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CLINICAL RESEARCH
Coronary heart disease

Sex differences in atheroma burden and endothelial function in patients with early coronary atherosclerosis

Seung Hwan Han^{1,2}, Jang Ho Bae^{1,3}, David R. Holmes Jr¹, Ryan J. Lennon⁴, Eric Eeckhout⁵, Gregory W. Barsness¹, Charanjit S. Rihal¹, and Amir Lerman^{1*}

¹Division of Cardiovascular Diseases, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA; ²Division of Cardiology, Gachon University of Medicine and Science, Incheon, Republic of Korea; ³Division of Cardiology, Knoryang University Hospital, Daejeon, Republic of Korea; ⁴Section of Biostatistics, Mayo Clinic, Rochester, MN, USA; and ⁵3 Cardiology Division, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

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Aims

Women and men have different clinical presentations and outcomes in coronary artery disease (CAD). We tested the hypothesis that sex differences may influence coronary atherosclerotic burden and coronary endothelial function before development of obstructive CAD.

Methods and results

A total of 142 patients (53 men, 89 women; mean \pm SD age, 49.3 \pm 11.7 years) with early CAD simultaneously underwent intravascular ultrasonography and coronary endothelial function assessment. Atheroma burden in the left main and proximal left anterior descending (LAD) arteries was significantly greater in men than women (median, 23.0% vs. 14.1%, $P = 0.002$; median, 40.1% vs. 29.3%, $P = 0.001$, respectively). Atheroma eccentricity in the proximal LAD artery was significantly higher in men than women (median, 0.89 vs. 0.80, $P = 0.04$). The length of the coronary segments with endothelial dysfunction was significantly longer in men than women (median, 39.2 vs. 11.1 mm, $P = 0.002$). In contrast, maximal coronary flow reserve was significantly lower in women than men (2.80 vs. 3.30, $P < 0.001$). Sex was an independent predictor of atheroma burden in the left main and proximal LAD arteries (both $P < 0.05$) by multivariate analysis.

Conclusion

Men have greater atheroma burden, more eccentric atheroma, and more diffuse epicardial endothelial dysfunction than women. These results suggest that men have more severe structural and functional abnormalities in epicardial coronary arteries than women, even in patients with early atherosclerosis, which may result in the higher incidence rates of CAD and ST-segment myocardial infarction in men than women.

Keywords

Atherosclerosis • Endothelium • Sex differences

Introduction

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in men and women in most developed nations.¹ Women and men have been shown to have different clinical presentations, prevalence, and clinical outcomes of CAD.^{2,3} Men present more often than women with ST-segment elevation myocardial infarction and have a higher prevalence of CAD adjusted to age.^{1,4–6} Women present more frequently than men, however, for the evaluation of chest pain and are more frequently hospitalized for it.² In addition, women who have CAD, especially younger

women, have more adverse outcomes after myocardial infarction and coronary artery bypass grafting surgery than men despite their documented lower angiographic disease burden.^{7–9} These findings indicate that sex may have an important influence on cardiovascular physiology and pathology.^{10,11} Thus, these differences may reflect a differential effect of sex on the pathogenesis of coronary atherosclerosis.

Measurement of atheroma burden by intravascular ultrasonography (IVUS)^{12–16} and assessment of endothelial function^{17–20} are useful tools for evaluating early features of coronary atherosclerosis. Current study was designed to test the hypothesis that

*Corresponding author. Tel: +1 507 255 4152, Fax: +1 507 255 2550, Email: lerman.amir@mayo.edu

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sex influences coronary atherosclerotic burden and coronary endothelial function before the development of obstructive CAD. We simultaneously investigated the sex differences in atheroma burden and coronary endothelial function in patients with early coronary atherosclerosis.

Methods

Study subjects

All patients had been referred for coronary angiography by an independent cardiologist for evaluation of CAD. From January 2001 to February 2007, patients without obstructive CAD (diameter stenosis, <30% by angiography) were prospectively enrolled in tests of endothelial function at Mayo Clinic in Rochester, MN, USA. Exclusion criteria for undergoing testing of endothelial function included presence of myocardial infarction; uncontrolled hypertension; valvular heart disease; cardiomyopathy; ejection fraction <40%; significant endocrine, hepatic, renal, or inflammatory disease; presentation with acute coronary syndrome; or refusal to participate. The Mayo Clinic Institutional Review Board approved the study, and informed consent was obtained from all participants.

For the current study, a subset of the group undergoing coronary endothelial function testing was selected to also undergo IVUS. Patients who had IVUS were selected randomly and did not sign a separate consent form for the IVUS study. Patients who had previous percutaneous coronary intervention or coronary artery bypass grafting surgery were further excluded for this study. Long-acting nitrates or calcium-channel blockers were withheld for 48 h before the study to allow assessment of baseline coronary physiology. Patients' demographic and laboratory data were obtained from the medical records.

Endothelial function assessment and coronary flow reserve

Coronary angiography was performed according to standard techniques using the femoral artery approach. Coronary endothelial function was assessed as previously described.^{21–22} Briefly, a Doppler guidewire (0.014" diameter, FloWire; Volcano Corp., Rancho Cordova, CA, USA) within a 2.2-F coronary infusion catheter (Ultrafuse; SciMed Life Systems, Maple Grove, MN, USA) was advanced and positioned in the middle portion of the left anterior descending (LAD) coronary artery. Velocity signals were obtained instantaneously from the Doppler wire by an online fast Fourier transform, and average peak velocity was determined. Intracoronary bolus injections of incremental doses (18–60 µg) of adenosine were administered until maximal hyperaemia was achieved, to evaluate endothelium-independent microvascular coronary flow reserve (CFR). CFR was calculated by dividing the average peak velocity after adenosine injection by the average peak velocity at baseline.

Subsequently, to assess endothelium-dependent function, the endothelium-dependent vasodilator acetylcholine was selectively infused into the LAD artery for 3 min at increasing concentrations (10^{-6} , 10^{-5} , and 10^{-4} M) to obtain effective coronary concentrations of 10^{-8} , 10^{-7} , and 10^{-6} M, respectively. Coronary artery diameter and average peak velocity were measured. After each infusion of acetylcholine, coronary blood flow was calculated from the Doppler-derived time velocity integral and vessel diameter as follows:

$$\pi \times \left(\frac{\text{coronary artery diameter}}{2} \right)^2 \times \left(\frac{\text{average peak velocity}}{2} \right).$$

Reproducibility of the measurements from our laboratory is $8 \pm 3\%$.

Percentage diameter change in the mid-LAD artery was measured in the segment 5 mm distal to the tip of the Doppler wire after each infusion. According to our previous studies, we defined coronary epicardial endothelial dysfunction as >20% diameter decrease in response to the maximum dose of acetylcholine compared with the coronary artery diameter measured during baseline angiography in the same artery location.²¹ Total length of endothelial dysfunction was defined as the sum of the lengths of all segments with endothelial dysfunction. Finally, endothelium-independent epicardial coronary artery function was determined by the change in coronary artery diameter in response to intracoronary nitroglycerine bolus injection (100 µg).

Coronary artery diameter and length were measured off-line by an independent investigator, who was unaware of the patient's sex and clinical characteristics, by using a quantitative coronary angiography program (Medis Corp., Leiden, The Netherlands) as previously described.²³ The reproducibility of the measurements from our laboratory is $5 \pm 2\%$.

Intravascular ultrasonography examination

After the assessment of coronary endothelial function and CFR, 200 µg intracoronary nitroglycerine was given, and IVUS imaging was performed according to previously described methods.^{23,24} A 2.9-F IVUS catheter with electronic scanning (Volcano Corp.) was advanced into the mid-distal LAD artery via a 0.014" guidewire, which was exchanged for a Doppler guidewire, and motorized pullback was performed at a speed of 0.5 to 1.0 mm/s. The IVUS image was recorded for later off-line analysis. External elastic membrane (EEM) area and lumen area were measured in the proximal, distal, and lesion sites of each left main and proximal LAD artery (from ostium to branching site of first diagonal) by an independent investigator blinded to the clinical data and endothelial function test results.

Atheroma (plaque plus media) area was calculated as EEM area minus lumen area. As previously recommended,²⁵ percentage atheroma burden was calculated as: (atheroma area/EEM area) × 100; atheroma eccentricity was calculated as: (maximum – minimum atheroma thickness)/maximum atheroma thickness. The proximal and distal site of each segment was selected as the image slice with the largest atheroma burden within 5 mm of the proximal and distal ends of each segment. The lesion site selected for analysis was the image slice with the largest percentage atheroma burden in each entire segment. Segments with poor image quality or large vessel diameter exceeding the IVUS scale, very short segments, and severely tortuous vessels were excluded from analysis.

Statistical analysis

Continuous variables were presented as mean ± SD for characteristics with appropriately near-symmetrical distributions or as median (interquartile range). Discrete data are presented as frequencies and percentages. Sex comparisons were evaluated with the Student's *t*-test, the Mann–Whitney *U*-test, or the Pearson χ^2 test. Multiple regression models were used to assess whether the differences between men and women were independent of other risk factors. Log transformations of glucose, triglyceride, and C-reactive protein (CRP) levels were used because of their skewed distributions. Because of the high correlation between body surface area (BSA) and sex ($r = 0.46$, $P < 0.001$), we standardized BSA values for men and women separately, such that the new variable had a mean of 0 and SD of 1 within each sex, and used that variable for modelling. Residual distributions were inspected by normal probability plots; if the plot showed clear deviation from normality, the base 2 log transform was

applied to the end point and the residuals were inspected again. The length of endothelial dysfunction was skewed because many patients had a length of 0 mm; thus, a multiple regression model for the length of endothelial dysfunction was analysed only in patients with endothelial dysfunction. Covariates for the models were selected by inspecting unadjusted linear associations. Variables significant at the 0.15 level were included in the model as potential confounders. All other hypothesis tests had a 0.05 significance level. All tests were two-sided.

Results

Patient characteristics and risk factors

Of the 638 patients who underwent endothelial function testing, 152 patients (23.8%) were randomly selected to also undergo IVUS during the study period. Ten of these patients were excluded on the basis of previous percutaneous coronary intervention; therefore, the cohort comprised 142 patients [53 men (37%), 89 women (63%)] who had a mean \pm SD age of 49.3 ± 11.7 years. The clinical characteristics of the patients are shown in Table 1. Fifty-four women (61%) were postmenopausal, 20 of whom (37%) were receiving hormone-replacement therapy before the present study. Men were significantly younger than women (45.2 ± 11.0 vs. 51.7 ± 11.5 years, $P = 0.001$) and had lower high-

density lipoprotein cholesterol levels (41.48 ± 10.33 vs. 57.98 ± 16.41 mg/dL, $P < 0.001$). Women had significantly larger body mass index (30.14 ± 6.78 vs. 28.23 ± 4.42 kg/m², $P = 0.04$), higher total cholesterol (190.44 ± 37.57 vs. 175.28 ± 36.48 mg/dL, $P = 0.02$) and CRP values [0.36 (0.14–0.90) vs. 0.16 (0.07–0.40) mg/dL, $P = 0.01$], and lower haemoglobin (12.96 ± 1.11 vs. 13.98 ± 0.4 g/dL, $P < 0.001$) and creatinine (0.91 ± 0.13 vs. 1.10 ± 0.21 , $P < 0.001$) values. Other parameters shown in Table 1 were not significantly different between men and women.

Intravascular ultrasonography findings

Some segments were excluded because of poor image quality (17 segments in the left main and three segments in the LAD arteries), large vessel diameter exceeding the IVUS scale (18 segments in the left main), very short segments (10 segments in the left main), or severely tortuous vessels (three segments in the LAD). A total of 97 IVUS findings in the left main artery (from 36 men and 61 women) and 136 IVUS findings in the proximal LAD artery (from 48 men and 88 women) remained for analysis. The EEM area, lumen area, and atheroma area of the lesion site in the left main artery were significantly larger in men (all $P < 0.05$; Table 2). Men also had significantly greater atheroma burden in the proximal site, distal site, and lesion site of the left main artery (all $P < 0.05$; Table 2, Figure 1A). In the proximal LAD

Table 1 Patient characteristics^a

Characteristic	Patients		P-value
	Men (n = 53)	Women (n = 89)	
Age (year)	45.2 \pm 11.0	51.7 \pm 11.5	0.001
Hypertension	30 (56.6)	44 (49.4)	0.41
Diabetes mellitus	3 (5.7)	10 (11.2)	0.27
Hyperlipidemia	32 (60.4)	54 (60.7)	0.74
Current smoking	8 (15.1)	10 (11.2)	0.20
Body mass index (kg/m ²)	28.23 \pm 4.42	30.14 \pm 6.78	0.04
Mean arterial pressure (mmHg)	96.56 \pm 14.00	99.29 \pm 15.23	0.31
Ejection fraction (%)	65.18 \pm 4.28	64.10 \pm 5.62	0.24
Creatinine (mg/dL)	1.10 \pm 0.21	0.91 \pm 0.13	<0.001
Haemoglobin (g/dL)	13.98 \pm 1.04	12.96 \pm 1.11	<0.001
Glucose (mg/dL)	94 (89–101)	94 (86–104)	0.56
Total cholesterol (mg/dL)	175.28 \pm 36.48	190.44 \pm 37.57	0.02
LDL cholesterol (mg/dL)	106.73 \pm 32.08	109.09 \pm 33.10	0.68
HDL cholesterol (mg/dL)	41.48 \pm 10.33	57.98 \pm 16.41	<0.001
Triglycerides (mg/dL)	129 (86–166)	98 (64–147)	0.05
C-reactive protein (mg/dL)	0.16 (0.07–0.40)	0.36 (0.14–0.90)	0.01
Medications			
Aspirin	31 (58.5)	45 (50.6)	0.36
β -Blockers	17 (32.1)	28 (31.5)	0.94
ACE inhibitors	13 (24.5)	17 (19.1)	0.44
Calcium-channel antagonists	20 (37.7)	35 (39.3)	0.85
Lipid-lowering agents	23 (43.4)	35 (39.3)	0.63

ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^aValues are no. of patients (%), mean \pm SD, or median (interquartile range).

Table 2 Intravascular ultrasonography findings in the left main and proximal left anterior descending arteries^a

Location	Left main artery		P-value	Proximal LAD artery		P-value
	Men (n = 36)	Women (n = 61)		Men (n = 48)	Women (n = 88)	
Proximal						
EEM area (mm ²)	24.2 (19.6–26.6)	19.7 (17.2–22.3)	<0.001	18.4 (16.2–20.2)	15.0 (12.9–16.8)	<0.001
Lumen area (mm ²)	19.7 (15.5–23.2)	17.7 (15.8–19.6)	0.06	13.5 (11.7–15.4)	12.4 (10.0–14.2)	0.01
Atheroma area (mm ²)	3.2 (1.8–5.0)	1.6 (1.1–2.9)	0.002	4.3 (2.1–6.0)	2.2 (1.1–3.6)	<0.001
Atheroma burden (%)	14.5 (8.4–20.5)	8.8 (5.6–14.8)	0.01	22.0 (11.8–34.3)	15.2 (8.1–23.9)	0.007
Distal						
EEM area (mm ²)	25.4 (21.2–27.5)	19.7 (18.0–21.9)	<0.001	16.8 (14.5–21.8)	15.3 (13.6–17.6)	0.01
Lumen area (mm ²)	18.8 (16.9–22.0)	17.6 (15.8–19.0)	0.006	12.9 (10.3–16.1)	12.0 (10.0–14.4)	0.17
Atheroma area (mm ²)	5.2 (1.9–8.3)	1.8 (1.2–3.7)	0.002	2.7 (1.4–7.5)	2.2 (1.3–4.6)	0.21
Atheroma burden (%)	19.4 (8.3–32.1)	9.4 (6.0–18.8)	0.02	18.4 (7.7–36.4)	17.9 (7.8–29.4)	0.56
Lesion						
EEM area (mm ²)	24.2 (20.0–27.1)	19.7 (17.4–22.1)	<0.001	17.1 (15.4–21.2)	15.1 (13.2–16.7)	<0.001
Lumen area (mm ²)	18.3 (15.4–21.0)	16.7 (14.7–18.5)	0.03	10.3 (8.7–12.2)	10.4 (8.3–12.3)	0.63
Atheroma area (mm ²)	5.8 (3.2–7.7)	2.8 (1.4–4.2)	<0.001	7.3 (4.8–8.7)	4.3 (2.3–6.2)	<0.001
Atheroma burden (%)	23.0 (14.3–32.4)	14.1 (8.1–21.8)	0.002	40.1 (30.3–49.7)	29.3 (18.2–42.4)	0.001
Atheroma eccentricity	0.91 (0.81–0.97)	0.90 (0.67–0.97)	0.24	0.89 (0.78–0.94)	0.80 (0.70–0.93)	0.04

EEM, external elastic membrane; LAD, left anterior descending artery.

^aValues are median (interquartile range).

artery, the EEM area and atheroma area of the lesion site were significantly larger in men (both $P < 0.001$; Table 2). Men also had significantly greater atheroma burden in the proximal site and lesion site of the proximal LAD artery than women (both $P < 0.01$; Table 2, Figure 1A). Atheroma eccentricity in the lesion site of the proximal LAD artery was also significantly greater in men than in women [0.89 (0.78–0.94) vs. 0.80 (0.70–0.93), $P = 0.04$; Table 2, Figure 1B].

Coronary endothelial function assessment

After intracoronary infusion of acetylcholine, the percentage change in mid-LAD artery diameter was similar between men and women (Table 3). However, the total length of coronary segments with endothelial dysfunction was significantly longer in men than in women [39.2 (0.0–71.6) vs. 11.1 (0.0–38.5) mm, $P = 0.002$; Table 3, Figure 2]. Even after correction for BSA, men had significantly longer coronary artery segments with endothelial dysfunction than women [17.9 (0.0–35.6) vs. 6.8 (0.0–22.6) mm/m², $P = 0.008$; Table 3]. The percentage change in artery diameter after intracoronary nitroglycerine infusion was not significantly different by sex (Table 3).

Coronary microvascular function test

Microvascular endothelial-dependent function, assessed by percentage change in coronary blood flow after intracoronary acetylcholine infusion, was not significantly different by sex (Table 3). In contrast, endothelium-independent microvascular function,

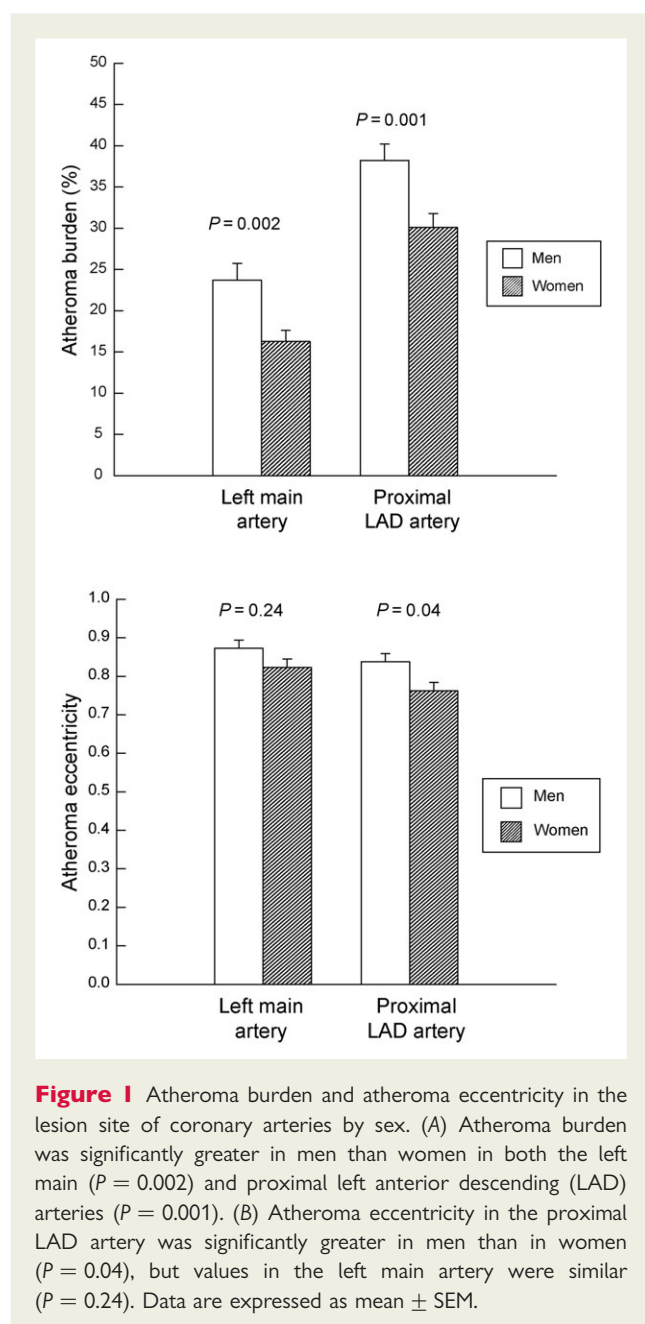
assessed by maximal CFR after intracoronary adenosine infusion, was significantly lower in women than in men (Table 3, Figure 3).

Independent predictors of atheroma burden in left main and proximal left anterior descending arteries

By univariate analysis, male sex, age, mean arterial pressure (per 10 mmHg), creatinine level (per 0.2 mg/dL), haemoglobin value, and log₂ triglyceride level were predictors of log₂ atheroma burden in the left main artery. Male sex, age, haemoglobin value, and log₂ triglyceride level were predictors of atheroma burden in the proximal LAD artery by univariate analysis (Table 4). By multivariate analysis, male sex was an independent predictor of log₂ atheroma burden in the left main artery [β -coefficient [95% confidence interval (CI)], 0.50 (0.03–0.97), $P = 0.04$] and atheroma burden in the proximal LAD artery [8.91 (2.52–15.30), $P = 0.007$] (Table 5).

Independent predictors of length of endothelial dysfunction and coronary flow reserve

Because the length of artery with endothelial dysfunction was 0 mm in 53 patients (37%), the multiple regression model for the length of endothelial dysfunction was performed including only patients with endothelial dysfunction ($n = 89$). Male sex and age were predictors of the length of the coronary segment with endothelial dysfunction by univariate analysis (Table 4). By multivariate analysis, male sex almost reached statistical significance as a predictor of the length of endothelial dysfunction [β -coefficient



(95% CI), 11.94 (-0.45 to 24.33), $P = 0.059$; Table 5]. Male sex, age, sex-standardized BSA, and creatinine (per 0.2 mg/dL), haemoglobin, \log_2 glucose, and \log_2 CRP values were predictors of \log_2 maximal CFR by univariate analysis (Table 4). Age ($P = 0.047$), sex-standardized BSA ($P = 0.002$), \log_2 glucose concentration ($P = 0.04$), and \log_2 CRP value ($P = 0.049$) were independent predictors of \log_2 maximal CFR by multivariate analysis (Table 5).

Discussion

To our knowledge, this is the first study to simultaneously investigate atheroma burden, endothelial function, and microvascular function by sex in patients with early coronary atherosclerosis. Our study showed that men with early atherosclerosis had more

severe atheroma burden, more eccentric atheroma, and more diffuse epicardial artery endothelial dysfunction than women. The current study supports a potential mechanism for the differential incidence and clinical presentations of CAD between men and women.

Age-adjusted CAD incidence rate and sudden cardiac death rate in men exceed those in women.^{1,5,26} In addition, men present more often than women with ST-segment elevation myocardial infarction.^{27,28} Women more frequently present with non-obstructive coronary arteries and non-Q-wave myocardial infarctions. In addition, aggressive revascularization management strategies have not been found to be effective in women, although they have a clear benefit in men.^{3,29-31} However, the potential mechanism for these differences in the clinical presentation of CAD is not known.

Recent clinical studies indicated that the level of plaque and plaque progression as measured by IVUS appeared to be related to future cardiovascular events,¹²⁻¹⁵ and IVUS-measured changes in atheroma burden was suggested as a surrogate cardiovascular end point.¹⁶ Our IVUS findings were similar to coronary angiographic findings in previous reports, which showed that women had lower rates of obstructive CAD at angiography when evaluated for symptoms suggestive of myocardial ischaemia.² Recently, Nicholls *et al.*³² reported that the atheroma volume in women with angiographic CAD is less than in men, despite the presence of more cardiovascular risk factors in women. Our study extends this previous observation and demonstrates that the difference in atheroma burden exists even at early stages of disease. Moreover, the women in the current study had more CAD risk factors in terms of age, body mass index, total cholesterol level, haemoglobin value, and CRP levels. Even accounting for different cardiovascular risk factors between sexes, male sex was an independent predictor of atheroma burden in left main and proximal LAD arteries by multivariate analysis. In addition, our study demonstrated that the morphology of atheroma was more eccentric in men than in women. Thus, our study shows that in patients with early CAD, men have greater atheroma burden and eccentric atheroma in coronary arteries than do women.

These findings may have clinical implications. Virmani *et al.*³³ previously reported that plaque rupture in patients with sudden coronary death was more frequent in men and that plaque erosion was more frequently found in young women. Our IVUS findings that men with early atherosclerosis had more severe atheroma burden and eccentric atheroma in coronary arteries suggest that these characteristics may contribute to a higher rate of plaque rupture.

Similar to anatomic findings such as atheroma burden measured by IVUS, functional measurements such as endothelium-dependent vasomotor function of the coronary and brachial arteries have been shown to predict long-term cardiovascular risk, including cardiac and cerebrovascular events.¹⁷⁻²⁰ In previous human studies, women had significantly improved flow-mediated dilator response to hyperaemia as measured by high-resolution ultrasonography of the brachial artery.^{34,35} Our study showed that men had significantly longer coronary segments with endothelial dysfunction. Thus, to our knowledge, this is the first study to show that

Table 3 Coronary epicardial and microvascular function^a

Measurement	Men (n = 53)	Women (n = 89)	P-value
Epicardial endothelial-dependent function			
Percentage change in mid-LAD diameter after acetylcholine IC	-10.6 (-26.0 to -1.3)	-10.1 (-22.4-0.4)	0.41
Length of the endothelial dysfunction (mm)	39.2 (0.0-71.6)	11.1 (0.0-38.5)	0.002
Length of endothelial dysfunction/BSA (mm/m ²)	17.9 (0.0-35.6)	6.8 (0.0-22.6)	0.008
Epicardial endothelial-independent function			
Percentage change in mid-LAD diameter after nitroglycerine IC	9.3 (2.5-20.4)	10.0 (0.0-18.7)	0.74
Microvascular endothelial-dependent function			
Percentage change in CBF after acetylcholine IC	27.3 (-22.5-98.7)	36.1 (-9.8-82.7)	0.89
Microvascular endothelial-independent function			
Maximal CFR after adenosine IC	3.30 (2.90-4.00)	2.80 (2.40-3.20)	<0.001

BSA, body surface area; CBF, coronary blood flow; CFR, coronary flow reserve; IC, intracoronary infusion.

LAD, left anterior descending artery.

^aValues are median (interquartile range).

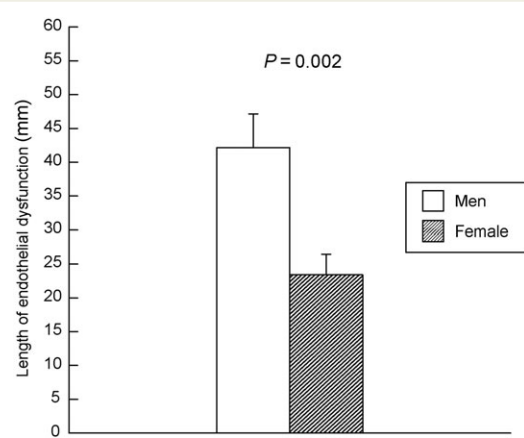


Figure 2 Graph shows the length of the coronary segments with epicardial endothelial dysfunction after intracoronary acetylcholine infusion. The segments were significantly longer in men than women ($P = 0.002$). Data are expressed as mean \pm SEM.

men have more segments with vascular injury and abnormal repair, which are at risk for the development of atherosclerosis and future cardiovascular events.

Taken together, our IVUS findings and results of functional studies show that men have more severe structural and functional abnormalities than women in epicardial coronary arteries. These abnormalities may explain the higher prevalence of CAD and the higher incidences of ST-segment elevation myocardial infarction and sudden cardiac death due to plaque rupture in men.

Although sex was not an independent predictor of maximal CFR by multivariate analysis, our study showed that women had significantly lower maximal CFR than men. These results are consistent with those of a previous report that showed women had slightly lower coronary vasodilatory reserve even with normal coronary angiographic results.³⁶ The endothelial-independent microvascular

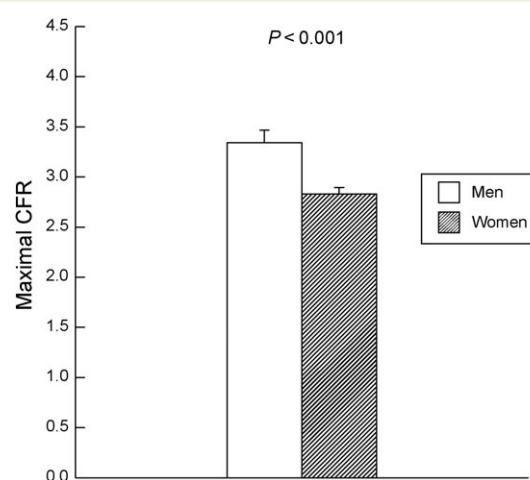


Figure 3 Maximal coronary flow reserve (CFR) after intracoronary infusion of adenosine was significantly decreased in women compared with men ($P < 0.001$). Data are expressed as mean \pm SEM.

dysfunction was an independent predictor of adverse outcomes in patients with mild CAD.³⁷ A recent clinical study reported that coronary microvascular dysfunction was not associated with markers of inflammatory or endothelial cell activation in women with chest pain in the absence of obstructive CAD.³⁸ This result is consistent with our current findings regarding the disparity between epicardial endothelial function and microvascular endothelial-independent function in women.³⁸ It has been suggested that the mechanism of myocardial ischaemia in women may be localized to the microvascular coronary arteries and that abnormal microvascular function may have prognostic implications.³⁸⁻⁴² Our microvascular function results support these observations.

Table 4 Predictors of atheroma burden in left main and proximal left anterior descending arteries, epicardial function, and microvascular function by univariate analysis^a

Parameter	Log ₂ atheroma burden in left main		Atheroma burden in proximal LAD		Length of coronary segments with endothelial dysfunction		Log ₂ maximal CFR	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Male sex	0.62 (0.23–1.01)	0.002	8.12 (2.80–13.44)	0.003	15.59 (3.76–27.42)	0.01	0.22 (0.10–0.34)	<0.001
Age (per year)	0.02 (0.001–0.04)	0.04	0.29 (0.07–0.52)	0.01	–0.61 (–1.14 to –0.08)	0.02	–0.01 (–0.01–0.003)	0.001
Diabetes mellitus	–0.46 (–1.18–0.26)	0.21	5.49 (–3.40–14.37)	0.22	–15.18 (–34.03–3.86)	0.12	0.01 (–0.20–0.23)	0.91
Current smoking	–0.19 (–0.76–0.37)	0.50	1.07 (–6.88–9.00)	0.79	–1.74 (–19.59–16.11)	0.85	–0.04 (–0.23–0.15)	0.68
BSA (per 0.25 m ²)	0.25 (0.04–0.47)	0.02	1.99 (–0.81–4.78)	0.16	4.21 (–2.94–10.81)	0.21	0.12 (0.06–0.19)	<0.001
Sex-standardized BSA	0.10 (–0.11–0.31)	0.34	0.09 (–2.57–2.76)	0.95	0.22 (–6.01–6.45)	0.95	0.08 (0.02–0.14)	0.01
BMI (per 5 kg/m ²)	0.09 (–0.07–0.25)	0.26	–0.15 (–2.29–1.99)	0.89	–2.10 (–7.21–3.02)	0.42	0.01 (–0.05–0.06)	0.85
MAP (per 10 mmHg)	0.18 (0.04–0.32)	0.01	0.19 (–1.61–2.00)	0.83	–3.38 (–7.41–0.65)	0.10	–0.04 (–0.08–0.01)	0.09
Creatinine (per 0.2 mg/dL)	0.28 (0.09–0.48)	0.005	2.50 (–0.29–5.28)	0.08	1.26 (–4.67–7.20)	0.67	0.07 (0.01–0.14)	0.03
Haemoglobin	0.32 (0.16–0.48)	<0.001	3.25 (1.09–5.41)	0.004	1.13 (–4.26–6.52)	0.68	0.06 (0.01–0.12)	0.02
Log ₂ glucose	0.12 (–0.51–0.76)	0.70	7.76 (–0.85–16.36)	0.08	–17.23 (–35.79–1.33)	0.07	–0.22 (–0.43 to –0.02)	0.03
Total cholesterol (per 40 mg/dL)	–0.01 (–0.22–0.21)	0.96	–0.10 (–2.89–2.69)	0.94	0.17 (–6.17–6.51)	0.96	–0.01 (–0.08–0.06)	0.80
LDL cholesterol (per 25 mg/dL)	–0.01 (–0.17–0.14)	0.87	–0.27 (–2.29–1.76)	0.80	0.71 (–3.90–5.32)	0.76	0.01 (–0.04–0.05)	0.83
HDL cholesterol (per 10 mg/dL)	–0.08 (–0.20–0.04)	0.20	–0.62 (–2.22–0.99)	0.45	–1.85 (–5.81–2.11)	0.36	–0.02 (–0.06–0.01)	0.22
Log ₂ triglyceride	0.28 (0.02–0.53)	0.03	3.25 (0.19–6.30)	0.04	1.40 (–5.95–8.75)	0.71	0.01 (–0.06–0.09)	0.76
Log ₂ CRP	0.03 (–0.06–0.13)	0.48	0.77 (–0.51–2.04)	0.24	–0.51 (–3.43–2.42)	0.73	–0.03 (–0.06 to –0.004)	0.02

BMI, body mass index; BSA, body surface area; CFR, coronary flow reserve; HDL, high-density lipoprotein; LAD, left anterior descending artery; LDL, low-density lipoprotein; MAP, mean arterial pressure.

^aValues are β-coefficient [95% confidence interval (CI)].

Table 5 Independent predictors of epicardial and microvascular function and atheroma burden in the left main and proximal left anterior descending arteries by multivariable analysis^a

Parameter	Log ₂ atheroma burden in left main artery		Atheroma burden in proximal LAD		Length of coronary segments with endothelial dysfunction		Log ₂ maximal CFR	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Male sex	0.50 (0.03–0.97)	0.04	8.91 (2.52–15.3)	0.007	11.9 (–0.45–24.3)	0.059	0.11 (–0.04–0.26)	0.13
Age (per year)	0.02 (0.003–0.04)	0.02	0.38 (0.15–0.60)	0.001	–0.28 (–0.87–0.31)	0.34	–0.005 (–0.011 to –0.00007)	0.047
MAP (per 10 mmHg)	0.14 (0.01–0.27)	0.03			–1.50 (–5.70–2.71)	0.48	–0.01 (–0.05–0.03)	0.59
Creatinine (per 0.2 mg/dL)	0.03 (–0.19–0.25)	0.78	–0.70 (–3.74–2.34)	0.65			0.02 (–0.05–0.10)	0.67
Haemoglobin	0.19 (0.02–0.36)	0.03	1.99 (–0.31–4.30)	0.09			0.006 (–0.05–0.06)	0.84
Log ₂ triglycerides	0.16 (–0.08–0.39)	0.19	1.79 (–1.14–4.72)	0.23				
Uric acid	0.01 (–0.11–0.13)	0.84					0.01 (–0.03–0.05)	0.59
Log ₂ glucose			5.43 (–2.78–13.6)	0.19	–8.91 (–29.9–12.2)	0.40	–0.21 (–0.41 to –0.01)	0.04
Diabetes mellitus					–6.11 (–27.2–15.0)	0.57		
Sex-standardized BSA							0.10 (0.04–0.16)	0.002
Log ₂ CRP							–0.03 (–0.06 to –0.0002)	0.049

BSA, body surface area; CFR, coronary flow reserve; CRP, C-reactive protein; LAD, left anterior descending artery; MAP, mean arterial pressure.

^aValues are β-coefficient [95% confidence interval (CI)].

Older age was an independent predictor of atheroma burden in the left main and proximal LAD arteries and of maximal CFR in our study. In this study, women were on an average 6.5 years older than men. Our results suggest that if we had enrolled more older men, sex differences in these parameters might be more clearly demonstrated.

The primary reason for the low incidence of CAD in premenopausal women compared with age-matched men may be related to the preventive effects of oestrogen, as was documented in animal studies of the antiatherosclerotic, antioxidative, and anti-inflammatory effects of oestrogen.^{43–45} One previous animal study⁴⁶ reported that basal release of nitric oxide from endothelium-intact aortic rings was greater in female rabbits than in male rabbits and was related to circulating oestradiol concentrations. Oestrogen administration in humans has been shown to enhance acetylcholine-stimulated increases in coronary blood flow.⁴⁷ In addition, oestrogen decreases cellular hypertrophy, smooth muscle cell migration, and lower collagen deposition in response to injury, which may lead to thinner fibrous plaque in women.⁴⁸ In our study, we did not find significant differences in coronary atheroma burden, endothelial function, or microvascular function between premenopausal women and postmenopausal women (data not shown). In addition, there were no significant differences in these parameters in postmenopausal women regardless of whether they were receiving oestrogen-replacement therapy (ERT, $n = 20$) or not ($n = 34$). Variable postmenopausal period, duration of ERT, and different starting ages of ERT after menopause may affect our results. Therefore, our study further suggests that the effects of long-term ERT on coronary atheroma burden, endothelial function, and microvascular function in postmenopausal women should be clarified in future large-scale clinical studies.

Androgens have been shown to affect the expression of atherosclerosis-related genes in men but not in women, perhaps contributing to the sex-related differences in CAD prevalence.⁴⁹ The variations in plasma sex hormones and receptor genes, acting with endothelial and vascular smooth muscle factors, may be potential links to the sex differences in vascular structure and function in ischaemic heart disease. Sex differences in CAD also may be caused by non-hormonal factors.⁵⁰

The current study has several limitations. It is possible that the extent of atheroma throughout the coronary tree is not uniform and that the extent of plaque demonstrated in one cross-section does not reflect the patient's plaque burden in general. However, men in our study had greater atheroma burden than women in several different sites, such as the proximal, distal, and lesion sites of the left main artery and the proximal and lesion sites of the proximal LAD artery.

We also cannot exclude the possibility of referral bias. Since the main goal of the study was to detect differences in atheroma, endothelial function, and microvascular function between sexes, however, we believe that the selection bias is minimal. Regarding a sex-based referral bias for coronary angiography, more recent studies have shown less of a sex-related difference in referral patterns for coronary angiography than earlier studies.^{51–53} Another limitation was the possibility of enrolling patients who had shown near-normal results of coronary angiography. However, the

segments that were near normal by coronary angiography usually had some degree of atheroma by the IVUS study. Therefore, IVUS rather than coronary angiography may provide more detailed information on the characteristics of atherosclerosis by sex. In this study, we investigated only LAD territory, since it is the largest artery and easier from a technical and risk standpoint; further study in the left circumflex and right coronary arteries will be warranted in the future. The analysis of several end points in this paper increases the chance (above the nominal rate of 5%) that some significant results reflect spurious associations.

In conclusion, the present study demonstrated that in early atherosclerosis, men have greater atheroma burden, more eccentric atheroma, and more diffuse epicardial endothelial dysfunction than women. These results suggest that men have more severe structural and functional abnormalities in epicardial coronary arteries than women, even in patients with early atherosclerosis. These factors may influence the higher incidence rates of CAD and ST-segment myocardial infarction in men than women.

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