**DIABETIC**
Medicine

# Non-invasive assessment of temporal changes in myocardial microvascular function in persons with type 2 diabetes and healthy controls

**Ida K. B. Rasmussen[1]** | **Philip Hasbak[2]** | **Bernt J. von Scholten[1]** | **Jens C. Laursen[1]** | **Emilie H. Zobel[1]** | **Lars Jorge Diaz[1]** | **Lene Holmvang[3]** | **Rasmus S. Ripa[2]** | **Peter Rossing[1,4]** | **Andreas Kjaer[2,4]** | **Tine W. Hansen[1]**

[1]Steno Diabetes Center Copenhagen, Copenhagen, Denmark

[2]Department of Clinical Physiology, Nuclear Medicine & PET and Cluster for Molecular Imaging, Rigshospitalet, Denmark

[3]Department of Cardiology, Rigshospitalet, Denmark

[4]University of Copenhagen, Copenhagen, Denmark

**Correspondence**
Ida Kirstine Bull Rasmussen, Steno Diabetes Center Copenhagen, Niels Steensens Vej 2, 2820 Gentofte, Denmark.
Email: ida.kirstine.bull.rasmussen.01@regionh.dk

## Abstract

**Background:** Cardiac Rubidium-82 ($^{82}$Rb) positron emission tomography/computed tomography (PET/CT) provides a measure of the myocardial blood flow and the myocardial flow reserve, which reflects the function of both large epicardial arteries and the myocardial microcirculation. Knowledge on changes in the myocardial microvascular function over time is lacking.

**Methods:** In this cohort study, we recruited 60 persons with type 2 diabetes and 30 non-diabetic controls, in 2013; all free of overt cardiovascular disease. All underwent a cardiac $^{82}$Rb PET/CT scan. In 2019, all survivors (n = 82) were invited for a repeated cardiac $^{82}$Rb PET/CT scan using the same protocol, and 29 with type 2 diabetes and 19 controls participated.

**Results:** Median duration between visits was 6.2 years (IQR: 6.1–6.3). In the total cohort, the mean age was 66.4 years (SD: 9.3) and 33% were females.

The myocardial flow reserve was lower in persons with type 2 diabetes compared to controls ($p = 0.002$) but there was no temporal change in the myocardial flow reserve in participants with type 2 diabetes: mean change: −0.22 (95% CI: −0.47 to 0.02) nor in controls: −0.12 (−0.49 to 0.25) or when comparing type 2 diabetes to controls: mean difference: −0.10 (95% CI: −0.52 to 0.31). The temporal reduction in stress-induced myocardial blood flow did not differ within the groups but was more pronounced in type 2 diabetes compared to controls: mean difference: −0.30 (95% CI: −0.55 to −0.04).

**Conclusion:** The myocardial microvascular function was impaired in persons with type 2 diabetes compared to controls but did not change significantly in either of the groups when evaluated over 6 years.

**KEYWORDS**
diabetes mellitus, clinical physiology, cardiovascular complications, microvascular function, imaging

# 1 | INTRODUCTION

Major advances in non-invasive imaging enable the investigation of the cardiac microcirculation. Quantitative cardiac positron emission tomography (PET) allows measurement of the myocardial blood flow (MBF) at rest and during pharmacologically induced hyperaemic conditions (stress). The ratio between the two is termed the myocardial flow reserve (MFR). A lower MFR can be a consequence of increased MBF at rest, a reduction in MBF during stress or both. Factors that increase the myocardial oxygen demand will increase the MBF at rest, whereas MBF during stress may be reduced because of diffuse atherosclerosis in the large epicardial arteries or dysfunction of the myocardial microcirculation.[1]

Measures of the myocardial microvascular function may provide additional and independent risk information beyond the extent of coronary atherosclerosis. The predictive value of the MFR has been evaluated for mortality in persons with diabetes (type 1 and type 2) referred for Rubidium-82 ($^{82}$Rb) PET myocardial perfusion scan due to chest pain and/or dyspnoea. The study demonstrated that impaired MFR (<1.6) was associated with a higher rate of cardiac death.[2]

As cardiac PET is often performed in combination with computed tomography (CT), it is also possible to estimate the coronary artery calcium score (CACS), a specific marker of the total atherosclerotic burden. Extensive prognostic data on CACS are available. Also, in asymptomatic persons with type 2 diabetes, CACS has been demonstrated as a strong predictor of cardiovascular events with the capacity to enhance prediction beyond established risk factors.[3,4]

In 2013–2014, we examined the prevalence of myocardial microvascular dysfunction and coronary artery calcification evaluated by cardiac $^{82}$Rb PET/CT myocardial perfusion scan in 60 persons with type 2 diabetes and in 30 healthy controls, all free of overt cardiovascular disease. We demonstrated an impaired myocardial microvascular function and a higher coronary artery calcification in the persons with type 2 diabetes compared to the healthy controls, and in the persons with type 2 diabetes and albuminuria compared to normoalbuminuria.[5]

However, knowledge on changes in myocardial microvascular function over time is lacking in persons with type 2 diabetes as well as in the general population.

Therefore, in the present study, we re-examined these participants with the aim to determine the changes over 6 years in myocardial microvascular function.

# 2 | METHODS

## 2.1 | Study population

As previously described, in 2013–2014, we recruited 60 persons with type 2 diabetes from the outpatient clinic at the Steno Diabetes Center Copenhagen, Denmark and 30 age- and sex-matched non-diabetic controls through newspaper advertisements. The persons with diabetes were stratified by albuminuria (≥30 mg/24 h; n = 30) and normoalbuminuria (<30 mg/24 h; n = 30). Participants were aged between 35 and 80 years and free of overt cardiovascular disease. All participants underwent a cardiac $^{82}$Rb PET/CT myocardial perfusion scan and a comprehensive assessment of other cardiovascular risk factors. Subjects were excluded if they had a history of coronary artery disease or other cardiovascular diseases, including stroke, uncontrolled arrhythmia, second- or third-degree atrioventricular block, sick sinus syndrome, clinic blood pressure >200/110 mmHg, kidney disease other than diabetic nephropathy, end-stage kidney disease, chronic obstructive pulmonary disease or asthma (defined as requirement of daily medical treatment) or if they were pregnant or lactating.

Survivors (n = 82) were invited to this re-examination in 2019. In total, 48 eligible persons, 29 with type 2 diabetes and 19 controls participated (response rate, 58.5%). The reasons for non-participation were intercurrent severe illness (n = 5), relocation (n = 1), none-responders (n = 8) or lack of interest (n = 20).

## 2.2 | Clinical measurements

Laboratory variables included HbA$_{1c}$, lipid profile and plasma creatinine which were measured by standard methods and estimated glomerular filtration rate (eGFR) calculated by the CKD-EPI equation.[6] Urine albumin-to-creatinine ratio (UACR) was measured in two morning urine samples by an enzyme immunoassay and calculated as the geometric mean of the two collections. Demographic characteristics, body mass index (kg/m$^2$), smoking status and a detailed medical history along with information on medical treatment were obtained at the visit at Steno Diabetes Center Copenhagen. Clinic blood pressure was measured after a 10-minute rest, using an appropriately sized cuff, and the mean of three measurements was calculated.[7] We also obtained a standard 12-lead resting electrocardiogram (ECG).

All persons attending the outpatient clinic at Steno Diabetes Center Copenhagen have regular ophthalmology examinations (approximately every 1–2 years) where retinal photography is taken through a dilated pupil by certified eye nurses and the images are graded by the certified eye nurses under the supervision of an ophthalmologist. Overall grading is defined as the highest stage diagnosed in either eye. Data regarding grading are stored in local electronic medical records that we accessed for the purpose of this study.

All measurements were performed similarly at the baseline and follow-up visit with the exception that albuminuria was measured in two 24-hour urine collections and estimated

as urinary albumin excretion rate (UAER) at the baseline visit.

## 2.3 | Cardiac PET/CT imaging

The cardiac $^{82}$Rb PET/CT myocardial perfusion scan was performed with similar equipment, protocol and analysed using identical software by the same single observer at the baseline and follow-up visit blinded to other characteristics.

The cardiac $^{82}$Rb PET/CT myocardial perfusion scans were performed using a hybrid PET/CT scan in 3D mode (Siemens Biograph mCT 128, Siemens, Munich, Germany) after administration of 1,100 MBq $^{82}$Rb (CardioGen-82, Bracco Diagnostics). The scans were performed at rest and during stress after adenosine infusion at 140 µg/kg/min for 6 min to induce maximum myocardial hyperaemia. The MBF was automatically calculated at rest and during stress using the Syngovia software (syngombf vb20a) with one-compartment tracer kinetic models for $^{82}$Rb and the extraction curve from Lortie et al.[8] Participants refrained from caffeine containing substances for at least 18 hours prior to the examination.

CACS was calculated as the sum of coronary artery calcium content in the three main coronary arteries by the method described by Agatston et al.[9] using semi-automated commercially available software (Syngovia 4.0).

The study was performed in compliance with the Declaration of Helsinki. All participants gave informed written consent, and the study protocol was approved by the Research Ethics Committee (H-19024534).

## 2.4 | Statistics

All participants with a baseline and a follow-up cardiac $^{82}$Rb PET/CT myocardial perfusion scan were included in the analysis. Continuous normal distributed variables are presented as mean and standard deviation (SD), and the non-normal distributed variables as median and interquartile range (IQR). Categorical variables are summarized as percentages. The non-normal distributed variables, including the CACS, were log2 transformed before analyses. A value of 1 was added to the CACS before log2 transformation, since the unequal distribution included values of zero.

Unpaired-samples $t$-test and the $\chi^2$ or Fisher's exact test were applied to assess whether baseline risk factors differed between participants with and without a follow-up examination.

Changes between the two visits were calculated using paired-samples t-tests for continuous variable and the McNemar's test for categorical variable. Group-wise comparison of changes between visits for participants with diabetes and controls was calculated using unpaired samples $t$-test and

$\chi^2$ test or Fisher's exact test, as appropriate. Analysis of covariance was applied to compare changes between visits among the participants with type 2 diabetes and the controls after adjustment for sex, baseline age, systolic blood pressure, eGFR and UAER. Analyses of MFR and MBF at rest and during stress were additionally adjusted for baseline heart rate. Owing to bias by indication, we did not include variables for medical treatment. Moreover, total cholesterol was not included because the participants with diabetes had lower levels than controls, likely due to lipid-lowering treatment.

Unadjusted and adjusted linear regression models were applied to ascertain the baseline characteristics or the change in risk factors between examinations that were associated with changes in myocardial microvascular function and CACS between the two visits in the total population. The adjustment included sex, baseline age, HbA1c, systolic blood pressure, eGFR, UAER and heart rate, as appropriate.

In all the analyses, the model assumptions were ascertained.

We performed a post-hoc power calculation to estimate the smallest effect size that we were able to detect given the sample size of 29 participants with type 2 diabetes and 19 controls. Achieving a power of 0.80 and an alpha of 0.05 and with the assumption of a standard deviation in delta MFR of 0.64 and 0.77 in type 2 diabetes and controls, respectively; and of 0.69 in the total cohort, the smallest effect we could detect was a MFR change equal to 0.35 in the participants with type 2 diabetes and 0.53 in the controls; and a difference in MFR change equals to 0.59 when comparing the groups.

Two-sided $p$ values $< 0.05$ were considered statistically significant. Statistical analyses were performed using R software (Solexc5_2020).

## 3 | RESULTS

The 48 participants with a follow-up examination had a higher eGFR ($p = 0.04$) and MFR ($p < 0.001$) and a lower CACS ($p = 0.02$) at the baseline examination compared to the 36 subjects who were known to be alive, but did not have a follow-up examination. The 48 participants were also younger ($p = 0.001$) and had a higher MFR ($p = 0.02$) than the participants who died before the follow-up examination. Table 1 presents the clinical characteristics at the baseline visit of participants with follow-up, without follow-up but known to be alive and the participants who died before follow-up, stratified by type 2 diabetes and controls.

### 3.1 | Clinical characteristics

At follow-up, the total population of 29 participants with type 2 diabetes and 19 controls had a mean age of 66.4 years (SD:

**TABLE 1** Clinical characteristics at the baseline visit of participants with follow-up, without follow-up but alive and participants who died before follow-up

| Characteristics | Diabetes | | | p, with follow-up vs. without but alive | p, with follow-up vs dead |
| | With follow-up (n = 29) | Without follow-up, but known to be alive (n = 25) | Dead (n = 6) | | |
| --- | --- | --- | --- | --- | --- |
| Female (%) | 27.6 | 40.0 | 33.3 | 0.39 | 1.0 |
| Age (years) | 60.7 (9.6) | 64.6 (7.7) | 69.9 (4.6) | 0.10 | 0.003 |
| Body mass index (kg/m$^2$) | 31.4 (5.0) | 32.0 (4.1) | 30.4 (4.0) | 0.62 | 0.58 |
| Systolic blood pressure (mmHg) | 138.1 (16.3) | 140.7 (20.0) | 135.0 (15.0) | 0.60 | 0.66 |
| Diastolic blood pressure (mmHg) | 80.5 (10.0) | 82.5 (9.2) | 71.8 (9.9) | 0.44 | 0.09 |
| HbA$_{1c}$ (mmol/mol) | 53 (10) | 60 (12) | 52 (10) | 0.03 | 0.79 |
| HbA$_{1c}$ (%) | 7.0 (0.9) | 7.6 (1.1) | 6.9 (0.9) | 0.03 | 0.79 |
| LDL cholesterol (mmol/l) | 2.3 (0.8) | 2.2 (1.0) | 1.6 (0.7) | 0.69 | 0.08 |
| Total cholesterol (mmol/l) | 4.4 (0.9) | 4.3 (0.9) | 3.9 (0.8) | 0.89 | 0.21 |
| eGFR (ml min$^{-1}$ 1.73 m$^{-2}$) | 81.1 (21.5) | 73.1 (25.6) | 64.2 (26.4) | 0.23 | 0.19 |
| Urinary albumin excretion rate (mg/24 h) | 27.3 [6.5, 135.6] | 31.0 [6.0, 151.0] | 37.0 [16.4, 462.1] | 0.91 | 0.44 |
| Current smokers (%) | 20.7 | 20.0 | 50.0 | 1.0 | 0.16 |
| Myocardial flow reserve | 2.6 (0.7) | 2.2 (0.8) | 1.5 (0.4) | 0.02 | 0.0003 |
| Coronary artery calcium score | 180 [22, 275] | 388 [41, 1025] | 167 [150, 1379] | 0.11 | 0.35 |

Data are %, mean (SD), geometric mean [IQR] (urinary albumin excretion rate) or median [IQR] (coronary artery calcium score). eGFR estimated glomerular filtration rate. p values for difference between participants with and without follow-up examination were calculated using unpaired samples t-test, the $\chi^2$ test or Fisher's exact test, as appropriate.

9.3) and 33% were females. The median duration of type 2 diabetes was 18.3 years (IQR: 11.2–21.5). None were smokers at the follow-up examination.

The median duration between examinations was 6.2 years (IQR: 6.1–6.3). Clinical characteristics of the participants at baseline and follow-up, for the type 2 diabetes and the control group, are presented in Table 2. Both groups had significantly lower LDL cholesterol, total cholesterol and triglycerides at follow-up compared to baseline. There was no significant change in level of albuminuria or in frequency/grading of retinopathy during follow-up. However, four of the six participants with non-proliferative retinopathy, had progressed from mild to moderate non-proliferative retinopathy. These four participants remained in the category as simplex retinopathy, even though they had some progression. At follow-up, all the participants with type 2 diabetes were treated with dietary modifications and oral glucose-lowering medication, moreover 69% received insulin.

## 3.2 | Change in cardiac PET/CT measurements between visits

Figure 1 presents the cardiac PET/CT measurements at baseline and at follow-up. MFR was lower in persons with type 2 diabetes compared to controls at baseline and at follow-up (baseline: type 2 diabetes: mean 2.6 [SD: 0.7], controls: 3.3 [SD: 0.7], $p = 0.002$; and follow-up: type 2 diabetes: 2.4 [SD: 0.6] controls: 3.2 [SD: 0.9], $p = 0.002$). However, change in MFR did not differ from baseline to follow-up in neither type 2 diabetes nor in controls (type 2 diabetes: mean difference: −0.22 [95% CI: −0.47 to 0.02] $p = 0.08$; controls: −0.12 [−0.49 to 0.25] $p = 0.51$) or between groups (mean difference: −0.10 [−0.52, 0.31] $p = 0.64$).

The participants with type 2 diabetes had a higher MBF at rest and a lower MBF during stress compared to the controls, albeit this difference was only significant for resting MBF at the baseline visit (type 2 diabetes: mean 1.2 [SD:

| Controls | | | | |
| --- | --- | --- | --- | --- |
| With follow-up (n = 19) | Without follow-up but known to be alive (n = 9) | Dead (n = 2) | p, with follow-up vs. without but alive | p, with follow-up vs dead |
| 42.1 | 44.4 | 0.0 | 1.0 | — |
| 59.4 (9.2) | 59.3 (12.1) | 66.7 (7.8) | 0.97 | 0.40 |
| 24.1 (3.4) | 25.6 (3.6) | 25.4 (0.8) | 0.18 | 0.21 |
| 124.5 (13.1) | 137.9 (23.4) | 142.5 (13.4) | 0.14 | 0.29 |
| 74 (7.8) | 82.3 (11.9) | 85.5 (7.8) | 0.08 | 0.26 |
| 36 (2) | 37 (2) | 35 (1) | 0.11 | 0.24 |
| 5.4 (0.2) | 5.5 (0.2) | 5.3 (0.1) | 0.11 | 0.24 |
| 3.2 (0.7) | 3.8 (0.6) | 3.7 (0.3) | 0.03 | 0.14 |
| 5.4 (0.8) | 5.7 (0.7) | 5.9 (0.3) | 0.34 | 0.17 |
| 87.6 (11.1) | 73.1 (11.5) | 80.3 (20.2) | 0.007 | 0.70 |
| 5.5 [5.0, 6.3] | 8.7 [4.9, 10.8] | 16.3 [14.0, 19.1] | 0.34 | 0.16 |
| 15.8 | 11.1 | 0.0 | 1.0 | — |
| 3.3 (0.7) | 2.6 (0.4) | 2.9 (1.2) | 0.001 | 0.70 |
| 0 [0, 54] | 81 [0, 205] | 296 [220, 372] | 0.08 | 0.32 |

0.3], controls: mean 1.0 [SD: 0.2], $p = 0.001$). Changes in MBF at rest and during stress did not differ from baseline to follow-up within the groups, although MBF during stress appeared to decline in type 2 diabetes, albeit not significant. When comparing type 2 diabetes to controls, we found a significant decline in MBF during stress in the participants with type 2 diabetes (mean difference: −0.30 [95% CI: −0.55 to −0.04] $p = 0.02$), and this difference remained significant after adjustment for sex, baseline age, systolic blood pressure, eGFR, UAER and heart rate ($p = 0.01$). Change in MBF at rest was similar between groups ($p = 0.29$).

CACS was higher in persons with type 2 diabetes compared to controls at baseline and at follow-up (baseline: type 2 diabetes: median 180 [IQR: 22–275], controls: 0 [IQR: 0–52] $p = 0.003$ and follow-up: type 2 diabetes: 560 [IQR: 139–959], controls: 18 [IQR: 0–108] $p < 0.001$) (Figure 1), and CACS increased within both groups (type 2 diabetes: mean difference: 512 [95% CI: 282–741] $p < 0.001$ and

controls: 45 [95% CI: 10–81] $p = 0.015$). Moreover, the increase was higher in persons with type 2 diabetes compared to controls (mean difference: 469 [95% CI: 192–744] $p = 0.002$). This difference remained significant after adjustment for sex, baseline age, systolic blood pressure, eGFR and UAER ($p = 0.03$).

### 3.3 | Variables correlated with changes in the cardiac PET/CT measurements

Overall, in the cohort, lower eGFR at baseline was associated with a more pronounced decline in MFR from baseline to follow-up ($p = 0.03$), but not after adjustment for sex, baseline age, HbA$_{1c}$, systolic blood pressure, UAER and heart rate ($p = 0.11$). Change in MFR was not associated with the duration of diabetes or with other risk factors at baseline or change in risk factors between examinations, and there was no association between change in MFR and change in CACS ($p = 0.88$).

**TABLE 2** Clinical characteristics of participants at baseline and follow-up

| Characteristics | Diabetes (n = 29) | | | Controls (n = 19) | | |
|---|---|---|---|---|---|---|
| | Baseline | Follow-up | p difference between visits | Baseline | Follow-up | p difference between visits |
| Female (%) | 27.6 | — | — | 42.1% | — | — |
| Age (years) | 60.7 (9.6) | 66.9 (9.5) | — | 59.4 (9.2) | 65.6 (9.3) | — |
| Body mass index (kg/m$^2$) | 31.4 (5.0) | 31.1 (4.2) | 0.32 | 24.1 (3.4) | 25.1 (4.0) | 0.002 |
| Systolic blood pressure (mmHg) | 138.1 (16.3) | 142.8 (15.0) | 0.08 | 124.5 (13.1) | 128.5 (12.4) | 0.05 |
| Diastolic blood pressure (mmHg) | 80.4 (10.0) | 79.0 (9.1) | 0.38 | 74 (7.8) | 75.4 (5.8) | 0.37 |
| HbA$_{1c}$ (mmol/mol) | 53 (10) | 57 (14) | 0.11 | 36 (2) | 37 (2) | 0.01 |
| HbA$_{1c}$ (%) | 7.0 (0.9) | 7.4 (1.3) | 0.11 | 5.4 (0.2) | 5.5 (0.2) | 0.01 |
| LDL cholesterol (mmol/l) | 2.3 (0.8) | 1.7 (0.6) | <0.001 | 3.2 (0.7) | 2.6 (0.6) | <0.001 |
| Total cholesterol (mmol/l) | 4.4 (0.9) | 3.6 (0.7) | <0.001 | 5.4 (0.8) | 5.1 (0.8) | 0.002 |
| Triglyceride (mmol/l) | 2.0 (1.0) | 1.5 (0.6) | 0.004 | 1.2 (0.5) | 0.9 (0.4) | 0.01 |
| eGFR (ml min$^{-1}$ 1.73 m$^{-2}$) | 81.1 (21.5) | 74.4 (23.4) | <0.001 | 87.6 (11.1) | 82.5 (10.4) | 0.002 |
| Urinary albumin excretion rate (mg/24 h)[a] | 27.3 [6.5, 145.0] | 8.5 [4.5, 145.5] | 0.18 | 5.5 [5.0, 6.5] | 4.0 [3.0, 6.5] | 0.02 |
| Retinopathy: none/simplex/ proliferative (%) | 69.0/20.7/10.3 | 69.0/20.7/10.3 | 1.0 | 0.0 | 0.0 | — |
| Current smokers (%) | 20.7 | 0.0 | — | 15.8 | 0.0 | — |
| RAAS inhibition treatment (%) | 86.2 | 86.2 | 1.0 | 0.0 | 5.3 | — |
| Beta-blocker treatment (%) | 10.3 | 6.9 | 0.20 | 0.0 | 5.3 | |
| Insulin treatment (%) | 55.2 | 69.0 | 0.04 | 0.0 | 0.0 | — |
| Metformin (%) | 96.6 | 69.0 | 0.01 | 0.0 | 0.0 | — |
| Dipeptidyl peptidase−4 inhibitor (%) | 10.3 | 13.8 | 1.0 | 0.0 | 0.0 | — |
| Sulfonylureas (%) | 3.4 | 3.4 | 1.0 | 0.0 | 0.0 | — |
| SGLT2 inhibitor treatment (%) | 0.0 | 38.0 | — | 0.0 | 0.0 | — |
| GLP−1 receptor agonist treatment (%) | 0.0 | 55.1 | — | 0.0 | 0.0 | — |
| Lipid-lowering treatment (%) | 93.1 | 93.1 | 1.0 | 0.0 | 10.5 | — |

Data are %, mean (SD) or geometric mean [IQR]. eGFR: estimated glomerular filtration rate, SGLT2: sodium-glucose co-transporter-2, GLP-1: Glucagon Like Peptide 1, RAAS: renin–angiotensin–aldosterone system.

p values for changes between visits were calculated using paired-samples t-tests for continuous variable and the McNemar's test for categorical variable.

[a]Urinary albumin creatinine rate (mg/g) for the follow-up visit.

Participants with lower eGFR at baseline had a more pronounced reduction in MBF during stress ($p < 0.001$). This association remained significant after adjustment for sex, baseline age, HbA$_{1c}$, systolic blood pressure, UAER and heart rate ($p < 0.001$). The temporal stress-induced reduction in MBF was also more pronounced with higher CACS at baseline ($p = 0.02$), but not after adjustment for other cardiovascular risk factors ($p = 0.11$). There were no associations between change in MBF at rest and other risk factors at baseline or change in risk factors between examinations.

## 3.4 | Sensitivity analyses

Three participants with type 2 diabetes (10%) had undergone percutaneous coronary intervention and one participant with type 2 diabetes had a stroke between baseline and follow-up examination. Two of the participants with percutaneous coronary intervention had a CV event and one participant had undergone the coronary intervention as a result of the cardiac PET/CT findings during the baseline examination. None of the controls had a CV event. To avoid the potential confounding effect of coronary intervention on the epicardial
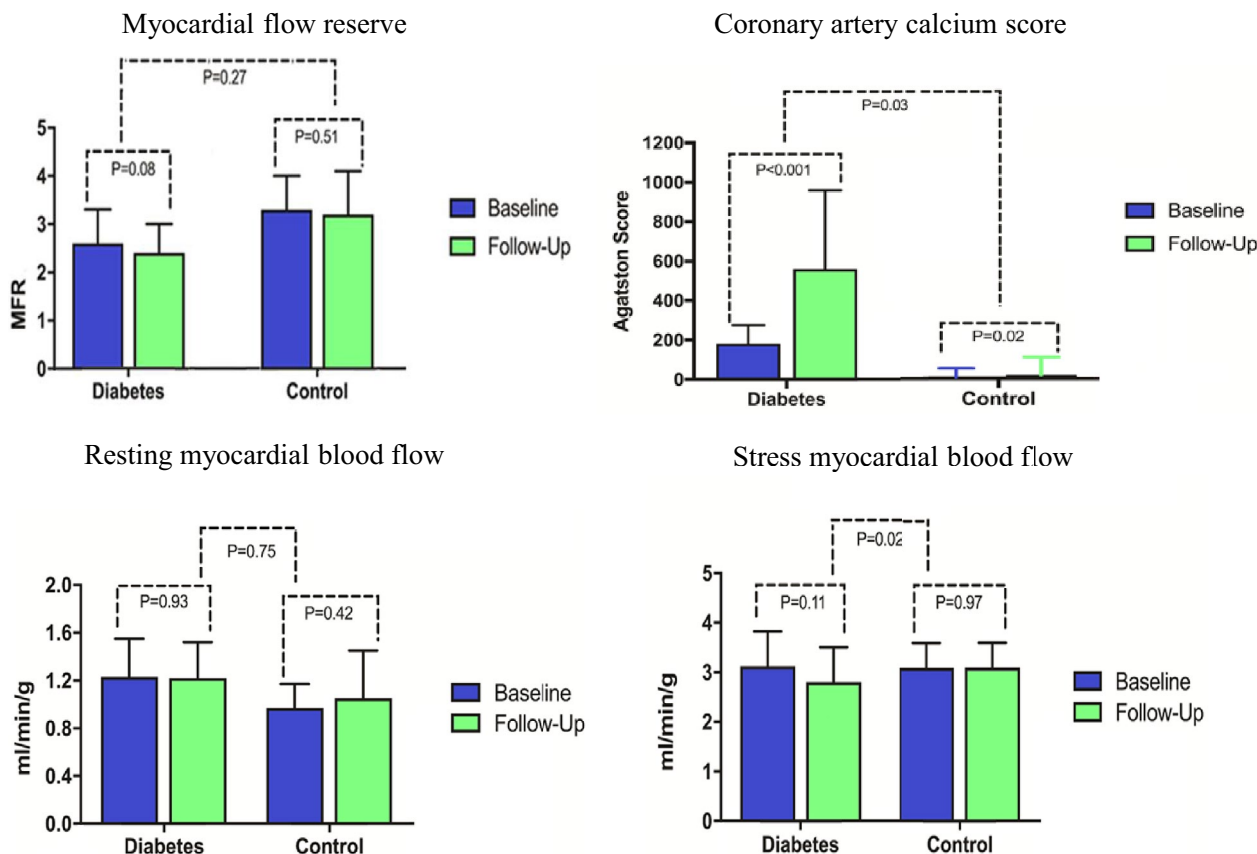
**FIGURE 1** PET/CT measurements at baseline and follow-up. The myocardial flow reserve and the myocardial blood flow at rest and during stress are presented as mean with standard derivation and the coronary artery calcium score is presented as median with interquartile range. P values for changes between visits were calculated using paired-samples t-tests and group-wise comparison of changes between visits for participants with diabetes and controls was calculated using unpaired samples t-test. Analysis of covariance was applied for the group-wise comparison to adjust for sex, baseline age, systolic blood pressure, estimated glomerular filtration rate and urinary albumin excretion rate. Analyses of myocardial flow reserve and the myocardial blood flow at rest and during stress were additionally adjusted for baseline heart rate.

arteries between visits, we performed a sensitivity analysis excluding the three participants who had coronary intervention between the visits. Results were confirmatory.

As a sensitivity analysis, we also performed additional adjustment for baseline total cholesterol in the comparison between participants with type 2 diabetes and controls for the changes in CACS between visits, which attenuated our findings slightly ($p = 0.07$).

## 4 | DISCUSSION

We re-examined 29 persons with type 2 diabetes and 19 controls with cardiac PET/CT myocardial perfusion scan to gain knowledge on changes in myocardial microvascular function over 6 years. The main findings were as follows: (a) the MFR did not change significantly in either of the two groups; (b) the MBF during stress declined more during the follow-up period in persons with type 2 diabetes compared to the controls; (c) lower baseline eGFR was associated with a more pronounced

stress-induced blood flow reduction and a decline in the MFR and (d) higher increase in CACS was associated with a more pronounced blood flow reduction during stress.

We could not demonstrate significant changes in any of the groups, and only the blood flow reduction during stress was more pronounced in the presence of type 2 diabetes. We speculate that this could indicate that significant changes in myocardial microvascular function occur earlier during the natural history of diabetes, as the baseline MFR in the persons with type 2 diabetes, with a median diabetes duration of 12.2 years (IQR: 5.1–14.9), was already significantly reduced compared to the controls at the baseline visit.[5] Moreover, the change in myocardial microvascular function might develop slower than expected, at least with the modern treatment, and the time span of 6 years may be too short to detect significant changes. In addition, other markers of microvascular damage (urine albumin excretion rate and diabetic retinopathy) did not exacerbate either in our study and participants were well treated (Table 1), although we might have been able to detect progression in retinopathy if the grading had been

more detailed. So, despite our findings with lack of change in the myocardial microvascular function over time were unexpected, it is in consistence, as neither of our microvascular outcomes changed. Other explanations for these distinct findings are the smaller sample size and bias related to selective follow-up, as discussed later.

A recent study suggested that reduced MFR in type 2 diabetes is caused by a combination of increased coronary perfusion at rest and decreased maximal perfusion during stress, evaluated by cardiac magnetic resonance.[10] In line with this, we found that the persons with type 2 diabetes had a significantly higher resting myocardial flow at the baseline examination when compared to the controls, and a similar trend was shown 6 years later at the re-examination. During follow-up, a more pronounced reduction in stress-induced MBF was observed in persons with type 2 diabetes compared to controls, suggesting that persons with type 2 diabetes over time develop progressive attenuation of stress-induced increase in MBF, signifying progression in myocardial microvascular dysfunction. The literature examining the prognostic significance of a reduced MBF during stress is conflicting.[11-13]

At baseline, a lower MFR was associated with a higher UAER and a lower eGFR, although only the association with UAER was independent of other risk factors.[5] At the re-examination, we could not demonstrate an association between changes in myocardial microvascular function and the baseline level of UAER or the change in albuminuria between visits. However, a lower baseline eGFR was associated with a more pronounced stress-induced blood flow reduction and a decline in MFR. This suggests that lower renal function is associated with development of cardiac microvascular dysfunction, especially with an attenuated ability to increase the myocardial blood flow during stress. This unifies a possible pathogenesis between cardiovascular and renal disease, and lower renal function may represent a current determinant of progression of myocardial microvascular dysfunction.

At the baseline examination, a lower MFR was associated with higher CACS independent of other risk factors.[5] At the re-examination, we demonstrated that a larger increase in CACS was associated with a greater myocardial blood flow reduction during hyperaemic conditions. This highlights the relation between coronary microvascular dysfunction and atherosclerosis in the epicardial arteries. However, we could not demonstrate an association between changes in MFR and CACS, suggesting that the increased atherosclerotic plaque burden in the coronary arteries was without haemodynamic significance and that the development of these functional and structural alterations might not be straightforward, and these measures might represent different pathophysiology and differences in time course and may thus provide complimentary prognostic information.

Notably, both groups had significantly lower LDL cholesterol, total cholesterol and triglycerides at follow-up compared to baseline, although there were no changes in the proportion receiving lipid-lowering treatment among the participants with type 2 diabetes and only two controls started treatment after the baseline examination. We speculate that the drop in lipids among the type 2 diabetes could be due to dose increase in lipid-lowering treatment between examinations, as 21% received a higher dose at the follow-up examination; moreover, 34.5% of the participants had also changed treatment from one statin drug to another, and the drug efficacy might differ among statins. Additionally, 55.1% received glucagon-like peptide-1 receptor agonists at the follow-up examination, which has also been reported to reduce LDL cholesterol. Other explanations could be that the participants have become more compliant with their lipid-lowering treatment after participating in the first examination, since they were informed on the level of the coronary artery calcium score.

# 5 | STRENGTHS AND LIMITATIONS

The strength of our study is the well-characterized cohort and the gold-standard measure of coronary microvascular function using cardiac $^{82}$Rb PET/CT myocardial perfusion scan, with similar equipment and protocol at both examinations. However, the results must be interpreted within the context of its potential limitations. First, the impact of selection bias. The persons who did not attend the re-examination were older, had lower kidney function and MFR and had higher CACS at baseline than the persons who participated. It is therefore possible that they had developed larger changes in myocardial microvascular function and coronary calcification, and non-participation may, consequently, have weakened the true progression in the population. Also, the second measurement may not be so different from the first due to rather stable condition of other risk factors over the two timepoints. Moreover, the limited sample size at the re-examination increases the likelihood of type II errors. Based on our post-hoc power calculation, we were able to detect a change in MFR of 0.35 in the participants with type 2 diabetes and of 0.53 in the controls; moreover, we could detect a difference in MFR change of 0.59 between groups. Consequently, we cannot reject the possibility of a smaller change. Larger prospective studies with a longer follow-up are therefore needed.

# 6 | CONCLUSION

The myocardial microvascular function was impaired in persons with type 2 diabetes compared to controls but did not change significantly in either of the groups when evaluated over 6 years. Impaired renal function may represent a risk factor for progression of myocardial microvascular dysfunction. Progression in coronary atherosclerosis was higher in persons with type 2 diabetes than controls and related to changes in myocardial blood flow during stress.

## 7 | DUALITY OF INTEREST

## 8 | CONTRIBUTION STATEMENT

IKBR acquired the data, performed the statistical analysis and drafted the manuscript. PH, RR, AK and PR conceived and designed the research, acquired the data, handled funding and supervision, and critically revised the manuscript for key intellectual content. JCL, EHZ, BJvS and LH acquired data and critically revised the manuscript for key intellectual content. LJD contributed to the revision with the post-hoc power calculation and critically edited the revised manuscript. TWH conceived and designed the research, acquired the data, performed the statistical analysis and critically revised the manuscript for key intellectual content. All authors have approved the final version of the manuscript. TWH is responsible for the integrity of the work as a whole.

## 9 | NOVELTY STATEMENT

What is already known?

- The myocardial microvascular function may provide additional and independent risk information beyond the extent of coronary atherosclerosis.

What this study has found?

When evaluated over 6 years:

- The myocardial microvascular function did not change significantly in persons with type 2 diabetes or in controls.
- The myocardial blood flow reduction during induced hyperaemic conditions was more pronounced in the presence of diabetes.

What are the clinical implications of the study?

- Persons with type 2 diabetes develop progressive attenuation in myocardial blood flow during induced hyperaemic conditions, signifying progression in myocardial microvascular dysfunction.

## ORCID
*Ida K. B. Rasmussen* https://orcid.org/0000-0001-6849-763X
*Jens C. Laursen* https://orcid.org/0000-0002-5036-8159

## REFERENCES
1. Schindler TH, Dilsizian V. Coronary microvascular dysfunction: clinical considerations and noninvasive diagnosis. *JACC Cardiovasc Imaging*. 2020;13(1 Pt 1):140-155.
2. Murthy VL, Naya M, Foster CR, et al. Association between coronary vascular dysfunction and cardiac mortality in patients with and without diabetes mellitus. *Circulation*. 2012;126(15): 1858-1868.
3. Elkeles RS, Godsland IF, Feher MD, et al. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: the PREDICT study. *Eur Heart J*. 2008;29(18):2244-2251.
4. Assante R, Acampa W, Zampella E, et al. Prognostic value of atherosclerotic burden and coronary vascular function in patients with suspected coronary artery disease. *Eur J Nucl Med Mol Imaging*. 2017;44:2290-2298.
5. von Scholten BJ, Hasbak P, Christensen TE, et al. Cardiac (82) Rb PET/CT for fast and non-invasive assessment of microvascular function and structure in asymptomatic patients with type 2 diabetes. *Diabetologia*. 2016;59(2):371-378.
6. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150: 604-612.
7. Theilade S, Joergensen C, Persson F, Lajer M, Rossing P. Ambulatory tonometric blood pressure measurements in patients with diabetes. *Diabetes Technol Ther*. 2012;14:453-456.
8. Lortie M, Beanlands RS, Yoshinaga K, Klein R, Dasilva JN, DeKemp RA. Quantification of myocardial blood flow with 82Rb dynamic PET imaging. *Eur J Nucl Med Mol Imaging*. 2007;34:1765-1774.
9. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15: 827-832.
10. Sørensen MH, Bojer AS, Pontoppidan JRN, et al. Reduced myocardial perfusion reserve in type 2 diabetes is caused by increased perfusion at rest and decreased maximal perfusion during stress. *Diabetes Care*. 2020;43(6):1285-1292.
11. Murthy VL, Naya M, Foster C, et al. Improved cardiac risk assessment with non-invasive measures of coronary flow reserve. *Circulation*. 2011;124(20):2215-2224.

12. Ziadi M, deKemp R, Williams K, et al. Impaired myocardial flow reserve on rubidium-82 positron emission tomography imaging predicts adverse outcomes in patients assessed for myocardial ischemia. *J Am Coll Cardiol*. 2011; 58(7):740-748.

13. Farhad H, Dunet V, Bachelard K, et al. Added prognostic value of myocardial blood flow quantitation in rubidium-82 positron emission tomography imaging, European heart journal cardiovascular. *Imaging*. 2013;14(12):1203-1210.