Sex Differences in Coronary Microvascular Function in Individuals with Type 2 Diabetes Mellitus

Short Running Title: Cardiac Sex Difference in Diabetes

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ABSTRACT:
Cardiovascular (CV) disease fatality rates are higher for women compared to men with diabetes despite lower rates of obstructive coronary artery disease (CAD). Impaired coronary flow reserve (CFR), the ratio of adenosine-stimulated to rest myocardial blood flow (MBF), is an indicator of coronary microvascular dysfunction and predicts major adverse CV events. We performed a post-hoc analysis to determine if there was a sex disparity in coronary microvascular dysfunction among 46 men and 27 women with well-controlled Type 2 DM and without clinical evidence of obstructive CAD. We found that women had a higher rest MBF, lower CFR, and worse diastolic function compared to men. Additionally, rest MBF was positively correlated with worse diastolic function in women. We previously showed mineralocorticoid blockade improved CFR in men and women with Type 2 DM, implicating aldosterone in the pathophysiology of coronary microvascular dysfunction. We therefore examined aldosterone levels and found that women had larger increases in aldosterone in response to an angiotensin-II infusion than did men. In conclusion, among individuals with Type 2 DM and good cardiometabolic control, women had worse myocardial perfusion and diastolic function compared to men. The greater aldosterone responsivity in women may be a mechanism for this sex effect.
INTRODUCTION:

Although women experience fewer cardiovascular (CV) events than men of the same age, this advantage erodes among individuals with Type 2 DM (1-3). Type 2 DM confers greater risk for coronary heart disease (CHD), myocardial infarction, and CHD-related death in women compared with men (4, 5). Yet, obstructive coronary artery disease (CAD) is less prevalent among women than men (6).

Coronary microvascular dysfunction is increasingly recognized as an important risk factor for CV events (7). A well-established indicator of the presence of coronary microvascular dysfunction is impaired coronary flow reserve (CFR). CFR is the ratio of maximal adenosine-stimulated (stress) to rest myocardial blood flow (MBF) and is assessed on positron emission tomography (PET). Impaired CFR is independently associated with major adverse CV outcomes (8). Murthy et al has shown that, regardless of sex, an increase in CFR of 10% was associated with 20% reduction in CV events (9).

Coronary microvascular dysfunction is more prevalent in individuals with diabetes mellitus and confers increased CV risk (10, 11). A study among individuals with Type 2 DM found that rates of cardiac death were comparable for individuals with obstructive CAD and for individuals without obstructive CAD but with impaired coronary vascular function (12).

In the present study, we investigated whether there was a sex disparity in coronary microvascular function among individuals with well-controlled Type 2 DM and without clinical evidence of obstructive CAD. We hypothesized that women with Type 2 DM would have lower CFR than men with Type 2 DM given the worse CV outcomes in women despite lower rates of obstructive CAD.
RESEARCH DESIGN AND METHODS:

We performed a post-hoc analysis of baseline data from a previously published double-blind, randomized, controlled study among individuals with Type 2 DM without clinical evidence of CAD. Our prior study demonstrated improvement in CFR after six months of mineralocorticoid receptor (MR) blockade as compared to treatment with placebo or hydrochlorothiazide (ClinicalTrials.gov NCT00865124) (13). Participants were aged 18-70 years and had well-controlled Type 2 DM. Exclusion criteria included any evidence of coronary, cerebrovascular, peripheral vascular, or renal disease based on history, physical, EKG and screening blood tests. The full list of inclusion and exclusion criteria has been previously published (13). Partners HealthCare Institutional Review Board approved the protocol, and all participants provided written informed consent.

Subjects completed a 3 month run-in phase where they were started on enalapril 20 mg daily and tapered off other anti-hypertensives. Amlodipine was added if systolic blood pressure (SBP) remained greater than 140 mmHg. Simvastatin 20 mg was added if LDL was greater than 100 mg/dL and the participant was not already on a statin. Anti-diabetic medications were adjusted to optimize glucose control.

After the 3 month run-in, participants were admitted to Brigham and Women’s Hospital Center for Clinical Investigation and completed a baseline assessment, which included echocardiography, cardiac PET scan, cardiac magnetic resonance imaging (MRI) scan and hormonal measurements using techniques previously described (14). All studies were performed with subjects in balance on a liberal sodium (250 mEq) diet. Stress (hyperemic) myocardial blood flow (MBF) and rest MBF from the left ventricle were obtained from cardiac PET scan and used to calculate CFR as previously described (14). Echocardiography was used to
determine E/e’. E/e’ is the ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e’) and is a measure of diastolic function. Normal E/e’ is a value less than 8 with greater than 15 being abnormal.

After subjects had been supine for 30 minutes, blood pressure was assessed every 5 minutes over a 30 minute period using an automated blood pressure device (dynamap). Morning blood was collected with subjects supine and fasting from midnight the previous night. To assess aldosterone response to a controlled stimulus, supine subjects were infused with angiotensin-II at 3 ng/kg/min for 60 minutes with measurement of serum aldosterone before and at the end of the infusion. The infusion rate was reduced to 1 ng/kg/min if SBP increased to greater than 180 mmHg or diastolic blood pressure increased to greater than 100 mmHg. Assays were performed as previously described (14); the lower limit of the aldosterone assay is 2.5 ng/dl. Any participant with imaging evidence of cardiac ischemia or prior myocardial infarction on late gadolinium enhancement cardiac MRI or cardiac PET Scan was excluded. This study used data obtained during this baseline, pre-randomization assessment.

STATISTICAL ANALYSIS:

The Shapiro-Wilk test was used to determine deviations from normality. The distribution of CFR and stress MBF were normally distributed while rest MBF (Shapiro-Wilk test p-value < 0.001) and diastolic function E/e’ (Shapiro-Wilk test p-value 0.01) were not normally distributed. On log transformation, both rest MBF and E/e’ had a log-normal distribution (Shapiro-Wilk test p-value 0.06 and p-value 0.51, respectively). Consequently, natural log transformation was applied to rest MBF and E/e’ before analysis and the coefficients were exponentiated and interpreted as percentage differences.
Multivariate regression was used to evaluate the primary outcome of sex differences in CFR. Covariates were chosen a priori to be the same as those used in our prior study and had been selected from among those associated with vascular function: hemoglobin a1c (HbA1c), body mass index (BMI), race, SBP, statin use, and age (13). The same multivariate model was used to assess sex differences in rest MBF, stress MBF, and diastolic function. Partial correlation was used to assess the relationship between rest MBF and diastolic function, retaining only those covariates contributing significantly to the model (race, HbA1c, and SBP). Other factors (HDL, fasting blood glucose, thiazolidinedione use) that differed between men and women at baseline did not influence our outcomes. Significance was considered for a 2-sided p-value ≤ 0.05. All statistical analysis was performed using Stata 15.1: College Station, TX: StataCorp LLC.

RESULTS:

A total of 73 study participants (63% men) were included in the analysis (see Table 1 for characteristics of the study population). Per the study protocol, all study participants were on the angiotensin-converting enzyme inhibitor enalapril and had excellent glycemic, blood pressure, and lipid management, per the 2012 American Diabetes Association guidelines (15).

Median rest MBF was 0.63 ml/g/min in men (IQR 0.57-0.73 ml/g/min) and 0.86 ml/g/min in women (IQR 0.71-0.95 ml/g/min) (Figure 1A). On multivariate regression, rest MBF was 35% higher in women compared to men, p-value < 0.001. In the model, increased age and SBP contributed to higher rest MBF. Rest MBF was 20% higher in those greater than 60 years old compared to those younger than 50 years old. For every increase of 10 mmHg in SBP, there was a 6% increase in rest MBF.
The median stress MBF was 1.83 ml/g/min in men (IQR 1.65-2.07 ml/g/min) and 2.17 ml/g/min in women (IQR 1.83-2.33 ml/g/min) (Figure 1B). After adjusting for covariates on multivariate regression, stress MBF was not significantly different between the sexes, p-value 0.08.

As CFR is the ratio of stress MBF to rest MBF, the disproportionately higher rest MBF compared to stress MBF in women versus men led to a lower CFR in women with Type 2 DM. The median CFR was 2.85 in men (IQR 2.41-3.46) compared to 2.38 in women (IQR 1.92-3.09), representing a 16% lower CFR in women, p-value 0.008 (Figure 1C).

Diastolic function was assessed with E/e’. Median E/e’ was 6.3 in men (IQR 5.4-7.5) and 7.3 in women (IQR 5.6-9.4), p-value 0.008 (Figure 1D). E/e’ was 18% higher (worse) in women compared to men. E/e’ was also found to be 6% higher for every 10 mmHg higher an individual’s SBP.

We then determined whether there was a correlation between rest MBF and diastolic function. While there was no significant correlation between rest MBF and diastolic function in the overall study population (p-value 0.08), among women there was a significant relationship. In women, higher rest MBF correlated with higher (worse) E/e’, controlling for race, HbA1c, and SBP, p-value 0.05 and correlation coefficient of 0.43 (Figure 2). There was no relationship between CFR and E/e’ in women, men, or the overall study population.

As abnormal activation of the renin-angiotensin-aldosterone system leads to myocardial damage and as MR blockade has been shown to improve CFR in men and women with Type 2 DM, we next examined aldosterone levels (13). There was no difference in baseline aldosterone levels between men (2.50 ng/dl, IQR 2.50-4.00 ng/dl) and women (2.50 ng/dl, IQR 2.50-3.16 ng/dl), p-value 0.644; however, peak angiotensin-II-stimulated aldosterone levels were lower in
men (7.66 ng/dl, IQR 5.68-11.22 ng/dl) than women (11.23 ng/dl, IQR 8.27-14.83 ng/dl), p-value 0.032. The aldosterone response to angiotensin-II (peak minus baseline) was smaller in men (median Δ4.77 ng/dl, IQR 2.72-7.35 ng/dl) than in women (median Δ7.85 ng/dl, IQR 5.76-10.28 ng/dl), p-value 0.027 (Figure 3). Statin use was associated with a smaller increase in aldosterone in response to angiotensin-II, consistent with our previous report that statin use reduced aldosterone production (16).

**DISCUSSION:**

This study demonstrated that among individuals with Type 2 DM in good cardiometabolic control and without clinical or imaging evidence of obstructive CAD, CFR is lower in women compared to men. To our knowledge, this is the first study to examine the impact of sex on MBF and coronary microvascular function in individuals with diabetes but without evidence of heart disease by symptoms and without evidence of obstructive cardiac disease by cardiac imaging (PET and MRI). Our observed difference of almost 0.5 in median CFR between women (2.38) and men (2.85) may be clinically relevant. In a large study of 1218 men and women referred for rest/stress cardiac PET because of concerns for suspected CAD (mean CFR 1.9), an increase in CFR by 0.2 was associated with a 10% decrease in major adverse cardiovascular events (9). However, large-scale studies are needed to determine the specific relationship between CFR and clinical outcomes in individuals without CAD symptoms and with diabetes.

Lower coronary microvascular function predicts CV mortality in men and women with diabetes (8). Our finding that coronary microvascular function is worse in women compared to men may contribute to the excess CV mortality that has been observed in women with diabetes.
as compared to men with diabetes (5). Taqueti et al showed that among patients with low CFR, women showed a higher frequency of non-obstructive CAD while men showed a higher frequency of severely obstructive CAD (17). Despite manifesting a lower burden of obstructive angiographic CAD, women had an elevated risk of CVD events (17).

Our data indicate that the reduction in CFR in women is driven in part by higher rest MBF. This increase in rest MBF does not appear benign as increases in rest MBF were associated with worse diastolic function. Further, a recent study demonstrated that increased rest MBF was an independent predictor of cardiac mortality (18). A key function of the coronary microvasculature is to adjust local blood flow to match tissue metabolic demands (19). While the cause for higher rest MBF in women is not known, it may be that rest MBF is increased in women due to an increase in rest cardiac metabolic demand, possibly related to increased mitochondrial dysfunction in women versus men. This phenomenon of higher rest MBF in women has also been demonstrated by others (9, 21), but these studies enrolled individuals with angina referred for cardiac stress testing.

Impaired CFR may involve remodeling of the microvasculature, altered endothelial function and smooth muscle dysfunction; however, the specific mechanisms involved in diabetes are not yet fully defined. Insulin resistant, obese rodents develop impaired coronary endothelium-dependent vasodilation and diastolic dysfunction, both of which are improved with blockade of the mineralocorticoid receptor (24). In male diabetic rodents, coronary vasoconstriction is enhanced and coronary vasodilation impaired; mineralocorticoid receptor blockade improves endothelium-dependent coronary vasodilatory function in addition to altering expression of genes involved in regulation of nitric oxide production and inflammation (24, 25). These preclinical studies suggest that excess mineralocorticoid receptor activity mediates
impairments in endothelium-dependent coronary vasodilation in diabetes. Further, in non-diabetic, hypertensive rodents, aldosterone damages the intramural coronary arterioles, induces perivascular inflammation and fibrinoid necrosis, causes cardiac inflammation, myocardial necrosis and fibrosis, and impairs mitochondrial function of cardiac fibroblasts (22, 26). Thus, it may be that excess mineralocorticoid receptor activation alters coronary microvascular function through effects on inflammation, fibrosis, mitochondrial function, vasoconstriction and vasodilation.

In an animal model of CV injury induced by low nitric oxide and angiotensin-II administration, mineralocorticoid receptor blockade markedly reduced coronary microvascular and myocardial injury to similar levels in male and female rodents. However, this model caused more aldosterone-mediated myocardial injury in female versus male rodents and this was due to angiotensin-II raising aldosterone to a higher level in female versus male rodents (23). Human females also show increased sensitivity to angiotensin-II; we showed increased angiotensin-II stimulated aldosterone in women versus men with hypertension (23). In humans, blockade of the receptor for aldosterone improved CFR in individuals with diabetes (13), suggesting that excess mineralocorticoid receptor activation is a key contributor to microvascular dysfunction in men and women with diabetes. In this study both men and women showed improvements with mineralocorticoid receptor blockade, but there was inadequate sample size (17 men and 6 women) to assess a sex effect. Our current finding that women with diabetes had increased angiotensin-II stimulated aldosterone versus men raises the possibility that, while activation of the mineralocorticoid receptor is an important contributor to impaired coronary microvascular function in both men and women with diabetes, the excess exposure to aldosterone in women may help explain the excess CVD incidence in women with diabetes.
Advantages of this study are that we carefully controlled cardiometabolic factors (blood pressure, lipids, and glucose) for the 3 months prior to the study. Fingerstick blood glucose prior to PET imaging was not elevated, which is important since elevated blood glucose at time of cardiac PET reduces coronary microvascular function. Additionally, this was a study in individuals without clinical or imaging evidence of obstructive CAD or scarring, so we are presumably examining early changes in diabetic CV disease.

Our study has several limitations. It is a relatively small, cross-sectional, post-hoc analysis and the original study was not designed to assess sex effects. However, our ability to detect significant sex differences suggests there is a strong signal. We cannot state that the observed decrease in coronary microvascular function in women will translate into increased CV morbidity and mortality. However, the association of increased rest MBF with reductions in diastolic function suggest the higher rest MBF is not benign. Further prospective randomized studies are warranted to identify additional mechanisms contributing to these sex disparities.

CONCLUSION:

These findings suggest that among individuals with Type 2 DM and good cardiometabolic control, women have more impairment in myocardial perfusion and diastolic function as compared to men. There may be unique pathophysiologic drivers in females leading to more extensive coronary microvascular dysfunction. One potential mechanism for this sex effect is dysregulated aldosterone production.

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CONFLICTS OF INTEREST:

All authors report no potential conflicts of interest relevant to this article.

AUTHOR CONTRIBUTIONS:

A.V.H., G.K.A., and M.F.D.C. were involved in developing the research question. G.K.A. procured funding. A.D.R. and R.G. recruited subjects. A.D.R., R.G., and G.K.A. helped in clinical management of study participants and conducted the study. R.Y.K. directed imaging and M.F.D.C. directed PET imaging and analysis. B.A.R. and A.V.H. performed the statistical analysis. A.V.H. and G.K.A. wrote the manuscript and R.Y.K., A.D.R., M.F.D.C, and R.G. reviewed/edited the manuscript.

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Dr. Gail Adler is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1. Characteristics of study population (n=73)

|                        | Men       | Women     | p-value |
|------------------------|-----------|-----------|---------|
| N                      | 46        | 27        |         |
| **Mean age (years)**   | 55 ± 7    | 52 ± 8    | 0.11    |
| **Race (n [%])**       |           |           | 0.19    |
| Caucasian              | 31 (67)   | 14 (52)   |         |
| African American       | 12 (26)   | 10 (37)   |         |
| Other                  | 3 (7)     | 3 (11)    |         |
| **BMI (kg/m^2)**       | 32.0 ± 4.6| 32.0 ± 4.8| 0.99    |
| **BP (mmHg)**          |           |           |         |
| Systolic               | 125 ± 12  | 126 ± 15  | 0.79    |
| Diastolic              | 76 ± 9    | 74 ± 10   | 0.37    |
| **Statin use (n [%])** | 36 (78)   | 21 (78)   | 0.96    |
| **Fasting laboratory data** |       |           |         |
| HbA1c (%)              | 6.8 ± 0.8 | 7.0 ± 0.7 | 0.26    |
| Blood glucose (mg/dL)  | 110 ± 20  | 121 ± 28  | 0.08    |
| Total cholesterol (mg/dL) | 148 ± 29 | 154 ± 34 | 0.42    |
| LDL (mg/dL)            | 83 ± 22   | 77 ± 27   | 0.31    |
| HDL (mg/dL)            | 41 ± 11   | 49 ± 12   | 0.01    |
| Triglycerides (mg/dL)  | 124 ± 69  | 124 ± 60  | 0.95    |
| Creatinine clearance rate (mL/min) | 74.8 ± 17.9 | 79.3 ± 20.6 | 0.32  |
| **Duration of diabetes (years)** | 7.9 ± 6.3 | 7.8 ± 6.5 | 0.93    |
| **Diabetes medications (n [%])** |       |           |         |
| Metformin              | 38 (78)   | 23 (79)   | 0.77    |
| Insulin                | 6 (12)    | 4 (14)    | 0.74    |
| Sulfonylurea           | 17 (35)   | 9 (32)    | 0.51    |
| Thiazolidinedione      | 0 (0)     | 3 (11)    | 0.05    |
| Glucagon-like peptide-1 analog | 2 (4)  | 3 (11)    | 0.35    |
| Dipeptidyl peptidase-4 inhibitor | 1 (2) | 0 (0)     | 0.64    |

*p-value < 0.05 between men and women

Data are expressed as mean ± SD for continuous variables and absolute numbers with percentages for categorical variables. Comparisons across sexes were performed using unpaired Student’s t-test, Fisher exact, and as χ² test for continuous, binary, and categorical variables, respectively.
Figure 1. Sex Differences in rest MBF, stress MBF, CFR, and Diastolic Function. A. Median rest MBF was 0.63 ml/g/min in men (IQR 0.57-0.73 ml/g/min) and 0.86 ml/g/min in women (IQR 0.71-0.95 ml/g/min), p-value < 0.001. B. Median stress MBF was 1.83 ml/g/min in men (IQR 1.65-2.07 ml/g/min) and 2.17 ml/g/min in women (IQR 1.83-2.33 ml/g/min), p-value 0.08. C. Median CFR was 2.85 in men (IQR 2.41-3.46) and 2.38 in women (IQR 1.92-3.09), p-value 0.008. D. Median diastolic function (E/e’) was 6.3 in men (IQR 5.4-7.5) and 7.3 in women (IQR 5.6-9.4), p-value 0.008. Higher E/e’ values indicate worse diastolic function. All p-values adjusted for HbA1c, BMI, race, systolic blood pressure, statin use, and age.

Figure 2. A. Partial correlation of log transformed rest MBF and log transformed diastolic function (E/e’) among women (partial coefficient of 0.43 and p-value of 0.05). Higher E/e’ values correspond with worse diastolic function. B. There was no significant partial correlation of log transformed rest MBF and log transformed diastolic function (E/e’) among men (partial coefficient of 0.0054 and p-value of 0.97).

Figure 3. Sex Differences in Aldosterone Response to Angiotensin-II (AngII). Men had smaller increases in aldosterone in response to Ang-II infusion (median ∆4.77 ng/dl, IQR 2.72-7.35 ng/dl) than did women (median ∆7.85 ng/dl, IQR 5.76-10.28 ng/dl), p-value 0.02.
A

Rest MBF (ml/g/min)

Men

Women

B

Stress MBF (ml/g/min)

Men

Women

C

CFR

Men

Women

D

Diastolic Function (E/e')

Men

Women

Diabetes
A

Women

B

Men

log diastolic dysfunction (E/e')

log rest MBF
Change in Aldosterone (ng/dl)

Men

Women

*