Reduced Myocardial Perfusion Reserve in Type 2 Diabetes Is Caused by Increased Perfusion at Rest and Decreased Maximal Perfusion During Stress

Martin H. Sørensen,[1,2] Annemie S. Bojer,[1,2] Julie R.N. Pontoppidan,[1] David A. Broadbent,[3,4] Sven Plein,[4] Per L. Madsen,[5,6] and Peter Gæde[1,2]

Diabetes Care 2020;43:1285–1292 | https://doi.org/10.2337/dc19-2172

OBJECTIVE

To examine differences in myocardial blood flow (MBF) at rest and during stress between patients with type 2 diabetes and control subjects, and to identify potential predictors of changes in MBF at rest and during stress.

RESEARCH DESIGN AND METHODS

A cross-sectional study was conducted of 193 patients with type 2 diabetes and 20 age- and sex-matched control subjects. Cardiovascular magnetic resonance was used to evaluate left ventricular structure and function and MBF at rest and during adenosine-induced stress. MBF was derived as the mean of the flow within all segments of a midventricular slice.

RESULTS

Patients with type 2 diabetes had higher global MBF at rest (0.81 ± 0.19 mL/min/g) and lower global MBF during stress (2.4 ± 0.9 mL/min/g) than control subjects (0.61 ± 0.11 at rest, 3.2 ± 0.8 mL/min/g under stress; both $P < 0.01$). Patients with macroalbuminuria had lower MBF during stress (1.6 ± 0.5 mL/min/g) than did patients with microalbuminuria (2.1 ± 0.7 mL/min/g; $P = 0.04$), who in turn had lower MBF during stress than did normoalbuminuric patients (2.7 ± 0.9 mL/min/g; $P < 0.01$). Patients with severe retinopathy had lower MBF during stress (1.8 ± 0.6 mL/min/g) than patients with simplex retinopathy (2.3 ± 0.7 mL/min/g; $P < 0.05$) and those who did not have retinopathy (2.6 ± 1.0 mL/min/g; $P < 0.05$). Albuminuria and retinopathy were associated with reduced MBF during stress in a multiple regression analysis. Stress-related MBF inversely correlated with myocardial extracellular volume ($P < 0.001$; $R^2 = 0.37$), a measure of diffuse myocardial fibrosis. A trend toward lower basal MBF was observed in patients treated with sodium–glucose cotransporter 2 inhibitors ($P = 0.07$).

CONCLUSIONS

Patients with type 2 diabetes have higher global MBF at rest and lower maximal MBF during vasodilator-induced stress than control subjects. Reduced MBF during stress is associated with diabetes complications (albuminuria and retinopathy) and is inversely correlated with diffuse myocardial fibrosis.

[1]Department of Cardiology and Endocrinology, Slagelse Hospital, Region Zealand, Denmark
[2]Institute of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
[3]Department of Medical Physics and Engineering, Leeds Teaching Hospitals NHS Trust, Leeds, U.K.
[4]Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, U.K.
[5]Department of Cardiology, Copenhagen University Hospital Herlev-Gentofte, Capital Region of Denmark, Denmark
[6]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Corresponding author: Martin H. Sørensen, mahso@regionsjaelland.dk

Received 30 October 2019 and accepted 7 February 2020

Clinical trial reg. no. NCT02684331, clinicaltrials.gov

The burden of cardiovascular disease among patients with type 2 diabetes is well known (1). Type 2 diabetes has long been considered a major risk factor for the development of coronary artery disease, and cardiovascular disease remains the leading cause of death among patients with type 2 diabetes (2,3). In addition, type 2 diabetes is associated with an increased risk of developing heart failure independent of coronary artery disease, age, and hypertension (4); this association has led to the hypothesis that a specific diabetic cardiomyopathy exists (5). Among several mechanisms, myocardial microangiopathy has been proposed as a cause of diabetic cardiomyopathy (6,7), and reduced myocardial perfusion reserve (MPR) in patients with type 2 diabetes has been demonstrated in several previous studies (8–10). We previously demonstrated that reduced MPR is associated with left ventricular (LV) diastolic dysfunction—a key characteristic of diabetic cardiomyopathy (11). Furthermore, patients with type 2 diabetes and albuminuria or retinopathy have lower MPR than patients with uncomplicated type 2 diabetes (10). MPR is defined as the ratio of maximal myocardial perfusion—usually measured during pharmacologically induced stress—to myocardial perfusion during resting conditions. Therefore, reduced MPR can be caused by either increased perfusion during rest or reduced perfusion during stress, but the contribution of either of the two mechanisms to reduced MPR in type 2 diabetes is still controversial. Previous studies trying to determine whether changes in perfusion at rest or during stress are responsible for the reduced MPR in type 2 diabetes have only used small sample sizes and have shown widely divergent results (12–14).

In this report, we present additional data on myocardial perfusion at rest and during pharmacologically induced stress in a large cohort of patients with type 2 diabetes (10). We hypothesized that increased perfusion at rest, decreased perfusion during stress, or both would be responsible for the reduction in MPR known to be associated with type 2 diabetes, and that changes in myocardial perfusion are associated with structural remodeling in the heart and with classic risk factors and complications related to type 2 diabetes. We used cardiac magnetic resonance (CMR) to quantify myocardial perfusion, using a protocol that also included imaging sequences to provide detailed assessments of cardiac function as well as structure and mapping sequences to allow quantitative assessment of intramyocardial diffuse fibrosis.

RESEARCH DESIGN AND METHODS

Recruitment, Study Design, and Participant Characteristics

The study was conducted at the Department of Cardiology and Endocrinology, Slagelse Hospital, and at the Department of Radiology, Næstved Hospital; was approved by the local ethics committee, Region Zealand, Denmark (SJ-490); and complied with the Declaration of Helsinki. All participants gave written informed consent. Detailed information about recruitment, inclusion and exclusion criteria, and the study design have been described before (10). In short, a total of 193 patients with type 2 diabetes were recruited from the Department of Endocrinology at Næstved, Slagelse, and Ringsted (NSR) Hospitals, along with 20 control subjects who did not have type 2 diabetes, hypertension, or coronary artery disease and were not receiving statin therapy. The study was a cross-sectional survey, and all participants underwent blood and urinary sampling, electrocardiography, echocardiography, CMR, and a clinical examination that measured height, weight, resting heart rate, and blood pressure. Medications; history of smoking, coronary artery disease, and hypertension; and duration of diabetes were collected from the electronic patient journal or reported by the patients themselves. Hypertension was defined as blood pressure >140/90 mmHg after 10 min of rest (measured in the clinic) or as having an active prescription of antihypertensive medication. Coronary artery disease was defined as angiographically verified coronary stenosis, previous myocardial infarction, or ischemic lesions on late gadolinium enhancement (LGE) CMR. In patients with type 2 diabetes, albuminuria was defined as a urinary albumin-to-creatinine ratio (ACR) >30 mg/day, and retinopathy was evaluated as present or absent on the basis of the patients' latest fundus photographs, which were routinely taken in the diabetes outpatient clinic. For subgroup analysis, albuminuria was divided into microalbuminuria (ACR 30–300 mg/day) and macroalbuminuria (ACR >300 mg/day), and retinopathy was categorized as simplex or severe (proliferative retinopathy, maculopathy, or previous laser photocoagulation). Autonomic nervous function was evaluated from orthostatic blood pressure measurements at 0.5, 1.5, 3, 5 and 7 min after standing up and from beat-to-beat variation. Autonomic neuropathy was diagnosed in patients with a decrease of 25 mmHg or more in any of the systolic blood pressure measurements (15) or with beat-to-beat variation over <4 bpm (16). Patients were stratified into the categories mono, dual, or combination therapy on the basis of their antidiabetes medication (one, two, or three or more antidiabetes drugs, respectively).

Echocardiography

Two-dimensional echocardiography was performed on a GE Vivid E9 cardiovascular ultrasound system with a GE Vivid S5 probe (GE Healthcare). LV diastolic function was measured through the apical four-chamber view. Peak E was defined as the highest early mitral inflow velocity and was measured by placing a pulsed-wave Doppler across the mitral valve. Septal and lateral mitral annular motion (e*) was measured by using tissue Doppler imaging and by placing a pulsed wave in the lateral and septal mitral annuli. E/e* was calculated from the mean of the lateral and septal e* values.

CMR Protocol

CMR was performed with an Avanto 1.5T scanner (Siemens, Erlangen, Germany) with spine and cardiac coils and electrocardiographic gating; patients were in the supine position. The CMR protocol has previously been published (10); for detailed information about sequence parameters and image acquisition we referred to that report. Patients were instructed to refrain from consuming caffeine in the 24 h before the scan. Data were acquired during a breath-hold at end expiration. From scouting images, the long and short axes of the LV were determined. LV and left atrial (LA) function were evaluated from a cardiac short-axis stack covering the entire heart, from the basis of the left atrium to the apex of the heart; one slice was obtained basal and apical to the heart in order to ensure full coverage.

Myocardial perfusion images were obtained from three short-axis slices (basal, midventricular, and apical levels) by using a saturation recovery pulse sequence with a spoiled gradient echo readout. A contrast dose of 0.075 mg/kg gadobutrol

Table 1—Clinical characteristics of patients with type 2 diabetes and control subjects

	Control subjects (n = 20)	Patients with diabetes (n = 193)
Age (years)	56 ± 10	59 ± 11
Male sex	15 (75)	137 (71)
BMI (kg/m^2)	25.3 ± 3.4	31.1 ± 4.6*
Duration of diabetes (years)	—	13 ± 8
Microalbuminuria	0 (0)	58 (30)*
Macroalbuminuria	0 (0)	12 (6)*
Simplex retinopathy	0 (0)	27 (14)*
Severe retinopathy	0 (0)	27 (14)*
Autonomic neuropathy	0 (0)	49 (25)*
Clinical SBP (mmHg)	132 ± 13	136 ± 19
Resting HR (bpm)	58 ± 10	72 ± 12*
Coronary artery disease	0 (0)	26 (13)
Hypertension	0 (0)	134 (69)
Active or former smoker	10 (50)	127 (66)
HbA_{1c} (% [mmol/mol])	5.4 ± 0.3 (35 ± 3)	7.9 ± 1.4 (63 ± 15)*
Serum creatinine (μmol/L)	81 ± 13	78 ± 26
LDL cholesterol (mmol/L)	3.0 ± 1.0	2.0 ± 0.9*
Total cholesterol (mmol/L)	5.2 ± 1.2	4.3 ± 1.1*
Medication		
ACE inhibitor/ARB	—	144 (75)
Statin	—	139 (72)
Metformin	—	160 (83)
SGLT-2 inhibitor	—	68 (35)
GLP-1 analog	—	76 (39)
Insulin	—	116 (60)
Monotherapy	—	28 (15)
Dual therapy	—	76 (39)
Combination therapy	—	89 (46)

Data are presented as the mean ± SD or n (%). Dash (—) indicates not applicable. ARB, angiotensin receptor blocker; GLP-1, glucagon-like peptide 1; HR, heart rate; SBP, systolic blood pressure. *$P <$ 0.05 vs. control subjects.

(Gadovist; Bayer AG) was administered at a rate of 5 mL/s, followed by 20 mL saline. A stress perfusion scan was performed during maximal vasodilation, which was stimulated by adenosine infusion at a dose of 140 μg/kg/min for 3 min. After at least a 10-min delay after stress perfusion imaging, with the patient at rest, a perfusion scan was performed in an identical slice location, following the same approach as for the stress perfusion scan.

T_1 maps were acquired in three short-axis slices, at similar positions as for the perfusion sequences, by using a shortened modified Look-Locker inversion recovery sequence during a breath hold. Native T_1 mapping was performed before the stress perfusion scan; T_1 mapping was performed 10 min after the contrast injection and just before the rest perfusion scan.

Following time of inversion scout sequence, LGE images were acquired using a phase-sensitive inversion recovery reconstruction sequence and were taken from the entire LV short-axis stack and from the two-, three-, and four-chamber views. LGE lesions were considered positive if they were present in two or more views.

CMR Data Analysis

CMR scans were analyzed with cmr42 version 5.5.1 (Circle Cardiovascular Imaging Inc., Calgary, Canada). Epicardial and endocardial borders were traced semi-automatically in the LV cine stack in order to calculate LV end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), ejection fraction, and mass. The maximal LA volume was calculated by manually tracing the left atrium in the LV end-systolic frame.

Myocardial blood flow (MBF) was quantified by using an in-house tool developed in MATLAB release 2015b (MathWorks, Natick, MA). The midventricular slice was analyzed (unless it was corrupted by artifact, in which case the basal slice was used). Regions of interest were drawn in the LV blood pool in the motion-corrected perfusion series and in the corresponding slice of the T_1 map. Endocardial and epicardial borders and the right ventricular insertion point were also defined on the motion-corrected perfusion series in order to allow segmentation based on the American Heart Association 16-segment model (17). From these regions, signal-time data and baseline blood T_1 were extracted. Baseline blood signal intensity was defined as the mean of values from the first three perfusion images. The nonlinear response of signal intensity to contrast agent concentration was corrected for on the basis of the baseline T_1 and signal intensity data (18). Data were cropped to the end of the first pass, identified visually from a plot of the LV blood signal-time data (the arterial input function). Fermi-constrained deconvolution (19) was performed on the cropped data to estimate mean MBF for each of the six myocardial segments.

Myocardial extracellular volume (ECV) was calculated from native and postcontrast myocardial T_1 times and hematocrit values, as previously described (20). ECV was calculated as the mean of values from the six midventricular segments. Any areas with infarction on LGE images were excluded.

Statistical Analysis

All statistical calculations were made in SAS Enterprise Guide version 7.15 (SAS Institute Inc., Cary, NC). Continuous variables were expressed as the mean ± SD and categorical variables, as percentages. Continuous variables were compared by using an unpaired two-tailed Student t test, and categorical variables were compared by using the χ^2 test or the Fisher exact test, as appropriate. Multiple groups were compared by using ANOVA. A general linear model was used for analyzing explanatory variables. Univariate analyses were performed to identify predictors for increased perfusion at rest and decreased perfusion during stress. Variables significant at a 10% level in the univariate analysis were included in a backward stepwise multivariable analysis based on a linear regression model. A two-tailed P value <0.05 was considered statistically significant.

RESULTS

Clinical characteristics of study participants are shown in Table 1. Patients with type 2 diabetes had higher MBF at rest

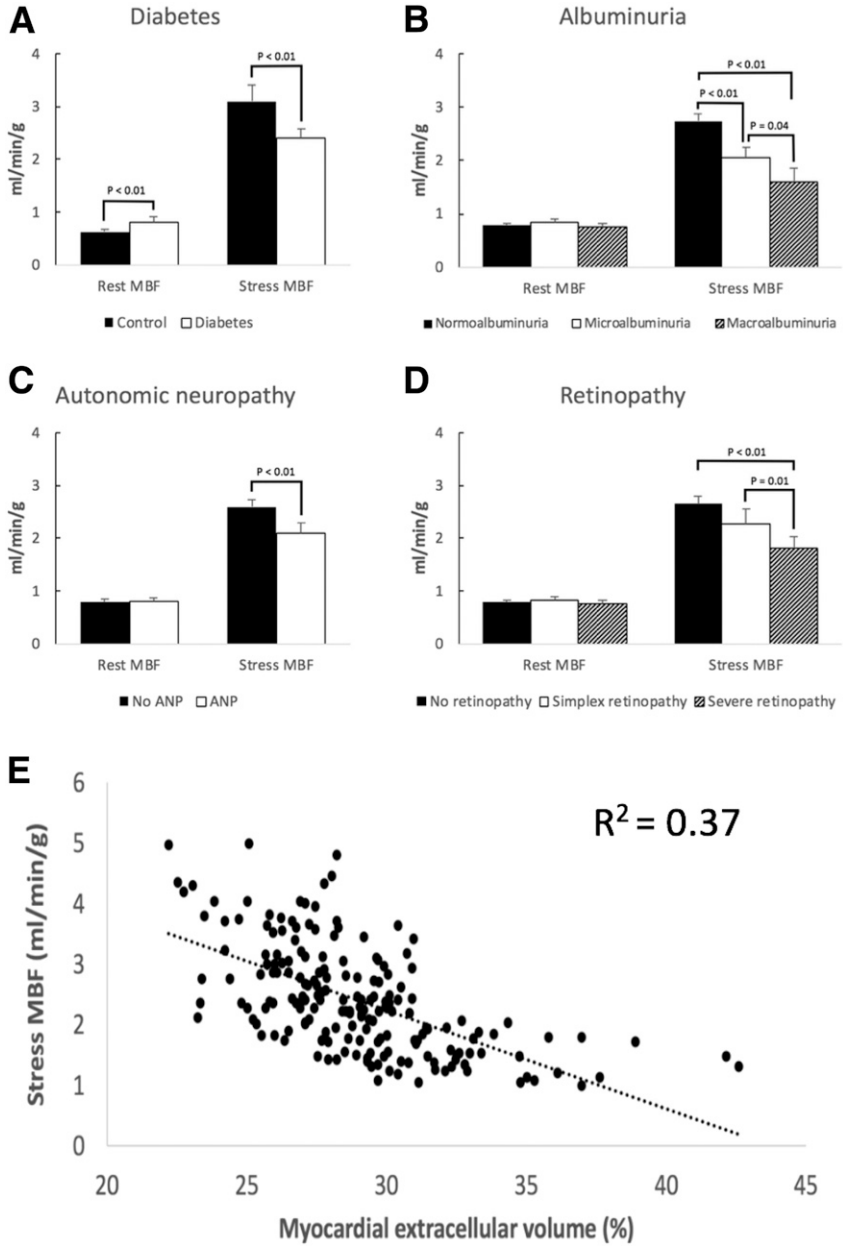

Figure 1—*A*: MBF at rest and during stress in patients with type 2 diabetes and in control subjects. *B–D*: MBF at rest and during stress in patients with type 2 diabetes with and without various diabetes complications: albuminuria (*B*), autonomic neuropathy (ANP) (*C*), and retinopathy (*D*). *E*: Correlation between MBF during stress and myocardial ECV in patients with type 2 diabetes.

(0.81 ± 0.19 mL/min/g) and lower MBF during stress (2.4 ± 0.9 mL/min/g) than control subjects (0.61 ± 0.11 mL/min/g at rest, 3.2 ± 0.8 mL/min/g during stress; $P < 0.01$ for both) (Fig. 1*A*). MBF during stress was significantly lower in patients with microalbuminuria (2.1 ± 0.7 mL/min/g) or with macroalbuminuria (1.6 ± 0.5 mL/min/g) than in normoalbuminuric patients (2.7 ± 0.9 mL/min/g; $P < 0.01$ for both), and patients with macroalbuminuria had significantly lower MBF during stress (2.1 ± 0.7 mL/min/g) than patients with microalbuminuria (1.6 ± 0.5 mL/min/g; $P = 0.04$) (Fig. 1*B*). Autonomic neuropathy was associated with a reduction in MBF under stress: 2.1 ± 0.7 mL/min/g in patients with the condition and 2.6 ± 0.9 mL/min/g in those without it ($P < 0.01$) (Fig. 1*C*). Patients with severe retinopathy had significantly lower MBF during stress (1.8 ± 0.6 mL/min/g) than patients with simplex retinopathy (2.3 ± 0.7 mL/min/g; $P = 0.01$) and patients without any signs of retinopathy on fundus photography (12.6 ± 1.0 mL/min/g; $P < 0.01$). The difference in MBF during stress between patients with simplex retinopathy (2.3 ± 0.7 mL/min/g) and patients without any signs of retinopathy (2.6 ± 1.0 mL/min/g) was of borderline significance ($P = 0.07$) (Fig. 1*D*). We observed no significant changes in MBF at rest in patients with type 2 diabetes who had and those who did not have complications.

Measurements of LV structure and function in patients with type 2 diabetes are shown in Table 2. Patients are presented in groups based on median MBF at rest and during stress. We found no differences in any of our measured parameters when comparing patients with MBF at rest below and above the median. Patients with MBF that was below the median during stress had higher E/e* (8.4 ± 2.9), LV mass (149 ± 40), LA maximal volume (101 ± 25), and ECV (30.7 ± 3.4%) than patients with MBF that was above the median during stress (E/e* 7.1 ± 2.5, LV mass 126 ± 32 g, LV maximal volume 91 ± 24 mL, and ECV 27.2 ± 2.1%; all $P < 0.05$). Differences in LV mass and LA volume remained significant after indexing to body surface area. We found no differences in EDV, ESV, SV, or LV ejection fraction between patients with MBF below or above the median during stress. No significant differences were observed for the use of sodium–glucose cotransporter 2 (SGLT-2) inhibitors, glucagon-like peptide 1 analogs, or insulin; however, we did see a trend toward higher use of SGLT-2 inhibitors in the group of patients with MBF below the median at rest ($P = 0.06$). In addition, between patients receiving monotherapy, dual therapy, or combination antidiabetes therapy, neither MBF at rest (0.86 ± 0.20, 0.81 ± 0.19, and 0.80 ± 0.18 mL/min/g, respectively; $P = 0.39$) nor MBF during stress (2.5 ± 0.9, 2.4 ± 1.0, and 2.4 ± 0.9 mL/min/g, respectively; $P = 0.77$) was significantly different.

Variables included in the multivariable linear regression model of patients with type 2 diabetes are presented in Table 3. In the univariable regression analysis, female sex, resting heart rate, autonomic neuropathy, serum creatinine, and treatment with SGLT-2 inhibitors correlated with MBF at rest ($P < 0.1$) and were included in the multivariable analysis. In the multivariable analysis, female sex ($P = 0.001$) and resting heart rate ($P = 0.045$) were significantly associated with increased MBF at rest. Age, sex, duration

Table 2—Relations between median MBF at rest and during stress, measurements of cardiac structure and function, and selected medications in patients with type 2 diabetes

	MBF at rest		MBF during stress	
	Below the median (n = 97)	Above the median (n = 96)	Below the median (n = 97)	Above the median (n = 96)
LVEF (%)	61 ± 7	62 ± 7	62 ± 9	63 ± 6
EDV (mL)	156 ± 34	152 ± 38	158 ± 38	150 ± 34
EDV index (mL/m^2)	72 ± 14	72 ± 16	73 ± 16	71 ± 13
ESV (mL)	63 ± 24	62 ± 22	62 ± 27	56 ± 18
ESV index (mL/m^2)	29 ± 10	28 ± 9	29 ± 12	26 ± 18
SV (mL)	94 ± 19	97 ± 22	95 ± 20	95 ± 21
LV mass (g)	143 ± 38	138 ± 37	149 ± 40	126 ± 32*
LV mass index (g/m^2)	65 ± 13	63 ± 15	68 ± 15	59 ± 11*
LA volume (mL)	98 ± 25	94 ± 25	101 ± 25	91 ± 24*
LA volume index (mL/m^2)	45 ± 11	44 ± 11	47 ± 11	43 ± 9*
Ischemic LGE	8	9	10	7
Mean E/e*	7.7 ± 2.9	7.9 ± 2.6	8.4 ± 2.9	7.1 ± 2.5*
ECV (%)	29.1 ± 3.3	28.7 ± 3.2	30.7 ± 3.4	27.2 ± 2.1*
Medication				
SGLT-2 inhibitors	40	28†	35	33
GLP-1 analogs	41	35	42	34
Insulin	57	59	62	54

Values are presented as mean ± SD or *n*. GLP-1, glucagon-like peptide 1; LVEF, left ventricular ejection fraction. *$P < 0.05$ vs. MBF below the median. †$P = 0.06$ vs. MBF below the median.

of diabetes, albuminuria, retinopathy, autonomic neuropathy, coronary artery disease, hypertension, smoking, and serum creatinine correlated with MBF during stress ($P < 0.1$). Age ($P = 0.001$), albuminuria ($P = 0.001$), retinopathy ($P = 0.001$), and coronary artery disease ($P = 0.02$) were significantly associated with reduced MBF during stress in the multivariable analysis.

Myocardial ECV and MBF during stress were inversely correlated ($R^2 = 0.37$; $P < 0.001$) (Fig. 1*E*). This correlation remained significant when the model was adjusted for age, sex, and known coronary artery disease ($P = 0.01$).

CONCLUSIONS

MPR impairment can be driven by either higher blood flow during rest or lower hyperemic blood flow during stress. However, the individual contribution of each factor in reducing MPR in patients with type 2 diabetes is still controversial. Some previous studies found either increased MBF at rest (13,21) or decreased MBF during stress (14,22) to be the sole contributor to reduced MPR in type 2 diabetes, whereas others found that small, insignificant changes in MBF both at rest and during stress added up to a significant decrease in overall MPR (9). One report even found MBF to be decreased during rest (12), emphasizing that this topic has not been fully explored and that more research is needed. Variations in previous findings may be explained by differences in study populations, the investigation modalities used, or simply chance; a common limitation of previous reports is the studies' relatively modest sample sizes, which make them susceptible to statistical errors. MPR has classically been considered the gold standard for examining myocardial perfusion, and most previous research investigating how myocardial perfusion is affected in a specific population or how changes in myocardial perfusion affect cardiac function has studied changes in MPR. In our study, we examined MBF at rest and during stress in a cohort of patients with type 2 diabetes that is, to our knowledge, the largest yet; we found that both increased basal MBF and reduced MBF during stress contribute to the reduced MPR seen in patients with type 2 diabetes. Having discovered that changes in MPR in patients with type 2 diabetes is indeed not monofactorial, we have investigated further the etiology of these changes and their influence on the heart in patients with diabetes. We are not aware of any previous studies that have examined this systematically.

A correlation has been demonstrated between insulin resistance and increased MBF at rest (21). Thus, as insulin is a known vasodilator, elevated plasma insulin concentrations may explain the increase in MBF at rest found in patients with type 2 diabetes. Glucose and lactate oxidation are decreased in the diabetic myocardium (23), so that it in turn relies more on fatty acid oxidation (24). Oxidation of fatty acids consumes a large amount of oxygen per molecule of ATP produced (25), resulting in a higher basal oxygen requirement and subsequently a higher basal MBF, as these are physiologically coupled. In our multiple regression analysis of patients with type 2 diabetes, we found that female sex was significantly associated with increased MBF during rest, which may be explained by a lower mean concentration of hemoglobin in women (26) that limits oxygen transportation capabilities and results in a higher basal MBF, which is necessary to meet myocardial oxygen demands. SGLT-2 inhibitors have been shown to reduce cardiovascular mortality and the incidence of new heart failure in patients with type 2 diabetes (27). The beneficial effect on mortality was already apparent within a few months of treatment with SGLT-2 inhibitors, but the exact mechanism responsible for this dramatic effect remains uncertain. We did not observe any significant changes in MBF related to SGLT-2 inhibition; we did,

Table 3—Univariable and multivariable linear regression models for patients with type 2 diabetes

	MBF at rest				MBF during stress			
	Univariable		Multivariable		Univariable		Multivariable	
	P	β	*P*	β	*P*	β	*P*	β
Age	0.08	0.12			<0.001	−0.42	0.001	−0.33
Female sex	<0.001	0.38	0.001	0.36	0.06	0.17		
BMI	0.54				0.90			
Duration of diabetes	0.37				<0.001	−0.27		
Resting heart rate	0.005	0.21	0.045	0.24	0.12			
Albuminuria	0.41				<0.001	−0.38	0.001	−0.37
Retinopathy	0.59				<0.001	−0.26	0.001	−0.28
Autonomous neuropathy	0.08	0.03			0.002	−0.23		
Coronary artery disease	0.31				0.001	−0.28	0.02	−0.14
Hypertension	0.29				0.002	−0.26		
Active or former smoker	0.64				0.001	−0.23		
HbA_{1c}	0.16				0.17			
Total cholesterol	0.17				0.45			
LDL cholesterol	0.14				0.61			
Serum creatinine	0.001	0.23			<0.001	−0.32		
SGLT-2 inhibitors	0.07	−0.17			0.79			
Insulin treatment	0.33	0.07			0.17	−0.1		

Factors with $P < 0.1$ in the univariable analysis were entered in the multivariable analysis. β values are presented for variables with $P < 0.01$ in the univariable analysis and variables that were significant in the final multivariable model.

however, see a trend toward MBF at rest being lower in patients treated with SGLT-2 inhibitors than in those who are not treated with SGLT-2 inhibitors. One explanation for the beneficial effect of SGLT-2 inhibitors is an improvement of cardiac energetics via the induction of mild ketosis and a shift toward oxidation of ketone bodies instead of free fatty acids (28). Oxidation of ketone bodies provides more ATP per oxygen molecule than does oxidation of fatty acids (29), resulting in an overall decrease in cardiac oxygen demand and consequently a lower basal MBF.

The microvascular complications retinopathy and albuminuria were associated with lower MBF during stress and remained significantly associated after adjusting for age and known coronary artery disease. Interestingly, our patients who had the most severe diabetes complications also had the lowest MBF during stress, suggesting that diabetes-related microvascular disease in the heart and other organs follows a similar disease pathway. The association between impaired MBF during stress and the classic complications in type 2 diabetes presented in our study may partly explain why previous studies reported only increased perfusion at rest to be the cause of reduced MPR in patients with type 2 diabetes, as those studies were performed either in patients without signs of micro- or macrovascular disease (21) or in populations of very young patients with type 2 diabetes, who are likely to be free of vascular complications (13).

Another striking observation in our study is the relationship between MBF during stress and myocardial ECV, which have previously been shown to correlate well with diffuse myocardial fibrosis. Increased diffuse myocardial fibrosis and ECV expansion are well known in patients with type 2 diabetes (30); to our knowledge, however, our study is the first to demonstrate a relationship between diffuse myocardial fibrosis and decreased maximal MBF. Although the observational design of our study prohibits us from commenting on causality, this apparent correlation is interesting. In the brain, it has been demonstrated that media thickening due to fibrinoid deposition and hypertrophy of connective tissue causes small-vessel disease, brain ischemia, and microinfarctions and that this process is promoted in patients with hypertension and type 2 diabetes (31). Our results support a similar theory whereby a buildup of fibrinoid tissue in the small vessels of the heart perhaps compromises vascular compliance and increases vascular resistance, ultimately leading to an overall decrease in maximal MBF. As this theory is, of course, only speculative, more research in this area would be needed before any conclusions could be made. Lower MBF during stress was associated with higher mean E/e*, and patients with MBF below the median during stress also had larger LV mass and LA volume. We previously demonstrated a similar relationship between these findings and reduced overall MPR (10). However, we cannot conclude whether decreased MBF during stress alone is responsible for these changes, or whether the combined effect of increased basal MBF and decreased MBF during stress depresses the overall MPR below a threshold necessary for maintaining normal cardiac diastolic function. Although still within normal ranges, these signs of subclinical diastolic dysfunction could be early steps in the development of diabetic cardiomyopathy and as such need to be investigated further. Previous reports have shown that both myocardial ECV (32) and MBF during stress (14) can be modulated by treatment with renin-angiotensin-aldosterone system blockers. Our study is not designed to investigate the longitudinal effects of specific interventions, but our findings indicate that it may be worthwhile to investigate how renin-angiotensin-aldosterone system blockers—perhaps in combination with SGLT-2 inhibitors—affect myocardial blood flow and to

examine whether changes in the myocardial microcirculation over time affect myocardial structure, function, and the risk of developing diabetic cardiomyopathy in patients with type 2 diabetes.

In conclusion, patients with type 2 diabetes had higher basal MBF and lower MBF during stress than a group of control subjects without type 2 diabetes. Reduced MBF during stress was associated with established coronary artery disease and the microvascular diabetes complications albuminuria and retinopathy. Furthermore, increased diffuse myocardial fibrosis in otherwise healthy myocardium was significantly correlated with decreased MBF during stress. Reduced basal MBF in patients treated with SGLT-2 inhibitors was of borderline significance.

Limitations

The cross-sectional design of our study is a limiting factor because it prohibits us from commenting on the timing of events. Coronary angiography was not carried out to exclude coronary artery disease and silent ischemia, as this would have been both logistically difficult and ethically inappropriate in our population of asymptomatic patients. Significant ischemia would have been detected from the stress perfusion scan, and, as such, we do not believe that undetected coronary artery disease had an important impact on our findings. However, any nonsignificant coronary artery stenosis or an artery system with balanced ischemia could be a confounding factor, since neither of these would necessarily be revealed from the CMR stress perfusion scan. ECV measured by CMR has been validated against histological samples from animal models and shown to correlate very well with diffuse interstitial fibrosis in the myocardium (33). Therefore, and for the similar reasons mentioned above, myocardial ECV findings were not confirmed histologically by myocardial biopsy.

Acknowledgments. The authors acknowledge staff in the Departments of Cardiology and Radiology at NSR Hospitals for their support in the execution of this study. Special thanks go to cardiac radiographers Anette Ohrt Elingaard-Larsen, Alice Holm Møller, Jakob Engelhardt Riis, and Karen Larsen (Department of Radiology, Næstved Hospital, Region Zealand, Denmark) for their expert assistance in performing the CMR scans.

Funding. This work was supported by the local research committee at NSR Hospitals, the regional research committee of Region Zealand (13-000849), and Hjerteforeningen (grant 16-R107-A6819-22020).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.H.S. collected and researched data and wrote the manuscript. A.S.B. collected data and reviewed and edited the manuscript. J.R.N.P. and S.P. reviewed and edited the manuscript. D.A.B. researched the data and reviewed and edited the manuscript. P.L.M. and P.G. designed the study and reviewed and edited the manuscript. M.H.S. and P.G. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Taylor KS, Heneghan CJ, Farmer AJ, et al. All-cause and cardiovascular mortality in middle-aged people with type 2 diabetes compared with people without diabetes in a large U.K. primary care database. Diabetes Care 2013;36:2366–2371
2. Poothullil JM. Diabetes and decline in heart disease mortality. JAMA 1999;282:1132
3. Moss SE, Klein R, Klein BEK. Cause-specific mortality in a population-based study of diabetes. Am J Public Health 1991;81:1158–1162
4. Gottdiener JS, Arnold AM, Aurigemma GP, et al. Predictors of congestive heart failure in the elderly: the Cardiovascular Health Study. J Am Coll Cardiol 2000;35:1628–1637
5. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. Am J Cardiol 1972;30:595–602
6. Yarom R, Zirkin H, Stämmler G, Rose AG. Human coronary microvessels in diabetes and ischaemia. Morphometric study of autopsy material. J Pathol 1992;166:265–270
7. Factor SM, Okun EM, Minase T. Capillary microaneurysms in the human diabetic heart. N Engl J Med 1980;302:384–388
8. Sari I, Soydinc S, Davutoglu V, Sezen Y, Aksoy M. Uncomplicated diabetes mellitus is equivalent for coronary artery disease: new support from novel angiographic myocardial perfusion-myocardial blush. Int J Cardiol 2008;127:262–265
9. Larghat AM, Swoboda PP, Biglands JD, Kearney MT, Greenwood JP, Plein S. The microvascular effects of insulin resistance and diabetes on cardiac structure, function, and perfusion: a cardiovascular magnetic resonance study. Eur Heart J Cardiovasc Imaging. 2014;15:1368–1376
10. Sørensen MH, Bojer AS, Plein S, Broadbent D, Madsen PL, Gæde P. Cardiac perfusion, structure, and function in type 2 diabetes mellitus with and without diabetic complications. Eur Heart J Cardiovasc Imaging. 23 October 2019 [Epub ahead of print]. DOI: 10.1093/ehjci/jez266
11. Lee MMY, McMurray JJV, Lorenzo-Almorós A, et al. Diabetic cardiomyopathy. Heart 2019;105:337–345
12. Cai X, Zhang S, Deng D, et al. Myocardial perfusion at rest in uncomplicated type 2 diabetes patients without coronary artery disease evaluated by 320-multidetector computed tomography: a pilot study. Medicine (Baltimore) 2018;97:e9762
13. Meyer C, Schwaiger M. Myocardial blood flow and glucose metabolism in diabetes mellitus. Am J Cardiol 1997;80:94A–101A
14. Hesse B, Meyer C, Nielsen FS, et al. Myocardial perfusion in type 2 diabetes with left ventricular hypertrophy: normalisation by acute angiotensin-converting enzyme inhibition. Eur J Nucl Med Mol Imaging 2004;31:362–368
15. Hilsted J. Decreased sympathetic vasomotor tone in diabetic orthostatic hypotension. Diabetes 1979;28:970–973
16. Nielsen FS, Rossing P, Bang LE, et al. On the mechanisms of blunted nocturnal decline in arterial blood pressure in NIDDM patients with diabetic nephropathy. Diabetes 1995;44:783–789
17. Cerqueira MD, Weissman NJ, Dilsizian V, et al.; American Heart Association Writing Group on Myocardial Segmentation and Registration for Cardiac Imaging. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Int J Cardiovasc Imaging 2002;18:539–542
18. Biglands J, Magee D, Boyle R, Larghat A, Plein S, Radjenović A. Evaluation of the effect of myocardial segmentation errors on myocardial blood flow estimates from DCE-MRI. Phys Med Biol 2011;56:2423–2443
19. Jerosch-Herold M, Wilke N, Stillman AE. Magnetic resonance quantification of the myocardial perfusion reserve with a Fermi function model for constrained deconvolution. Med Phys 1998;25:73–84
20. Iles L, Pfluger H, Phrommintikul A, et al. Evaluation of diffuse myocardial fibrosis in heart failure with cardiac magnetic resonance contrast-enhanced T1 mapping. J Am Coll Cardiol 2008;52:1574–1580
21. Picchi A, Limbruno U, Focardi M, et al. Increased basal coronary blood flow as a cause of reduced coronary flow reserve in diabetic patients. Am J Physiol Heart Circ Physiol 2011;301:H2279–H2284
22. Yokoyama I, Momomura S, Ohtake T, et al. Reduced myocardial flow reserve in non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 1997;30:1472–1477
23. Chatham JC, Seymour AML. Cardiac carbohydrate metabolism in Zucker diabetic fatty rats. Cardiovasc Res 2002;55:104–112
24. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. Cardiovasc Res 1997;34:25–33
25. Taegtmeyer H, McNulty P, Young ME. Adaptation and maladaptation of the heart in diabetes: part I: general concepts. Circulation 2002;105:1727–1733
26. Waalen J, Felitti V, Beutler E. Haemoglobin and ferritin concentrations in men and women: cross sectional study. BMJ 2002;325:137
27. Zinman B, Wanner C, Lachin JM, et al.; EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2015;373:2117–2128
28. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal

outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care 2016;39:1115–1122

29. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. Prostaglandins Leukot Essent Fatty Acids 2004;70:309–319

30. Wong TC, Piehler KM, Kang IA, et al. Myocardial extracellular volume fraction quantified by cardiovascular magnetic resonance is increased in diabetes and associated with mortality and incident heart failure admission. Eur Heart J 2014;35:657–664

31. Caplan LR. Lacunar infarction and small vessel disease: pathology and pathophysiology. J Stroke 2015;17:2–6

32. Swoboda PP, McDiarmid AK, Erhayiem B, et al. Diabetes mellitus, microalbuminuria, and subclinical cardiac disease: identification and monitoring of individuals at risk of heart failure. J Am Heart Assoc 2017;6:1–11

33. Zeng M, Zhang N, He Y, et al. Histological validation of cardiac magnetic resonance T_1 mapping for detecting diffuse myocardial fibrosis in diabetic rabbits. J Magn Reson Imaging 2016;44:1179–1185