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ORIGINAL RESEARCH

Effects of Age and Sex on Systemic Inflammation and Cardiometabolic Function in Individuals With Type 2 Diabetes

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BACKGROUND: Systemic inflammation, aging, and type 2 diabetes (T2D) lead to varying degrees of cardiovascular dysfunction and impaired aerobic exercise capacity. This study evaluates the impact of inflammation and sex differences on coronary and peripheral vascular function and exercise capacity in older individuals with and without T2D.

METHODS: Older individuals (aged≥65 years) underwent biochemical and tissue inflammatory phenotyping, cardiopulmonary exercise testing, cardiovascular magnetic resonance imaging, and vascular reactivity testing. Correlation and regression analyses determined the effects of systemic inflammation, older age, and sex on cardiovascular health, stratified by T2D status.

RESULTS: For the 133 recruited individuals (44% women; median age, 71±7 years, 41% with T2D), the presence of T2D most significantly increased the white blood cell count (P=0.004; P.adj.=0.140) among markers of systemic inflammation. White blood cell count was comparable in men and women. Hyperemic myocardial blood flow and flow-mediated and flow-independent nitroglycerin–induced brachial artery dilation were significantly impaired in men but not women with T2D. Peak oxygen consumption during exercise was lower with T2D (P=0.021), and overall reduced in women compared with men (P=0.002). Across all participants, both peak oxygen consumption during exercise and hyperemic myocardial blood flow were significantly impaired with increased white blood cell count. Women showed more adverse myocardial remodeling assessed by extracellular volume than men (P=0.008), independently of T2D status.

CONCLUSIONS: The pathophysiological manifestations of T2D on vascular function and aerobic exercise capacity are distinct in older men and women, and this may reflect underlying differences in vascular and myocardial aging in the presence of T2D.

Key Words: aerobic exercise capacity ■ cardiovascular aging ■ inflammatory biomarkers ■ sex differences ■ type 2 diabetes

Cardiovascular disease (CVD) is a leading cause of death among older individuals with type 2 diabetes (T2D).[1,2] Given its role as a shared pathophysiological hallmark of aging, diabetes, and CVD, systemic inflammation has been a major target of ongoing efforts to mitigate CVD risk in diabetes.[3,4] Most effects of systemic inflammation on the vasculature manifest phenotypically at the level of the microvasculature, where increased oxidative stress, immune system activation, and epigenetic factors converge to

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CLINICAL PERSPECTIVE

What Is New?

- This study reveals distinct sex-specific impacts of type 2 diabetes (T2D) on coronary and peripheral vascular function and aerobic exercise capacity in older individuals with well-controlled cardiovascular disease risk factors; older men with T2D are at higher risk for impaired vascular function compared with women, whereas older women, even without T2D, exhibit greater impairment in exercise capacity and myocardial remodeling.
- Systemic inflammation, represented by white blood cell count, impairs coronary vascular function and exercise capacity independent of T2D in older men and women.

What Are the Clinical Implications?

- Management of cardiovascular dysfunction in older individuals with T2D should consider inflammation and sex-specific differences in disease manifestation; a sex-specific approach in clinical examinations, therapeutic strategies, and lifestyle modifications may improve cardiovascular outcomes in older patients with T2D.

Nonstandard Abbreviations and Acronyms

CPET	cardiopulmonary exercise testing
ECV	extracellular volume
FMD	flow-mediated dilation
hMBF	hyperemic myocardial blood flow
LGE	late gadolinium enhancement
MBF	myocardial blood flow
NID	nitroglycerin-induced vasodilation
T2D	type 2 diabetes

impair microvascular function across coronary and noncoronary beds.[5,6] These effects are amplified with aging.[7]

The multifaceted changes in cardiovascular structure and function with aging, termed *cardiovascular aging*, reflect a complex interplay among cumulative clinical risk factors (eg, obesity, diabetes), modifiable lifestyle traits (eg, physical activity, diet), and inflammation.[8] Yet comprehensive imaging-based studies in older individuals at risk for advanced microcirculatory cardiovascular conditions (particularly those with diabetes) are limited. In this context, cardiovascular magnetic resonance (CMR) offers a capability to assess myocardial blood flow (an index of microcirculatory coronary function) alongside broad organ- and tissue-level myocardial phenotyping (left ventricular [LV] mass and volumes, tissue fibrosis) relevant to cardiovascular aging.

It remains uncertain whether optimal medical management of CVD risk factors in individuals with T2D effectively preserves vascular function as compared with age-matched individuals without T2D, and whether systemic inflammation impairs vascular function similarly in older individuals with and without T2D. Addressing this issue is essential, as the impact of systemic inflammation on vascular function in these specific populations remains uncertain. Additionally, although data indicate increased risk of CVD among women after menopause,[9,10] there are few studies that focus specifically on women who are ≥10 years postmenopausal.[11]

The goal of this study was to investigate the effects of T2D, advanced age, and sex on coronary and peripheral vascular function, systemic inflammation, and cardiopulmonary exercise capacity. We hypothesized that (1) inflammation impairs microvascular function and exercise capacity in older individuals who have optimal medical management of cardiovascular risk factors independent of T2D status; and (2) older age and T2D show differential effects in men and women.

To that end, we performed extensive cardiovascular phenotyping using innovative techniques including (1) biochemical assays of cytokines, chemokines, and proinflammatory mediators of immune activation and inflammation in blood serum and skin biopsies[12,13]; (2) CMR imaging for assessment of ventricular volumes and function, tissue characterization by T1 mapping,[14–16] and rest and stress myocardial perfusion imaging for myocardial blood flow quantification; (3) cardiopulmonary exercise testing (CPET); and (4) vascular reactivity of the brachial artery and skin. CPET measures peak oxygen consumption (V_{O_2}) during exercise, a measure of cardiovascular fitness, and provides information about cardiopulmonary and muscle physiology.[17]

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

We prospectively recruited individuals age ≥65 years, with and without T2D, through local clinics and community advertisements. The diagnosis of T2D followed published American Diabetes Association criteria.[18] Individuals with contraindications to CMR, LV systolic

dysfunction (defined as LV ejection fraction <50% by any imaging modality within 1 year before enrollment) and prior ST-segment–elevation myocardial infarction by clinical history or electrocardiographic Q waves (in at least 2 contiguous leads) were excluded. Individuals with hemoglobin A_{1c} ≤6.5% and without previous diagnosis of diabetes were enrolled as healthy volunteers. Additional exclusion criteria were current cigarette or tobacco use, unstable cardiac conditions, severe chronic obstructive pulmonary disease, use of oral anticoagulant therapy, glomerular filtration rate <50 mL/min, prior hemorrhagic or ischemic stroke, and any other condition possibly preventing completion of the CPET study protocol.[19]

The initial study visit included a review of medical history, physical examination, completion of dietary and physical activity questionnaires assessing baseline self-reported metabolic equivalents (METs),[20] urine collection, blood draw, vascular reactivity testing, and skin biopsy. A subsequent study visit within 2 weeks included CMR and CPET. A 50-mL blood sample (≈35 mL plasma, ≈10 mL serum, and a buffy coat in a PAXgene tube) was collected at the screening and follow-up visits. Blood was processed via ultracentrifugation and stored at −80 °C. The study was approved by the Institutional Review Board of the Beth Israel Deaconess Medical Center, and written informed consent was obtained from all subjects.

Stress CMR

CMR studies were performed after an overnight fast and 24-hour abstinence from caffeine intake with a 3 Tesla magnetic resonance imaging scanner (Siemens Medical Solutions, Erlangen, Germany) with a dedicated 18-element phased body array. The CMR protocol included (1) assessment of ventricular volumes and function with steady-state free precession cine imaging; (2) first-pass contrast-enhanced T1-weighted myocardial perfusion imaging in 3 short-axis slices (basal, mid, apical levels) through the LV; (3) T1 mapping with a modified Look-Locker imaging sequence before and after gadolinium contrast administration for assessment of extracellular volume expansion, a marker of diffuse fibrosis; and (4) imaging of late gadolinium enhancement (LGE) to detect any myocardial scar.[14–16] A contrast bolus dosage of 0.06 mmol of gadopentetic acid–gadolinium-diethylenetriaminepentacetate (Magnevist; Berlex Inc.) was injected ≈5 seconds from the start of the perfusion scan, and a total of 80 images were acquired per slice. Rest perfusion imaging was performed first, followed 20 minutes later by stress perfusion imaging after inducing hyperemia by bolus injection of 0.4 mg/5 mL of regadenoson over 10 seconds (Astellas Pharma US, Inc., Northbrook, IL). After both stress and rest perfusion imaging, an additional gadolinium dose was injected to reach a total of 0.15 mmol/kg before postcontrast T1 mapping.

Cine, perfusion, modified Look-Locker imaging, and LGE images were analyzed with QMASS CMR image analysis software version 9.3 (Medis Medical Imaging Systems, Leiden, The Netherlands) by tracing the endocardial and epicardial contours avoiding the blood pool. Myocardial blood flow (MBF) was quantified in mL/min per g for 16 myocardial segments in 3 short-axis slices. Using modified Look-Locker imaging T1 mapping, segmental myocardial R1 (1/T1) was plotted against blood R1, to determine the extracellular volume (ECV) fraction using the (same day) measured blood hematocrit. Global longitudinal strain of the left ventricle was quantified by feature tracking on long-axis cine images (QStrain; Medis Medical Imaging Systems, Leiden, The Netherlands).

Cardiopulmonary Exercise Testing

An intravenous catheter was placed in an arm vein and 20 cc of blood was collected at rest. Subjects then exercised on a Lode-Corival semirecumbent stationary cycle ergometer using a ramp protocol (10–15 watts per stage) until peak volitional fatigue, unable to maintain >40 revolutions per minute on isokinetic ergometer or respiratory exchange ratio >1.20. This ergometer was calibrated and maintained by the hospital's Clinical Engineering Department on an annual basis. Per laboratory policy, the study was terminated if there was a clinical concern for potential harm to the subject (ie, hemodynamic compromise, malignant ectopy, angina, or ST-segment depression/elevation). At peak exercise, a second sample of 20 cc of blood was collected. Continuous ventilatory expired gas analysis was obtained (Medgraphics Ultima CPX Metabolic Stress Testing system, Minneapolis, MN). The oxygen and carbon dioxide sensors were calibrated using gases with known oxygen and carbon dioxide concentrations before each test. The flow sensor was calibrated with a 3-L syringe before each test. The V_{O_2} (mL/kg per min) calibration technique is based on the manufacturer's recommendation. Carbon dioxide output (L/min), and minute ventilation (L/min) were collected throughout the exercise test.[21] The respiratory exchange ratio was calculated by dividing the carbon dioxide output by V_{O_2}. The chronotropic index for CPET was calculated as difference of peak heart rate during exercise and resting heart rate, normalized by the age-adjusted expected heart response, defined as 220−age (years)−resting heart rate.

Vascular Function Testing

Endothelial function was assessed by measuring flow-mediated dilation (FMD) in accordance with published guidelines.[22] Briefly, brachial artery diameter

was measured before and during reactive hyperemia using high-resolution ultrasound with a 10.0-MHz linear array transducer and an Aloka Prosound 7 system (Hitachi Aloka Medical Ltd, Tokyo, Japan). Reactive hyperemia was induced by inflating a pneumatic tourniquet distal to the brachial artery to 50 mm Hg above systolic blood pressure for 5 minutes and then deflating it rapidly. FMD was expressed as the percentage change between baseline and postocclusive artery diameter. For nitroglycerin-induced vasodilation (NID), a third and fourth ultrasound of the brachial artery were performed after 20 minutes of supine rest and again 4 minutes after administration of 400 μg of sublingual nitroglycerin. NID was expressed as the percentage change between baseline and post-nitroglycerin brachial artery diameter.[22–24] NID was not performed in 19 subjects with T2D and 17 without T2D due to contraindication to nitroglycerin administration.

Skin blood flow was measured in a temperature-controlled environment before and after iontophoresis of acetylcholine (endothelium-dependent) and sodium nitroprusside (endothelium-independent) using laser Doppler flowmetry (DRT4 Laser Doppler Blood Flow Monitor; Moor Instruments Ltd, Millwey, Devon, England).[25] The percentage change in skin blood flow after acetylcholine and sodium nitroprusside administration compared with baseline were used as measures of microcirculatory function.

Skin Biopsy

Two 3-mm punch skin biopsies at the volar aspect of the forearm were performed for each participant. The specimen was placed directly into a Cryomold prefilled with optimum cutting temperature compound (Tissue-Tek) solution, stored at −80 °C and used for immunohistochemistry and immunofluorescent measurements to analyze mast cell degranulation and macrophage polarization.

Serum Biomarkers

Peripheral venous blood was collected under fasting conditions (after an 8- to 12-hour overnight fast), with immediate centrifugation, aliquots, and storage at −80 °C. Selected serum CVD and metabolic risk biomarkers were processed using MilliporeSigma (Burlington, MA) bead-based multiplex assays using the Luminex technology (Luminex Corporation, Austin, TX). Panels for CVD markers, cytokine markers, and matrix metalloproteinases were run on the blood serum samples.

Statistical Analysis

The primary end points were differences in inflammatory markers, vascular reactivity, cardiac function, and exercise capacity between older individuals with and without T2D. Data were expressed as mean±SD for normally distributed data or median with interquartile range for nonnormally distributed data. Between-group comparisons were performed using Student's *t* test for normally distributed data or nonparametric tests (Mann–Whitney) when data were not normally distributed. Between-group comparisons of categorical variables were conducted using Pearson's χ^2 test or Fisher's exact test. Relationships between quantitative variables were assessed with Pearson's correlation test or Spearman's rank correlation as applicable. Previous studies have highlighted sex differences in coronary microvascular function and myocardial glucose metabolism in individuals with and without T2D.[9,26] Based on this evidence, we included in linear models for MBF, CPET, and peripheral vascular function an interaction between sex and T2D status. The effects of T2D, sex and their interaction were analyzed with linear regression analysis, followed by post hoc pairwise comparisons of marginal means using Tukey's method for adjustment of *P* values (R-package "emmeans"). Peak V_{O_2} was analyzed with a linear regression model that included age and sex as predictors, as with widely used models for prediction of peak V_{O_2}.[27] Additional predictors were T2D status and chronotropic index to adjust for chronotropic incompetence, the interactions of body mass index (BMI) and chronotropic index with sex, and white blood cell count (WBC; log-transformed) as inflammatory markers, with the strongest correlation with both hyperemic MBF and peak V_{O_2}. A similar multivariable linear regression model was built for analyzing peak O_2 pulse, but here the chronotropic index, BMI, age, and myocardial ECV were added as predictors to determine any effects of myocardial remodeling on stroke volume. Chronotropic index, BMI, and age are predictors in the validated equations developed by Wasserman et al for prediction of peak V_{O_2}.[28] Visual and formal checks of various linear model assumptions (normality of residuals, normality of random effects, linear relationship, homogeneity of variance, multicollinearity) were performed with the R package "performance" (R Foundation for Statistical Computing, Vienna, Austria). Multicollinearity was assessed by calculating the variance inflation factor. The standardized residuals were examined for normal distribution by visual inspection of a Q-Q plot and using the Shapiro–Wilk test for normality. Checks for (non)constant error variance (heteroscedasticity) were performed with the Breusch–Pagan test. Lack of fit (eg, missed nonlinearities) was examined with partial-residual plots.[29]

The study was originally designed to detect a "moderate effect size" of 0.5 (Cohen's *d*) for coronary hyperemic MBF and peripheral FMD. For 80% power and α=0.05, this required ≈100 studies, equally split between the 2 groups for T2D status. Due to unforeseen

circumstances, including the COVID-19 pandemic, we could complete only 75 CMR studies. We estimate that our power, based on the same assumptions as used for the original power calculation, was reduced from 80% to ≈65%.

A P value of <0.05 was considered statistically significant, and P values of $0.05 \leq P < 0.1$ were considered indicative of a trend and marginally significant. Statistical analysis was performed using the R environment (R version 4.1.2) and the Minitab Statistical Package Version 21.3 (Minitab Inc., State College, PA).

RESULTS

Study Population Baseline Characteristics

We enrolled 133 individuals, 54 with T2D and 79 without T2D (Table 1). There were no differences in age, sex, race, or ethnicity between the groups. T2D participants had higher BMI (P=0.11) and waist-to-hip ratio (P=0.007), lower diastolic blood pressure (P=0.021), and lower total and low-density lipoprotein cholesterol (both P<0.0001). Statin and antihypertensive medication use were significantly higher among individuals with T2DM compared with controls (P<0.0001 for both). There were no differences between controls and T2D in baseline self-reported METs for either vigorous or moderate exercise or all activities. There were no differences in METs between men and women.

CMR Results

Due to COVID-19 restrictions, CMR was performed in a subset of 85 subjects (30 T2D subjects). There were no differences in age or sex between subjects that underwent CMR and those who did not. We did not observe significant differences for any CMR measurements related to T2D status, including rest MBF and hyperemic myocardial blood flow (hMBF) (Table 2; see Table S1 for CMR measurements stratified by sex). Rest MBF was overall higher in women compared with men (P=0.028), also when normalized by the rate pressure product. Hyperemic MBF was 0.39 mL/min per g lower in men with T2D (P=0.039; Table S1) compared with men without T2D, but the difference became nonsignificant with adjustment by age and log-transformed WBC (P=0.158; Figure 1B). Within the T2D group hMBF was lower in men compared with women (P=0.029; Figure 1B). The results from the multivariable model for hMBF (Tables S2) indicate that hMBF was significantly reduced with increasing age (P=0.003), and with higher WBC (log-transformed, P=0.004). Additionally, there was a significant interaction effect between male sex and T2D (P=0.034). The negative relationship of hMBF with the WBC in this model and shown in the correlation plot in Figure 1C demonstrates that systemic inflammation is associated with a decrease of hMBF independent of T2D status. Interleukin-13, an anti-inflammatory type 2 cytokine with a putative protective role against atherosclerosis, correlated positively with hMBF (r=0.31; P=0.014; Figure 1D). The ECV was higher in women (P=0.008), and T2D did not have a significant effect (P=0.48). The LV ejection fraction at rest correlated negatively with ECV in women (r=−0.43; P=0.011) but not in men (r=−0.43; P=0.83; see Figure S1). Only 1 participant without T2D had evidence of myocardial scar by LGE in the LV.

CPET Results

CPET was performed in a cohort subset of 59 subjects (20 with T2D) due to COVID restrictions. There were no differences in age or sex between the subjects that underwent CPET and those who did not. The respiratory exchange ratio averaged 1.12±0.11, correlated with BMI (r=−0.49; P<0.001), and both T2D and sex had no significant effect on respiratory exchange ratio. Peak V_{O_2} was lower with T2D (17.5±4.8 with T2D versus 20.7±5.0; P=0.002), mostly because of the effect of T2D in women (14.2±2.9 with T2D versus 19.7±4.1 mL/min per kg; P=0.004 for comparison of marginal means; Figure 2A). The chronotropic index reached during CPET was similar in individuals with and without T2D. The correlation of peak V_{O_2} with the chronotropic index was noticeably different in men (r=0.56; P=0.001) compared with women (r=0.21; P=0.31; Figure 2B). The difference between men and women of the correlation of peak V_{O_2} and chronotropic index was reflected in a significant interaction of sex and chronotropic index (P=0.006) in the linear regression model for peak V_{O_2} (Table S4 with estimates for the regression coefficients). The linear model for peak V_{O_2} accounts for >50% of the observed variability and showed a trend for peak V_{O_2} to be lower in women (P=0.059) and a significant decrease of peak V_{O_2} with advancing age (P=0.001) and increasing WBC (P=0.026).

Peak O_2 pulse, an index of stroke volume, was significantly lower in women compared with men (P<0.001), and T2D had no significant effect (P=0.12). Peak O_2 pulse correlated strongly with the stroke volume measured at rest with CMR (r=0.69; P<0.001), and with the myocardial ECV (r=−0.36; P=0.015), a marker of diffuse myocardial fibrosis, which was previously shown to be associated with diastolic dysfunction in patients with T2D.[30] The associations of ECV (P=0.015) and chronotropic index (P=0.041) with peak O_2 pulse were also significant in a multilinear regression model for peak O_2 pulse shown in Table S5. In this model, there was no significant association of peak O_2 pulse with the WBC.

The increase of the serum inflammatory markers interleukin-6 and interleukin-8 during CPET (value right

Table 1. Participant Baseline Characteristics

Variable	No T2D, N=79*	T2D, N=54*	*P* value†
Female sex	31/79 (39)	27/54 (50)	0.219
Age, y	71 (67–76)	70 (68–74)	0.940
BMI, kg/m^2	27.56±5.23	30.12±5.74	0.011
Waist-to-hip ratio	0.95±0.07	0.98±0.07	0.007
Body surface area, m^2	1.85±0.23	1.84±0.23	0.828
Systolic blood pressure, mm Hg	129.39±17.57	131.35±15.98	0.509
Diastolic blood pressure, mm Hg	76.78±10.59	72.48±10.12	0.021
Heart rate, bpm	64.42±14.19	68.18±17.97	0.387
RPP, mm Hg, bpm/1e4	0.93±0.21	0.93±0.19	0.962
History of hypercholesterolemia	8/41 (20)	9/23 (39)	0.088
History of hypertension	9/41 (22)	10/23 (43)	0.071
Tobacco use, current/past	4/41 (9.8)	1/23 (4.3)	0.646
Years of T2D		15.0 (8.0, 20.0)	
Insulin, μIU/mL	6.20 (4.15–9)	10.15 (6.33–20)	<0.001
Fasting glucose, mg/dL	90 (84–95)	115 (93–145)	<0.001
HOMA-IR	1.68±1.15	6.00±10.60	0.006
Hemoglobin A_{1c}	5.50 (5.40–5.80)	7.00 (6.60–7.80)	<0.001
Creatinine, mg/dL	0.82 (0.71–1.01)	0.84 (0.70–0.97)	0.939
Sodium, mmol/L	141 (139–142)	141 (139–142)	0.466
Potassium, mmol/L	4.20 (4.00–4.60)	4.40 (4.10–4.50)	0.653
eGFR, mL/min 1.73	77.77±14.37	77.00±18.96	0.826
Total cholesterol, mg/dL	187 (164–216)	153 (137–177)	<0.001
Triglycerides, mg/dL	95 (72–113)	102 (65–143)	0.356
LDL cholesterol, mg/dL	106 (81–134)	76 (61–93)	<0.001
HDL cholesterol, mg/dL	60 (47–76)	55 (43–68)	0.090
Blood urea nitrogen, mg/dL	17.83±6.38	18.02±7.41	0.892
WBC, 1e6/mL	5.50 (4.90–7)	6.50 (5.40–8)	0.004
RBC, 1e6/μL	4.47 (4.33, 5)	4.58 (4.27, 5)	0.813
Blood hematocrit, %	41.67±3.77	39.74±3.28	0.002
Microalbumin–creatinine ratio	7.55 (3.93–18.98)	12.55 (7.15–42.05)	0.038
C-reactive protein, mg/dL	1.69 (0.73–3)	1.33 (0.80–2)	0.718
Statins	21/79 (27)	44/54 (81)	<0.001
Insulin	0/79 (0)	16/54 (30)	<0.001
Oral antidiabetic	0/79 (0)	45/54 (83)	<0.001
Sodium–glucose cotransporter 2 inhibitor	0/79 (0)	1/54 (1.9)	0.406
GLP-1 agonist	0/79 (0)	4/54 (7.4)	0.025
Aspirin	18/79 (23)	25/54 (46)	0.004
β Blocker	7/79 (8.9)	11/54 (20)	0.057
Diuretic	6/79 (7.6)	9/54 (17)	0.104
ACEi	1/79 (1.3)	1/54 (1.9)	>0.999
Calcium channel blocker	9/79 (11)	6/54 (11)	0.960
ARB	13/79 (16)	23/54 (43)	<0.001
Omega-3	6/79 (7.6)	2/54 (3.7)	0.472
Race			
American Indian/Alaska Native	0/14 (0)	1/27 (3.7)	
Asian	5/14 (36)	3/27 (11)	
Black or African American	7/14 (50)	17/27 (63)	

(*Continued*)

Table 1. Continued

Variable	No T2D, N=79*	T2D, N=54*	*P* value†
Not provided	1/14 (7.1)	3/27 (11)	
White	1/14 (7.1)	3/27 (11)	
Ethnicity			0.395
Hispanic/Latino	2/79 (2.5)	3/54 (5.6)	
Not Hispanic/Latino	77/79 (97)	51/54 (94)	

ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide 1; HDL, high-density lipoprotein; HOMA-IR, Homeostasis Model Assessment–Insulin Resistance; IQR, interquartile range; LDL, low-density lipoprotein; RBC, red blood cell count; RPP, rate pressure product; SGLTi, sodium–glucose cotransporter 2 inhibitors; T2D, type 2 diabetes; and WBC, white blood cell count.

*Data are expressed as n/N (%), median (IQR), or mean±SD.

†Pearson's χ^2 test; Wilcoxon rank-sum test; Welch 2-sample *t* test; Fisher's exact test.

after CPET baseline value) was larger in participants with T2D than without T2D. The serum inflammatory marker interleukin-6 changed by 6% (0% to 41%) in T2D compared with –2% (–12% to 9%) in participants without T2D (*P*=0.054 for Wilcox test). For interleukin-8, the corresponding changes were 10% (6%–18%) with T2D versus 3% (–3% to 11%) without T2D (*P*=0.04 for Wilcox test).

Vascular Reactivity Results

FMD in the brachial artery, a measure of vascular endothelial function, was lower in individuals with T2D compared with controls (Table 2 and Table S6). There was a significant interaction between T2D and sex in their effects on FMD: The predicted FMD was 4.8% lower in men with T2D compared with women with T2D (*P*=0.023; Table S6). Similarly, NID, a measure of vascular smooth muscle function, was reduced by –8.1 (95% CI, –15 to –1.1) (*P*=0.025 in Table S7; *P*=0.005 in Figure S2B) in men with T2D compared with male controls, but there was no effect of T2D in women (*P*=0.997; Figure S2B). No differences were observed in the forearm skin vasodilation (endothelium-dependent and -independent) between T2D and controls. NID correlated with METs for vigorous plus moderate exercise (*r*=0.362; *P*=0.018) and METs for all activities (*r*=0.281; *P*=0.079). There was a weak and marginally significant correlation between NID and hMBF as well as FMD and hMBF (NID: *r*=0.353; *P*=0.055; FMD: *r*=0.23; *P*=0.083). Peak V_{O_2} did not correlate significantly with FMD (*r*=0.20; *P*=0.178) and NID (*r*=–0.31; *P*=0.2). WBC had no significant effects on neither FMD nor NID in univariable or multivariable regression models (Tables S6 and S7, respectively).

Serum and Skin Inflammatory Markers

WBC was increased in individuals with T2D (*P*=0.004), mostly because WBC was higher in women with T2D compared with female controls (*P*=0.001), while in men there was no significant effect of T2D (*P*=0.474). A 2-way ANOVA model for the WBC confirmed this interaction between T2D and sex (*P*=0.039), in addition to the main effect of T2D (*P*=0.003). Interleukin-5 was lower in participants with T2D (*P*=0.047 from Wilcoxon rank-sum test; Table S8). BMI correlated in participants with T2D with WBC (*r*=0.34; *P*=0.014). Growth differentiation factor 15 was higher in individuals with T2D (*P*=0.012), and 2-way ANOVA model for growth differentiation factor 15 confirmed the effect of T2D (*P*=0.008), while sex had no significant effect (*P*=0.21). No other major differences were observed between the groups among the inflammatory cytokines, adhesion molecules, and growth factors in Table S8. No differences were observed in the forearm skin biopsies in factors that included mast cell degranulation, number of vessels, macrophages, and round inflammatory cells (data not shown).

DISCUSSION

By using state-of-the-art, detailed inflammatory phenotyping, noninvasive cardiac imaging, and cardiopulmonary exercise testing, this observational study of older (aged >65 years) individuals explored the independent contributions of T2D, age, sex, and inflammation on cardiovascular health. The present findings support our first hypothesis that inflammation contributes to impaired coronary microvascular function and exercise capacity in older individuals independently of T2D status. Elevated WBCs were significantly associated with reduced coronary blood flow and exercise capacity, consistent with prior evidence linking inflammation to adverse cardiovascular outcomes, such as reduced epicardial flow and myocardial perfusion, increased thromboresistance, and higher incidence of heart failure and death.[31] Our study extends these findings by demonstrating inflammatory impacts in older individuals with and without T2D. The observed transient rise in interleukin-6 and interleukin-8 following

Table 2. Cardiac MRI Parameters, Vascular Reactivity, and Cardiopulmonary Testing

Variable	Non-T2D	T2D	
Cardiac MRI parameters	**N=55***	**N=30***	***P* value†**
LV EDVi, mL/m^2	70±15.0	69±12.8	0.649
LV ESVi, mL/m^2	28±8.2	28±8.3	0.881
LV ejection fraction, %	61±5.7	60±7.6	0.521
LV global longitudinal strain, %	−23±4.9	−22±4.0	0.828
LV mass index, g/m^2	50±13.6	50±14.1	0.981
LV mass/EDV ratio, g/mL	0.72±0.19	0.75±0.26	0.677
Cardiac index, L/min per m^2	2.79±0.63	2.66±0.57	0.355
RV ESVi, mL/m^2	30.46±6.55	28.75±8.65	0.351
RV EDVi, mL/m^2	66.97±13.12	62.22±16.18	0.173
RV ejection fraction, %	54.38±5.37	53.91±5.94	0.719
Rest MBF, mL/min per g	0.81±0.18	0.80±0.22	0.909
Rest MBF (RPP-cor.), mL/min per g	0.91±0.23	0.88±0.27	0.741
Hyperemic MBF, mL/min per g	1.96±0.53	1.77±0.48	0.148
Myocardial perfusion reserve	2.47±0.69	2.36±0.72	0.581
Myocardial native T1, ms	1120±47	1111±38	0.423
ECV	0.28±0.04	0.29±0.03	0.420
Any LV LGE	1 (2.0%)	0 (0%)	>0.999
Vascular reactivity	**N=67***	**N=40***	
Resting brachial artery diameter, mm	4.2±0.8	4.7±0.8	0.003
FMD, % of increase over baseline	6.5±5.3	4.0±5.0	0.018
NID, % of increase over baseline	9.8±4.8	5.9±7.4	0.040
Acetylcholine-induced skin vasodilation, % of increase over baseline	50±65	41±84	0.531
Sodium nitroprusside–induced skin vasodilation, % of increase over baseline	77±71	73±78	0.772
Cardiopulmonary testing	**N=39***	**N=20***	
Peak V_{O_2}, mL/min per kg	20.7±5.0	17.5±4.8	0.022
Peak V_{O_2}/WR, mL/min per W	9.8±1.8	8.7±1.7	0.017
Peak respiratory exchange ratio	1.11±0.12	1.12±0.10	0.741
O_2 pulse peak	12.1±3.8	10.5±3.1	0.098
VE-VCO_2 slope	27.8±3.6	28.2±3.3	0.643
Chronotropic index	0.7±0.3	0.8±0.2	0.451

V_{O_2}/WR, Oxygen consumption (mL/min)/Work rate (watts) shows the relationship of oxygen consumption to work performed. Respiratory exchange ratio, the volume of carbon dioxide produced to oxygen consumed (also known as respiratory quotient). VE/V_{CO_2} slope, minute ventilation to carbon dioxide produced, a prognostic marker for either cardiac, pulmonary, or both as limiting factors such as when there is ventilatory perfusion mismatch often associated with increased cardiac wedge pressure, pulmonary hypertension, or pulmonary causes. ECV, extracellular volume; EDV, end-diastolic volume; EDVi, end-diastolic volume index; ESVi, end-systolic volume index; FMD, flow-mediated dilation; LGE, late gadolinium enhancement; LV, left ventricular; MBF, myocardial blood flow; MRI, magnetic resonance imaging; RPP, rate pressure product; and RV, right ventricular.

*Data are expressed as n/N (%) or mean±SD.

†Pearson's χ^2 test; Welch 2-sample *t* test; Fisher's exact test.

exercise in participants with T2D further confirms that inflammation may exacerbate cardiopulmonary exercise intolerance in T2D. Contrary to coronary microvascular function, peripheral vascular function did not correlate with inflammatory markers such as the WBC or proinflammatory cytokines like interleukin-13.

Our study also confirms the second hypothesis of a sex dimorphism of T2D with respect to coronary and peripheral vascular function as well as exercise capacity. Men showed significantly greater impairments in coronary hyperemic (hMBF) and peripheral vascular function (brachial FMD and NID), compared with their female counterparts. A multivariable model for hMBF confirms the sex dimorphism of T2D (interaction term T2D:male sex in Table S2). This finding aligns with earlier studies indicating that men with diabetes are more susceptible to peripheral arterial and microvascular impairment than women of similar age.[32,33] In contrast, cardiopulmonary exercise capacity (peak V_{O_2}) showed a trend in the multivariable model to be lower in women, irrespective of T2D status (Table S4).

Interestingly, coronary microvascular function in women with T2D was relatively preserved compared with men. Female sex was associated in previous studies with higher hMBF compared with men.[34,35] While the effect of T2D on hMBF was not found to be significant in a subcohort aged 45 to 85 years from the Multi-Ethnic Study of Atherosclerosis, the authors did not report on any possible interaction of T2D with sex.[35] A significant effect of T2D on coronary vascular function (hMBF and coronary perfusion reserve) was previously reported for premenopausal women, but hMBF and coronary perfusion reserve were still higher compared with older (≈15 years) postmenopausal women without diabetes.[36] This latter finding suggests that older age and change in menopause status can have effects on hMBF in women of similar magnitude as T2D. In a positron emission tomography study by Haas et al of individuals with T2DM and good cardiometabolic control, both rest and hyperemic MBF were higher in women (aged 52±8 years) compared with men of similar age, yet the perfusion reserve was lower in women (menopausal status was not specified).[9] In contrast, the present study shows a similar myocardial perfusion reserve in older men and (postmenopausal) women without T2D, but hMBF was significantly lower in men with T2D. Our results suggest that menopause does not blunt the differences of hMBF and coronary reactivity seen in premenopausal women compared with men. Any benefits of better vascular function in women may also lessen the impact of T2D on coronary vascular function in women after menopause and result in a more pronounced effect of T2D on coronary microvascular function in men compared with women.

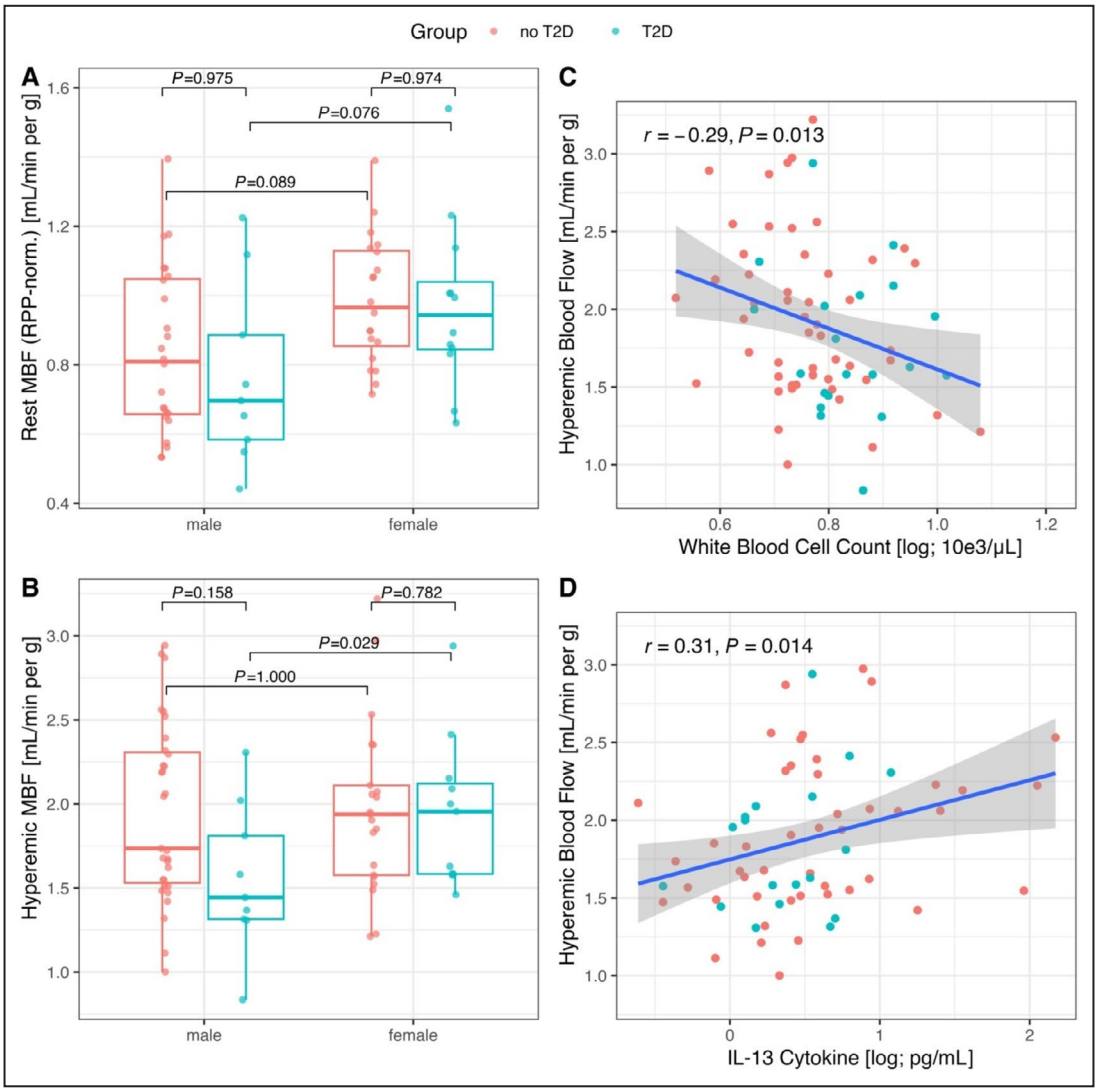

Figure 1. Changes in myocardial blood flow by T2D status and markers of inflammation.
A, Rest MBF was overall higher in women compared with men (P=0.018 for t test). Rest MBF, normalized by rate pressure product, was higher in women (P=0.002), with simultaneous adjustment by age (P=0.018), T2D (P>0.9), and the white blood cell count (log-transformed; P=0.15). The brackets show adjusted P values from post hoc pairwise comparisons of the marginal means based on the linear regression model summarized in Table S3. **B**, MBF during hyperemia induced by regadenoson was lower in men with T2D (P=0.034), but not in women (P=0.3), with simultaneous adjustment by age (P=0.003) and log-transformed WBC (P=0.004). The brackets show adjusted P values from post hoc pairwise comparisons of the marginal means based on the linear regression model summarized in Table 2. **C**, The WBC was significantly higher in individuals with T2D (5.50 [4.90–7] vs 6.50 [5.40–8] pg/mL; P=0.004) and correlated negatively with myocardial blood flow during hyperemia (r=−0.29; P=0.013). **D**, Interleukin-13, an anti-inflammatory type 2 cytokine with a putative protective role against atherosclerosis was not significantly different between participants with and without T2D (P=0.34) but correlated positively with hyperemic (“stress”) MBF (r=0.31; P=0.014). IL-13 indicates interleukin-13; MBF, myocardial blood flow; RPP, rate pressure product; T2D, type 2 diabetes; and WBC, white blood cell count.

An impaired MBF may be confounded by the presence of obstructive coronary lesions.[15,37] We addressed this potential confounding by excluding participants with tobacco use and examining LGE in the LV. Only 1 participant without T2D showed LGE, suggesting that obstructive coronary lesions were not a major factor. The consistency of a male-specific effect of T2D on hMBF, and brachial artery FMD and NID, and the low probability of simultaneous flow-limiting stenosis in both vascular beds without symptoms indicate that obstructive disease was not a major confounder.

The measures of peripheral endothelial and smooth muscle vascular function obtained in our study (FMD and NID) provide evidence for similar male-specific effects of T2D, as was the case for the coronary hyperemic response. The multivariate regression models for

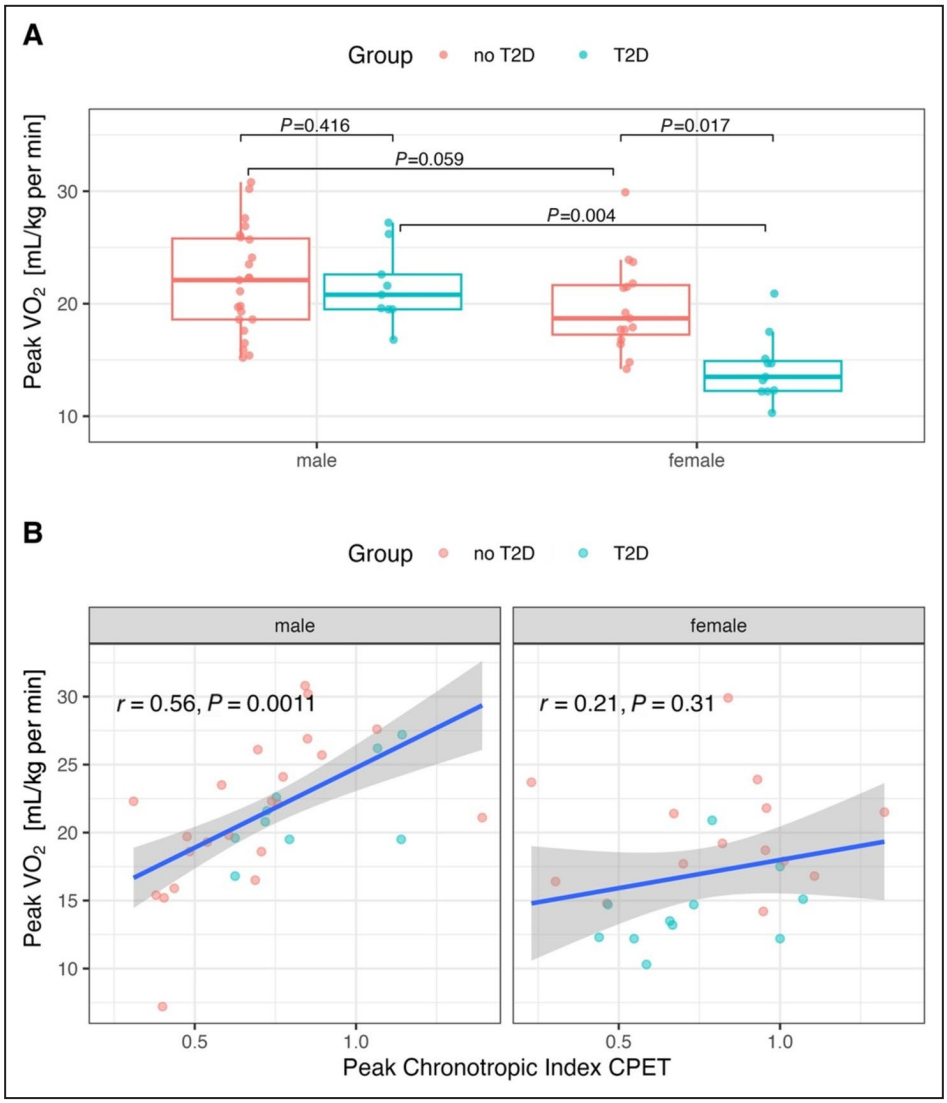

Figure 2. Cardiopulmonary exercise testing.
A, Peak Vo_2 was reduced by T2D in women (*P*=0.017), but there was no T2D-related difference in men (*P*=0.416). The brackets show adjusted *P* values from post hoc pairwise comparisons of the marginal means based on the multivariate linear regression model summarized in Table S4, which includes the age, chronotropic index, and log-transformed WBC as predictors in addition to sex and T2D status. **B**, In men, peak Vo_2 correlated significantly with the chronotropic index (*P*=0.56, *P*=0.001), but in women the correlation was weaker and not significant (*r*=0.21; *P*=0.31). The difference between men and women of the correlation of peak Vo_2 and chronotropic index was reflected in a significant interaction of sex and chronotropic index (*P*=0.006) in the linear regression model for peak Vo_2 (Table S4). The chronotropic index is defined as the difference of peak heart rate during CPET and the resting heart rate, normalized by the age-adjusted expected heart response, defined as 220—age (y)—resting heart rate. CPET indicates cardiopulmonary exercise testing; T2D, type 2 diabetes; Vo_2, oxygen consumption; and WBC, white blood cell count.

brachial artery FMD and NID showed a sex dimorphism of T2D with respect to peripheral vascular function. In contrast with coronary function, WBC had no significant effect on FMD and NID. The univariate effect of T2D on brachial artery FMD and NID, and the absence of a univariate effect of T2DM on hMBF could suggest that T2D had a stronger effect on the peripheral than the coronary circulation. This would be consistent with the previous finding that among patients with T2D and among nondiabetic subjects the risk of adverse cardiovascular events conferred by peripheral artery disease was higher than the risk conferred by coronary artery disease.[38] Also, diabetes appears to be associated with increased risk of peripheral artery disease in

patients with and without established coronary artery disease,[39] suggesting that diabetes may have a more pronounced effect in the peripheral vasculature compared with the coronary vasculature.

In contrast with the relatively preserved vascular function in women with T2D compared men with T2D, the women in this study presented lower exercise tolerance reflected by lower peak V_{O_2} and peak O_2 pulse. The lower exercise tolerance in women may be mediated by their higher levels of adverse myocardial remodeling (ECV), which were associated with a decrease of peak O_2 pulse (Table S5) and LV ejection fraction (Figure S1) in women. These findings align with prior studies showing increased susceptibility to heart failure with preserved ejection fraction in women, driven by increases in myocardial fibrosis and diastolic dysfunction.[30,40] Diffuse myocardial fibrosis can impair augmentation of relaxation velocity as heart rate increases during exercise.[41] This suggests that diffuse myocardial fibrosis may cause diastolic dysfunction with exertion not detectable at rest. The stroke volume fails to rise with heart rate, and patients experience dyspnea and fatigue. As ECV was significantly higher in female participants compared with men, effects of diffuse fibrosis on cardiac function may manifest more readily in older women. The prevalence of LV diastolic dysfunction markedly increases after menopause and may lead to heart failure.[42] Additionally, the multivariable model for peak V_{O_2} predicted that WBC was negatively associated with peak V_{O_2}, suggesting a link between reduced exercise capacity and systemic inflammation. T2D may exacerbate the effects of inflammation as acute maximal exercise effort caused a larger transient increase in interleukin-6 and interleukin-8 in T2D compared with controls.

Previous studies in individuals with both type 1 and 2 diabetes have shown that cardiac autonomic neuropathy leads to inability to increase heart rate during exercise was associated with a reduced peak V_{O_2}.[43] During exercise, the healthy heart increases both stroke volume and heart rate. When the cardiac contractility reserve is impaired (eg, with incipient heart failure), peak V_{O_2} fails to increase with heart rate as the stroke volume becomes the limiting factor. In our study, peak V_{O_2} in women did not increase significantly with the chronotropic index (Figure 2B), suggesting that any chronotropic incompetence would be less of a limiting factor than in men. The sex-related difference in the relation of peak V_{O_2} with chronotropic index was confirmed in a multivariable analysis (Table S4).

Although the women in our study have gone through menopause, the effects of prolonged estrogen exposure earlier in life may still contribute to the sex differences in cardiovascular complications at later stages. This hypothesis is supported by multiple studies, including recent meta-analyses that have reported an increased risk of CVD and heart failure in women who experience early menopause.[44–47] Additionally, women with premature ovarian insufficiency or early cessation of menstruation are at higher risk for developing T2D.[48] However, most longitudinal studies focus on changes occurring around the time of menopause,[11] and more research is needed to understand cardiovascular risks in women during late menopause.

There is growing evidence of shared molecular pathways between ovarian aging and aging in other systems, including the cardiovascular system. For instance, epigenetic modifications, such as blood DNA methylation patterns linked to accelerated aging, have been identified as potential mechanisms underlying early menopause.[49] Noncoding RNAs and "female key driver genes" have also been proposed as mediators of the link between aging, the immune system, and diabetes, potentially explaining sex differences in CVD risk and heart failure.[50–53]

In this study, we did not focus on genetic or epigenetic markers but instead examined a wide range of inflammatory markers as potential biomarkers linking T2D, aging, and CVD. We found only a few differences in inflammatory markers between men and women and patients with and without T2DM (see Table S8): WBC was significantly higher in women with T2D compared with men with T2D, while there was no difference between men and women without T2D. Further research is needed to clarify the mechanisms underlying the observed sex differences in older individuals with and without T2D.

Apart from increased WBC and lower interleukin-5 levels among individuals with T2D, there were no major differences between T2D and controls regarding serum inflammatory markers. Growth differentiation factor 15 was higher among those with T2D; however, this is likely explained by metformin increasing growth differentiation factor 15 levels.[54] We found no differences between T2D and controls in skin inflammatory markers, which stands in contrast with studies in younger cohorts reporting an increased number of skin inflammatory cells compared with controls without diabetes.[55,56] Our findings provide evidence that there is no significantly increased skin inflammation in older individuals with T2D.

Together, these findings highlight the complex, sex-specific interactions between aging, inflammation, and T2D in cardiovascular health. The differential effects of T2D by sex underscore the importance of adopting tailored cardiovascular assessments and management strategies, particularly in older individuals where T2D and vascular aging present unique clinical challenges. These findings raise the question of whether sex-specific tests, such as prioritizing exercise tests for women and vascular reactivity tests for men, are needed to more accurately assess cardiovascular

outcomes in older individuals. Further research, particularly longitudinal studies, is needed to better understand the mechanisms driving sex-specific cardiovascular outcomes and to test such tailored approaches. If validated in larger studies, our findings could justify broader physiological testing and additional attention to reducing inflammation in older individuals with T2D, including those with well-managed comorbidities, and support the implementation of more targeted, sex-specific assessments.

Our study has several limitations. Owing to the constraints imposed by the COVID-19 pandemic, not all participants could complete the entire range of planned examinations, including CPET and CMR. The number of participants with T2D was lower than the number of participants without T2D; however, the number of individuals studied was sufficient to provide adequate statistical power for group comparisons. Our study population had a mean duration of diabetes of 15 years and a mean hemoglobin A_{1c} of 7%, which reflects good glycemic control for an older population.[57] Our subjects with T2D had very prevalent use of statins and antihypertensive medications, indicating that cardiovascular comorbidities were well controlled. With respect to diabetes management, at the time we conducted our study, glucagon-like peptide 1 receptor agonists and sodium–glucose cotransporter 2 inhibitors were not used as commonly as they are now. Given their cardiovascular benefits in T2DM,[58,59] our results might underestimate the cardiovascular health of T2D participants currently using these drugs. Our findings may not be generalizable to those with diabetes of much longer or shorter duration, worse glycemic control, or suboptimal management of cardiovascular comorbidities. Finally, our data on physical activity was self-reported. Although we used a validated physical activity assessment,[60] there could be bias in self-reported physical activity data by sex and/or by T2D/control status.

CONCLUSIONS

In older individuals whose CVD risk factors are managed according to contemporaneous guidelines, T2D has a sex-specific effect on coronary and peripheral vascular phenotypes, impairing vascular function and reactivity more in men than in women. In contrast, aerobic exercise capacity was more impaired in women, compared with men, independent of T2D status, and women showed higher levels of adverse myocardial remodeling that may result in exercise-induced myocardial dysfunction. Our findings suggest that the pathophysiological manifestations of T2D on vascular function and exercise capacity are distinct in older men and women, and this may reflect underlying differences in vascular and myocardial aging in the presence of T2D. Systemic inflammation contributed to reduced coronary microcirculatory function and cardiopulmonary exercise capacity independent of T2D status. A possible clinical implication of this finding could be that using lifestyle or other interventions to reduce inflammation in patients with T2D could improve cardiopulmonary capacity and microcirculatory function and reduce cardiovascular risk.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S8
Figures S1–S2

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