Cardiac Imaging

Inflammation is Related to Coronary Flow Reserve Detected by Positron Emission Tomography in Asymptomatic Male Twins

CME

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Inflammation is Related to Coronary Flow Reserve Detected by Positron Emission Tomography in Asymptomatic Male Twins

Objectives   This study sought to examine the relationship between inflammation and coronary microvascular function in asymptomatic individuals using positron emission tomography (PET) and assessment of coronary flow reserve (CFR).

Background   Coronary microvascular dysfunction is an early precursor of coronary artery disease (CAD) thought to result from endothelial cell activation and inflammation, but data are limited.

Methods   We examined 268 asymptomatic male monozygotic and dizygotic twins. Plasma biomarkers of inflammation and endothelial cell activation included C-reactive protein (CRP), interleukin (IL)-6, white blood cell count (WBC), vascular cell adhesion molecule (VCAM)-1, and intercellular adhesion molecule (ICAM)-1. Blood flow quantitation was obtained with [^{13}N] ammonia PET at rest and after adenosine stress. CFR was measured as the ratio of maximum flow to baseline flow at rest; abnormal CFR was defined as a ratio <2.5. A summed stress score for visible perfusion defects was calculated.

Results   In within-pair analyses, all biomarkers, except VCAM-1, were higher in twins with lower CFR than their brothers with higher CFR ($p < 0.05$). This was observed in the entire sample, as well as within pairs discordant for a CFR of <2.5. Associations persisted after adjusting for summed stress score and CAD risk factors. In contrast no biomarker, except IL-6, was related to the summed stress score of visible defects.

Conclusions   Even in asymptomatic subjects, a decrease in coronary microvascular function is accompanied by a systemic inflammatory response, independent of CAD risk factors. Our results, using a controlled twin design, highlight the importance of coronary microvascular function in the early phases of CAD.   (J Am Coll Cardiol 2011;57: 1271–9) © 2011 by the American College of Cardiology Foundation

Coronary microvascular dysfunction refers to abnormal regulation of the coronary microcirculation resulting in reduced myocardial blood flow with increased demand that is not explained by disease in the epicardial coronary arteries (1). It is thought to result from vasomotor dysregulation or endothelial dysfunction of the small coronary arterioles and represents 1 of the earliest signs of coronary atherosclerosis (1,2). Although no technique enables the direct visualization of the coronary microcirculation in humans in vivo, in the absence of coronary artery disease (CAD), coronary microvascular disease manifests as impaired coronary flow reserve (CFR), an index of coronary vasodilator capacity (2,3).

Among individuals without evidence of CAD and myocardial disease, coronary microvascular dysfunction is typically attributed to traditional coronary risk factors (smoking, hypertension, hyperlipidemia, and diabetes and insulin-resistant states), but the underlying pathophysiology is unclear (1,4). Functional abnormalities of the microcirculation have been proposed, including endothelial and/or vascular smooth muscle cell dysfunction, as well as extravascular influences, such as autonomic dysfunction (4). Vascular inflammation and endothelial cell activation have been suggested as plausible pathophysiological mechanisms, since they have also been linked to vascular function in the periphery (5,6). For the coronary circulation, however, data in humans are limited and inconsistent (7,8). Part of the difficulties are due to the selected patient samples that have been evaluated using invasive angiographic methods and Doppler technology. Whether a relationship between in-

flammation and CFR exists in asymptomatic individuals is fundamentally unexplored.

The purpose of our study was to examine the relationship between inflammation and CFR in a controlled study of asymptomatic middle-aged male twins. CFR, measured by positron emission tomography (PET), is currently the most established noninvasive method of assessing microvascular function, and can be used in asymptomatic subjects (2,3). Because inflammation is a key mechanism through which several risk factors increase CAD risk (9), and a reduced CFR has been linked to CAD risk factors (10–15) and with adverse long-term prognosis (16,17), we were interested in examining whether the relationship between inflammation and CFR was independent of CAD risk factors. Twins provide unique opportunities to study the association between inflammation and CFR while controlling for shared environmental or genetic factors, because twin siblings share genes (50%, on average, if dizygotic, and all if monozygotic), maternal factors, and early familial environment.

Methods

Subjects. The THS (Twins Heart Study) is an investigation of psychological, behavioral, and biological risk factors for subclinical cardiovascular disease using twins (18,19). Twins were selected from the VET (Vietnam Era Twin) Registry (20), which includes 7,369 middle-aged male–male twin pairs, both of whom served in the U.S. military during the time of the Vietnam War. THS included 180 twin pairs, 93 monozygotic and 87 dizygotic, who were born between 1946

and 1956. Zygosity information was determined by DNA typing. Two groups of twin pairs were randomly sampled from the VET Registry: 1 group included twins discordant for a lifetime history of major depression, and in a second group, neither twin had a history of depression. All twins were examined at the Emory University General Clinical Research Center between March 2002 and March 2006; their medical history was updated at the examination. The protocol was approved by the Institutional Review Board at Emory University, and informed consent was obtained from all subjects.

PET protocol. Subjects underwent imaging of myocardial blood flow (MBF) with PET nitrogen 13 [^{13}N] ammonia at rest and following pharmacological (adenosine) stress during a single imaging session. All twins were admitted to the GCRC in the late morning or early afternoon on the day prior to the PET scan, and stayed overnight in the GCRC facility. They all received a similar low-fat dinner, and then remained fasting until the PET scan was completed the following morning. They were instructed to abstain from smoking and drinking alcoholic or caffeinated beverages and from eating any foods other than what was served to them. All medications were withheld the morning of the PET scan. The imaging protocol was performed by personnel unaware of the twins' medical history or other subject characteristics.

PET scanning was performed in 2-dimensional mode using a CTI ECAT Exact 47 (921) camera (5-mm resolution) (Siemens, Knoxville, Tennessee). Initially, a 2- to 3-mCi dose of [^{13}N] ammonia was injected, and a 4-min static scan was collected and reconstructed without any corrections to verify subject position. Then, rest and pharmacological stress (adenosine) ammonia imaging was performed. The rest and stress imaging protocols were identical except that a 4-min infusion of adenosine (0.14 mg/kg/min) was started 2 min prior to the ammonia injection for the stress imaging session. 20 mCi of [^{13}N]ammonia were injected, and a 5-min, 31-frame dynamic acquisition was started (12 frames × 5 s, 3 frames × 20 s, and 1 frame × 300 s). Data were collected in 47 planes 3.375-mm thick covering a range of 16 cm. Immediately after the conclusion of the dynamic sequence, a 15-min gated acquisition was started. Finally, transmission data were collected for 5 min in the windowed mode using germanium 68 (^{68}Ge) rods for segmented attenuation correction. The process was repeated, including a second transmission scan, for the stress study. Images were reconstructed with filtered back projection using a Hann filter cutoff at 1 cycle/cm. The electrocardiogram was monitored continuously, and blood pressure and heart rate measurements were taken before, during (every minute), and after the adenosine infusion. The rate-pressure product was calculated as the mean systolic blood pressure during adenosine infusion multiplied by the mean heart rate during adenosine infusion divided by 100.

PET flow measures. To calculate CFR, measurements of MBF at rest and during adenosine hyperemia were obtained. The last frame of the dynamic sequence was used as a template for sectorial region-of-interest analy-

sis. The input function was generated by drawing a region of interest in the left ventricular chamber on a mid-ventricular slice, and flow was calculated (expressed in milliliters per minute per gram of tissue) using established methodology (21,22). The left ventricle was sampled radially from 40 different angles, and 40 samples of flow were obtained for each short-axis slice. The resulting hundreds of samples were grouped into 20 segments.

Our main outcome was the overall measure of CFR for the entire myocardium (across all 20 regions), defined as the ratio of maximum flow during stress to flow at rest. Secondarily, regional CFR was also calculated (i.e., left anterior descending, left circumflex, and right coronary artery territory). Absolute increase in hyperemic blood flow, calculated as the difference between maximum flow during stress and flow at rest, was also examined.

Myocardial perfusion defect score. In addition to flow measures, we constructed a summation score describing the number and the severity of visible perfusion defects across the 20 segments of acquisition. In each segment, the defect severity was quantified on a 4-point scale and subsequently summed up across the 20 segments, yielding a total score. Separate scores were obtained for the rest (summed rest score) and stress (summed stress score) scans. A reversible defect score (summed difference score) was obtained by subtracting the rest score from the stress score. These scores, and a dichotomous indicator of perfusion abnormalities (defined as a summed stress score ≥4 [23]), represented secondary perfusion outcomes.

Assessment of inflammatory biomarkers. Blood was drawn in the morning prior to the PET scan after an overnight fast. Plasma samples were separated and stored at −80°C until analysis. We measured biomarkers of inflammation and endothelial cell activation, including C-reactive protein (CRP), interleukin (IL)-6, white blood cell count (WBC), and 2 soluble cell adhesion molecules, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1. CRP was measured with the Beckman Coulter High Sensitivity C-Reactive Protein assay on the Synchron LX-20 analyzer (Beckman Coulter, Brea, California); the inter- and intra-assay precision of this test is <5%. IL-6, VCAM-1, and ICAM-1 were assessed using commercially available ELISA kits from R and D Systems (Minneapolis, Minnesota). Inter- and intra-assay variability for these assays is <10%. WBC count was measured with the Beckman Coulter LH 750 hematology analyzer. All biochemical assays for each twin pair were processed in the same analytical run.

Abbreviations and Acronyms

CAD = coronary artery disease

CFR = coronary flow reserve

CRP = C-reactive protein

ICAM = intercellular adhesion molecule

IL = interleukin

MBF = myocardial blood flow

PET = positron emission tomography

VCAM = vascular cell adhesion molecule

WBC = white blood cell count

Other measurements. A medical history and a physical exam were obtained from all twins. Concentrations of glucose and plasma lipids were measured from overnight fasting blood samples using standardized methods. Physical activity was assessed with a modified version of the Baecke Questionnaire of Habitual Physical Activity used in the ARIC (Atherosclerosis Risk in Communities) study (24), yielding a global score of physical activity at work, during sports and nonsports activities.

Statistical analysis. CFR and inflammatory biomarkers were mainly analyzed as continuous variables; inflammatory biomarker levels had a skewed distribution and were log transformed for analysis. All p values were corrected for the correlation between co-twins using generalized estimating equations or mixed-effects models with a random intercept for each pair, depending on variable distribution.

The association between inflammatory markers and microvascular dysfunction was first examined in the whole sample of twins, treating twins as separate observations. In these analyses, all twins were examined regardless of whether their brother was available for analysis, and CFR was categorized according to quartiles. Next, we performed comparisons within twin pairs, where inflammatory marker levels were compared between brothers who differed in CFR. Within-pair differences in CFR were examined in 3 ways. First, we expressed the within-pair difference in CFR as a continuous variable quantifying the individual twin variation from the twin pair average; this formulation is analogous to fitting the absolute difference between the twins in a pair (25). Second, we simply classified each twin as having either a higher or a lower CFR than his brother. Finally, we examined discordant twin pairs for a CFR <2.5, which has been suggested as a cut point for microvascular dysfunction, being the lower limit of normal flow reserve in arteries free of obstructive CAD (26). The within-pair effects are inherently controlled for demographic, shared familial, and early environmental influences; in addition, daily activities and other environmental factors during the examination day were controlled since twin pairs were examined together. Because twin pairs were raised together, and monozygotic pairs share 100% of their genetic material, any association between CFR and inflammatory markers within monozygotic pairs cannot be ascribed to genes or early family environment such as parental influences, socioeconomic status, and behaviors learned while growing up. Dizygotic pairs also share familial factors, but on average, only share 50% of their genetic material. All analyses used mixed effects regression models and accounted for the twin pair clustering by using a random effect term for each pair. Because the dependent variables were log transformed, and the association was inverse, we expressed the results as percentage difference of the nontransformed values by using the following formula: $[1 - (\exp^{\beta})] \times 100$ (%); where β is the regression coefficient and $\exp^{\beta}$ returns the exponential value of the parameter.

Because we were interested in microvascular dysfunction, we adjusted for presence of perfusion abnormalities. Analyses were further adjusted for factors that were significantly different according to CFR status, or that were a priori considered potentially important confounders, including body mass index, previous history of CAD, study year, and the Framingham risk score, which incorporates age, low-density-lipoprotein cholesterol, high-density lipoprotein cholesterol, blood pressure, diabetes mellitus, and smoking (27). Sensitivity analyses were conducted by adjusting for habitual physical activity (Baecke score), cardiac medications (statins, aspirin, beta-blockers, and angiotensin-converting enzyme inhibitors), rate-pressure product during the adenosine infusion, history of major depression (which was a criterion for inclusion in a subsample), and by adjusting for individual CAD risk factors rather than including them in the Framingham risk score. We also repeated the analyses after excluding a few individuals who developed CAD between 1990 and the examination date. All analyses were conducted using SAS software version 9.1 (SAS Institute, Cary, North Carolina). Significance level was set at 0.05, 2-sided.

Results

Study sample. Of the 360 twins in THS, 57 were excluded because of incomplete quantitative MBF data, and 35 were excluded because of a previous history of CAD, leaving 268 twins in the analysis: 122 complete pairs (74 monozygotic and 48 dizygotic) and 24 unpaired twins.

The mean CFR in the entire sample was 2.68, with a standard deviation of 0.83. Within the 122 pairs, when each twin was classified as having a higher or a lower CFR than his brother, both twins had similar levels of risk factors, medical history, and medication use, with the possible exception of smoking (Table 1). The Framingham risk score was also similar within pairs. The mean CFR was 3.11 in the twins with higher CFR and 2.32 in their matched brothers with lower CFR.

Imaging and hemodynamics. Less than 30% of the sample had a summed stress score ≥4, which would indicate a significant perfusion abnormality; this proportion was the same comparing twins with higher and lower CFR within pairs (Table 2). The overall perfusion defect score, either during adenosine stress or at rest, was also similar, as were hemodynamic responses during adenosine infusion, including maximum systolic blood pressure, maximum heart rate, and the average and maximum rate-pressure products (Table 2).

Inflammatory biomarkers and CFR. In the overall sample, inflammatory and endothelial activation biomarkers showed a decreasing trend from the lowest to the highest quartile of CFR, which was more pronounced for CRP, IL-6, and WBC than for soluble cell adhesion molecules (Fig. 1). Biomarker levels were expressed as geometric means calculated from log-transformed data. Arithmetic means show a larger spread of data; for example, for CRP,

Table 1	**Within-Pair Comparison of Subject Characteristics According to CFR Status**		
	Twin With Lower CFR **(n = 122)**	**Twin With Higher CFR** **(n = 122)**	**p Value**
Age, yrs	54.0 (53.5–54.6)	54.0 (53.5–54.6)	—
Systolic blood pressure, mm Hg	130 (126–132)	130 (127–132)	0.74
Diastolic blood pressure, mm Hg	81.2 (79.2–83.2)	80.6 (78.7–82.6)	0.59
LDL cholesterol, mg/dl	124 (118–130)	127 (121–133)	0.44
HDL cholesterol, mg/dl	38.7 (37.1–40.3)	39.6 (37.8–41.4)	0.32
Diabetes	8.20	8.26	0.99
Body mass index, kg/m^2	28.8 (28.0–29.6)	29.2 (28.4–30.0)	0.34
Current smoker	22.9	15.6	0.10
Framingham Risk Score	5.76 (5.39–6.14)	5.70 (5.31–6.09)	0.76
Physical activity (Baecke) score	7.36 (7.02–7.70)	7.45 (7.15–7.74)	0.67
Number of alcoholic beverages in typical week	4.79 (3.27–6.32)	5.49 (3.51–7.48)	0.58
Taking ACE inhibitors	10.7	9.84	0.83
Taking statins	22.1	17.2	0.24
Taking beta-blocker medications	4.92	4.10	0.71
Taking aspirin	16.4	19.7	0.51
Taking antidepressants	16.4	12.3	0.35
CFR	2.32 (2.21–2.42)	3.11 (2.95–3.26)	<0.001

All data are reported either as mean (95% confidence interval) or % of subjects. p values are obtained from mixed models for continuous variables or generalized estimating equations (GEE) for categorical variables. p values for inflammatory biomarkers are based on log-transformed data.

ACE = angiotensin-converting enzyme; CFR = coronary flow reserve; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

the arithmetic means going from the lowest to the highest quartile of CFR were 6.10, 2.94, 2.95, and 1.58 mg/l.

The association was similar when CFR was analyzed as a continuous variable, in the entire myocardium, and in specific vascular territories (Table 3). For each unit of increasing CFR in the entire myocardium, CRP decreased 17.0% (p = 0.03), IL-6 decreased 14.5% (p = 0.01), WBC decreased 4.5% (p = 0.02), ICAM decreased 3.5% (p = 0.08), and VCAM decreased 3.1% (p = 0.22). All inflammatory biomarkers, except VCAM-1, were inversely associated with the CFR in all vascular territories. Although absolute maximal flow was not significantly related to inflammation, maximal flow increase from baseline showed similar relationships: p = 0.02 for CRP, p = 0.003 for IL-6, p = 0.005 for WBC, p = 0.10 for ICAM, and p = 0.30 for VCAM. In contrast, none of the biomarkers, except IL-6, were correlated

with detectable myocardial perfusion abnormalities as measured by the summed stress score (data not shown).

Within pairs, all inflammatory biomarkers were higher in the twins with lower CFR than in their siblings with higher CFR (Table 4). Of 122 complete twin pairs, 50 pairs were discordant, for a CFR of <2.5. Values of all biomarkers except VCAM-1 were significantly higher in twins with a CFR <2.5 than their brothers with a CFR of 2.5 or higher. For example, CRP was 65% higher and IL-6 was 54% higher in the twin with CFR <2.5 than in his brother with CFR ≥2.5. These associations remained statistically significant and fundamentally unchanged after adjusting for the Framingham risk score, body mass index, and summed stress score (Table 5). Adjustment for education, marital status, habitual physical activity, rate-pressure product during the adenosine infusion, medications (statins, beta-blockers, aspirin), and history of major depression did not materially change the results. Adjusting for individual risk factors, rather than including them in the Framingham risk score, also did not substantially change the results. Results were similar after stratification by zygosity, indicating that genetic factors do not play an important role in this association.

Results were also similar when subjects with significant perfusion abnormalities (a summed stress score ≥4) were excluded. These results indicate that global myocardial perfusion, rather than perfusion in specific coronary territories, was reduced in twins with higher inflammation.

Discussion

Using PET myocardial perfusion imaging in a sample of twins without symptomatic CAD, we found a robust

Table 2	**Within-Pair Comparison of Myocardial Perfusion Imaging Data According to CFR Status**		
	Twin With Lower CFR **(n = 122)**	**Twin With Higher CFR** **(n = 122)**	**p Value**
Abnormal perfusion (summed stress score ≥4), n (%)	33 (27.3)	33 (27.3)	1.00
Summed stress score	2.75 (0.55)	2.50 (0.44)	0.66
Summed rest score	0.82 (0.28)	0.73 (0.21)	0.76
Summed difference score	1.93 (0.45)	1.77 (0.37)	0.77
Average rate-pressure product during adenosine	92.5 (2.4)	88.4 (2.1)	0.12
Maximal rate-pressure product during adenosine	115 (3)	110 (3)	0.16

Unless otherwise indicated, all data are reported as mean (SE) values. p values are obtained from mixed models for continuous variables or generalized estimating equations for categorical variables, including a random effect for pair.
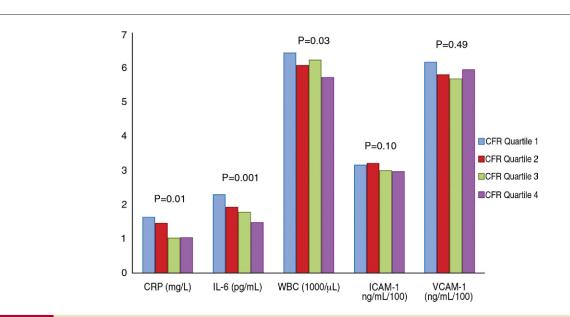
CFR = coronary flow reserve.

Figure 1 **Geometric Means of Inflammatory Biomarkers According to Quartiles of CFR**

Quartile ranges: quartile 1: coronary flow reserve (CFR) 3.04 to 6.84; quartile 2: CFR 2.59 to 3.03; quartile 3: CFR 2.10 to 2.58; quartile 4: CFR 1.06 to 2.09. p values refer to tests for trend. CRP = C-reactive protein; ICAM = intercellular adhesion molecule; IL = interleukin; VCAM = vascular cell adhesion molecule; WBC = white blood cell count.

association linking biomarkers of systemic inflammation and endothelial cell activation with lower CFR. We also found that this association is independent of CAD risk factors, as well as of familial and genetic factors shared by the twins. In addition, the relationship was independent of perfusion defects, suggesting that the microvascular circulation plays a role. Although no direct causality can be inferred from our data, our results are suggestive of a mechanistic relationship between inflammation and microvascular function and highlight the importance of inflammatory processes in the early phases of CAD.

The role of inflammation in the pathogenesis of atherosclerosis has been widely recognized for many years (28,29). In addition to extensive experimental research, clinical studies have found a link between peripheral vascular function and inflammation (5,6). However, data from the peripheral circulation cannot necessarily be extrapolated to the coronary circulation (30), for which much less is known. Two studies found an attenuated CFR in patients with

chronic inflammatory conditions, including rheumatoid arthritis and systemic lupus erythematosus, but without significant coronary stenosis, compared with controls (31,32). However, 2 additional studies of patients with chest pain and no obstructive CAD were not able to demonstrate a relationship between invasive angiographic assessment of CFR and levels of inflammatory biomarkers (7,8); although, in 1 of these, endothelial function measured with administration of intracoronary acetylcholine was correlated with CRP (7). These contrasting findings may be due to selected study samples that included symptomatic patients referred for medical evaluation, and/or inadequacies in the selection of control groups. No previous investigation explored the relationship between inflammation and CFR in asymptomatic individuals from the community.

A link between inflammation and coronary microvascular function may result from early atherosclerotic changes, microvascular endothelial or smooth muscle dysfunction, and/or inflammation of the coronary microvasculature.

Table 3 **Percentage Decrease in Inflammatory Biomarkers per 1-U Increase in CFR**

	Entire Myocardium		Left Anterior Descending		Circumflex		Right Coronary Artery	
	%	p Value	%	P Value	%	p Value	%	p Value
CRP, mg/l	17.0	0.03	16.6	0.03	18.5	0.009	15.4	0.05
IL-6, pg/dl	14.5	0.01	13.6	0.008	16.1	<0.001	13.3	0.01
WBC, 1,000/μl	4.5	0.02	4.7	0.01	4.3	0.01	4.5	0.02
ICAM-1, ng/ml	3.5	0.08	2.9	0.12	2.5	0.17	2.7	0.16
VCAM-1, ng/ml	3.1	0.22	2.5	0.27	1.5	0.48	2.4	0.31

Percentage decrease in biomarkers is per 1-U increase in coronary flow reserve (CFR), in the entire myocardium, and in regional coronary artery territories. Values are derived from mixed models with log-transformed biomarker data. Percentages are calculated from regression coefficients.

CRP = C-reactive protein; ICAM = intercellular adhesion molecule; IL = interleukin; VCAM = vascular cell adhesion molecule; WBC = white blood cell count.

Table 4	Unadjusted Within-Pair Differences in Plasma Inflammatory Biomarker Levels Based on CFR Discordance Status							
	Inflammatory Markers (n = 122 Twin Pairs)				Inflammatory Markers (n = 50 Twin Pairs)			
	Twin With Lower CFR	Twin With Higher CFR	Within-Pair Difference (%)	p Value	Twin With CFR <2.5	Twin With CFR ≥2.5	Within-Pair Difference (%)	p Value
CRP, mg/l	1.40 (1.11)	1.10 (1.12)	21.0	0.04	1.67 (1.18)	1.01 (1.18)	65.4	0.006
IL-6, pg/dl	2.04 (1.07)	1.65 (1.07)	18.7	0.02	2.65 (1.11)	1.72 (1.11)	54.2	0.002
WBC, 1,000/μl	6.35 (1.02)	5.87 (1.02)	7.5	0.005	6.44 (1.04)	5.89 (1.04)	9.3	0.04
ICAM-1, ng/ml	323 (1.02)	302 (1.02)	6.6	0.02	336 (1.04)	303 (1.04)	11.0	0.02
VCAM-1, ng/ml	614 (1.03)	573 (1.03)	6.7	0.04	603 (1.05)	580 (1.05)	4.0	0.46

Values are geometric mean (SE) unless otherwise indicated. CFR is defined as the ratio of myocardial blood flow during adenosine to flow at baseline. All values in table are derived from mixed models with random intercept for pair.
Abbreviations as in Table 3.

Adenosine-induced hyperemic response incorporates both endothelium-dependent and endothelium-independent pathways (33). Endothelium-derived nitric oxide is a key modulator of vascular tone and plays an important role in the regulation of the coronary microcirculation (34). Inhibition of the endothelial nitric oxide synthase by intravenous infusion of L-N^G-monomethyl arginine reduces the vasodilatory response to adenosine by about 25% as measured by PET, suggesting a role of the endothelium (35,36). Although the influence of inflammatory processes on coronary microvascular physiology and pathology has been less studied, our study suggests that similar processes are involved. Increased inflammation in the artery wall may result from oxidative stress due to reduced endothelium-derived nitric oxide. For example, oxidative stress is critical for the activation of nuclear factor-kappa B (NF-κB) (37), a transcription factor that increases the expression of pro-inflammatory cytokines, chemokines, and cell adhesion molecules through angiotensin-II stimulation. Increased inflammation may also reflect injury of the arterial wall in the early phases of the atherosclerotic process (33).

Although our study subjects were asymptomatic and without a prior history of CAD, 27% of them showed perfusion defects by PET imaging. This relatively high prevalence of perfusion abnormalities is not surprising given that CAD risk factors, such as smoking, obesity, and hypertension, were common in this sample. The prevalence

of myocardial perfusion abnormalities in asymptomatic subjects at risk (either because of family history or because of risk factors) has been reported to be between 20% and 50% (38–40). Therefore, our data are consistent with previous published reports. These perfusion abnormalities may be due to pre-clinical, mild, or diffuse coronary artery narrowing, or to endothelial dysfunction preceding luminal narrowing. Indeed, adenosine-induced hyperemia is a mixture of both endothelial-dependent and -independent pathways (33).

In contrast to their robust relationship with CFR, none of the inflammatory biomarkers, except IL-6, were associated with perfusion defects. These results clearly demonstrate that the link between inflammation and CFR in our study was driven by microvascular dysfunction rather than epicardial coronary stenoses. The association, however, could be an epiphenomenon of risk factors such as hypercholesterolemia, hypertension, diabetes, and smoking, which have been linked to perfusion abnormalities even in the absence of coronary obstruction (10–15). We were able to demonstrate that CAD risk factors did not explain the link between microvascular function and inflammation. As a whole, our data highlight the importance of inflammation in the early phases of the atherosclerotic process in asymptomatic individuals, because microvascular dysfunction appears to precede the development of frank CAD (41). These results may also have implications for prevention, since microvas-

Table 5	Adjusted Within-Pair Differences in Plasma Inflammatory Biomarker Levels Based on CFR Discordance Status							
	Inflammatory Markers (n = 122 Twin Pairs)				Inflammatory Markers (n = 50 Twin Pairs)			
	Twin With Lower CFR	Twin With Higher CFR	Within-Pair Difference (%)	p Value	Twin With CFR <2.5	Twin With CFR ≥2.5	Within-Pair Difference (%)	p Value
CRP, mg/l	1.42 (1.10)	1.11 (1.10)	22.2	0.03	1.77 (1.17)	1.09 (1.17)	62.9	0.006
IL-6, pg/dl	2.05 (1.07)	1.65 (1.07)	19.4	0.02	2.63 (1.11)	1.74 (1.11)	51.2	0.003
WBC, 1,000/μl	6.38 (1.02)	5.89 (1.02)	7.6	0.003	6.42 (1.04)	5.94 (1.04)	8.2	0.06
ICAM-1, ng/ml	322 (1.02)	301 (1.02)	6.5	0.02	332 (1.04)	302 (1.04)	9.7	0.04
VCAM-1, ng/ml	610 (1.03)	570 (1.03)	6.6	0.049	605 (1.05)	580 (1.05)	4.3	0.44

Values are geometric mean (SE) unless otherwise indicated. CFR is defined as the ratio of myocardial blood flow during adenosine to flow at baseline. All values in table are derived from mixed models with random intercept for pair. All estimates adjusted for perfusion defect score, Framingham risk score (including age, current smoking, systolic blood pressure, LDL cholesterol, HDL cholesterol, and diabetes), body mass index, and study year. Adjusting for additional covariables, including education, marital status, habitual physical activity, rate-pressure product during the adenosine infusion, and medications (statins, beta-blockers, aspirin), did not materially change the results.
Abbreviations as in Tables 1 and 3.

cular dysfunction is potentially reversible and is associated with future cardiovascular events (16,17).

Study limitations. Our study is cross-sectional, thus limited in the ability to discern the temporal order between inflammation and CFR. Furthermore, as in any study, sample variability in biomarker assays may increase random error. However, all assays used in this study have acceptable levels of precision. Nonetheless, it is possible that the lack of significant findings for ICAM- and VCAM-1 is in part due to random error. Because our twins were all middle-aged male military veterans, caution should be used in generalizing our results to women or to younger or older individuals. On the other hand, using a co-twin design, we were able to control for many unmeasured risk factors including maternal factors, early familial environment, and genetic influences, in addition to a comprehensive set of measured CAD risk factors. Finally, CFR measurement based on nitrogen 13 [^{13}N] ammonia PET perfusion imaging is currently limited to institutions with onsite cyclotrons, which may limit clinical utility. It is likely that new methods will allow the measurement of MBF using agents that do not require a cyclotron in the near future. Until these techniques become available, PET with ^{13}N remains the gold standard method for the noninvasive assessment of CFR.

Conclusions

We found that in asymptomatic middle-aged subjects, a decrease in coronary microvascular function is associated with increased systemic inflammatory response. Given the demonstrated importance of inflammation in the pathogenesis of CAD, our results, using a controlled design of matched twin pairs, support the pathogenic role of inflammation in human microvascular dysfunction.

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