Heart

Differences in Myocardial Structure and Coronary Microvasculature Between Men and Women With Coronary Artery Disease

Duncan J. Campbell, Jithendra B. Somaratne, Alicia J. Jenkins, David L. Prior, Michael Yii, James F. Kenny, Andrew E. Newcomb, Darren J. Kelly, Mary Jane Black

Abstract—Women younger than 75 years with stable angina or acute coronary syndrome have higher cardiac mortality than similarly aged men, despite less obstructive coronary artery disease. To determine whether the myocardial structure and coronary microvasculature of women differs from that of men, we performed histological analysis of biopsies from nonischemic left ventricular myocardium from 46 men and 11 women undergoing coronary artery bypass graft surgery who did not have previous cardiac surgery, myocardial infarction, heart failure, atrial fibrillation, or furosemide therapy. The 2 patient groups had similar clinical characteristics, apart from a lower body surface area (BSA) in women (P=0.0015). Women had less interstitial fibrosis than men (P=0.019) but similar perivascular fibrosis. Arteriolar wall area/circumference ratio, a measure of arteriolar wall thickness, was 47% greater in women than men (P=0.012). Cardiomyocyte width and diffusion radius were positively correlated, and capillary length density was negatively correlated with BSA (P<0.05). Whereas cardiomyocyte width, capillary length density, diffusion radius, and cardiomyocyte width/BSA ratio were similar for men and women, women had a greater diffusion radius/BSA ratio (P=0.0038) and a greater diffusion radius/cardiomyocyte width ratio (P=0.027). Women also had lower vascular endothelial growth factor (VEGF) receptor-1 levels (P=0.048) and VEGF receptor-1/VEGF-A ratio (P=0.024) in plasma. We conclude that women with extensive coronary artery disease have greater arteriolar wall thickness and diffusion radius relative to BSA and to cardiomyocyte width than men, which may predispose to myocardial ischemia. Additional studies of larger numbers of women with less extensive coronary artery disease are required to confirm these findings. (Hypertension. 2011;57:186-192.) ● Online Data Supplement

Key Words: gender ■ ischemic heart disease ■ myocardial fibrosis ■ coronary microvasculature

Ischemic heart disease of women is different from that of men.1,2 Women with stable angina referred for angiographic evaluation are less likely to have significant coronary artery disease,3 yet women younger than 75 years with stable angina have a higher coronary standardized mortality ratio than men.4 Moreover, when women present with an acute coronary syndrome, they are less likely to have an ST-segment elevation myocardial infarction and more likely to have unstable angina,5 and although they are less likely to have significant coronary artery disease,6,5 women less than 75 years of age with myocardial infarction have a higher risk of coronary death than men.3,6–8 Evidence for a greater role for coronary microvascular dysfunction in ischemic heart disease of women is the report that retinal arterial narrowing, a marker of lower hyperemic myocardial blood flow and perfusion reserve,9 predicted coronary events in women, but not in men.10 To investigate the possibility that the different characteristics of ischemic heart disease in men and women reflect differences in myocardial structure and coronary microvasculature, we examined myocardial fibrosis, cardiomyocyte size, capillary length density, diffusion radius, and arteriolar dimensions in biopsies from nonischemic left ventricular (LV) myocardium from men and women undergoing coronary artery bypass graft surgery. Our finding that women had greater arteriolar wall thickness and diffusion radius relative to body surface area (BSA) and to cardiomyocyte width led us to also study angiogenesis-related markers in this patient population.

Methods

The St. Vincent’s Health Human Research Ethics Committee approved this research, and all patients gave written informed consent. For details of the St. Vincent’s Health Cardiac Tissue Bank and biopsy of the nonischemic LV myocardium, see the online Data Supplement.

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Study Patients
The 2 patient groups were of similar age and had similar extent of coronary artery disease (Table 1). Four men and 2 women did not have angina before surgery. Of the remaining patients, the median duration of angina was 6 months (range 1 to 360) in men (n=42) and 24 months (range 1 to 240) in women (n=9). Of the 42 men with angina, 24 had stable angina, 24 had angina of <2 months duration or increasing in severity, and 16 had rest pain before surgery. Of the 9 women with angina, 2 had angina of <2 months duration or increasing in severity, and 7 had rest pain before surgery. Men and women had similar clinical characteristics except that women had a lower BSA and lower hemoglobin. There were no differences in therapies between men and women.

Men and women had similar hemodynamics at the time of surgery (Table 1). In particular, men and women had similar LV filling pressures, as indicated by pulmonary capillary wedge pressure. Among the patients who had preoperative transthoracic echocardiography, the 2 patient groups had similar LV ejection fraction (median, interquartile range; men: 0.61, 0.56 to 0.66, n=61; women: 0.61, 0.56 to 0.66, n=66; P=0.94), LV end-diastolic diameter (men: 4.9, 4.6 to 5.2 cm, n=42; women: 4.4, 4.2 to 5.0 cm, n=5; P=0.14), septal wall thickness (men: 1.1, 0.8 to 1.2 cm, n=19; women: 1.0, 0.8 to 1.2 cm, n=6; P=0.82), posterior wall thickness (men: 1.0, 0.8 to 1.2 cm, n=20; women: 1.0, 0.9 to 1.1 cm, n=6; P=1.0), mitral Doppler velocity E/A wave ratio (men: 1.0, 0.9 to 1.2, n=22; women: 0.8, 0.7 to 1.2, n=7; P=0.26), mitral valve deceleration time (men: 232, 192 to 266 ms, n=26; women: 207, 191 to 236 ms, n=7; P=0.42), early diastolic peak velocity of the septal mitral annulus, E' (men: 5.9, 4.6 to 6.9 cm/s, n=22; women: 5.8, 5.5 to 7.3 cm/s, n=6; P=0.80), and E/E' ratio (men: 12, 9 to 14, n=25; women: 13, 9 to 15, n=6; P=0.73).

Histology
Women had less total and interstitial fibrosis than men, but similar perivascular fibrosis (Figure 1 and Table 2). Because of the trend for arterioles of women to have greater mean diameter than the arterioles of men, arteriolar dimensions were analyzed for arterioles of mean diameter 20 to 80 µm, and women had 47% greater arteriolar wall area/circumference ratio, indicative of a greater arteriolar wall thickness. There were no differences between men and women in cardiomyocyte width, capillary length density, or diffusion radius. Cardiomyocyte width, capillary length density, and diffusion radius of men were each correlated with BSA, and cardiomyocyte width was correlated with diffusion radius (Figure 2). Similarly, significant correlations existed for body weight and lean body mass, whereas correlations with height and body mass index were not statistically significant (data not shown).

BSA of women was 90% of that of men, and lean body mass of women was 72% of that of men (Table 1). The cardiomyocyte width/BSA ratio was similar for men and women, whereas the cardiomyocyte width/lean body mass ratio of women was greater than that of men (Table 2). There was evidence for a reduced capillary length density in women, relative to BSA, lean body mass, and cardiomyocyte width, in that women had a greater diffusion radius/BSA ratio, diffusion radius/lean body mass ratio, and diffusion radius/cardiomyocyte width ratio than men.

Plasma Levels of Advanced Glycation End Products and Angiogenesis-Related Biomarkers
Given the greater diffusion radius relative to BSA, lean body mass, and cardiomyocyte width in women, we measured the levels of advanced glycation end products (AGEs) and angiogenesis-related biomarkers in plasma collected from these patients before surgery (Table S1, available in the online Data Supplement). Compared with men, women had 28% lower vascular endothelial growth factor receptor (VEGFR)-1 levels (P=0.048) associated with a nonsignificant 74% increase in VEGF-A levels, and the VEGFR-1/VEGF-A ratio was 48% lower in women (P=0.024).

Discussion
Several mechanisms have been proposed for the greater cardiac mortality of women with stable angina and acute coronary syndrome than similarly aged men, despite less obstructive coronary artery disease. These include the smaller coronary arteries, abnormal coronary reactivity, microvascular dysfunction, and a predisposition to plaque erosion and distal embolization in women. The myocardium normally extracts most of the oxygen in arterial blood, and the importance of the coronary microvasculature to the ischemic vulnerability of the myocardium is well recognized. The greater arteriolar wall area/circumference ratio, indicative of increased arteriolar wall thickness, was associated with a parallel trend for greater arteriolar diameter and was consistent with vascular hypertrophy rather than eutrophic remodeling of the arteriolar vessel wall. Tone and remodeling of resistance vessels are highly interrelated, and arteriolar hypertrophy may predispose women to a greater coronary vasoconstrictor or lesser vasodilator response to vasoactive agents. Impaired coronary dilator response to acetylcholine predicts cardiovascular events, and impaired coronary microvascular reactivity to adenosine predicts adverse outcomes in women evaluated for suspected...
Table 1. Characteristics of Male and Female Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males (n=46)</th>
<th>Females (n=11)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>64 (57–73)</td>
<td>67 (47–73)</td>
<td>0.61</td>
</tr>
<tr>
<td>Left main stenosis &gt;50%, n (%)</td>
<td>24 (52%)</td>
<td>2 (18%)</td>
<td>0.051</td>
</tr>
<tr>
<td>One vessel stenosis &gt;70%, n (%)</td>
<td>10 (22%)</td>
<td>4 (36%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Two vessel stenosis &gt;70%, n (%)</td>
<td>24 (52%)</td>
<td>4 (36%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Three vessel stenosis &gt;70%, n (%)</td>
<td>11 (24%)</td>
<td>2 (18%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Patients with occluded coronary artery, n (%)</td>
<td>17 (37%)</td>
<td>3 (27%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Coronary collaterals, Rentrop grade 2 or 3, n (%)</td>
<td>22 (48%)</td>
<td>5 (45%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Wall motion abnormality, n (%)</td>
<td>5 (11%)</td>
<td>1 (9%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Previous percutaneous transluminal coronary angioplasty, n (%)</td>
<td>7 (15%)</td>
<td>0 (0%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Coronary artery conduits/patient, n</td>
<td>3 (3–4)</td>
<td>2 (2–4)</td>
<td>0.17</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29 (26–31)</td>
<td>31 (26–35)</td>
<td>0.19</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>64 (58–67)</td>
<td>46 (44–51)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>2.0 (1.9–2.1)</td>
<td>1.8 (1.7–1.9)</td>
<td>0.0015</td>
</tr>
<tr>
<td>Clinical risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>10 (21%)</td>
<td>5 (45%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Metabolic syndrome (nondiabetic), n (%)</td>
<td>17 (37%)</td>
<td>4 (36%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Preadmission SBP, mm Hg</td>
<td>129 (122–143)</td>
<td>131 (124–146)</td>
<td>0.66</td>
</tr>
<tr>
<td>Preadmission DBP, mm Hg</td>
<td>76 (70–81)</td>
<td>75 (70–79)</td>
<td>0.42</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>31 (67%)</td>
<td>8 (73%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Use of tobacco, ever, n (%)</td>
<td>28 (61%)</td>
<td>5 (45%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Fasting plasma total cholesterol, mmol/L</td>
<td>3.3 (2.8–3.8)</td>
<td>3.8 (2.9–4.2)</td>
<td>0.49</td>
</tr>
<tr>
<td>Fasting plasma LDL cholesterol, mmol/L</td>
<td>1.9 (1.5–2.3)</td>
<td>2.1 (1.6–2.8)</td>
<td>0.35</td>
</tr>
<tr>
<td>Fasting plasma HDL cholesterol, mmol/L</td>
<td>0.9 (0.8–1.1)</td>
<td>0.9 (0.8–1.0)</td>
<td>0.83</td>
</tr>
<tr>
<td>Fasting plasma triglyceride, mmol/L</td>
<td>1.3 (1.1–2.0)</td>
<td>1.7 (1.4–1.8)</td>
<td>0.42</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>6.1 (5.4–6.6)</td>
<td>5.6 (5.0–6.0)</td>
<td>0.10</td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol/L</td>
<td>65 (36–100)</td>
<td>57 (45–91)</td>
<td>0.88</td>
</tr>
<tr>
<td>β cell function from HOMA2-%B</td>
<td>69 (52–91)</td>
<td>88 (62–113)</td>
<td>0.18</td>
</tr>
<tr>
<td>Insulin sensitivity from HOMA2-%S</td>
<td>81 (53–148)</td>
<td>92 (64–120)</td>
<td>0.75</td>
</tr>
<tr>
<td>Insulin resistance from HOMA2-IR</td>
<td>1.20 (0.7–1.9)</td>
<td>1.10 (0.8–1.6)</td>
<td>0.80</td>
</tr>
<tr>
<td>Plasma NT-proBNP, pmol/L</td>
<td>12 (5–27)</td>
<td>8 (5–17)</td>
<td>0.79</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>14.7 (13.8–15.3)</td>
<td>12.7 (11.3–13.3)</td>
<td>0.0007</td>
</tr>
<tr>
<td>eGFR, ml/min per 1.73 m²</td>
<td>75 (62–83)</td>
<td>65 (62–71)</td>
<td>0.12</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.2 (0.8–4.5)</td>
<td>3.7 (1.2–4.4)</td>
<td>0.36</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
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<tr>
<td>ACE inhibitor therapy, n (%)</td>
<td>24 (52%)</td>
<td>7 (64%)</td>
<td>0.74</td>
</tr>
<tr>
<td>ARB therapy, n (%)</td>
<td>11 (24%)</td>
<td>4 (36%)</td>
<td>0.46</td>
</tr>
<tr>
<td>ACEI and/or ARB therapy, n (%)</td>
<td>34 (74%)</td>
<td>10 (91%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Statin therapy, n (%)</td>
<td>40 (87%)</td>
<td>8 (73%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Aspirin therapy, n (%)</td>
<td>42 (91%)</td>
<td>10 (91%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Calcium antagonist therapy, n (%)</td>
<td>10 (22%)</td>
<td>5 (45%)</td>
<td>0.14</td>
</tr>
<tr>
<td>β-blocker therapy, n (%)</td>
<td>33 (72%)</td>
<td>10 (91%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Long-acting nitrate therapy, n (%)</td>
<td>10 (22%)</td>
<td>4 (36%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Thiazide or indapamide therapy, n (%)</td>
<td>10 (22%)</td>
<td>5 (45%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Intraoperative hemodynamics immediately after induction of anesthesia</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Central venous pressure, mm Hg</td>
<td>8 (6–11)</td>
<td>9 (6–11)</td>
<td>0.87</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>10 (8–12)</td>
<td>10 (6–11)</td>
<td>0.38</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>5.0 (4.0–6.2)</td>
<td>4.0 (3.4–4.6)</td>
<td>0.016</td>
</tr>
<tr>
<td>Cardiac index, L/min per m²</td>
<td>2.5 (2.0–3.0)</td>
<td>2.3 (1.9–2.4)</td>
<td>0.18</td>
</tr>
<tr>
<td>Left ventricular stroke work, g.m per beat</td>
<td>78 (67–91)</td>
<td>69 (58–74)</td>
<td>0.20</td>
</tr>
<tr>
<td>Left ventricular stroke work index, g.m.m⁻² per beat</td>
<td>39 (32–45)</td>
<td>40 (34–43)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are expressed as median (25th to 75th percentiles) or number (percentage). One male and one female with left main stenosis >50% did not have other vessel stenosis >70%. Coronary collaterals were scored according to Rentrop et al.47 Lean body mass was calculated according to Boer.48 HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; HOMA, Homeostasis Model Assessment calculator version 2.2; eGFR, estimated glomerular filtration rate calculated using the Modification of Diet in Renal Disease study equation; NT-proBNP, amino-terminal-pro-B-type natriuretic peptide.
ischemia. Several studies indicate that women have a higher coronary microvascular tone than men. These include the prediction of incident coronary events by smaller retinal arterial caliber, a marker of lower hyperemic myocardial blood flow, in women but not in men, the more frequent occurrence of impaired coronary flow reserve in response to intracoronary adenosine in women, and the more frequent occurrence of coronary microvascular spasm in women.

Because the diffusion radius was correlated with BSA and cardiomyocyte size, we expressed diffusion radius not only in absolute terms but also as diffusion radius/BSA and diffusion radius/cardiomyocyte ratios. The greater arteriolar wall area/circumference ratio, diffusion radius/BSA ratio, and diffusion radius/cardiomyocyte width ratio of women may contribute to their greater vulnerability to myocardial ischemia. Consistent with this speculation is the increased risk of all-cause cardiac death associated with LV hypertrophy, with its associated increased diffusion radius, and the greater impact of LV hypertrophy on survival in women than in men without significant coronary disease, although not in patients with coronary artery disease.

A previous autopsy study reported loss of cardiomyocytes and cardiomyocyte hypertrophy in association with aging in men but not in women, and at the age of the patients in our study, there was a trend for men to have a greater cardiomyocyte width than women. However, another recent small autopsy study reported similar cardiomyocyte size and capillary length density in men and women without coronary artery disease. Our finding that cardiomyocyte width correlated with body size was consistent with the establishment of cardiomyocyte number in infancy, with subsequent increases in body size and LV mass accompanied by cardiomyocyte enlargement. After puberty, LV mass is higher in men than in women, and despite the higher BSA of men, the LV mass/BSA ratio is higher in men. In addition to body size and gender, hemodynamic load is a major influence on LV mass, but there were no differences in blood pressure, hemodynamics, or antihypertensive medication use between men and women in our study. Given the lower LV mass/BSA ratio reported in women and the lower BSA of women in this study, if women and men had similar numbers of cardiomyocytes one would predict that women would have smaller cardiomyocytes than men. Our finding of a trend for smaller cardiomyocyte width in women was consistent with this prediction.

Our finding of a correlation between cardiomyocyte width and diffusion radius was consistent with the critical role of cardiomyocyte size in determining capillary length density and diffusion radius in the adult because of the limited potential for myocardial angiogenesis in adults. In addition, we showed that capillary length density and diffusion radius were each correlated with body size. However, the greater diffusion radius/BSA ratio of women could not be explained by a greater cardiomyocyte size because of the trend for women to have smaller cardiomyocyte width than men. Moreover, the use of medications that may influence myocardial angiogenesis, such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers, was similar for men and women in this study.

Alterations in myocardial capillary density may be a consequence of changes in factors that control angiogenesis or, alternatively, may cause a reactive change in angiogenesis factors. The role of AGEs in angiogenesis is complex, with both inhibition and stimulation of angiogenesis described. However, we found no difference between men and women in plasma levels of AGEs or soluble receptor for AGEs. VEGF-A is a major regulator of angiogenesis, and the soluble form of VEGFR-1 functions as a circulating VEGF-A antagonist by preventing VEGF-A binding to VEGFR-2, the main VEGF mediating angiogenesis. VEGF-A levels are increased in both acute and chronic myocardial ischemia and predict cardiac risk, whereas there are reports of both increased and decreased VEGFR-1 levels following myocardial infarction. The reduced VEGF-R1 levels and VEGFR-1/VEGF-A ratio and nonsignificant 74% increase in VEGF-A levels in women, compared with men, in our study may therefore reflect their possible predisposition to ischemia attributable to their arteriolar hypertrophy and their greater diffusion radius relative to BSA and cardiomyocyte width.

Although sex-related differences in myocardial remodeling have been described, the role of sex hormones in cardiac fibrosis is uncertain. Inhibition of cardiac fibroblast growth by estrogens is unlikely to apply to our study because most women were postmenopausal and none were taking estrogens. In contrast to our finding of lower myocardial intersti-
tial fibrosis in women with coronary artery disease, men and women with aortic stenosis were recently reported to have similar myocardial fibrosis, and an autopsy study also reported similar collagen content of myocardium from men and women. Moreover, experimental studies showed no effect of androgens on cardiac mass or fibrosis of wild-type mice, although androgens increased cardiac mass and fibrosis of mice lacking the gene encoding guanylyl cyclase-A. We found no association between myocardial fibrosis and any echocardiographic or hemodynamic parameter for the 46 men in this study (data not shown), and the functional significance of lower myocardial interstitial fibrosis in women is unknown.

Table 2. Histology of LV Biopsies of Male and Female Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males (n=46)</th>
<th>Females (n=11)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardium area per section, mm²</td>
<td>3.4 (2.9–5.6)</td>
<td>3.6 (2.4–4.4)</td>
<td>0.69</td>
</tr>
<tr>
<td>Total fibrosis, %</td>
<td>2.1 (1.4–2.7)</td>
<td>1.1 (1.0–1.8)</td>
<td>0.011</td>
</tr>
<tr>
<td>Interstitial fibrosis, %</td>
<td>1.4 (1.1–2.1)</td>
<td>0.8 (0.6–1.2)</td>
<td>0.019</td>
</tr>
<tr>
<td>Perivascular fibrosis ratio, µm²/µm²</td>
<td>1.7 (1.1–2.4)</td>
<td>1.8 (1.2–2.8)</td>
<td>0.76</td>
</tr>
<tr>
<td>Cardiomyocyte width, µm</td>
<td>23 (21–25)</td>
<td>21 (20–22)</td>
<td>0.055</td>
</tr>
<tr>
<td>Cardiomyocyte width/BSA ratio, µm²/m²</td>
<td>11 (10–13)</td>
<td>12 (10–13)</td>
<td>0.50</td>
</tr>
<tr>
<td>Cardiomyocyte width/lean body mass ratio, µm/kg</td>
<td>0.36 (0.33–0.39)</td>
<td>0.45 (0.40–0.49)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Arterioles per section, n</td>
<td>4 (3–5)</td>
<td>4 (3–5)</td>
<td>0.58</td>
</tr>
<tr>
<td>Arterioles/mm² myocardium area, n</td>
<td>1.0 (0.7–1.2)</td>
<td>1.0 (0.8–1.6)</td>
<td>0.49</td>
</tr>
<tr>
<td>Mean arteriolar diameter, all arterioles, µm</td>
<td>37 (25–49)</td>
<td>44 (37–49)</td>
<td>0.12</td>
</tr>
<tr>
<td>Arteriolar wall area/circumference ratio, arterioles 20–80 µm mean diameter, µm²/µm</td>
<td>4.5 (3.7–6.1)</td>
<td>6.6 (5.2–7.0)</td>
<td>0.012</td>
</tr>
<tr>
<td>Capillary length density, mm/mm³</td>
<td>1226 (1021–1555)</td>
<td>1141 (1015–1484)</td>
<td>0.70</td>
</tr>
<tr>
<td>Diffusion radius, µm</td>
<td>16 (14–18)</td>
<td>17 (15–18)</td>
<td>0.70</td>
</tr>
<tr>
<td>Diffusion radius/BSA ratio, µm²/m²</td>
<td>8.0 (7.3–8.5)</td>
<td>9.0 (8.4–9.9)</td>
<td>0.0038</td>
</tr>
<tr>
<td>Diffusion radius/lean body mass ratio, µm/kg</td>
<td>0.25 (0.23–0.28)</td>
<td>0.35 (0.32–0.39)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diffusion radius/cardiomyocyte width ratio, µm/µm</td>
<td>0.67 (0.62–0.78)</td>
<td>0.78 (0.70–0.84)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Data are expressed as median (25th to 75th percentiles). Myocardium area per section excludes epicardium. We did not attempt to analyze arterioles in the longitudinal section, and only arterioles in an approximate cross-section or oblique section with diameters (average of maximum and minimum diameter of each arteriole) of 12–151 µm were counted for estimation of arteriolar density and analyzed for perivascular fibrosis. The arteriolar wall area/circumference ratio was measured for arterioles with diameters of 20–80 µm for 45 men and 11 women. Capillary length density and diffusion radius were measured for 45 men.

Figure 2. Correlations between cardiomyocyte width and BSA (A), diffusion radius and BSA (B), capillary length density and BSA (C), and diffusion radius and cardiomyocyte width (D) in males (open symbols; n=46) and females (filled symbols; n=11). Also shown are the regression lines for men (dotted lines) and women (dashed lines). The Spearman rank correlation coefficients are for men alone. The Spearman rank correlation coefficients for women alone did not achieve statistical significance.
This study had a number of limitations. It had limited sample size because of the need for a myocardial biopsy from each patient and the smaller proportion of women undergoing coronary artery bypass graft surgery. Another limitation was the inherent selection bias caused by the sampling of patients presenting for coronary artery bypass graft surgery, and it is not known whether our findings apply to patients with less extensive coronary artery disease. However, the key differences in characteristics of ischemic heart disease between men and women occur in those with coronary artery disease, and in a separate study, we found similar myocardial fibrosis, cardiomyocyte width, capillary length density, diffusion radius, and arteriolar dimensions in aortic stenosis patients with and without coronary artery disease (data not shown). Although men and women had similar extent of coronary lesions, there was, however, a borderline significant greater proportion of men with left main stenosis that may have precipitated surgery independent of coexisting microvascular dysfunction more than other coronary lesions. To avoid the effect of coronary stenoses on myocardial structure and the microvasculature, we took particular care to collect biopsies from the same epicardial region of the LV myocardium without evidence of ischemia or wall motion abnormality, which was proximal to significant flow-limiting coronary stenoses and collateral. However, it cannot be guaranteed that the biopsies were from healthy and comparable parts of myocardium of different patients, as subclinical perfusion disturbances might not be obvious, and another limitation of this approach is that we do not know if the data obtained apply to other regions of the myocardium. Moreover, we had echocardiographic data for only a limited number of patients, although we had ventriculograms and hemodynamic data, including LV filling pressures, for all patients.

Perspectives
The coronary microvasculature makes a greater contribution to ischemic heart disease in women than in men, impacting on their quality of life and leading to premature myocardial infarction and death. We found that women with extensive coronary artery disease presenting for coronary artery bypass graft surgery had greater arteriolar wall thickness and diffusion radius relative to BSA and to cardiomyocyte width than men. If these differences in coronary microvasculature were to exist in women with less severe coronary artery disease, they may contribute to the greater vulnerability of women to myocardial ischemia and may thereby explain in part the different presentation of ischemic heart disease in women from that of men. These findings could also help explain why women tolerate obstructive coronary artery disease poorly. Additional studies of larger numbers of women with less extensive coronary artery disease are required to confirm these findings. Improved understanding of the mechanisms of myocardial arteriolar hypertrophy and the regulation of myocardial capillary length density and diffusion radius may lead to new therapies that reduce the burden of ischemic heart disease not only in women but also in men.

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Disclosures
The authors declare that there is no duality of interest associated with this manuscript.

References


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Differences in myocardial structure and coronary microvasculature between men and women with coronary artery disease

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Methods:

St. Vincent's Health Cardiac Tissue Bank
The St. Vincent's Health Cardiac Tissue Bank was established for the purpose of investigating myocardial mechanisms of heart failure. St. Vincent's Health is a tertiary referral hospital and most patients were referred from other suburban or country hospitals. Patients recruited to the Tissue Bank were unselected apart from the requirement for informed consent, and the exclusion of patients with previous cardiac surgery or who were at particularly high surgical risk. Between April 2005 and November 2008, 323 patients scheduled for cardiac surgery were recruited to the Tissue Bank. These included patients undergoing coronary artery bypass graft surgery, valve surgery, or the combination of coronary artery bypass graft and valve surgery. St. Vincent's Health performs approximately 450 cardiac surgical procedures with cardiopulmonary bypass each year. The 323 patients recruited to the Cardiac Tissue Bank were only a small proportion of the total number of potential patients. This was because only two of the four surgeons collected biopsies, many patients were recruited to other research studies and were therefore unavailable for recruitment to the Tissue Bank, the need for consent by the patient and their family, and the availability of staff to obtain consent and to attend theatre for the collection and processing of biopsies and plasma and the collection of data.

All blood samples were collected from the radial artery cannula of fasted patients before anesthesia and plasma stored at -80°C. All patients had Swan-Ganz catheters inserted before surgery that provided measures of pulmonary artery and pulmonary capillary wedge pressures and cardiac output recorded immediately after induction of anesthesia.

A partial-thickness wedge-shaped biopsy was taken during surgery, immediately after cardioplegia, from a region of the lateral wall of the left ventricle near the base of the heart, between the territories of the left anterior descending and circumflex arteries, that was free of any macroscopic pathology, without evidence of ischemia or wall motion abnormality on pre-operative or intra-operative imaging studies. The biopsy was excised with an elliptical incision and was approximately 3 mm in width, 5-10 mm in length, and 5 mm in depth. The biopsy was immediately rinsed in ice-cold normal saline with 20 mmol/L KCl to ensure cardiomyocytes and vessels were relaxed. The inner 2 mm of the biopsy was excised and cut into 2 portions; one portion was placed in RNAlater RNA stabilization reagent (Qiagen, Valencia, CA) and stored at -80°C for subsequent RNA extraction, and one portion was frozen in liquid nitrogen and stored at -80°C for subsequent proteomics studies. The remainder of the biopsy, including the epicardium, was cut into 2 portions; one portion was immersion fixed in 4% paraformaldehyde and embedded in paraffin, and the other portion was frozen in Optimum Cutting Temperature compound (Tissue-Tek, Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands) for frozen section. Paraffin- and frozen-embedded tissues were embedded with the epicardium on one side; however, it was not possible to determine the orientation of cardiomyocytes and capillaries in the tissue before embedding.

Transthoracic echocardiography was performed either by the referring hospital or by St. Vincent's Health. However, for some patients who did not have echocardiography performed by the referring hospital, there was insufficient time after admission to St. Vincent's Health to perform echocardiography before surgery. However, all patients had ventriculograms performed.
Metabolic syndrome was defined according to the Adult Treatment Panel III (ATP III) guidelines. For patients in whom abdominal circumference was not measured, based on the relationship between abdominal circumference and body mass index (BMI), those with BMI $\geq 30$ kg/m$^2$ were considered to exceed the abdominal circumference threshold of the ATP III guidelines, and those with BMI $< 25$ kg/m$^2$ were considered to be below the abdominal circumference threshold, whereas for patients with BMI 25-30 kg/m$^2$, abdominal circumference was not classified. A patient had diabetes if a history of diabetes was evident from use of glucose-lowering medications and/or insulin or if fasting plasma glucose was $\geq 7$ mmol/L.

Biochemistry
Blood HbA1c and plasma levels of glucose, insulin, lipids, creatinine, and C-reactive protein (high sensitivity assay) were measured by St. Vincent's Health Pathology using routine clinical methods. Amino-terminal-pro-B-type natriuretic peptide was measured by electrochemiluminescence immunoassay using an Elecsys instrument (Roche Diagnostics, Basel, Switzerland).

Carboxymethyl lysine (CML) was measured by ELISA (Microcoat, Penzberg, Germany). Low molecular weight fluorophore (LMWF) levels were measured by fluorescence spectroscopy. Angiogenesis factors in EDTA plasma were measured by ELISA using kits from Uscn Life Science Inc., Wuhan, P.R. China for vascular endothelial growth factor (VEGF)-B, and from R&D Systems Inc., Minneapolis, MN for VEGF-A, VEGF receptor (VEGFR)-1, VEGFR-2, angiopoietin-1, angiopoietin-2, Tie-1, Tie-2, fibroblast growth factor (FGF) acidic, FGF basic, endostatin, placental growth factor (PLGF), and hepatocyte growth factor (HGF).

Menopausal status was confirmed by measurement of plasma levels of ethinyl estradiol, follicle stimulating hormone, and luteinizing hormone by St. Vincent's Health Pathology.

Histological analysis
All histological analyses were performed blind to patient group allocation. Twenty sequential 4 µm sections were cut from each paraffin block and sections 1 and 20 were stained with picrosirius red for analysis of interstitial and perivascular fibrosis and arteriolar dimensions by quantitative morphometry of digitized images of the whole section (Aperio Technologies, Inc., CA). Myocardial interstitial collagen density was calculated using the positive pixel count algorithm as the area of collagen staining expressed as a percentage of the total myocardial tissue area, after excluding perivascular fibrosis and the epicardium.

Arterioles were identified by the presence of a layer of media and immunohistochemical staining for elastin showed the blood vessels were relaxed. The tissue was immersion fixed and the arterioles were usually oval in shape because of deformation and/or because they were cut at an oblique angle. We did not attempt to analyze arterioles in longitudinal section, and only arterioles in approximate cross-section or oblique-section were counted for estimation of arteriolar density and analyzed for perivascular fibrosis; these arterioles had diameters (average of maximum and minimum diameter of each arteriole) of 12-151 µm. Both picrosirius red-stained sections 1 and 20 were analyzed with care to avoid counting the same arteriole twice. Arterioles were analyzed by planimetry of the digitized image. The inner and outer vessel
borders, and the outer border of the perivascular fibrosis, were carefully traced manually, and the Aperio program automatically calculated the circumference and area of each region. Perivascular fibrosis was calculated as the ratio of the area of perivascular fibrosis to the total vessel area (area of vessel wall plus lumen).\(^5\) Arteriolar wall area/circumference ratio was measured for arterioles with average diameters of 20-80 \(\mu m\), which represented 86% of all arterioles counted.

Cardiomyocyte width, determined on 4 \(\mu m\) sections of paraffin-embedded tissue (one section per patient) stained for reticulin,\(^6\) was the mean of >100 measurements for each section of the shortest diameter of cardiomyocyte profiles containing a nucleus.

Capillary length density, which is the length of capillaries per unit volume of tissue, was determined by analysis of 4 \(\mu m\) sections of paraffin-embedded tissue (one section per patient) immunostained for CD31 (mouse anti-human CD31 monoclonal antibody, Dako Denmark A/S, Glostrup, Denmark) using standard stereological techniques.\(^7\)\(^-\)\(^10\) Each tissue section was systematically imaged over the entire tissue face using 40x objective (SPOT Insight 4 Meg FW Color Mosaic camera, model 14.2, Diagnostic Instruments, Inc., MI). Each image was opened in Image-Pro Plus image analysis software (SciTech, Australia) and in each field of view 4 unbiased counting frames (120 \(\mu m\) x 120 \(\mu m\)) were superimposed over the section (within the 4 corners of the field); within each unbiased counting frame there was a 40 \(\mu m\) orthogonal grid (9 squares per unbiased counting frame). There were two lines of exclusion for each counting frame, one horizontal running along the bottom and one vertical running along the left border of the counting frame. Capillary length density was calculated using the equation: Length density = \(2 \cdot \sum C / \sum Pt \cdot 0.0016mm^2\), where \(C\) = the number of capillaries found within the counting frame, excluding those that touched a line of exclusion, \(Pt\) = the number of grid points lying on tissue (maximum of 9 per counting frame), and 0.0016 \(mm^2\) = area associated with each grid point.\(^8\)\(^,\)\(^9\) Diffusion radius was determined according to the formula: Diffusion radius=\(\sqrt{(1 / \pi \times \text{length density})}\).\(^8\)\(^,\)\(^10\) As the orientation of cardiomyocytes and capillaries in the biopsies was not known at the time of embedding, and there was only one biopsy per patient, the mean capillary length density for each patient group represents the mean of estimates from sections with capillaries in different orientations.

**Statistical analysis**

Percentages were presented for discrete variables and medians with 25th and 75th percentiles for continuous variables. Differences between groups were tested with \(\chi^2\) or Fisher’s exact tests for discrete variables and Mann-Whitney U tests for continuous variables. Correlation between variables was estimated from the Spearman rank correlation coefficient. Calculations were performed using Statview 5.0.1 statistical software (SAS Institute Inc.) and a \(P\) value of less than 0.05 was considered to indicate statistical significance.

**References**


Table S1. Plasma levels of advanced glycation end-products and angiogenesis-related biomarkers in male and female patients undergoing coronary artery bypass graft surgery.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th>Females</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=46</td>
<td>n=11</td>
<td></td>
</tr>
<tr>
<td>CML (µmol/L)</td>
<td>2.2 (1.8, 2.4)</td>
<td>2.2 (1.7, 2.3)</td>
<td>0.53</td>
</tr>
<tr>
<td>LMWF (AU/mL)</td>
<td>2.5 (2.1, 3.2)</td>
<td>2.8 (2.0, 3.1)</td>
<td>0.90</td>
</tr>
<tr>
<td>sRAGE (pg/mL)</td>
<td>573 (455, 744)</td>
<td>667 (464, 1199)</td>
<td>0.36</td>
</tr>
<tr>
<td>VEGF-A (pg/mL)</td>
<td>23 (14, 30)</td>
<td>40 (12, 45)</td>
<td>0.27</td>
</tr>
<tr>
<td>VEGF-B (pg/mL)</td>
<td>&lt;15</td>
<td>&lt;15</td>
<td></td>
</tr>
<tr>
<td>VEGFR-1 (pg/mL)</td>
<td>117 (80, 184)</td>
<td>84 (36, 124)</td>
<td>0.048</td>
</tr>
<tr>
<td>VEGFR-1/VEGF-A ratio (pg/pg)</td>
<td>4.6 (3.2, 12.6)</td>
<td>2.4 (1.1, 4.6)</td>
<td>0.024</td>
</tr>
<tr>
<td>VEGFR-2 (pg/mL)</td>
<td>6661 (5754, 7752)</td>
<td>6630 (6386, 7257)</td>
<td>0.76</td>
</tr>
<tr>
<td>Angiopoietin-1 (pg/mL)</td>
<td>3707 (2510, 5730)</td>
<td>2678 (1298, 5650)</td>
<td>0.33</td>
</tr>
<tr>
<td>Angiopoietin-2 (pg/mL)</td>
<td>1366 (1090, 1875)</td>
<td>1554 (1196, 2510)</td>
<td>0.22</td>
</tr>
<tr>
<td>Tie-1 (ng/mL)</td>
<td>36 (29, 41)</td>
<td>36 (29, 43)</td>
<td>0.70</td>
</tr>
<tr>
<td>Tie-2 (ng/mL)</td>
<td>16 (14, 18)</td>
<td>15 (12, 17)</td>
<td>0.35</td>
</tr>
<tr>
<td>FGF acidic (pg/mL)</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td></td>
</tr>
<tr>
<td>FGF basic (pg/mL)</td>
<td>8.3 (5.1, 10.6)</td>
<td>5.9 (4.5, 8.7)</td>
<td>0.23</td>
</tr>
<tr>
<td>Endostatin (ng/mL)</td>
<td>90 (75, 113)</td>
<td>102 (75, 119)</td>
<td>0.56</td>
</tr>
<tr>
<td>PLGF (pg/mL)</td>
<td>10.8 (8.3, 16.1)</td>
<td>8.2 (7.1, 11.5)</td>
<td>0.070</td>
</tr>
<tr>
<td>HGF (pg/mL)</td>
<td>768 (580, 983)</td>
<td>670 (580, 885)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Data shown as median (25th, 75th percentile), statistical comparisons by Mann-Whitney U tests. CML, carboxymethyl lysine; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; LMWF, low molecular weight fluorophore; PLGF, placental growth factor; sRAGE, soluble receptor for advanced glycation end-products; Tie-1 and Tie-2 are angiopoietin receptors 1 and 2, respectively; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.