Journal of the American College of Cardiology
© 2010 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 55, No. 25, 2010
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.01.054

Coronary Microvascular Reactivity to Adenosine Predicts Adverse Outcome in Women Evaluated for Suspected Ischemia

Results From the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) Study

Carl J. Pepine, MD,* R. David Anderson, MD,* Barry L. Sharaf, MD,† Steven E. Reis, MD,‡ Karen M. Smith, MD,* Eileen M. Handberg, PHD,* B. Delia Johnson, PHD,‡ George Sopko, MD, MPH,§ C. Noel Bairey Merz, MD‖

Gainesville, Florida; Providence, Rhode Island; Pittsburgh, Pennsylvania; and Los Angeles, California

Objectives We investigated whether coronary microvascular dysfunction predicts major adverse outcomes during follow-up among women with signs and symptoms of ischemia.

Background Altered coronary reactivity occurs frequently in women evaluated for suspected ischemia, and the endothelium-dependent component is linked with adverse outcomes. Possible links between endothelium-independent microvascular coronary reactivity and adverse outcomes remain uncertain.

Methods As part of the National Heart, Lung and Blood Institute-sponsored WISE (Women's Ischemia Syndrome Evaluation), we investigated relationships between major adverse outcomes and baseline coronary flow reserve (CFR) after intracoronary adenosine in 189 women referred to evaluate suspected ischemia.

Results At a mean of 5.4 years, we observed significant associations between CFR and major adverse outcomes (death, nonfatal myocardial infarction, nonfatal stroke, or hospital stay for heart failure). An exploratory receiver-operator characteristic analysis identified CFR <2.32 as the best discriminating threshold for adverse outcomes (event rate 26.7%; and ≥ 2.32 event rate 12.2%; $p = 0.01$). Lower CFR was associated with increased risk for major adverse outcomes (hazard ratio: 1.16, 95% confidence interval: 1.04 to 1.30; $p = 0.009$). This held true among the 152 women without obstructive coronary artery disease (CAD) (hazard ratio: 1.20, 95% confidence interval: 1.05 to 1.38; $p = 0.008$). The CFR significantly improved prediction of adverse outcomes over angiographic CAD severity and other risk conditions.

Conclusions Among women with suspected ischemia and atherosclerosis risk factors, coronary microvascular reactivity to adenosine significantly improves prediction of major adverse outcomes over angiographic CAD severity and CAD risk factors. These findings suggest that coronary microvessels represent novel targets for diagnostic and therapeutic strategies to predict and limit adverse outcomes in women. (Women's Ischemia Syndrome Evaluation [WISE]; NCT00000554) (J Am Coll Cardiol 2010;55:2825–32) © 2010 by the American College of Cardiology Foundation

Women with chest discomfort and other findings suggesting myocardial ischemia are diagnostic and therapeutic challenges, due in part to low likelihood for obstructive coronary artery disease (CAD) and costs of care related to

From the *Division of Cardiovascular Medicine, University of Florida College of Medicine, Gainesville, Florida; †Division of Cardiology, Rhode Island Hospital, Providence, Rhode Island; ‡Cardiovascular Institute, University of Pittsburgh, Pittsburgh, Pennsylvania; §Division of Cardiovascular Disease, National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health, Bethesda, Maryland; and the ‖Heart Institute, Cedars-Sinai Medical Center, Los Angeles, California. This work was supported by contracts from the NHLBI, nos. N01-HV-68161, N01-HV-68162, N01-HV-68163, N01-HV-68164, grants U0164829, U01 HL649141, U01 HL649241, T32HL69751; GCRC grant MO1-RR00425 from the National Center for Research Resources; and grants from the Gustavus and Louis Pfeiffer Research Foundation, Danville, New Jersey; The Ladies Hospital Aid Society of Western Pennsylvania, Pittsburgh, Pennsylvania; QMED, Inc., Laurence Harbor, New Jersey; and The Women's Guild of Cedars-Sinai Medical Center, the Edythe L. Broad Women's Heart Research Fellowship; and the Barbra Streisand Women's Cardiovascular Research and Education Program, Cedars-Sinai Medical Center, Los Angeles, California. Dr. Pepine has received research grants and an educational grant from Pfizer. Dr. Bairey Merz has served as a consultant for Strategy Group, BSP, and Kendle Internation, Inc.; has served on the advisory board of Novartis; has served on the External Review Panel of Karolinska Institute; was the NHLBI DSMB chair of the University of Pittsburgh; has performed grant reviewing for Pfizer and the NHLBI; has received lecture honorarium from Northwestern University, University of California, Davis, Abbott Laboratories, CV Therapeutics, Boehringer Ingelheim, American College of Physicians, ProMedica, Mayo Clinic, and Merck; and owns stock in Boston Scientific, Medtronic, Johnson & Johnson, and Teva Pharmaceuticals.

Manuscript received March 17, 2009; revised manuscript received January 13, 2010, accepted January 18, 2010.

Abbreviations and Acronyms

CAD = coronary artery disease
CFR = coronary flow reserve
CHF = congestive heart failure
CI = confidence interval
CV = cardiovascular
HR = hazard ratio
LV = left ventricular
MBF = myocardial blood flow
MI = myocardial infarction
ROC = receiver-operator characteristic

repeated testing, hospital stay, and disability (1). Although knowledge of mechanisms explaining these findings is limited, impaired coronary reactivity (endothelium- and non–endothelium-dependent) has been proposed to contribute (2–10). The endothelium-dependent component has been linked to risk factors and proinflammatory processes promoting atherosclerosis (8,9) as well as adverse clinical outcomes (5,7,10). Although the non–endothelium-dependent component has received less attention, the concept that patients with risk factors might have evidence for reduced coronary flow reserve (CFR) is not new (11–17). There is increasing interest in this microvascular response, as recently reviewed elsewhere (18), and in particular the response among women (8). For example, hypercholesterolemia abolishes the voltage-dependent K+ channel contribution to adenosine-mediated smooth muscle relaxation, in both endothelium-intact and -denuded coronary arterioles, in a sex-specific manner (19,20). Vascular smooth muscle cells undergo alterations in phenotype in response to physiological and pathophysiological stimuli like hypertension and diabetes, which are highly prevalent in post-menopausal women, as well as estrogen receptor alpha expression (21,22).

Myocardial perfusion alterations during adenosine-induced vasodilation are not infrequent in the absence of significant epicardial CAD (23,24). Although there has been long interest in microvascular ischemia, most work has focused on the endothelium-dependent component (25–28), but adenosine-related vascular smooth muscle alterations do not necessarily correlate with dysfunctional endothelium (8,20,29). Thus, additional information on adenosine-related coronary microvascular reactivity would facilitate an improved understanding of processes underlying these vascular alterations in women. If these alterations contribute to adverse outcomes, they potentially offer an important target for risk stratification and evaluation of preventive treatments among these women, particularly now that coronary microvascular reactivity can be readily assessed noninvasively (30–32).

Accordingly, we investigated the relationship between adenosine-coronary reactivity at baseline and adverse outcomes during follow-up in women referred for coronary angiography.

Methods

The WISE (Women's Ischemia Syndrome Evaluation) study is a National Heart, Lung and Blood Institute-sponsored study aimed at improving diagnostic evaluation and understanding of pathological mechanisms of ischemic heart disease in women, and protocol details—including selection criteria—have been previously published (33). Site institutional review boards approved the study, and each participant provided written informed consent. Women ages 18 to 84 years undergoing clinically-indicated angiograms were enrolled, underwent a variety of testing, and were followed for clinical outcomes. A subgroup of 189 women from the Universities of Florida and Pittsburgh sites also had evaluation of coronary reactivity to adenosine. Their selection criteria also included informed consent for this additional testing, absence of stenosis warranting coronary revascularization, and an appropriate coronary segment for Doppler flow testing.

Baseline evaluation included physical examination and collection of clinical and laboratory data (Table 1). Inflammatory markers were measured in a subgroup of 134 women from blood frozen on site at −70°C and analyzed at a core laboratory with validated techniques. Qualitative and quantitative coronary angiographic analyses were done by a core laboratory masked to patient data (34). Any ≥50% diameter stenosis was defined as obstructive CAD, 20% to 49% as mild CAD, and <20% as no CAD. A CAD severity score was defined as an aggregate of percent stenosis, extent and location of stenosis, and degree of collateral vessels (34).

Coronary reactivity testing was performed in a stenosis-free area of the left anterior descending coronary artery (n = 138) when possible, with the left circumflex artery as a secondary choice. A Doppler-tipped guidewire (0.014-inch FloWire, JOMED/Cardiometrics, Mountain View, California) was advanced through the diagnostic catheter. Once a stable velocity signal was obtained, baseline recordings were made. Intracoronary bolus injections of 18 μg of adenosine (Adenocard, Fujisawa USA, Deerfield, Illinois), a predominantly non–endothelium-dependent microvascular dilator, were administered into the left main coronary artery (35). At least 3 injections were done to ensure that a stable average peak coronary flow velocity was obtained after adenosine, with return to baseline flow velocity documented before each bolus. Pulsed-wave Doppler flow spectra were used to calculate time-averaged peak velocity. Recordings were analyzed at a core laboratory (University of Florida) masked to all other data, and CFR was defined as the ratio of average peak velocity after adenosine to average baseline velocity just before adenosine. As this measure correlated closely ($r = 0.87$, $p < 0.001$) with volumetric flow (35), it was used to represent CFR.

To access the possible influence of left ventricular (LV) hypertrophy on CFR, 39 of these women without coronary stenosis had quantitative analysis of echocardiograms performed with a standardized protocol within several days of CFR measurements according to American Society of Echocardiography recommendations. These analyses included measurement of LV mass (2-dimensional echocardiography) determined by an anatomically validated short-axis area length method (36), mass index, and an index of

Table 1 Baseline Characteristics

Characteristic	All Women (n = 189)	CFR <2.32 (n = 74)	CFR ≥2.32 (n = 115)	p Value
Age, yrs	55 ± 10	58 ± 10	54 ± 10	0.02
Yrs since last menses	16 ± 11	19 ± 12	14 ± 10	0.004
White/Caucasian	83	88	80	0.16
Body mass index (kg/m^2)	31.2 ± 7.4	31.2 ± 6.3	31.2 ± 8.0	0.67
SBP (mm Hg)	136 ± 20	139 ± 23	134 ± 18	0.08
DBP (mm Hg)	77 ± 10	77 ± 9	76 ± 11	0.93
Family history of premature CAD	69	71	67	0.61
History of				
Diabetes	21	26	18	0.24
Hypertension	57	58	56	0.79
Dyslipidemia	50	54	48	0.51
Current smoking	19	16	20	0.50
Past smoking	37	39	35	0.57
Menopause	76	81	72	0.18
Blood assays				
Total cholesterol (mg/dl)	185 ± 44	188 ± 45	183 ± 42	0.39
HDL cholesterol (mg/dl)	52 ± 12	53 ± 12	52 ± 13	0.84
LDL cholesterol (mg/dl)	107 ± 37	111 ± 40	104 ± 36	0.16
Triglycerides (mg/dl)	141 ± 141	148 ± 118	136 ± 155	0.72
Plasma glucose (mg/dl)	105 ± 48	108 ± 52	102 ± 44	0.34
Hemoglobin (g/dl)	13.0 ± 1.4	13.0 ± 1.6	13.0 ± 1.3	0.35
Serum creatinine (mg/dl)	0.8 ± 0.5	0.8 ± 0.3	0.8 ± 0.6	0.35
Serum amyloid A (mg/dl)	1.0 ± 2.3	1.1 ± 2.6	1.0 ± 2.1	0.06
C-reactive protein (mg/l)	7.8 ± 11.6	8.8 ± 13.7	7.2 ± 10.2	0.22
Interleukin-6 (pg/ml)	3.7 ± 2.8	3.9 ± 3.0	3.6 ± 2.6	0.72
Medication use, currently				
Aspirin	50	57	46	0.14
Statin	16	19	15	0.47
ACE inhibitor	24	27	23	0.51
Beta-blocker	29	36	24	0.06
Hormone use, ever				
Hormone replacement	56	53	58	0.53
Oral contraceptives	60	60	59	0.88
Ejection fraction	64.7 ± 9.6	64.5 ± 10.9	64.9 ± 8.8	0.71
CAD				
None (<20% stenosis)	51	46	54	
Mild (20%–49% stenosis)	30	28	32	
Obstructive (≥50% stenosis)	19	26	15	0.11
CAD severity score, median (IQR)	5.0 (5.0–9.2)	7.5 (5.0–11.2)	5.0 (5.0–8.0)	0.036

Data expressed as mean ± SD or percentage unless otherwise noted.

ACE = angiotensin-converting enzyme; CAD = coronary artery disease; CFR = coronary flow reserve; DBP = diastolic blood pressure; HDL = high-density lipoprotein; IQR = interquartile range; LDL = low-density lipoprotein; SBP = systolic blood pressure.

LV concentric remodeling (end-diastolic ratio of short-axis myocardial to cavity area). All echocardiography measurements were done at a core laboratory masked to patient data.

Adverse outcomes during follow-up. During protocol-directed yearly follow-up, records of women reporting an event were reviewed by an events committee and tabulated as death; nonfatal myocardial infarction (MI); nonfatal stroke; and hospital stay for congestive heart failure (CHF), angina, and other vascular events. Women sustaining multiple events were counted only once and by the initial event. Those with death, nonfatal MI, nonfatal stroke, or CHF hospital stay were categorized as having major adverse outcomes. Categorization of deaths as cardiovascular (CV) was made only when documentation definitely confirmed that death was due to CV causes.

Statistical analyses. Values are expressed as mean ± SD or percentages as indicated, and the *t* test or chi-square analysis, where appropriate, was used to evaluate differences among groups. The CAD angiographic severity score was non-normally distributed and was expressed as medians and interquartile ranges with differences among groups evaluated by the Kruskal-Wallis test. To determine the CFR value best predictive of major adverse outcomes (death, MI, stroke, or hospital stay for CHF), we generated a receiver-operator characteristic (ROC) curve from the PROC LOGISTIC function in SAS (SAS Institute, Cary, North

Carolina), which calculates area under the curve, indicated by the c-statistic, with the trapezoidal rule. The CFR value corresponding to the point on the curve closest to 100% sensitivity and specificity was matched, first by visual means and then by verification runs of incremental cut-points near this value. The discriminating threshold by ROC analysis was then used to categorize women into low versus high CFR groups. Kaplan-Meier analysis was used to compare time to adverse event by CFR group in all women and in women without obstructive CAD. Multivariate Cox proportional hazards regression was used to examine the role of baseline characteristics, including the natural logs of both the CAD severity score and CFR (logCFR × 10) on adverse outcomes. Baseline characteristics (Table 1) were chosen for entry into multivariable Cox models on the basis of their discrimination between low and high CFR as well as on univariate associations with adverse outcomes of $p < 0.20$. A combination of forward and backward selection procedures was used to aid in determining the best model of independent predictors. This was followed by forcing potential confounders, including drugs used at baseline and during follow-up, into the models and determining their effect on the relationship of interest. The likelihood ratio test was used to compare the incremental goodness of fit of nested models. All tests were 2-sided, and $p \leq 0.05$ was considered statistically significant. All analyses were performed with SAS software version 9.1 (SAS Institute).

Results

Baseline characteristics. Pertinent characteristics of the women are summarized in Table 1. Their mean age was 55 ± 10 years; most were white; about three-quarters were post-menopausal; and more than one-half were obese (body mass index ≥30 kg/m^2), had a history of hypertension or dyslipidemia, had a family history of premature heart disease, or had ever used hormone replacement or oral contraceptive pills. A history of diabetes was present in approximately one-fifth. Approximately one-half were currently taking aspirin; approximately one-quarter were currently taking angiotensin-converting enzyme inhibitors; less than one-third were currently taking beta-blockers; and approximately one-fifth were currently taking statins.

Angiographic CAD, echocardiography LV mass, and CFR. Most (152 or 81%) of the women had either no or <50% obstructive CAD (Table 1), and lower CFR was weakly associated with higher CAD severity score ($r_s = -0.15$, $p = 0.04$). In comparison of groups with obstructive, mild, or no angiographic CAD, the CFRs were 2.3 ± 0.7, 2.5 ± 0.7, and 2.6 ± 0.7, respectively ($p = 0.09$ for trend). Regression analyses and comparisons of subgroups with normal versus elevated LV mass, mass index, and end-diastolic ratio of short-axis myocardial to cavity area showed no significant relationships between LV mass variables and CFR (data not shown).

Adverse outcomes by risk factors and CFR. During follow-up (mean 5.4 years), 79 women (42%) had an adverse outcome, and in total there were 138 events, including 11 deaths (Table 2). Thirty-four of these 79 women had major adverse outcomes (death, MI, stroke, or hospital stay for CHF). Note that 25 of the women without obstructive CAD had major adverse outcomes.

All analyses summarized in the following text were done with only major adverse outcomes. Substituting definite CV deaths for all-cause deaths produced very similar results.

Among all women in this cohort, those with major adverse events during follow-up had at baseline higher systolic blood pressure ($p = 0.01$), C-reactive protein ($p = 0.038$), and interleukin-6 ($p = 0.02$) levels and CAD severity scores ($p = 0.0009$) and were more frequently post-menopausal ($p = 0.02$) compared with those without events. Similar trends were found among the subgroup without obstructive CAD, but only post-menopausal status ($p = 0.025$) and higher CAD severity score ($p = 0.0005$) reached statistical significance, due to smaller sample size with fewer events.

By ROC analysis, a CFR <2.32 provided the best prediction, with a sensitivity of 62% and specificity of 65%, for major adverse outcomes (CFR <2.32 outcomes rate 27.0% and ≥2.32 outcomes rate 12.2%, chi-square = 6.73, $p = 0.010$). The area under the curve was 0.63 (95% confidence interval: 0.53 to 0.73). Similar results were obtained when restricting the analysis to women without obstructive CAD: women with low CFRs experienced significantly more major adverse outcomes (CFR <2.32 outcome rate 26.6% vs. 9.3%, chi-square = 5.84; $p = 0.016$). Women with low CFR were older ($p = 0.02$) and had a higher CAD severity ($p = 0.036$) versus those with high CFR. There was a significant decline over time in freedom from major events for women with lower (<2.32) CFR compared with those with higher CFR ($p = 0.003$) (Fig. 1), which remained consistent after adjusting for

Table 2 Index (First) and Total Adverse Events During Follow-Up

Events	Number of Index (First) Events	Total Number of Events
Major events		
Death	8	11
Hospital stay for nonfatal event		
MI	3	7
CHF	6	18
Stroke	8	10
Other events		
PCI	12	23
CABG	1	4
Angina	36	52
Other vascular event	5	13
Total (%)	79 (42%)	138*

*Total (n = 138) events in 79 women.

CABG = coronary artery bypass graft surgery; CHF = congestive heart failure; MI = myocardial infarction; PCI = percutaneous coronary intervention.

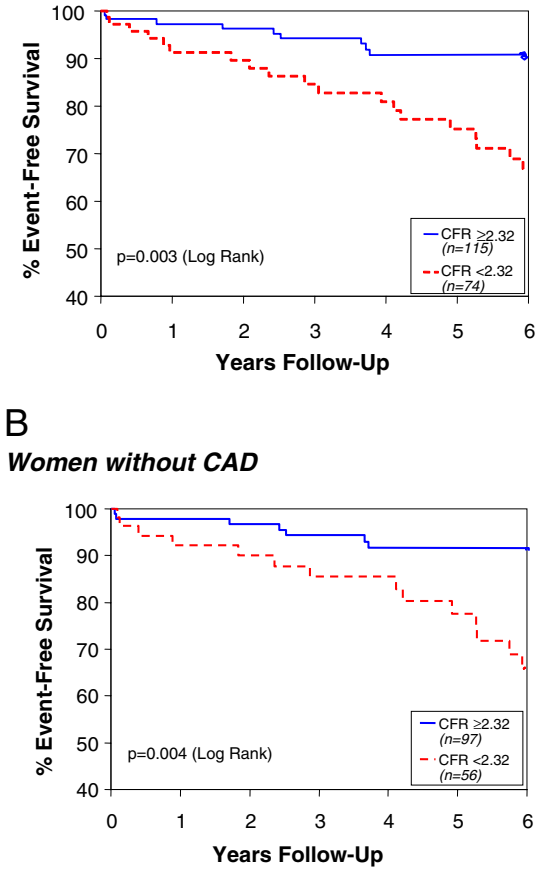

Figure 1 **CFR and Event-Free Survival**

Coronary flow reserve (CFR) and event-free survival among all women **(A)** and those without coronary artery disease (CAD) **(B)**. Data represent unadjusted Kaplan-Meier curves for absence of death, nonfatal myocardial infarction, nonfatal stroke, or hospital stay for congestive heart failure during follow-up.

risk factors (systolic blood pressure, CAD severity, age, diabetes, and smoking). The Cox proportional hazard-adjusted time-to-event curves are similar to the unadjusted Kaplan-Meier curves shown in Figure 1, and the p values remain significant.

When used as a continuous variable, low CFR also significantly predicted increased risk for major adverse outcomes (Table 3). For each 0.1-U decrease in logCFR, the relative risk for major adverse outcomes increased by 20% among women without obstructive CAD.

Multivariate Cox regression modeling (Table 4) identified only logCFR ($p = 0.043$), log CAD severity score ($p = 0.058$), and systolic blood pressure ($p = 0.012$) as independent predictors of major adverse outcome. When multiple CAD risk conditions (e.g., age, history of diabetes, and smoking) were forced into the model, potentially overfitting the model, CFR remained a significant predictor ($p = 0.038$), whereas the effect of log CAD severity was attenuated. No other risk factors contributed (data not shown). When any statin, angiotensin-converting enzyme inhibitor, and beta-blocker use were forced into the models, use of these drugs was neither a significant predictor of adverse outcome nor an influence on relationship between CFR and adverse outcome. In the combined model, the likelihood ratio test determined that adding CFR significantly improved prediction of major adverse outcomes over the other variables (chi-square = 4.37; $p = 0.036$).

Discussion

Many women presenting with ischemic-type symptoms and signs do not have obstructive CAD but have impaired coronary reactivity to adenosine, which has the potential to limit myocardial flow, yet an association with adverse outcome is unclear. Most reports did not examine endothelium-independent microvascular responses, few patients had major serious events (most were revascularization), relatively few women were included, and/or sex-specific data were not reported.

Nitenberg et al. (3) published CV outcome in hypertensive and diabetes patients without CAD using cold-pressor testing with epicardial coronary diameter measurements, which is related both to endothelial- and non–endothelial-dependent mechanisms, but no sex stratification or flow responses were reported. Halcox et al. (2) found a significant association between acetylcholine responses and outcomes. Although trends were noted for nitroprusside and adenosine, they concluded in this underpowered study that endothelium-independent responses were not predictive of outcome (2). Al Suwaidi et al. (6) reported the prognostic value of the response to adenosine and nitroprusside but simply stated that CFR response to adenosine was significantly lower in those with endothelial dysfunction, implying that endothelial-dependent and -independent mechanisms show similar responses.

Other studies indicate that positron emission tomography measures of absolute myocardial blood flow (MBF) and CFR or myocardial perfusion reserve (1,2,5,7,10,14) might be abnormal in individuals with risk factors without apparent CAD. Overall, these reports document reduced coronary reactivity in individuals with a greater coronary risk factor burden. Except for a report (5) suggesting that impaired MBF responses to cold-pressor testing were associated with increased risk of events, the prognostic value of traditional clinical risk assessment versus myocardial perfusion reserve by positron emission tomography cannot be compared because of lack of follow-up data. Additionally, other studies (37) have demonstrated that maximum MBF and CFR might be impaired in myocardial territories supplied by arteries that do not appear obstructed in patients with obstructive CAD elsewhere. Finally, reduced MBF reserve in patients with hypertrophic (9) or dilated cardiomyopathy (29) has predictive value for prognosis. Thus, the

Table 3 Decreasing LogCFR and Risk for Adverse Outcomes

Outcome	Hazard Ratio	95% CI	p Value
All women			
Major adverse outcome*	1.16	1.04–1.30	0.009
CV death, nonfatal MI, nonfatal stroke, or hospital stay for CHF	1.15	1.02–1.30	0.019
CV death, nonfatal MI, or hospital stay for CHF	1.18	1.03–1.36	0.018
Women without obstructive CAD			
Major adverse outcome*	1.20	1.05–1.38	0.008
CV death, nonfatal MI, nonfatal stroke, or hospital stay for CHF	1.19	1.03–1.37	0.020
CV death, nonfatal MI, or hospital stay for CHF	1.23	1.03–1.47	0.021

Unadjusted Cox regression analyses. *Death, nonfatal MI, nonfatal stroke, or hospital stay for CHF.
CI = confidence interval; CV = cardiovascular; other abbreviations as in Tables 1 and 2.

overall topic of coronary microvascular dysfunction and its clinical implications has increasing interest (11–13,15–18,38–43).

A better understanding of adverse outcomes associated with dysfunctional microvessels could help to clarify the pathophysiology of ischemic heart disease in women and perhaps identify new targets for both diagnostic testing and therapeutic intervention.

Our findings indicate that endothelium-independent CFR is a predictor of major adverse outcomes in the women studied. The link between CFR and major adverse outcomes remained, regardless of presence or absence of obstructive CAD or multiple risk conditions. The low but significant association of this component of coronary reactivity with CAD severity is intriguing, because atherosclerotic plaque is usually localized to conduit arteries and those selected for flow measurement did not have flow-limiting stenoses. Furthermore, among the 152 women without any obstructive stenoses, the link with adverse outcomes remained significant. Although adenosine receptors are present in both endothelial and smooth muscle cells, it is unlikely that dilation of conduit arteries, through either an endothelial or smooth muscle mechanism, could explain our findings. Thus, the location of the defect exposed by adenosine in these women is most likely at the microvascular level. This might represent an early manifestation of vascular defects underlying ischemic heart disease in these women, with the potential to contribute to subsequent major adverse outcomes even in the absence of conduit vessel obstruction. Indeed, women in the WISE cohort, including those without obstructive CAD, had a surprisingly high risk for major adverse events during follow-up (44,45). A possible conclusion from this analysis is that nonobstructive CAD (≤49% diameter stenosis) in women is perhaps of greater importance than can be surmised merely from speculation about conduit artery hemodynamic impairment (or lack thereof). Such findings seem to be associated with evidence of microvascular disease and a poorer outcome. Presence of such findings in women might warrant CFR measurements to refine estimates of prognosis. Alternatively, a lower percentage narrowing threshold (or CAD score) might be identified in such women that might be useful for prognostic purposes (even if not useful for intervention purposes).

It would be highly desirable to have a simple reliable physiologic measure to identify women at highest risk for adverse outcomes, and this measure could be altered coronary microvascular reactivity. If proven so in other studies, this could lead to more targeted treatment and the reactivity measurement could be noninvasively performed and followed to assess response to treatment and prevention strategies.

Other evidence implicates the coronary microcirculation to explain some findings in ischemic heart disease that are observed in women (46). In the absence of conduit vessel obstruction, this includes myocardial metabolic, electrocardiographic, and scintigraphic evidence for ischemia (47); histologic evidence for small vessel disease (48); the predictive value of brain natriuretic peptide and C-reactive protein for adverse

Table 4 Multivariate Modeling of Major Events

	Model 1		Model 2		Model 3	
Predictor	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
LogCFR	1.15 (1.02–1.30)	0.018	1.13 (1.004–1.27)	0.043	1.14 (1.01–1.29)	0.038
SBP	1.02 (1.005–1.04)	0.011	1.02 (1.004–1.04)	0.012	1.02 (1.001–1.04)	0.035
Log CAD severity	—	—	1.68 (0.98–2.88)	0.058	1.61 (0.92–2.81)	0.10
Age	—	—	—	—	1.00 (0.96–1.04)	0.90
History of diabetes	—	—	—	—	1.44 (0.65–3.20)	0.37
Ever smoked	—	—	—	—	1.22 (0.60–2.46)	0.58

Cox regression analysis. When interpreting these tables, the hazard ratios (HRs) reflect the percentage change in events for every 0.1-U increment in the logCFR. Adjusting for 1 covariate at a time among other variables in Table 1 did not substantially change the relationship between CFR and major adverse outcome.
SBP = systolic blood pressure; other abbreviations as in Tables 1 and 2.

outcomes (49); and findings of microvascular obstruction in women dying with acute coronary syndromes (50). Even more important is the question: could microvascular dysfunction deteriorate into or perhaps promote macrovascular disease?

Study limitations. Although this study represents the largest group of women with microvascular reactivity and follow-up data reported, we evaluated a relatively select cohort with ischemic-type symptoms and multiple risk conditions prompting referral for angiography. This indication bias limits generalization of results. Unknown factors, including individual variability in dose responses or in vascular smooth muscle effects of similar CAD risk conditions, could potentially affect CFR. Although adenosine-induced CFR increases are mediated largely via smooth muscle relaxation, endothelium-dependent mechanisms could contribute (51). It is likely that the 18-μg intracoronary adenosine dose might not have achieved near maximal hyperemia in every patient. Similar adenosine doses provide near maximal increase in flow in 90% to 92% of cases (52), and larger doses used for fractional flow reserve measurement to assess coronary stenosis severity might provide different results. Left ventricular hypertrophy might influence coronary microvascular reactivity; however, quantitative echocardiographic analysis from a subgroup of these women without CAD suggested that low CFR could not be explained by left ventricular hypertrophy. We used only blood flow velocity measurements, but we have previously shown in an analysis from WISE that this measure agrees closely with volumetric flow reserve (35). Finally, despite the relatively large sample of women in this cohort, the low number of major events limits statistical power and creates the risk of over-fitting the models when adding covariates. However, despite this potential problem, the addition of such covariates in no case affected the relationship between CFR and adverse outcomes.

Conclusions

In women undergoing coronary angiography to further evaluate suspected ischemia, a limited coronary microvascular response to adenosine is associated with increased risk for major adverse outcomes even in the absence of significant obstructive CAD. This finding supports the need for more investigation of altered coronary smooth muscle reactivity and the smaller vessels in women with suspected ischemia. Long-term follow-up of new cohorts of women should help to determine whether coronary microvascular dysfunction and its link with adverse outcomes can be confirmed and modified.

Reprint requests and correspondence: Dr. Carl J. Pepine, Division of Cardiovascular Medicine, University of Florida College of Medicine, 1600 Southwest Archer Road/Box 100277, Gainesville, Florida 32610-0277. E-mail: pepincj@medicine.ufl.edu.

REFERENCES

1. Shaw LJ, Merz CN, Pepine CJ, et al. The economic burden of angina in women with suspected ischemic heart disease: results from the National Institutes of Health–National Heart, Lung, and Blood Institute–sponsored Women's Ischemia Syndrome Evaluation. Circulation 2006;114:894–904.
2. Halcox JP, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002;106:653–8.
3. Nitenberg A, Pham I, Antony I, Valensi P, Attali JR, Chemla D. Cardiovascular outcome of patients with abnormal coronary vasomotion and normal coronary arteriography is worse in type 2 diabetes mellitus than in arterial hypertension: a 10 year follow-up study. Atherosclerosis 2005;183:113–20.
4. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101:1899–906.
5. Schindler TH, Nitzsche EU, Schelbert HR, et al. Positron emission tomography-measured abnormal responses of myocardial blood flow to sympathetic stimulation are associated with the risk of developing cardiovascular events. J Am Coll Cardiol 2005;45:1505–12.
6. Al Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948–54.
7. von Mering GO, Arant CB, Wessel TR, et al. Abnormal coronary vasomotion as a prognostic indicator of cardiovascular events in women: results from the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation 2004;109:722–5.
8. Bottcher M, Botker HE, Sonne H, Nielsen TT, Czernin J. Endothelium-dependent and -independent perfusion reserve and the effect of L-arginine on myocardial perfusion in patients with syndrome X. Circulation 1999;99:1795–801.
9. Chan NN, Colhoun HM, Vallance P. Cardiovascular risk factors as determinants of endothelium-dependent and endothelium-independent vascular reactivity in the general population. J Am Coll Cardiol 2001;38:1814–20.
10. Schindler TH, Hornig B, Buser PT, et al. Prognostic value of abnormal vasoreactivity of epicardial coronary arteries to sympathetic stimulation in patients with normal coronary angiograms. Arterioscler Thromb Vasc Biol 2003;23:495–501.
11. Di Carli MF, Janisse J, Grunberger G, Ager J. Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. J Am Coll Cardiol 2003;41:1387–93.
12. Pitkanen OP, Nuutila P, Raitakari OT, et al. Coronary flow reserve is reduced in young men with IDDM. Diabetes 1998;47:248–54.
13. Pitkanen OP, Raitakari OT, Niinikoski H, et al. Coronary flow reserve is impaired in young men with familial hypercholesterolemia. J Am Coll Cardiol 1996;28:1705–11.
14. Schelbert HR. Coronary circulatory function abnormalities in insulin resistance: insights from positron emission tomography. J Am Coll Cardiol 2009;53:S3–8.
15. Yokoyama I, Momomura S, Ohtake T, et al. Reduced myocardial flow reserve in non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 1997;30:1472–7.
16. Yokoyama I, Murakami T, Ohtake T, et al. Reduced coronary flow reserve in familial hypercholesterolemia. J Nucl Med 1996;37:1937–42.
17. Yokoyama I, Ohtake T, Momomura S, Nishikawa J, Sasaki Y, Omata M. Reduced coronary flow reserve in hypercholesterolemic patients without overt coronary stenosis. Circulation 1996;94:3232–8.
18. Camici PG, Crea F. Coronary microvascular dysfunction. N Engl J Med 2007;356:830–40.
19. Heaps CL, Bowles DK. Gender-specific K(+)-channel contribution to adenosine-induced relaxation in coronary arterioles. J Appl Physiol 2002;92:550–8.
20. Heaps CL, Tharp DL, Bowles DK. Hypercholesterolemia abolishes voltage-dependent K+ channel contribution to adenosine-mediated relaxation in porcine coronary arterioles. Am J Physiol Heart Circ Physiol 2005;288:H568–76.
21. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004;84:767–801.

22. Pepine CJ, Nichols WW, Pauly DF. Estrogen and different aspects of vascular disease in women and men. Circ Res 2006;99:459–61.
23. Verna E, Ceriani L, Giovanella L, Binaghi G, Garancini S. "False-positive" myocardial perfusion scintigraphy findings in patients with angiographically normal coronary arteries: insights from intravascular sonography studies. J Nucl Med 2000;41:1935–40.
24. Wieneke H, Zander C, Eising EG, Haude M, Bockisch A, Erbel R. Non-invasive characterization of cardiac microvascular disease by nuclear medicine using single-photon emission tomography. Herz 1999;24:515–21.
25. Cannon RO III. Microvascular angina and the continuing dilemma of chest pain with normal coronary angiograms. J Am Coll Cardiol 2009;54:877–85.
26. Mohri M, Koyanagi M, Egashira K, et al. Angina pectoris caused by coronary microvascular spasm. Lancet 1998;351:1165–9.
27. Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990;81:491–7.
28. Zeiher AM, Drexler H, Saurbier B, Just H. Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. J Clin Invest 1993;92:652–62.
29. Bottcher M, Madsen MM, Refsgaard J, et al. Peripheral flow response to transient arterial forearm occlusion does not reflect myocardial perfusion reserve. Circulation 2001;103:1109–14.
30. Lanza GA, Buffon A, Sestito A, et al. Relation between stress-induced myocardial perfusion defects on cardiovascular magnetic resonance and coronary microvascular dysfunction in patients with cardiac syndrome X. J Am Coll Cardiol 2008;51:466–72.
31. Pennell DJ. Perfusion abnormality, normal coronaries, and chest pain. J Am Coll Cardiol 2008;51:473–5.
32. Tona F, Caforio AL, Montisci R, et al. Coronary flow velocity pattern and coronary flow reserve by contrast-enhanced transthoracic echocardiography predict long-term outcome in heart transplantation. Circulation 2006;114:I49–55.
33. Merz CN, Kelsey SF, Pepine CJ, et al. The Women's Ischemia Syndrome Evaluation (WISE) study: protocol design, methodology and feasibility report. J Am Coll Cardiol 1999;33:1453–61.
34. Sharaf BL, Pepine CJ, Kerensky RA, et al. Detailed angiographic analysis of women with suspected ischemic chest pain (pilot phase data from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation [WISE] Study Angiographic Core Laboratory). Am J Cardiol 2001;87:937–41.
35. Reis SE, Holubkov R, Lee JS, et al. Coronary flow velocity response to adenosine characterizes coronary microvascular function in women with chest pain and no obstructive coronary disease. Results from the pilot phase of the Women's Ischemia Syndrome Evaluation (WISE) study. J Am Coll Cardiol 1999;33:1469–75.
36. Reichek N, Helak J, Plappert T, Sutton MS, Weber KT. Anatomic validation of left ventricular mass estimates from clinical two-dimensional echocardiography: initial results. Circulation 1983;67:348–52.
37. Chauhan A, Mullins PA, Taylor G, Petch MC, Schofield PM. Both endothelium-dependent and endothelium-independent function is impaired in patients with angina pectoris and normal coronary angiograms. Eur Heart J 1997;18:60–8.
38. Cecchi F, Olivotto I, Gistri R, Lorenzoni R, Chiriatti G, Camici PG. Coronary microvascular dysfunction and prognosis in hypertrophic cardiomyopathy. N Engl J Med 2003;349:1027–35.
39. Neglia D, Michelassi C, Trivieri MG, et al. Prognostic role of myocardial blood flow impairment in idiopathic left ventricular dysfunction. Circulation 2002;105:186–93.
40. Sambuceti G, Parodi O, Marzullo P, et al. Regional myocardial blood flow in stable angina pectoris associated with isolated significant narrowing of either the left anterior descending or left circumflex coronary artery. Am J Cardiol 1993;72:990–4.
41. Tsagalou EP, Anastasiou-Nana M, Agapitos E, et al. Depressed coronary flow reserve is associated with decreased myocardial capillary density in patients with heart failure due to idiopathic dilated cardiomyopathy. J Am Coll Cardiol 2008;52:1391–8.
42. Uren NG, Marraccini P, Gistri R, de Silva R, Camici PG. Altered coronary vasodilator reserve and metabolism in myocardium subtended by normal arteries in patients with coronary artery disease. J Am Coll Cardiol 1993;22:650–8.
43. Yokoyama I, Ohtake T, Momomura S, et al. Hyperglycemia rather than insulin resistance is related to reduced coronary flow reserve in NIDDM. Diabetes 1998;47:119–24.
44. Johnson BD, Shaw LJ, Pepine CJ, et al. Persistent chest pain predicts cardiovascular events in women without obstructive coronary artery disease: results from the NIH-NHLBI-sponsored Women's Ischaemia Syndrome Evaluation (WISE) study. Eur Heart J 2006;27:1408–15.
45. Gulati M, Cooper-DeHoff RM, McClure C, et al. Adverse cardiovascular outcomes in women with nonobstructive coronary artery disease: a report from the Women's Ischemia Syndrome Evaluation Study and the St James Women Take Heart Project. Arch Intern Med 2009;169:843–50.
46. Pepine CJ, Kerensky RA, Lambert CR, et al. Some thoughts on the vasculopathy of women with ischemic heart disease. J Am Coll Cardiol 2006;47:S30–5.
47. Buffon A, Rigattieri S, Santini SA, et al. Myocardial ischemia-reperfusion damage after pacing-induced tachycardia in patients with cardiac syndrome X. Am J Physiol Heart Circ Physiol 2000;279:H2627–33.
48. Mosseri M, Yarom R, Gotsman MS, Hasin Y. Histologic evidence for small-vessel coronary artery disease in patients with angina pectoris and patent large coronary arteries. Circulation 1986;74:964–72.
49. Wiviott SD, Cannon CP, Morrow DA, et al. Differential expression of cardiac biomarkers by gender in patients with unstable angina/non-ST-elevation myocardial infarction: a TACTICS-TIMI 18 (Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis In Myocardial Infarction 18) substudy. Circulation 2004;109:580–6.
50. Burke AP, Farb A, Malcom GT, Liang Y, Smialek J, Virmani R. Effect of risk factors on the mechanism of acute thrombosis and sudden coronary death in women. Circulation 1998;97:2110–6.
51. Buus NH, Bottcher M, Hermansen F, Sander M, Nielsen TT, Mulvany MJ. Influence of nitric oxide synthase and adrenergic inhibition on adenosine-induced myocardial hyperemia. Circulation 2001;104:2305–10.
52. Jeremias A, Whitbourn RJ, Filardo SD, et al. Adequacy of intracoronary versus intravenous adenosine-induced maximal coronary hyperemia for fractional flow reserve measurements. Am Heart J 2000;140:651–7.

Key Words: adverse outcomes ■ ischemia ■ microcirculation ■ women.