compared with the diabetic participants most likely because the controls were not receiving lipid-lowering treatment. In analyses restricted to the participants with diabetes, we applied additionally adjustment for HbA$_{1c}$, LDL cholesterol and diabetes duration, and for the analyses of retinopathy also for UACR.

# Results

## Clinical characteristics

Characteristics for the total cohort are presented in *Table 1*. The 90 participants included 37 (41%) females and mean ± SD age was 59.3 ± 9.5 years. Compared to the persons with NORMO the

**Table 2**   **Cardiac PET/CT and albuminuria**

| Variables | Controls (n = 30) | Normoalbuminuria (n = 30) | Macroalbuminuria (n = 30) | P-value controls vs. normoalbuminuria | P-value normoalbuminuria vs. macroalbuminuria |
|---|---|---|---|---|---|
| 24-h systolic blood pressure (mmHg) | 127 ± 13 | 135 ± 9 | 138 ± 11 | 0.009 | 0.26 |
| 24-h diastolic blood pressure (mmHg) | 79 ± 8 | 78 ± 6 | 77 ± 5 | 0.88 | 0.48 |
| 24-h pulse pressure (mmHg) | 48 ± 10 | 57 ± 6 | 61 ± 9 | 0.0002 | 0.04 |
| Heart rate (bpm) | 61 ± 8 | 69 ± 12 | 72 ± 12 | 0.003 | 0.41 |
| Myocardial blood flow rest (mL/g/min) | 1.1 ± 0.25 | 1.0 ± 0.25 | 1.2 ± 0.35 | 0.82 | 0.01 |
| Myocardial blood flow stress (mL/g/min) | 3.0 ± 0.53 | 3.0 ± 0.51 | 2.5 ± 0.82 | 0.73 | 0.004 |
| MFR | 3.0 ± 0.79 | 3.1 ± 0.79 | 2.1 ± 0.92 | 0.74 | <0.0001 |
| MFR <2.5 (%) | 17 | 23 | 77 | 0.52 | <0.0001 |
| CAC score[a] | 0 (0–81) | 72 (22–247) | 263 (23–1315) | 0.028 | 0.17 |
| CAC score >300 (%) | 7 | 17 | 44 | 0.71 | 0.026 |

Data are expressed as total numbers in percent, mean ± SD, or geometric mean (IQR). P-values from independent samples t-test, $\chi^2$ test, and Mann–Whitney U test[a] or Fisher's exact test.

CAC, coronary artery calcium; MFR, myocardial flow reserve.

controls had lower 24-h systolic blood pressure, HbA$_{1c}$, and eGFR but higher LDL cholesterol and UACR. Persons with MACRO had longer diabetes duration and lower eGFR compared with persons with NORMO. Of the 60 participants with diabetes, 7 (12%) had known CAD (five had a history of percutaneous coronary intervention and two had coronary artery bypass graft). These participants were all in the group with MACRO. Simplex retinopathy was present in 16 (53%) and proliferative retinopathy in 3 (10%) of the persons with NORMO. Simplex retinopathy was present in 8 (27%) and proliferative retinopathy in 21 (70%) of the persons with MACRO. In the total population, the mean left ventricular ejection fraction at rest was 64.2% with a range of 39–85%.

## Cardiac PET/CT in controls and in Type 1 diabetes stratified by urinary albumin excretion

The MFR and the frequency of reduced MFR (<2.5) were similar in persons with NORMO and in controls (3.1 ± 0.79 vs. 3.0 ± 0.79 and 23 vs. 17%, $P \geq 0.52$). Persons with MACRO had a lower MFR and the frequency of reduced MFR was higher when compared with persons with NORMO (2.1 ± 0.92 vs. 3.1 ± 0.79 and 77 vs. 23%, $P < 0.0001$). When we adjusted the MFR for CAC score, MFR remained lower and the frequency of reduced MFR higher in persons with MACRO compared to NORMO ($P \leq 0.001$).

The CAC score was higher in persons with NORMO compared to controls [72 (22–247) vs. 0 (0–81), $P = 0.028$], and comparable between persons with MACRO and NORMO [263 (23–1315) vs. 72 (22–247), $P = 0.17$]. The frequency of high CAC was similar in persons with NORMO and the controls (17 vs. 7%, $P = 0.71$), but higher in persons with MACRO compared to NORMO (44 vs. 17%, $P = 0.026$). Results are summarized in *Table 2*.

After adjustment for sex, age, 24-h systolic blood pressure, eGFR, and smoking, MFR remained lower and the frequency of reduced MFR higher in persons with MACRO compared to NORMO ($P \leq 0.0077$, *Figure 1A*), and these differences persisted after additional
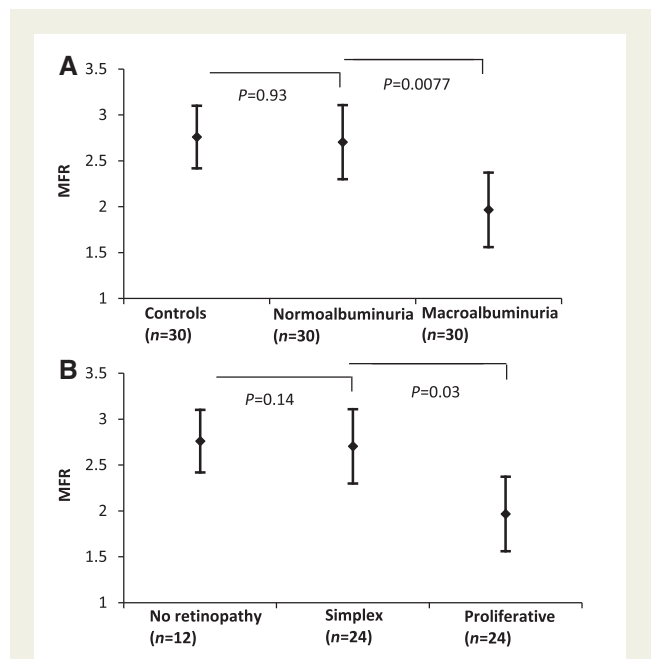


**Figure 1** Estimated marginal means with 95% CIs for myocardial flow reserve (MFR) adjusted for sex, age, 24-h systolic blood pressure, eGFR, and smoking according to groups [(A) Controls, normoalbuminuria and macroalbuminuria; (B) Retinopathy].

adjustment for HbA$_{1c}$, LDL cholesterol, and diabetes duration ($P \leq 0.038$). The CAC remained higher in the persons with NORMO compared to controls after adjustment ($P = 0.03$), but the difference between persons with MACRO and NORMO lost significance.

The myocardial blood flow during stress were similar in persons with NORMO and in controls (3.0 ± 0.51 vs. 3.0 ± 0.53 mL/g/min, $P = 0.73$). Persons with MACRO had lower myocardial blood flow during stress compared with persons with NORMO (2.5 ± 0.82 vs. 3.0 ± 0.51 mL/g/min, $P = 0.004$). Results are summarized in *Table 2*.

**Table 3** Cardiac PET/CT and retinopathy

| Variables | No retinopathy (n = 12) | Simplex (n = 24) | Proliferative (n = 24) | P-value no retinopathy vs. simplex | P-value simplex vs. proliferative |
|---|---|---|---|---|---|
| MFR | 3.3 ± 0.77 | 2.8 ± 0.84 | 2.1 ± 0.97 | 0.11 | 0.02 |
| MFR <2.5 (%) | 8 | 46 | 75 | 0.03 | 0.04 |
| CAC score[a] | 45 (0–435) | 72 (23–247) | 299 (51–1651) | 0.42 | 0.25 |
| CAC score >300 (%) | 25 | 14 | 48 | 0.64 | 0.04 |

Data are expressed as total numbers in percent, mean ± SD, or geometric mean (IQR). *P*-values from independent samples *t*-test, $\chi^2$ test, and Mann–Whitney *U* test[a] or Fisher's exact test.
CAC, coronary artery calcium; MFR, myocardial flow reserve.

The difference between persons with MACRO and NORMO lost significance following adjustment ($P = 0.19$).

A total of 10 participants had ischaemia on the PET/CT, seven of which were known with CAD. All 10 had reversible ischaemia (Nine with MACRO and one healthy control), median (IQR) extent was 24 (14–29)%. In five of these participants, irreversible ischaemia (fixed perfusions defects) was also observed, all had MACRO and the median extent was 19 (16–25)%.

## Cardiac PET/CT in persons with Type 1 diabetes stratified by retinopathy stage

In persons with simplex retinopathy ($n = 24$) compared to persons without retinopathy ($n = 12$) MFR was comparable (2.8 ± 0.84 vs. 3.3 ± 0.77, $P = 0.11$), while reduced MFR was more frequent (46 vs. 8%, $P = 0.03$). In persons with proliferative ($n = 24$) compared to simplex retinopathy the MFR was lower (2.1 ± 0.97 vs 2.8 ± 0.84, $P = 0.02$) and the frequency of reduced MFR was higher (75 vs. 46%, $P = 0.04$). The CAC score was comparable between stages of retinopathy, but high CAC was more frequent in persons with proliferative compared to simplex retinopathy (48 vs. 14%, $P = 0.04$). Results are summarized in *Table 3*.

The MFR remained lower in persons with proliferative compared to simplex retinopathy after initial adjustment ($P = 0.03$, *Figure 1B*), but lost significance after further adjustment for $HbA_{1c}$, LDL cholesterol, diabetes duration, and UACR ($P = 0.27$). The differences in frequency of reduced MFR and high CAC between retinopathy groups lost significance after adjustment.

## Variables correlated with MFR and CAC

In the total population, MFR was negatively correlated with CAC ($R^2 = 0.20$, $P < 0.0001$, *Figure 2A*), UACR ($R^2 = 0.25$, $P < 0.0001$, *Figure 2B*), age ($R^2 = 0.07$, $P = 0.01$), 24-h systolic blood pressure ($R^2 = 0.05$, $P = 0.04$), and positively correlated with eGFR ($R^2 = 0.22$, $P < 0.0001$, *Figure 2C*). In analyses restricted to the persons with diabetes, MFR was negatively correlated with CAC ($R^2 = 0.15$, $P = 0.004$), UACR ($R^2 = 0.25$, $P < 0.0001$), age ($R^2 = 0.07$, $P = 0.04$), and diabetes duration ($R^2 = 0.19$, $P = 0.0005$), and positively correlated with eGFR ($R^2 = 0.22$, $P = 0.0002$).

In the total population, CAC was negatively correlated with eGFR ($R^2 = 0.06$, $P = 0.02$) and positively correlated with UACR ($R^2 = 0.05$,
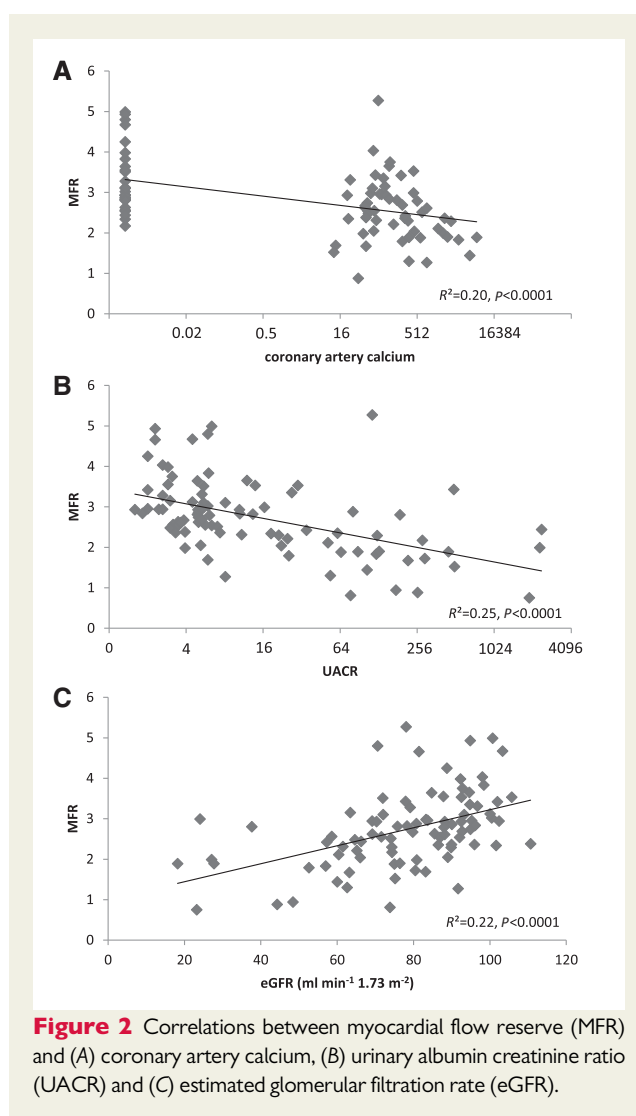
**Figure 2** Correlations between myocardial flow reserve (MFR) and (*A*) coronary artery calcium, (*B*) urinary albumin creatinine ratio (UACR) and (*C*) estimated glomerular filtration rate (eGFR).

$P = 0.04$), age ($R^2 = 0.21$, $P < 0.0001$), and 24-h systolic blood pressure ($R^2 = 0.10$, $P = 0.004$). In the diabetic population, CAC was positively correlated with age ($R^2 = 0.30$, $P < 0.0001$) and diabetes duration ($R^2 = 0.17$, $P = 0.002$) but not UACR ($R^2 = 0.009$, $P = 0.50$).

## Variables association with MFR in adjusted analyses

In the total cohort, higher UACR ($P = 0.02$), higher age ($P = 0.009$), lower eGFR ($P = 0.03$), and smoking ($P = 0.02$) were associated with lower MFR in multivariate linear regression ($R^2 = 0.39$). In analyses restricted to the persons with diabetes, higher UACR ($P = 0.02$), female sex ($P = 0.02$), higher age ($P = 0.05$), and longer diabetes duration ($P = 0.02$) were independently associated with lower MFR ($R^2 = 0.54$).

## Variables association with CAC in adjusted analysis

Higher age and 24-h systolic blood pressure were associated with higher CAC in multivariate linear regression ($R^2 = 0.34$) in the total cohort ($P \leq 0.03$). Further inclusion of MFR added to the model ($R^2 = 0.41$) and was significantly associated with higher CAC ($P = 0.004$). In analyses restricted to the persons with diabetes, longer diabetes duration ($P = 0.03$) and higher age ($P = 0.0002$) were associated with higher CAC ($R^2 = 0.44$). Further inclusion of MFR added to the model ($R^2 = 0.46$), but was not significantly associated with CAC ($P = 0.22$).

## Additional analysis

To avoid the potential confounding effect of epicardial stenosis on MFR, we performed a sensitivity analysis excluding all participants with known CAD or reversible and/or irreversible ischaemia revealed by cardiac PET ($n = 10$). MFR was $3.0 \pm 0.79$; $3.1 \pm 0.79$, and $2.3 \pm 0.89$ in controls, in persons with NORMO, and in persons with MACRO, respectively. These results are presented in Supplementary data online, Table S1. The results were confirmatory as the differences in MFR and in MFR <2.5 between normoalbuminuric participants and controls were non-significant, and the differences between macroalbuminuric and normoalbuminuric participants were significant ($P \leq 0.002$). After adjustment for sex, age, 24-h systolic blood pressure, eGFR, and smoking, MFR remained lower and the frequency of reduced MFR higher in persons with MACRO compared to NORMO ($P \leq 0.03$). After additional adjustment for HbA$_{1c}$, LDL cholesterol, and diabetes duration, the difference in MFR lost significance ($P = 0.10$), however, the difference in reduced MFR (<2.5) persisted ($P = 0.007$).

# Discussion

The main findings in this study were (i) the myocardial microvascular function was comparable in the healthy controls and the persons with Type 1 diabetes and NORMO; but impaired in the presence of MACRO and also in the presence of another microvascular complication: retinopathy; (ii) the coronary calcification was high in persons with Type 1 diabetes, but was to a larger extent explained by age and diabetes duration than presence of albuminuria. Our findings signify that microvascular impairment occurs in multiple microvascular beds and we detected microvascular injury in the heart which potentially affects how heart disease in Type 1 diabetes should be understood and treated.

Very little data on myocardial blood flow in Type 1 diabetes has been published until now. Older studies in a limited number of diabetic persons (mix of Types 1 and 2) referred for coronary arteriography have shown reduced MFR in persons with diabetes compared to persons without diabetes.[20–22] The ability to measure MFR non-invasively by cardiac PET allows examinations of larger and less selected populations. In 2783 consecutive patients referred for cardiac PET Murthy et al.[23] demonstrated that the rate of cardiac death for the diabetes patients ($n = 1172$, type of diabetes not specified) without CAD but with impaired MFR was comparable to that for non-diabetic patients with CAD. The cardiac mortality rate for persons with diabetes but without CAD and preserved MFR was very low. This emphasizes impaired MFR as a powerful, independent predictor of cardiac death in diabetes.

In this study by Murthy et al., the patients had symptoms of chest pain and/or dyspnoea and more than 60% had previous cardiovascular disease. Therefore, the median values for MFR was very low (1.6 for the patients with diabetes and 1.9 for the non-diabetics) and the cut-off for impaired MFR was chosen accordingly. In our population, we anticipated that the prevalence of cardiovascular disease was considerable lower, and therefore, that the median MFR was higher. In accordance with our expectations, the median value for MFR was 2.7 in our study.

Knowledge about the myocardial microvascular function in Type 1 diabetes is sparse. Our study showed that myocardial microvascular function was comparable in the healthy controls and the persons with Type 1 diabetes and NORMO. Pitkänen et al.[6] reported impaired MFR quantified by cardiac PET in 12 young men with Type 1 diabetes and NORMO compared with 12 healthy matched volunteers ($3.76 \pm 1.69$ vs. $5.31 \pm 1.86$, $P < 0.05$). Similarly, a study including 35 young subjects with diabetes (18 with Type 1 and 17 with Type 2) and 11 age-matched healthy controls, showed that the MFR was comparable in the subjects with Type 1 and Type 2 diabetes, but lower than in the controls.[24] In comparison, our study population consisted of older patients with a longer duration of diabetes, which may account in part for the different results.

In our study, myocardial microvascular function was impaired in the presence of MACRO and this association persisted after adjustment for traditional cardiovascular risk factors including the variables associated with lower MFR in our cohort (higher UACR, female sex, higher age, and longer duration of diabetes). MFR may capture both epicardial artery disease as well as microvascular disease. When we used CAC score as a co-variate (in absence of direct athero-quantification such as CT-angiogram-derived plaque burden) to test whether there was a true difference in microvascular disease between the participants with MACRO compared to NORMO, MFR remained lower and the frequency of reduced MFR higher in persons with MACRO compared to NORMO. To further disentangle if the association between albuminuria and MFR simply reflected existing clinically diagnosed CAD, we performed a separate set of analysis excluding the 10 participants with known CAD and/or ischaemia revealed by cardiac PET/CT. Our results were confirmatory indicating that there is a separate microvascular injury in the heart in Type 1

diabetes and it is found especially in the persons with further microvascular damage. Our findings in Type 1 diabetes are concordant with results from a study published in 2004 that demonstrated impaired MFR, measured by cardiac PET, in 16 persons with Type 1 diabetes with microangiopathy as compared to 12 without ($P < 0.05$). Microangiopathy was defined as presence of non-proliferative diabetic retinopathy ($n = 14$), microalbuminuria ($n = 2$), and/or peripheral neuropathy ($n = 10$).[25] We have previously reported impaired MFR in persons with Type 2 diabetes and albuminuria (UAER $\geq$30 mg/24 h, $n = 30$) as compared to NORMO ($n = 30$).[7] Recently, these results have been confirmed in a larger study including 118 patients with Type 2 diabetes demonstrating that MFR decreased progressively in relation to higher urinary albumin excretion.[26]

Knowledge about the myocardial microvascular function in Type 1 diabetes and the link to retinopathy is also limited. A cardiac PET study in 21 young men with Type 1 diabetes and NORMO found no difference in MFR in participants with or without retinopathy nor between patients with diabetes without retinopathy and 12 non-diabetic controls ($P \geq 0.2$).[5] Comparable results were shown in another small study including 12 young men with Type 1 diabetes and NORMO; here, MFR was similar in the presence of mild background retinopathy ($n = 5$) as in patients without retinopathy.[6] However, these results are in contrast to a small study ($n = 29$) in Type 2 diabetes demonstrating reduced MFR, measured by cardiac catheterization, in the presence of retinopathy compared with no retinopathy ($1.9 \pm 0.4$ vs. $2.8 \pm 0.3$, $P < 0.01$). Data on renal function or albumin excretion were not presented.[27] We demonstrated an association between impaired microcirculation in the heart and proliferative retinopathy. In contrast to the two small studies in young men with Type 1 diabetes, our study population was larger, older, and with longer diabetes duration.

Since diabetic retinal disease often coexists with diabetic renal disease, it has been difficult to discern their individual association to cardiovascular disease in Type 1 diabetes. Our study was intentionally stratified by albuminuria and the association between MFR and retinopathy was partly explained by the albumin excretion rate, signifying that retinopathy had no independent association to the myocardial microcirculation. Ninety-seven percent of the persons with MACRO in our study had retinopathy underlining the close relation between retinal and renal disease in Type 1 diabetes.

CAC is an accepted non-invasive measure of atherosclerotic burden. Our findings of higher CAC in people with Type 1 diabetes than in non-diabetic control subjects are in concordance with the results from the Coronary Artery Calcification in Type 1 Diabetes Study.[28] We report comparable CAC score between persons with MACRO and NORMO, however, investigating the association dichotomized at a CAC score >300, we found a higher frequency of elevated CAC score in persons with MACRO compared to NORMO. It may be that there is an association between CAC and albuminuria in Type 1 diabetes, but we might have limited power to detect it because of the skewed distribution of CAC scores.

## Clinical implications

Our findings suggest microvascular myocardial damage is common in Type 1 patients and can be identified with MACRO. How best to treat the microvascular damage in the heart remains to be established in future studies in contrast to the macrovascular damage known to respond to antihypertensive and lipid-lowering medication.

## Strengths and limitations

The strength of this study is that to our knowledge this is the first evaluation of MFR and CAC in Type 1 diabetes in a cohort stratified by albuminuria by design. Potential limitations include that (i) the power calculation was based on differences in MFR between albuminuric groups and not retinopathy; and (ii) the cross-sectional nature of our study precludes the assessment of causal relationships.

# Conclusion

In persons with Type 1 diabetes, we have demonstrated (i) impaired MFR in persons with MACRO compared to NORMO; and (ii) impaired MFR in persons with proliferative compared to simplex retinopathy. Our findings support the hypothesis that albuminuria reflects widespread vascular damage in Type 1 diabetes and that microvascular impairment occurs in multiple microvascular beds. Prospective studies are needed to establish the role of MFR in cardiovascular disease risk prediction in Type 1 diabetes.

# Supplementary data

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

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