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Endothelial Function Predicts Progression of Carotid Intima-Media Thickness

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Background—Endothelial dysfunction develops early and has been shown to predict the development of clinical complications of atherosclerosis. However, the relationship between early endothelial dysfunction and the progression of arterial disease in the general population is unknown. We investigated endothelial dysfunction, risk factors, and progression of carotid intima-media thickness (cIMT) in late-middle-aged individuals at low to intermediate cardiovascular risk in a prospective study between 1997 and 2005.

Methods and Results—Brachial artery flow-mediated dilatation and cIMT were measured in 213 nonsmoking British civil servants recruited from a prospective cohort (Whitehall II study). Participants (age, 45 to 66 years) were free of clinical cardiovascular disease and diabetes mellitus. Risk factors and Framingham Risk Score were determined at baseline. cIMT was repeated 6.2 ± 0.4 years later. At baseline, age, blood pressure, low-density lipoprotein cholesterol, and Framingham Risk Score correlated with cIMT. However, only flow-mediated dilatation, not risk factors or Framingham Risk Score, was associated with average annual progression of cIMT. This relationship remained significant after adjustment for risk factors whether entered as separate variables or as Framingham Risk Score. Further adjustment for waist circumference, triglycerides, and employment grade had no significant effect.

Conclusions—Systemic endothelial function was associated with progression of preclinical carotid arterial disease over a 6-year period and was more closely related to cIMT changes than conventional risk factors. Thus, the relationship between endothelial dysfunction and adverse outcome is likely to be due not only to destabilization of established disease in high-risk populations but also to its impact on the evolution of the atherosclerotic substrate. Flow-mediated dilatation testing provides an integrated vascular measure that may aid the prediction of structural disease evolution and represents a potential short- to intermediate-term outcome measure for evaluation of preventive treatment strategies. (***Circulation***. 2009;119:1005-1012.)

Key Words: atherosclerosis ■ endothelium ■ nitric oxide ■ risk factors

Atherosclerosis is a multifactorial disease that begins in childhood and typically evolves over a long preclinical phase until it results in morbidity and mortality, usually from middle age onward.[1] Several recognized risk factors, including low-density lipoprotein (LDL) cholesterol, diabetes mellitus, hypertension, and smoking, have been shown to have a causal role.[2–4] They are predictive of cardiovascular outcome and are amenable to safe and effective interventions that improve prognosis.[5–9] However, atherosclerosis also develops in a significant number of individuals with relatively modest risk factor profiles,[10] highlighting the need for measures that improve the prediction of disease progression and risk stratification.

The vascular endothelium has a central role in the maintenance of vascular health.[11,12] By recognizing and respond-

ing to local physical and chemical signals, endothelial cells are able to influence vasomotor, thrombotic, inflammatory, and cellular proliferation pathways, thus affecting both the short- and long-term biology of the vessel wall. Endothelial dysfunction (ED) has been shown to predict clinical outcome in high-risk cohorts, including the elderly,[13] patients with peripheral vascular disease, hypertensive patients, and those undergoing cardiac catheterization.[14–17] This may be due to enhancement of local inflammation and a prothrombotic tendency in the arterial wall, which may increase risk by promoting plaque instability and arterial thrombosis.

In addition, we and others have shown that ED may develop decades before clinical presentation in patients with conventional risk factors (RFs) such as hypercholesterolemia, diabetes, and smoking and novel RFs such as obesity, infection, and inflammation.[18–23] The development of arterial ED with reduced nitric oxide bioavailability may alter unfa-

vorably the balance between vascular injury and repair and thus promote conditions favoring lesion development. However, the impact of ED on the progression of structural arterial disease in the preclinical population has not been evaluated prospectively.

Endothelial function and structural arterial changes can be measured noninvasively with high-resolution ultrasound to measure brachial artery flow-mediated dilatation (FMD) and carotid artery intima-media thickness (cIMT), respectively.[24] We have used these techniques in a well-characterized prospective cohort of middle-aged British civil servants (the Whitehall II study) to evaluate the role of the endothelium in the evolution of structural arterial disease.

Methods

Study Population
The Whitehall II study population consists of 10 308 nonindustrial civil servants who have been followed up approximately every 2.5 years since entry between 1985 and 1988.[25] We recruited 282 participants into a vascular substudy investigating the relationship between metabolic RFs and preclinical vascular phenotype. Participants were selected at the phase 5 (1997 to 1999) evaluation of the Whitehall II study from among nonsmokers who had no history of diabetes mellitus or clinical cardiovascular disease identified at this phase. These participants underwent assessment of cardiovascular disease RFs (blood pressure, fasting glucose, total cholesterol, LDL and high-density lipoprotein [HDL] cholesterol, triglycerides, and waist circumference), cardiovascular disease status, and measures of body habitus FMD and cIMT at baseline. Reassessment of cIMT was undertaken 6.2 ± 0.4 years later at phase 7 (2003 to 2005). We excluded 7 individuals in whom it was impossible to acquire IMT data of adequate quality for analysis at baseline and 62 who did not return for a follow-up assessment of IMT, leaving 213 participants for analysis.

RF Measurement
Blood pressure was measured twice in subjects in the sitting position after 5 minutes of rest with the Hawksley random-zero sphygmomanometer. Waist circumference (smallest circumference at or below the costal margin) was measured with participants unclothed in the standing position with a fiberglass tape measure at 600-g tension.

Fasting plasma glucose, total cholesterol, HDL cholesterol, and triglyceride levels were determined as previously reported.[26] LDL was calculated from the Friedewald formula.[27] For each participant, the predicted 10-year risk (Framingham Risk Score [FRS]) of a cardiovascular event was calculated from age, sex, systolic blood pressure, and total and HDL cholesterol levels.[4]

Vascular Measurements
All studies were performed in a temperature-controlled (22°C to 26°C) vascular laboratory by 2 trained operators after a 10-minute rest. All participants abstained from food and caffeinated drinks for ≥4 hours, and those treated with antihypertensive therapy refrained from taking their medication for ≥48 hours.

Brachial Artery Vasomotor Function
Brachial artery vasomotor function was assessed with high-resolution ultrasound as previously reported,[28] with arterial diameter analysis done with an automated edge detection system (Brachial Tools, Iowa City, Iowa). FMD was expressed as a percentage from baseline. The overall coefficient of variation for repeated measures of FMD in our laboratory is <11%.

Carotid Artery IMT
Longitudinal images of the common carotid artery 1 cm proximal to the bifurcation in which the far wall intima-media interface (M-line) was clearly defined were magnified and recorded on videotape for later analysis. The distance between the leading edge of the intima and the media-adventitia interface was measured with ultrasonic calipers. Three measurements were taken in both the right and left common carotid arteries, and a mean IMT was calculated as previously described.[29,30] cIMT was assessed on 2 occasions: a baseline analysis at phase 5 (1997 to 1999) and a follow-up evaluation at phase 7 (2003 to 2005). The annual rate of progression of cIMT (ΔcIMT per year) was determined by subtracting the cIMT at phase 5 from the cIMT at phase 7 and then dividing this value (expressed in micrometers) by the time interval in years between the phase 5 and 7 evaluations for each participant. The overall coefficient of variation for repeated measures of cIMT in our laboratory is <5%.

Statistical Analysis
The analysis data set was almost complete, with only blood pressure (1 missing value), HDL and LDL (27 missing), and waist measurements (44 missing) having any missing values. These missing values were replaced with RF data from phase 3 (1991 to 1993) to provide a complete data set. The distributions of cIMT and the annual rate of cIMT progression were both normally distributed. Contemporaneous data were used to evaluate cross-sectional relationships between RFs and measures of vascular function and structure at baseline. RFs and FMD at phase 5 also were used to explore relationships with ΔcIMT per year. These associations were initially described by tabulating mean RFs by quartiles of cIMT and progression of cIMT. Univariate between-group comparisons were made with t tests or Mann-Whitney tests for nonnormally distributed data. Bivariate correlations between parameters were assessed with the Pearson or Spearman correlation coefficient for normally or nonnormally distributed variables, respectively.

Linear regression analysis was used to assess the relationships between baseline cIMT or annual rate of progression of cIMT and RFs, including FMD. These relationships were fitted by use of least-squares regression with cIMT or annual rate of progression as the dependent variable. Additional analyses confirmed that no evidence existed (all $P>0.70$) for any departures from the assumption of linearity. The associations of cIMT and progression of cIMT with the RFs were summarized through the use of the regression coefficient and β coefficient showing the change (on the unstandardized and standardized scales) in the outcome associated with a unit change in the RFs. Because no evidence was found that the relationships differed by sex, men and women were pooled in the analyses when possible. Each RF relationship, adjusted for age and sex, was first assessed separately (Tables 1 and 2). When adjusting for age and sex, we allowed the relationships of each RF with age to differ between the sexes. The analyses for the effects of waist circumference and HDL were done in men and women separately because the mean levels of these RFs showed large sex differences. The relationship between the annual rate of progression of cIMT and FMD was examined further with multiple linear regression (Table 3) to assess the effect of additional adjustment for the Framingham cardiovascular disease RFs (age, sex, blood pressure, smoking, diabetes mellitus, and total and HDL cholesterol) entered into the model as both individual variables and the FRS. Further adjustments to the model were made for waist circumference (as a measure of abdominal obesity) and triglyceride levels, which are widely recognized as the principal emerging clinical markers of cardiometabolic risk not accounted for by the Framingham variables.

The value of using FMD to predict an adverse progression of cIMT, defined as having an annual rate of cIMT progression in the highest 25% of the distribution, was assessed with receiver-operating characteristic curves. The sensitivity and specificity of using a cut point of FMD to predict the upper quartile of cIMT progression were calculated. They were used to determine the cut point for FMD that gave the best (defined as the point at which the sum of sensitivity plus specificity was greatest) prediction of cIMT progression. Analyses were performed with SPSS version 14.0 (SPSS Inc, Chicago, Ill) and SAS version 8.1 (SAS Institute, Inc, Cary, NC).

Table 1.   Total Sample Characteristics and Association of cIMT at Baseline (Phase 5: 1997 to 1999) With RFs

		cIMT Quartiles				Regressions of cIMT vs RFs		
	Total Sample (n=213)	Q1 (Lowest) (n=50)	Q2 (n=61)	Q3 (n=47)	Q4 (Highest) (n=55)	Regression Coefficient* (SE), μm	P	β† (SE), μm
Age, ‡ y	55.9±5.5	54.1 (0.76)	55.8 (0.69)	56.2 (0.78)	57.4 (0.73)	5 (2)	0.01	26 (11)
Female gender, ‡ %	39.4	46.0	41.0	36.2	34.6	−26 (22)	0.24	−26 (22)
Waist, § cm								
Men	87.7±10.1	84.4 (2.2)	88.7 (1.9)	88.5 (2.0)	87.5 (1.9)	0.6 (1.5)	0.69	7 (17)
Women	80.8±13.3	81.5 (2.4)	78.9 (2.2)	81.2 (2.7)	84.0 (2.6)	0.9 (1.1)	0.45	10 (13)
SBP, § mm Hg	119±15.6	117 (2.2)	117 (1.9)	119 (2.2)	122 (2.1)	1.1 (0.7)	0.13	16 (11)
DBP, § mm Hg	75.5±9.5	74.4 (1.4)	74.7 (1.2)	76.4 (1.4)	76.7 (1.3)	0.8 (1.1)	0.45	8 (11)
Cholesterol, § mmol/L	5.76±0.97	5.62 (0.14)	5.80 (0.12)	5.81 (0.14)	5.81 (0.13)	16.3 (11.1)	0.14	16 (11)
HDL, § mmol/L								
Men	1.36±0.32	1.47 (0.07)	1.27 (0.06)	1.38 (0.06)	1.36 (0.06)	−15 (46)	0.74	−6 (17)
Women	1.61±0.41	1.70 (0.08)	1.66 (0.07)	1.49 (0.09)	1.55 (0.08)	−4 (36)	0.92	−1 (14)
Triglycerides, § mmol/L	1.19±0.71	1.04 (0.10)	1.29 (0.09)	1.26 (0.10)	1.16 (0.10)	−0.9 (15.0)	0.95	−1 (11)
LDL, § mmol/L	3.83±0.94	3.64 (0.13)	3.85 (0.12)	3.86 (0.14)	3.93 (0.13)	21 (11)	0.06	20 (11)
Fasting glucose, § mmol/L	5.30±1.18	5.34 (0.17)	5.23 (0.15)	5.07 (0.17)	5.54 (0.16)	7.5 (9.0)	0.40	9 (11)
FMD, § mm	0.23±0.12	0.20 (0.02)	0.24 (0.02)	0.24 (0.02)	0.22 (0.02)	93 (88)	0.29	11 (11)
FMD, § %	5.17±2.97	4.33 (0.41)	5.49 (0.37)	5.70 (0.42)	5.13 (0.39)	6.0 (3.4)	0.10	18 (11)

Q indicates quartile; SBP, systolic blood pressure; and DBP, diastolic blood pressure. Values are mean±SD or median (SE) as appropriate.

*Regression coefficient shows the change in cIMT associated with a unit change in the RF.

†β Coefficient shows the change in cIMT associated with a 1-SD change in the RF (regression coefficient shown for gender).

‡Regression and β coefficients for age and gender are mutually adjusted. Coefficients for gender are for women vs men.

§Adjusted for age and gender.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Participants

Two participants were receiving treatment with statins at phase 5, and 27 individuals were being treated with antihypertensive agents. These individuals were included in the primary analysis (Table 1).

Of the 62 participants who attended at baseline but did not attend for follow-up vascular assessment at phase 7, 35 attended for general assessment as part of the main Whitehall II study but chose not to accept the written and follow-up telephone invitation to reattend for vascular assessment, 5 formally withdrew from the study, 2 died, 2 were lost to follow-up, 9 requested a home visit for their general phase 7 assessment and were unable to attend the vascular laboratory, and 9 chose not to attend for any follow-up assessment at phase 7. Baseline diastolic blood pressure was higher in those who did not return for follow-up vascular assessment (79.1±10.1 versus 75.4±9.5 mm Hg; P=0.009), but all other RFs assessed at baseline, including FRS and FMD, were similar to the RFS of those who reattended at phase 7.

Vascular Structure and Function

Baseline Vascular Measures

Men had slightly lower FMD (4.95±2.92% versus 5.58±3.03%; P=0.18) and higher cIMT (707 versus 674 μm; P=0.13) than women at their baseline assessment.

Relationships Between RFs and Baseline Vascular Measures

Age (r=−0.23, P<0.001) was inversely correlated with FMD. Waist circumference (P=0.02) and both systolic and diastolic blood pressures (both P<0.001) also were inversely associated with FMD after adjustment for age and sex. Lipid parameters and fasting glucose were not associated with FMD.

Age, systolic blood pressure, total cholesterol, and LDL cholesterol were correlated with baseline cIMT. Only the relationships between cIMT and age and LDL cholesterol remained after adjustment for age and sex (Table 1). FMD, waist circumference, and other associated metabolic RFs, including HDL, triglycerides, and fasting glucose levels were not correlated with cIMT. FRS was correlated strongly with baseline cIMT (r=0.24, P<0.001) and inversely with FMD (r=−0.27, P<0.001).

Annual Rate of Progression of cIMT

The mean annual rate of progression of cIMT (the Figure) was 0.012±0.028 mm/y and was similar in men and women. It was correlated with age (r=0.16, P=0.02), FMD (r=−0.19, P=0.006), and blood pressure (systolic blood pressure: r=0.13, P=0.07: diastolic blood pressure: r=0.15, P=0.03). After adjustment for age and sex, FMD and diastolic blood pressure were the only parameters of those assessed at baseline, including FRS, that remained associated with progression of cIMT (Table 2).

The relationship between FMD and progression of cIMT remained after adjustment for FRS variables, whether entered

Table 2.   Association of ΔcIMT per Year Between Phases 5 and 7 With WFs

	ΔcIMT per Year Quartiles				Regressions of ΔcIMT per Year vs RFs		
	Q1 (Lowest) (n=52)*	Q2 (n=53)	Q3 (n=55)	Q4 (Highest) (n=53)	Regression Coefficient,† μm	P	β,‡ μm
Age,§ y	54.9 (0.75)	55.7	55.6	57.5	0.8 (0.3)	0.02	4.4 (1.9)
Gender,§ (% women)	38.5	37.7	49.1	32.1	0.6 (3.9)	0.88	0.6 (3.9)
Employment grade, % low‖	19.4	16.6	23.9	12.3	3.4 (5.4)	0.53	3.4 (5.4)
Waist,‖ cm							
Men	85.6 (2.0)	90.1	91.2	83.3	0.03 (0.26)	0.90	0.4 (3.1)
Women	83.0 (2.5)	82.4	80.2	79.8	−0.20 (0.21)	0.34	−2.4 (2.5)
SBP,‖ mm Hg	118 (2.1)	116	121	120	0.19 (0.12)	0.12	3.0 (1.9)
DBP,‖ mm Hg	74.7 (1.3)	75.0	75.7	76.7	0.39 (0.20)	0.05	3.7 (1.9)
Cholesterol,‖ mmol/L	5.83 (0.13)	5.86	5.61	5.76	−2.8 (2.0)	0.15	−2.8 (1.9)
HDL,‖ mmol/L							
Men	1.32 (0.06)	1.29	1.31	1.52	8.2 (8.0)	0.31	3.1 (3.0)
Women	1.60 (0.08)	1.46	1.67	1.68	0.0 (6.7)	0.99	0.0 (2.5)
Triglycerides,‖ mmol/L	1.17 (0.09)	1.47	1.19	0.94	−2.9 (2.7)	0.28	−2.1 (1.9)
LDL,‖ mmol/L	3.92 (0.13)	3.94	3.70	3.76	−3.2 (2.0)	0.12	−3.0 (1.9)
Fasting glucose,‖ mmol/L	5.12 (0.16)	5.55	5.22	5.30	0.7 (1.6)	0.67	0.8 (1.9)
FMD,‖ mm	0.24 (0.02)	0.24	0.24	0.19	−34 (16)	0.03	−4.1 (1.9)
FMD,‖ %	5.67 (0.40)	5.34	5.39	4.29	−1.6 (0.6)	0.01	−4.7 (1.9)

Abbreviations as in Table 1. Values are mean (SE) when appropriate.

*Regression coefficient shows the change in progression of cIMT associated with a unit change in the RF.

†β Coefficient shows the change in progression of cIMT associated with a 1-SD change in the RF. Regression coefficient is shown for gender (women vs men) and employment grade (lowest vs highest).

‡SEM applies to the means in all 4 quartiles, so it is shown only here.

§Regression and β coefficients for age and gender are mutually adjusted.

‖Adjusted for age and gender.

into the model as individual variables or as the composite FRS (Table 3). Further adjustment for waist circumference and triglycerides, the other modifiable markers of cardiometabolic risk, in addition to FRS variables, did not affect the relationship between FMD and progression of cIMT (β=−5.0, P=0.01). Employment grade was not significantly associated with FMD, cIMT, or progression of IMT, and adjustment for grade had very little effect on the results.

Table 3.   Association of Baseline FMD (%) With ΔcIMT (mm/y) Between Phases 5 and 7 and the Effect of Adjustment for Cardiovascular Disease RFs and FRS

Model (Adjustments)	Regression Coefficient* (SE), μm	P	β† (SE), μm
Model 1 (age and gender)	−1.6 (0.6)	0.01	−4.7 (1.9)
Model 2 (Individual FRS components‡)	−1.5 (0.7)	0.03	−4.3 (2.0)
Model 2a (FRS)	−1.6 (0.6)	0.01	−4.8 (1.9)
Model 2b (FRS+waist+triglycerides)	−1.7 (0.6)	0.01	−5.0 (1.9)

The relationship between FMD and ΔcIMT per year was of a similar strength when FMD was expressed as an absolute change (mm) and percentage change in diameter.

*Regression coefficient shows the change in progression of cIMT associated with a unit change in the RF.

†β Coefficient shows the change in progression of cIMT associated with a 1-SD change in the RF.

‡Model 2 is based on the FRS risk factors (age, gender, systolic blood pressure, cholesterol, HDL, diabetes mellitus) entered separately.

Reactive hyperemia (the flow stimulus for FMD) was not associated with cIMT progression (P=0.14), and adjustment for reactive hyperemia did not alter the strength or significance of the relationship between FMD and cIMT progression (β=−4.8 μm/y; P=0.02). Brachial artery diameter (P=0.02), but not carotid artery diameter (P=0.78), was associated with cIMT progression.

The association between FMD and cIMT progression was similar if the 27 patients who were treated with antihypertensive drugs and the 2 patients receiving statin therapy were omitted from the analysis (data not shown).

Receiver-operating characteristic analysis exploring the ability of baseline data to predict a rate of cIMT progression in the highest quartile of the distribution, demonstrated areas under the curve of 0.539 for FRS and 0.632 for FMD. Combining both FMD and FRS data did not provide incremental predictive power over FMD alone (area under the curve=0.633). A value of FMD ≤4.10% provided the best discriminatory ability, at which point sensitivity was 58%, specificity was 65%, positive predictive value was 36%, and negative predictive value was 83% for the prediction of those likely to have a rate of cIMT progression in the top quartile of the distribution.

Discussion

Endothelial function is known to predict clinical events in high-risk subjects. We now demonstrate for the first time that
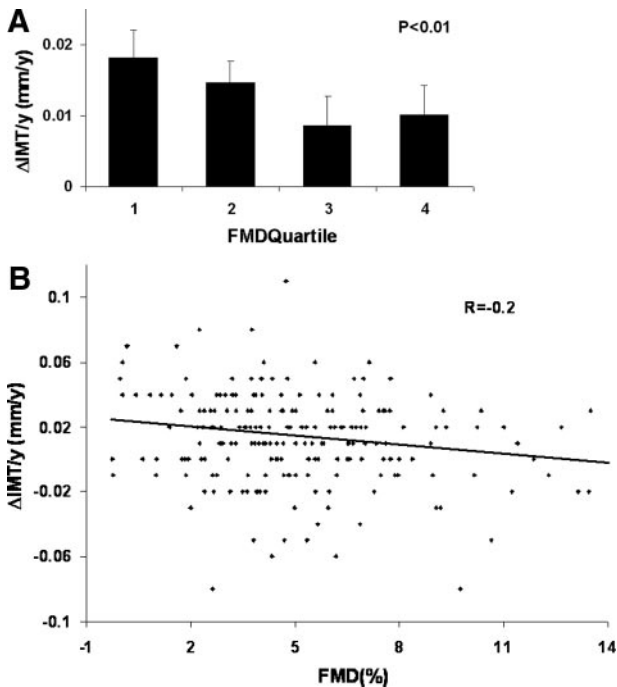
Figure. More rapid progression of carotid structural disease is seen in those with worse baseline endothelium-dependent vasodilatation. All data are unadjusted. Top, Relationship between FMD of the brachial artery divided into quartiles (1 indicates the lowest quartile; 4, highest FMD; annual ΔIMT is expressed in mm/y). Bottom, Scatterplot demonstrating annual change in cIMT vs FMD.

impaired endothelial function is associated with more rapid progression of structural arterial disease in a general, middle-aged population. This relationship was more robust than, and independent of, the impact of conventional RFs and the FRS. Endothelial function testing with FMD may therefore have a role in identifying those individuals at higher risk of disease progression and may serve as an appropriate end point for short- to intermediate-term trials of preclinical intervention.

A dysfunctional endothelium may contribute both to destabilization of established disease and to initiation and progression of arterial disease. Dynamic vascular changes are implicated in the activation of atherosclerotic disease, which leads to plaque destabilization and acute clinical events. The dysfunctional vascular endothelium has been implicated in this biology through the production of factors that adversely affect vascular tone, cellular adhesion, vessel wall inflammation, and the local thrombotic balance. Measures of endothelium-dependent vasomotor function are strongly linked to prognosis, even after accounting for the impact of conventional RFs, in higher-risk subjects.[13–17,31] Mechanisms implicated in plaque destabilization are likely to be predominantly responsible because the majority of subjects probably had significant atheroma burden at the time of endothelial function testing.

Although previous studies have implicated ED as a key determinant of disease progression by demonstrating significant association with the burden of atherosclerotic disease and its RFs,[19,32–36] no previous study has explored prospectively the relationship between ED and evolution of structural

disease. Thus, our study was designed to explore whether endothelial function testing was able to identify individuals in the preclinical setting who may be at greater risk of developing structural atherosclerosis, the substrate for future clinical cardiovascular events.

FMD provides a clinically relevant estimate of local vascular nitric oxide bioavailability.[12] Reduced nitric oxide in the context of ED results in attenuation of its antimigratory, antiproliferative, and antiinflammatory functions. In addition, the dysfunctional endothelium is more permeable to atherogenic lipoproteins and expresses increased quantities of proinflammatory cytokines, chemokines, and adhesion molecules, which enhance leukocyte recruitment to the arterial wall. ED also results in increased local synthesis and generation of the vasoactive growth factors endothelin-1 and angiotensin II. Together, these processes create the ideal milieu for the initiation and progression of atherosclerotic disease.

We conducted this study in the Whitehall II study population, which is a well-characterized, prospective cohort study of British civil servants who have been under active follow-up since 1985.[25] We purposely chose participants who were at low to intermediate risk as determined by their FRS and baseline cIMT levels because more cardiovascular events eventually occur in these subjects than in high-risk subjects as a result of their greater numbers in the population.[10] Although most of the participants in our study were low to intermediate risk, our findings were similar regardless of whether the higher-risk individuals receiving statins and antihypertensive drugs were included in our analysis. More detailed analysis of vascular risk in low- to intermediate-risk patients is of particular interest because those at high risk by conventional assessment methodology would already be considered candidates for aggressive preventive measures.[37] Despite the fact that men had slightly greater cIMT at baseline than women, the rate of cIMT progression was similar in both sexes. This was not surprising given that cardiovascular risk is known to increase markedly in women after menopause, following relative resistance to clinical atherosclerotic disease during premenopausal life.[19] Our data reflect this, with women demonstrating a lower "accumulated" cIMT burden than men by their mid 50s, which incorporates predominantly premenopausal life, but a similar rate of disease progression over subsequent years.

Smokers were excluded from the study because of the well-recognized difficulties in accurately quantifying the level of exposure and the more variable acute influence of smoking on the results of dynamic vascular endothelial function testing. We did not assess how circulating biomarkers of inflammation relate to vascular dysfunction and disease progression. These parameters, however, may have only limited incremental predictive value.[38]

The careful characterization of the participants and their RF profiles and the meticulous conduct of the vascular testing procedures[28] are both likely to have contributed to our ability to characterize the relationships between RFs and vascular measures and between functional and structural measures during follow-up, even in our relatively small study population.

The expected relationships were observed between RFs and vascular measures at baseline, with the FRS best predicting the presence of structural disease. Although we were surprised not to see a relationship between FMD and cIMT at baseline, this finding is most likely explained by the fact that cIMT, as a structural marker, reflects the impact of lifetime RF exposure, whereas FMD has more proximal influences. Juonala et al[36] have previously demonstrated a small but highly significant relationship between FMD and cIMT (adjusted β cIMT$=-0.006\pm0.002$ mm per 1-SD difference in FMD) in a cross-sectional study of $>$2000 young (31.6$\pm$5.0 years of age) individuals. Our findings could be explained either by the considerable difference in age between their cohort and ours or by the lower statistical power of our smaller prospective study to detect this relatively weak relationship. FMD and cIMT provide complementary information on vascular status and should not be compared directly. FMD is a dynamic measure that reflects the impact of both acute and chronic influences on endothelial function and appears to be a more integrated indicator of current arterial status than individual RF levels. In contrast, cIMT is a more stable measure of accumulated structural changes in the arterial wall. Although reflecting both fibromuscular hyperplasia and intimal and subintimal changes of atheroma, this measure is reliably associated with both coronary atheroma and adverse clinical events.[24,39,40]

We carefully characterized the key biological determinants of clinical disease and endothelial function at baseline and examined their relationship to disease progression. Of these, FMD was the most closely associated with change in cIMT over a 6-year period. This finding provides novel evidence that is consistent with the concept of endothelial function as a causal determinant of atherogenesis, although our prospective follow-up data cannot confirm definitively the cause and effect relationship between the 2 variables. Although some major RFs were related to cIMT progression, this was less clear after multivariable analysis. This is likely to reflect the fact that each individual RF is only partially responsible for the development of arterial wall changes rather than a direct measure of overall adverse biological processes involved in disease development. Similarly, when RF data are presented according to quartile of cIMT progression (Table 2), waist circumference and triglyceride levels tended to be lower and HDL levels tended to be higher in those with most rapid progression. This is contrary to epidemiological data showing associations between abdominal obesity with metabolic syndrome RF characteristics and cardiovascular disease. LDL cholesterol levels were no different. Waist circumference, triglycerides, HDL, and LDL were not consistently associated with cIMT progression in all analyses, suggesting that type 1 error is the most likely explanation of these modest and unexpected associations. In contrast, the consistency of the association between lower FMD and greater cIMT progression across all of our series of analyses suggests that this relationship is robust. Notably, receiver-operating characteristic analysis confirmed the superiority of FMD over conventional risk assessment for the identification of those at an increased risk of more rapid cIMT progression. An FMD level of ≤4.1% was most informative for predicting acceler-

ated cIMT progression. The sensitivity and specificity of this threshold value to identify rapid progressors were both ≤65%, and the positive predictive ability was only 35%. This is in keeping with our previous findings that the negative predictive value of the test results was most powerful.[16] Our data do not currently support a role for FMD testing in routine cardiovascular risk assessment, but wider evaluation with standardized, high-quality testing protocols is warranted.[28]

Reactive hyperemia was not associated with cIMT progression and did not influence the relationship between FMD and cIMT progression, suggesting that it is the endothelial vasomotor response to flow that is linked to the progression of structural arterial abnormalities. Of note, brachial artery diameter was associated with cIMT progression. In keeping with the reported relationship between brachial artery diameter and cardiovascular prognosis, remodeling of the brachial artery may be related to evolution of clinical vascular disease.[13] This issue is somewhat confounded by the fact that the carotid artery diameter was not associated with cIMT progression in our study. Further work is required to clarify these relationships.

We studied preclinical individuals whose arterial wall biology and structure are more likely to be reversible and in whom intervention is most likely to have the greatest benefit on lifetime cardiovascular risk. Although our conclusions may apply only to low- to intermediate-risk individuals and further evaluation of this association between FMD and progression of structural disease in higher risk cohorts is required, ED has repeatedly been shown to be associated with a worse cardiovascular prognosis in such patients.[11–17,31]

Sixty-two participants were unable or unwilling to return for follow-up vascular assessment at phase 7. Other than diastolic blood pressure, which was slightly higher in these participants, all other baseline RFs, including FRS and FMD, were similar to those in participants who reattended. Although a borderline association was observed between diastolic blood pressure and IMT progression after age and sex adjustment, it is unlikely that our conclusions would have been instrumentally altered by the absence of these subjects, but a small influence cannot be excluded.

RF data that were missing at baseline were imputed from the participants' phase 3 data so that no subjects were excluded from the multivariable analysis. This technique optimizes the power of the study and avoids the possibility of any selection bias inherent in selecting only those subjects with complete data. The proportion of imputed data was low ($<$6% of the total RF data). These phase 3 data used for the imputation are our best estimates of the subjects' current RF levels, and this method has been used in previous reports from the Whitehall II study.[41] Analyses using the same models in which the subjects with incomplete RF profiles were excluded still demonstrated a significant association between FMD and IMT progression despite the lower statistical power and potential for confounding (data not shown).

We have shown for the first time that endothelial function as assessed by FMD predicts progression of structural carotid arterial disease even after taking into account the underlying RF profile. This result suggests that the combination of cIMT and FMD provides a measure of both current disease burden

and likelihood of progression. Our findings complement the previously reported role of endothelial function testing to predict risk of acute clinical events in higher-risk subjects.[12–17] Given the excellent reproducibility of FMD testing under carefully controlled experimental conditions,[28] FMD would therefore represent an attractive measure of vascular status for the assessment of intervention strategies in both the earlier and later stages of atherosclerotic disease.

Disclosures
Professor Deanfield is a British Heart Foundation chair holder; has been on the speakers' bureau for Pfizer, AstraZeneca, Merck Sharp & Dhome, sanofi aventis, Novartis, and Takeda; and has received honoraria for consultancy work from Pfizer, Novartis, sanofi aventis, Roche, and Danone. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Atherosclerosis is a multifactorial disease that begins long before its clinical manifestations. A need exists for measures that improve prediction of disease progression and risk stratification from the "preclinical" phase. Our findings in a group of middle-aged civil servants demonstrate, for the first time, that a noninvasive measure of endothelial function is associated with evidence of progression of carotid intima-media thickness over 6 years. This finding adds evidence that endothelial dysfunction not only is associated with activity of established atherosclerosis and risk of clinical events but also is likely to be involved in earlier disease development. Carotid intima-media thickness and flow-mediated dilatation together thus provide a measure of current disease burden and likelihood of progression. Flow-mediated dilatation testing is an attractive outcome measure for valuation of preventive treatments, as well as for use in patients with established clinical disease.